Mutagenesis and Directed Evolution in CHO Cells to Improve Bio-Production Characteristics

A thesis submitted for the degree of PhD

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Abstract

The purpose of this project was to investigate techniques that may be successfully applied to improve the characteristics of a bioprocess relevant mammalian cell line such as CHO (Chinese Hamster Ovary).

The first technique explored in this thesis investigates the possibility of gene targeting and knockout using an AAV (adeno associated virus) viral vector. A custom vector was constructed comprised of an insertion cassette flanked by homologous 'arm' sequences generated from the region of genomic DNA being targeted. Infecting cells with this virus at an MOI of over 2×10^5 particles per cell, we have demonstrated that this unique genomic locus can be successfully targeted with foreign DNA through homologous recombination in CHO cells, with a targeting frequency of 3.1% calculated in this instance. While single allele knockouts were detected using a DHFR targeting vector, a number of alternative techniques implemented did not successfully isolate homozygous negative mutants, however further work based on the data provided in this study could successfully generate null mutants.

The second technique applied is a variation of the directed evolution approach. A gene (TBP) was first selected that is known to be involved at a high level in the global cellular transcriptional machinery and subjected to random mutagenesis to generate a mutant library. When transfected this created a heterogeneous pool of diverse clones with a variety of phenotypes. An environmental stress was then applied for a number of passages to select for clones with suitable advantageous mutations, which were then isolated and characterised.
Using this technique we successfully isolated a number of mutant clones with average maximum viable cell densities 53% greater than a control cell line when placed through the same stress selective procedure. This improvement in growth coupled with additional benefits to late culture stage viability contributed to an increase in accumulated integrated viable cell density (viable cell-hours) of over 44%. A transcriptional profiling experiment was also carried out to investigate the underlying cellular mechanisms that may have contributed to these advantageous metabolic changes.

Abbreviations

aa	-	Amino acid
AAV	-	Adeno Associated Virus
AIVCD	-	Accumulated integrated viable cell density
bp	-	Base pair
BSA	-	Bovine serum albumin
cDNA	-	Complementary DNA
CHO	-	Chinese Hamster Ovary
CMV	-	Cytomegalovirus
DHFR	-	Dihydrofolate reductase
DIG	-	Digoxigenin
DMEM	-	Dulbecco's modified Eagle's medium
DMSO	-	Dimethyl sulphoxide
DNA	-	Deoxyribonucleic acid
dNTP	-	Deoxynucleotide triphosphate
DSB	-	Double stranded break
EDTA	-	Ethylene diamine tetracetic acid
ELISA	-	Enzyme linked immunosorbent assay
ER	-	Endoplasmic reticulum
GFP	-	Green fluorescent Protein
gTME	-	Global transcription machinery engineering
HEK	-	Human embryonic kidney
HPRT	-	Hypoxanthine-guanine phosphoribosyltransferase
HRP	-	Horseradish peroxidase
HT	-	Hypoxanthine/thymidine
Ig	-	Immunoglobulin
IMS	-	Industrial methylated spirits
ITR	-	Inverted terminal repeat
IVT	-	In vitro transcription
kb	-	Kilobase
LB	-	Luria-Bertani
min(s)	-	Minute(s)
MOI	-	Multiplicity of infection
mRNA	-	Messenger RNA
MTX	-	Methotrexate
NHEJ	-	Non-homologous end joining
PBS	-	Phosphate buffered saline
PCR	-	Polymerase chain reaction
PolyA	-	Polyadenylation

RMCE	-	Recombinase-mediated cassette exchange
RNA	-	Ribonucleic acid
RNAi	-	RNA interference
ROS	-	Reactive oxygen species
rpm	-	Revolutions per minute
S/MAR	-	Scaffold/matrix attachment region
SEAP	-	Secreted alkaline phosphatase
Sec(s)	-	Second(s)
siRNA	-	Short interfering ribonucleic acid
SV40	-	Simian vacuolating virus 40
TAF	-	TBP associated factor
TALEN	-	Transcription activator-like nucleases
TBP	-	TATA-Binding protein
TE	-	Tris-EDTA
TV	-	Trypsin versene
UCOE	-	Ubiquitous chromatin opening element
UHP	-	Ultra high purity
UPR	-	Unfolded protein response
v/v	-	Volume to volume
w/v	-	Weight per volume
ZFN	-	Zinc finger nuclease

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Section 1.0

Introduction

1.1 Introduction

In the last 35 years, genetic engineering technology has advanced from early methods used to construct artificial plasmids and generate the first recombinant organisms, to modern techniques that allow for the manipulation of a host cells genetic content with the aim of producing large amounts of recombinant therapeutic proteins. Modern medicine has chiefly relied on small molecule drugs to treat illnesses and disease. However, due to the power of protein biopharmaceuticals, the industrial focus is beginning to shift from small molecule drugs to more powerful recombinant protein therapies. Due to the proliferation of these therapies, there is a constant effort to improve production titres as a means of reducing the cost to manufacture these drugs, in turn leading to a wider availability of these treatments which have now been proven as important tools in dealing with a range of chronic illnesses.

Since the approval of the first recombinant protein therapy to be produced in mammalian cells over two decades ago, the industry has seen a drastic increase in the yields of batch production processes used to generate protein therapeutics. This increase has driven yields from milligrams per litre to titres of the order of grams per litre (Zhou et al. 1997). A number of factors have contributed to this rise in productivity. These include improvements to the physical formats used to propagate cells, optimisation of the culture media components, selection for cell lines/clones that show improved production characteristics as well as genetic manipulation of these target cell lines in order to increase levels of recombinant protein synthesis.
Early, basic recombinant proteins could be produced by expressing the requisite coding sequence in simple organisms (one of the first commercial applications of this technology involved the production of recombinant insulin in *E. coli* cells). However, with the advent of more complex proteins requiring intricate post translational modifications and the necessity for more efficacious therapeutic products, a more robust host was necessary leading to the widespread use of mammalian cell lines we see today.

While a variety of mammalian host cells have been or are currently being used to commercially produce therapeutic recombinant proteins, CHO (Chinese Hamster Ovary) has stood out for many years due to its range of positive traits necessary for large scale biopharma production. High growth, productivity and ease of adaptability to necessary conditions such as suspension growth make these cells a very attractive host cell line, with a majority of biopharmaceuticals currently being produced using CHO. With this in mind, it stands to reason that any improvements that could be consistently produced in CHO cells would have a positive influence on a wide range of commercial processes. Enhancements made to key properties of these cells in areas such as growth, density, lifespan and productivity have the impact of making these processes more cost effective, allowing new waves of biopharmaceuticals to be produced at a lower cost and ultimately reach a wider range of patients for whom these treatments may have a drastic positive impact.

This project was carried out as an attempt to explore alternative ways to improve the characteristics of these host cells. While a number of traditional techniques have proven to be successful in the past (transgene over expression, refinement of target gene expression vectors etc), we set out to explore alternative molecular biological techniques and apply them in CHO, with the ultimate objective of providing a means to improve their performance in an industrial setting.

1.2 Aims

This thesis consists of two distinct strategies, both with the aim of investigating techniques that may be successfully applied in CHO lines as a means of improving their industrially relevant characteristics.

The first strategy investigated involved the use of an adenoviral vector (AAV) for gene targeting. In industrial cells this approach has a number of potential uses including the targeted disruption of a selected gene, as well as the introduction of a gene of interest into a region of known transcriptional activity to improve productivity.
To exploit the process of homologous recombination within cells, first we create a viral vector with homologous targeting 'arms'; sequences identical to the genomic locus we wish to insert an exogenous gene or expression cassette. When introduced into the cell, these homologous arms then allow for targeted recombination to take place, and the vector becomes inserted at the intended site. This process has been demonstrated using simpler vectors, however the use of AAV has been shown to produce targeting rates in excess of those gained with traditional plasmid based recombination. While alternative techniques exist for gene knockout and gene targeting, AAV currently presents itself as an attractive alternative as being more effective than RNAi for the total elimination of an unwanted gene's effects, as well as being relatively inexpensive compared to novel methods such as engineered nucleases.
This study tests the hypothesis that gene targeting using AAV for the purpose of homologous recombination is a viable method for introducing exogenous DNA and disrupting native gene sequences in CHO cells. This will involve the characterisation of transduced cells for both targeted and off-target insertions, as well as screening successfully targeted cells for homozygous (-/-) null mutants.

The second technique investigated attempted to replicate a directed evolution technique described as 'global transcriptional machinery engineering', notably applied in bacterial (Alper and Stephanopoulos 2007) and yeast systems (Alper et al. 2006) in order to improve relevant phenotypic traits such as tolerance to alcohol. A gene

known to be involved at a high level in the transcriptional machinery is selected and mutated to create a diverse library. This is subsequently transfected into CHO to create a heterogeneous pool of clones, each expressing its own compliment of mutant sequences causing a unique transcriptomic shift in each cell.

The next phase of this technique involves the application of an environmental pressure or external stress to the population. In doing this we allow natural selection to take place, eliminating cells with detrimental changes and enriching for cells that have integrated advantageous mutants. With subsequent rounds of stress, we would expect these artificially adapted cells to gradually outgrow their counterparts in culture, leading to a population of cells more resistant to the environmental pressure and generating a more effective phenotype.

Natural selection works to select beneficial features from heterogeneous populations. Our hypothesis posits that an artificial increase in heterogeneity (in this case, a mutant gene library), followed by a suitable selection procedure will allow for the selection of individual clones more suited to an industrial growth environment. This study will examine the phenotypic effects of both the mutant library and selective stress procedure (the two main features of this approach) in transfected CHO cells by investigating key characteristics such as growth, viability and productivity. Transcriptomic profiling will also be used as a means of investigating the genetic mechanisms behind potentially beneficial changes in advantageous selected cell lines.

1.3 Mammalian Cell Line Engineering

Mammalian cell engineering has undergone a considerable amount of progress in the past decade as mammalian cell lines have established themselves as the predominant method for biopharmaceutical production. CHO cells in particular have been subject to a high level of research due to their attractive basic characteristics for protein production (fast growth, ability to grow in suspension, ability to apply post-translational modifications etc). As such, a vast majority of recombinant pharmaceuticals (estimates as high as 60-70%) are produced in these cellular factories, with titres as high as 5 grams per litre being achieve, orders of magnitude greater than those achieved decades ago (Wurm 2004).

Engineering methods undertaken to modify and improve cells such as these have themselves evolved from basic optimisation of growth conditions to complex modifications of metabolic targets to maximise their potential for productivity. Here we will examine this range of techniques for industrial cell line engineering and highlight the changes in approaches used; from basic techniques such as gene amplification, to the search for relevant gene targets and related regulatory sequences with significant impact on behaviour, through to the whole cell 'directed evolutionary' techniques that are now used to improve cellular fitness on a genome wide level.

1.3.1 Environmental optimisation

Before the use of any dedicated genetic intervention, media optimisation is an essential basic step in any bioprocess. For laboratory scale work, a vast number of media formulations exist for different cell lines and purposes, while many companies will invest a large amount of effort generating highly specific proprietary media formulations by perfecting the concentrations of carbohydrates, peptides and other media supplements to ensure maximum cell viability and productivity rates for industrial cell lines.

Animal derived serum was commonly used as an additive in cell culture media, due to the complex spectrum of macromolecules and growth factors present that supplemented cell growth. However, serum is commonly removed from most modern industrial bioprocesses due to immunogenic and pathological concerns regarding possible contaminants, as well as a batch to batch variability that does not afford tight regulation over components' concentrations. This is now overcome using strictly defined media additives, with key proteins found in serum such as albumin, insulin and transferrin now replaced by purified recombinant forms (Grillberger et al. 2009) that have shown to be effective replacements for animal derived products in optimisation experiments (Keenan et al. 2006).
Aside from media formulation, culture method is also a basic environmental factor that may be modified. These can range from basic adherent monolayer cell cultures, to the common 'cell suspension' bioreactor in batch or fed-batch configurations, to even more complex systems such as perfusion cultures in which cells grow attached to porous scaffolds allowing the removal of media while keeping the cells in place.

While modifications to the cellular environment such as the media or choice of culture method can have positive impacts on the overall productivity of the cell, genetic engineering of these cells is currently the main focus of the majority of experimentation in order to improve protein producing characteristics, as the gains made by approaches involved in environmental optimisation begin to plateau. By altering the genetic content of the cell, by means of introducing heterologous sequences into the cell or altering/eliminating the expression of native genes, we can begin to improve the performance of the cell beyond its natural basic capacity.

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1.3.2 Basic engineering methods

1.3.2.1 Plasmid vectors

The most basic forms of mammalian cell engineering often involve the transfection of plasmids, circular DNA molecules encoding a gene or sequence of interest, generally driven by a constitutive promoter if the gene of interest is to be expressed. Once transfected into a cell (for example using a common lipisomal based delivery system) this will allow for expression of the target gene whose gross effect on the cell can then measured. This effect can be measured transiently or by generating stably expressing cells using a suitable antibiotic selection marker to induce integration into the genome.

Modern plasmids (circular genetic components originally isolated from bacterial cells) are generally engineered to contain a number of key features making them attractive to use as vectors. These include a constitutive strong promoter to drive transgene expression (such as common viral promoters SV40 or CMV), an antibiotic resistance marker to promote integration into a host genome, as well as a multiple cloning site for digestion and insertion of an experimental transgene or sequence.

While plasmid transfection is not the only vector used for introducing exogenous sequences into cells (viral vectors are also used extensively for different purposes), they are generally the most popular due to the relative ease with which they can be manipulated, amplified and delivered into cells. This technique of gene delivery has been the standard tool for elucidating the function of numerous genes in many cultured cell lines for decades and is the basis for a large portion of cell line engineering.

1.3.2.2 RNAi-based gene knockdown

Conversely, the effects of gene silencing and the removal of a genes effect from the cell can be examined through the use of short interfering (siRNA) molecules. A relatively recent discovery in molecular biology, this mode of naturally occurring post-transcriptional gene regulation was first described in plant cells (Hamilton and Baulcombe 1999) and soon confirmed as present in mammalian cells (Elbashir el al. 2001).

siRNAs are small, double stranded RNA molecules that play an important role in post translational silencing of genes via the RNA interference (RNAi) pathway. In this mechanism of gene regulation double stranded RNAs are transcribed and then subsequently processed by an endoribonuclease known as Dicer into short (21-25 bp long) molecules that become incorporated into a protein complex known as the RNAi induced silencing complex (RISC). This activated RISC can then recognise mRNA sequences based on sequence specific homology with the short RNA sequence contained within, and target them for degradation prior to translation, reducing the levels of expressed protein in the cell.
To transiently examine the effects of the knockdown of a particular gene, artificially generated siRNAs can be transfected directly into the cell. These can be enzymatically generated in vitro using a purified Dicer enzyme and double stranded template oligos or, more commonly, they are chemically synthesized and bought commercially. This type of transient effect found with transfected siRNA generally lasts for no more than 3-5 days (Amarzguioui, Rossi, and Kim 2005). In order to overcome the short lifespan of single doses of siRNA, constant transcription of these molecules can be artificially induced within the cell using vectors stably generating RNAi effector molecules to generate a more consistent and prolonged effect within the cell. Vectors are created in a similar fashion to those designed to over express a target protein, using polymerase II or III promoters to drive expression of a cassette producing short hairpin RNAs (shRNA) that are in turn processed and integrated in the RISC complex to produce a reduction in target gene translation (Wu 2009). Selectable antibiotic markers can also be used to induce stable vector integration and generate cell lines with persistent gene knockdown effects.

These two basic methods of engineering based on the overexpression or repression of target genes form the basis of a wide assortment of engineering strategies in mammalian cell lines. A range of applications based on these strategies and tools exist, allowing for a number of different means to improve cell characteristics. Outlined below are some of these various applications and how they are used to improve industrial mammalian cell lines.

1.3.3 Gene amplification

Many basic engineering strategies to date have focused on increasing the amount of cellular transcription as a means of increasing the final protein titre, such as increasing gene copy numbers or improvements to expression vectors that improve mRNA production. Gene amplification is a well known and relatively easy method of increasing the expression levels of a target gene, often a gene encoding a product of value to be expressed. This is carried out by first introducing a vector encoding the target gene into the genome paired with a selectable marker that can be used to subsequently amplify the construct. Once a stably transfected cell line is developed, an inducer molecule is added in gradually increasing concentrations, such as an antibiotic or other cytotoxic compound. The negative effect of this molecule is mitigated by the selectable marker gene, which multiplies in frequency within the genome in response to the addition of higher inducer concentrations. Any genetically linked sequences such as the target gene are also then multiplied, leading to increased target mRNA expression levels and, ultimately, greater product yield (Omasa 2002).

1.3.3.1 DHFR amplification

The most common of these systems is the *dhfr* gene amplification system, in which a DHFR deficient cell line is transfected with the *dhfr* gene linked to a target cDNA. Methotrexate (MTX), a toxic inhibitor of DHFR, is used as an inducer at increasing concentrations with amplified copy numbers of over 1000 frequently reported using *dhfr* amplification system (Crouse, McEwan and Pearson 1983; Kaufman and Sharp 1982). However, relative gene expression levels can vary from little or no increase, up to 10-30 times the level prior to amplification. This is due in part to phenomena such as variety in chromosomal integration positions, as well as imperfect copies of the amplified cassette generating non functional mRNA. Genes maintained at high copy numbers may be subject to instability however, with reductions in gene copy number observable when the inducer molecule is removed for extended periods (Johnston, Beverley and Schimke 1983). This is a reasonably simple technique that can be employed to maximise productive output, with little or no optimisation necessary beyond the steady increase in the concentrations of inducer molecule.

1.3.3.1 GS amplification

A similar system also commonly used in CHO is the Glutamine Synthetase (GS) amplification system. In the same way that MTX is found to inhibit DHFR, the compound methionine sulfoximine (MSX) was discovered as an antagonist of GS, with natural cellular resistance to its effects achieved through amplification of the locus encoding the GS gene (Sanders and Wilson 1984). This was exploited to generate the GS amplification system, in which the gene of interest is linked to a recombinant GS gene. Addition of MSX causes amplification of the GS gene as well as co-amplification of the linked gene of interest, ultimately increasing target product expression and titre. This system was also found to have the advantage of requiring fewer rounds of selection with increases of MSX inducer for sufficient amplification, often gaining maximum benefits after a single round (Brown et al. 1992).While high copy numbers similar to the *dhfr* amplification system are reported (Sanders et al. 1987), the relative instability of clones generated compared to the *dhfr* system has been cited as a concern (Jun et al. 2006) as well as its status as a proprietary technology requiring a license from its owner Lonza.

1.3.4 Apoptosis prevention

Apoptosis is defined as the mechanism for programmed cell death, a natural process in living organisms that has been extensively studied and many of its causes and their associated pathways have been well characterised. Therefore, the genes and proteins involved have become prime targets for genetic regulation in order to mitigate the destructive effects apoptotic stimuli might have on cultured cells in a bioprocess. By preventing or reducing the effects of apoptosis in a commercial process, we can expect to increase the length of a viable production culture, thus leading to an increase in product titre. Its has been shown that in the later stages of batch culture the vast majority of cell death is caused by apoptosis rather than the necrotic effects of the culture environment (Goswami et al. 1999). This fact has lead to the development of novel methods of suppressing cell death via the alteration in the levels of pro- or anti-apoptotic genes with the aim of maintaining the viability of a batch culture, thus leading to an overall increase in final product yield.

1.3.4.1 Anti-apoptotic gene expression

A number of studies have examined the effect of apoptosis related genes. One example demonstrated the generation of an apoptosis resistant CHO cell line by stably over expressing *bcl*-2 (Lee and Lee 2003), an anti-apoptotic gene that inhibits the apoptotic effects of caspase proteins. This study attempted to overcome the apoptotic effect of sodium butyrate (NaBu), a molecule shown to increase expression of foreign proteins at the cost of cell viability. It was confirmed that stable over expression of *bcl*-2 could improve total cell viability and mitigate the negative effects of NaBu, leading to an increase in recombinant antibody production.

The Bcl-2 gene family also contains a number of evolutionarily related genes with comparable functions that have been targeted for cell engineering purposes. Overexpression of Bcl-2 related proteins such as Bcl-xL (Kim et al. 2009; Majors et al. 2008) and Mcl-1 (Majors et al. 2009) have shown to repress caspase activity and prolong culture viability in recombinant CHO cell lines. An experiment involving the synergistic co-expression of Bcl-2 and c-Myc was also carried out. This study showed that Bcl-2 could successfully mitigate the apoptotic effects of overexpressed c-Myc, while retaining its positive effect on cell proliferation (Ifandi and Al-Rubeai 2005).

1.3.4.2 Pro-apoptotic gene knockdown

In logical contrast, studies have demonstrated that knockdown of genes that assist in apoptotic pathways also proves useful in delaying the effects of programmed cell death. One such study investigated the downregulation of pro-apoptotic genes Bax and Bak using an RNA interference method (Lim et al. 2006). A CHO cell line expressing anti Bax and Bak siRNA was generated with a 90% reduction in expression of these genes. These cells were then subjected to a number of chemical stimuli and physical conditions known to promote apoptosis. These included stimuli commonly found in industrial batch conditions such as depletion of nutrients and high osmolarity due to waste build-up. In all cases, the knockdown CHO line demonstrated greater resistance to apoptosis and higher viable cell densities than the control, leading to a longer culture lifespan and ultimately a higher recombinant protein titre. This effect has been replicated in a number of other experiments, with different apoptotic genes targeted for knockdown. One example used data from a previous gene profiling experiment that identified apoptosis signalling genes. 4 of these targets (FADD, FAIM, ALG-2 and *Requiem*) were stably silenced in an Interferon producing CHO cell line. This gene silencing led to a reduction in apoptosis in both batch and fed-batch culture configurations, with a reported increase in Interferon yields of up to 2.5 times higher (Wong et al. 2006).

A number of disadvantages with the stable siRNA method have been reported. These include difficulty in isolating clones with sufficient knockdown, lack of long term stability in these clones as well as the possibility for non specific interference effects (Lim et al. 2006). Transfection of increasing numbers of vectors to affect multiple targets may also place a transcriptional burden on the cell that may otherwise cause metabolic bottlenecks or eventual net loss of productivity. This would suggest that absolute gene knockout would be preferable for genes known to have a definitive positive outcome when their effects are removed from the cell. Evidence of this difference in efficacy has been shown in a comparative study involving Sam68, an RNA binding protein implicated in a number of cellular processes (Taylor, Resnick and Shalloway 2004). A knockout cell line was compared to a constitutive siRNA knockdown cell line, with the conclusion drawn that the knockout cell line provided more accurate results than the siRNA knockdown cells (Li et al. 2010).

1.3.5 Cell cycle arrest

Similar in approach to interventions designed to prevent apoptosis, a commonly used strategy in modern industrial processes is the use of cell cycle arrest to promote both an increase in specific productivity as well as a delay in the onset of apoptosis. This has been demonstrated as a proven method of directing cells energy expenditure from metabolic processes involved in growth and cell division to those involved in production and secretion of target recombinant products, employing methods such as temperature shift and induction of cytostatic proteins (Kaufmann et al. 1999; Fussenegger, Mazur and Bailey 1997).

1.3.5.1 Temperature shift

The most common iteration of this technique is the use of thermal shock in order to harness the positive effects of native temperature-inducible proteins. Numerous experiments to date have documented the positive effects of temperature shift on cell productivity. One illustrative study was carried out on CHO cells in which the surrounding media temperature was reduced from 37°C to 30°C after an initial growth phase (Kaufmann et al. 1999). Epitomising the effect found at the heart of the temperature shift method, this technique demonstrated a halt to the cell cycle in the G1 phase prior to mitotic division in a majority of cells, leading to an increase in specific productivity as well as a reduction in waste products such as lactate and ammonia. This approach demonstrates the biphasic growth pattern, where cell growth is allowed to reach high densities then is halted by temperature shift, channelling the cells resources towards generation of the target product. This increase in productivity has been repeatedly demonstrated as effective in various CHO cell lines. Examples of other studies successfully implementing a biphasic culture include Fox et al. 2006 (90% increase in product titre at 32°C), Fogolín et al. 2004 (3.2 fold increase in productivity at 33°C combined with PYC2 overexpression) and Chen et al. 2004 (47% increase in productivity in a high density perfusion culture system).

While the ultimate effect of this technique is well established, the intracellular processes that govern the temperature shift response are as of yet not entirely elucidated, but recent studies carried out have begun to uncover a number of

differentially expressed protein and RNA molecules that are implicated in the resulting change in metabolism during temperature shift (Kumar et al. 2008, Gammell et al. 2007). These targets may prove useful for rational engineering efforts to harness the positive effects of these temperature inducible molecules.

1.3.5.2 Inducible systems

Many cell engineering methods describe the use of constitutive expression of molecules designed to continuously produce or, in the case of siRNAs, reduce the levels of a given protein within the cell. However, in some cases the persistent metabolic change may not be the ultimate goal, with temporal control over gene expression at an appropriate point in the growth culture existing as an attractive alternative in some cases. This is relevant with regard to genes critical to cell growth and proliferation for example, that may be detrimental to product formation once a suitable cell density has been reached. In instances like these, the use of inducible systems is indicated as being an advantageous approach. Temperature shift remains a common tool for cell cycle arrest in industry today, however the energy and engineering requirements necessary to repeatedly heat or cool large volumes (thousands of litres) carry inherent costs and difficulties. That may be alleviated by alternative strategies, such as the use of molecule induced expression systems to provoke cell cycle arrest. This has been achieved using a number of inducible systems with positive results.

An example of a system used in examining the benefits of regulated expression can be seen in the ‘tet-off’ tetracycline responsive expression system, a prevalent method of controlling gene expression. It is regulated by a fusion protein, the tetracycline transactivator (tTA), containing two halves. One half is derived from a tetracycline responsive repressor protein found in *E. coli*, while the other half is an activator of transcription derived from the Herpes Simplex VP16 protein. In the ‘tet-off’ configuration, this chimeric transactivator protein binds to the ‘tetO’ operator sequence in the absence of tetracycline, activating a local promoter sequence and driving transcription of any upstream gene. The addition of tetracycline to the cellular medium inactivates this switch and prevents transcription.

This configuration typifies the construction of most artificial inducible systems, with a protein 'switch' (responsive to a specific inducer molecule) coupled to a domain capable of regulating transcription when the system is activated.

This system was used in a study (Fussenegger, Mazur and Bailey 1997) to regulate the expression of both a reporter gene and one of a number of cytostatic proteins involved in the cell cycle such as p21, p27 as well as a mutant version of the p53 gene capable of arresting cell cycle progression, but unable to induce apoptosis. Cell proliferation was halted upon depletion of tetracycline from the media, leading to roughly a fourfold increase in SEAP production relative to a constitutive control producer. A greater proportion of substrate converted to desirable product was noted within the process, as well as a reduction in the amount of waste product generated such as lactate and ammonia.

Another study investigated the effect of an inducible antisense c-*jun* gene in murine erythroleukemia cells, the effect of which is the down-regulation of c-*jun* and an inhibition of the cell cycle (Kim et al. 2000). It was shown that induction once logarithmic growth had begun could prolong protein production at high cell viabilities for double the time period of control cells. This strategy also carries the advantage of reducing the levels of apoptotic cells releasing destructive enzymes into their environment, degrading the product within the surrounding medium and having a detrimental effect on the quality and quantity of the recombinant product being manufactured. A similar effect was seen in a study in which an inducible CHO cell line was generated using a system responsive to the insect hormone ecdysone (Figueroa et al. 2007). Induction of the anti-apoptotic proteins E1B-19K and AVEN in tandem was carried out 2 days into fed cultures, resulting in increased viability and total productivity due to operational times 5 days longer than control cell lines.

The idea of a temporally controllable system (for example, one that can be activated once a process reaches a required cell density) is an attractive one. The downside to the use of such chemical inducible systems on an industrial scale remains the cost of large volumes of inducer molecule if a drug sensitive system is used, and its possible complication of downstream processing and purification. However, with the potential for further optimisation and reduction in the necessary concentrations of inducer molecule techniques such as these may still prove useful in the creation of an optimised cell line for industrial scale protein production.

1.3.6 Metabolic engineering

Improvements in expression systems have allowed for greater levels of transcription and, to an extent, the amount of translated product within the cell. However, experiments have shown that secreted protein levels do not improve when mRNA reaches a saturation point (Barnes, Bentley and Dickson 2004). Great increases in transcription may also lead to diminishing returns, as cellular machinery may struggle to process and secrete large amounts of transcribed mRNA leading to translational attenuation and protein degradation as a result of the Unfolded Protein Response (UPR) (Schroder 2006). This is one of a number of key biological bottlenecks to overcome as increasingly higher levels of production are required to satisfy the demand likely to be placed on the biopharma industry as protein drugs become more prolific. This has lead some research to focus on developing and improving post transcriptional processes as well as the cells metabolic ability to cope with the synthesis and processing of large amounts of recombinant product.

1.3.6.1 Product formation and processing

Many experiments carried out to date have demonstrated that augmenting the machinery of the cell can remove inefficiencies and bottlenecks from the processes, therefore boosting the output of the cell. In some cases this has been achieved by overexpressing proteins such as chaperones and enzymes that take part in key protein processing pathways. For example, a study carried out in IgG producing insect cells demonstrated that overexpression of the protein chaperone BiP could generate a 2.5 fold increase in IgG secretion (Whiteley, Hsu and Betenbaugh 1997). BiP, a protein responsible for preventing protein aggregation prior to correct folding and processing within the cell, was shown to reduce the levels of improper association and aggregation between heavy and light antibody chains within the cell, allowing for a greater level of secretion into the medium of properly folded antibody. A similar experiment was carried out overexpressing Protein Disulphide Isomerise (PDI) in CHO cells producing two different products (Mohan et al. 2007). While one product was shown to have a marked increase in expression (15-27%), the other product showed no increase. This was possibly caused by a difference in the amount of disulphide bonds (the site of PDI’s catalytic action) between products as different

proteins will exert different stresses on the cell, or perhaps that the basal level of PDI is sufficient for the processing of some products with no bottleneck to be overcome with increased expression.

A related study investigated the effects of both BiP and PDI proteins' effects in IgG producing CHO (Borth et al. 2005). While increases in PDI did indeed allow for less retention of IgG heavy chain, and ultimately greater secretion of the active product, these results also demonstrated a reduction in product secretion with BiP upregulation in contrast to previous results, such as that of the insect derived cell model. Reasons for this result have been speculated on based on modelling of cellular pathways, including limiting factors such as concentrations of free ATP and other co-chaperones (Khan, Schroder 2008). As the elucidation of the functions and interactions of these processing pathways are still ongoing, conflicting reports as to the efficacy of interventions are to be expected, especially with regards to differing cell lines and products being expressed. Also, due to the complex interactions and feedback loops that affect these pathways, it is likely that a more coordinated regulation of the expression of a selection of metabolic proteins such as chaperones, foldases and glycosylation enzymes would be necessary for a reproducible effect on the output of mammalian producer cells.

1.3.6.2 Waste forming processes

The targeting of genes involved in the production of waste products has also proven successful in reducing the secretion of these detrimental compounds as well as improving target protein production. For example, a study was described in which a copy of a CHO lactate dehydrogenase gene (LDH-A) was disrupted using homologous recombination (Chen et al. 2001). Following a 50% reduction in LDH activity, this led to a 2 fold increase in specific antibody production as well as a 30% increase in total cell density. A more recent study used siRNA in CHO cells to downregulate LDH-A as well as pyruvate dehydrogenase kinases involved in the TCA cycle (Zhou et al. 2011). This combined RNAi approach allowed for greater reduction in target expression, leading to a 90% reduction in lactate production and a 75% increase in specific productivity.

1.3.6.3 Targeting of regulatory genes

Another approach to achieve an improved metabolism that is being explored is to modulate the expression of targets that ultimately control activity of a number of genes themselves rather than target single, limited function proteins such as those involved in protein folding, secretion or waste production. This approach seeks to simplify the levels of change that needs to be affected on a biological system by targeting the expression of small numbers of regulatory genes responsible in turn for the expression of a wider range of useful genes. The metabolic strain that is placed on a cell when multiple beneficial genes are overexpressed using strong constitutive promoters may ultimately have negative effects on the phenotype. This may limit the amount of direct engineering of individual genes possible, with an eventual maximum negating any prior benefit due to the hijacking or overloading of the cells transcriptional and translational machinery by the same targets intended to improve their action.

This approach of targeting high level regulatory genes was followed in a study examining the effects of XBP1. A gene analysis study was carried out comparing *xbp1* deficient cells with wild type plasma cells (Shaffer et al. 2004). This gene, a transcription factor known to be implicated in the regulation of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) related genes as well as the cells secretory pathway, was shown to have a broad improvement on phenotype including increased overall cell size, ER surface area and total protein synthesis, leading to a speculation of its role a ‘master regulator’. This effect was confirmed in CHO cells (Tigges and Fussenegger 2006), whereby overexpression of transgenic XBP1 provided a boost to the post translational processing capacity of production CHO lines, in some cases up to 5-fold.

Similarly, a study was carried out in which the serine/threonine kinase mTOR was overexpressed in CHO (Dreesen and Fussenegger 2011). The mTOR pathway is known to integrate a wide range of intra-cellular signals relating to translation, energy, growth, survival and proliferation, and was chosen as an engineering target based on its involvement in this myriad of cellular processes. Ultimately the overexpression of recombinant human mTOR in CHO was found to have a beneficial impact in the areas of specific productivity and secretion, cell size, proliferation and viability, increasing productivity four-fold compared to a parental control cell line.

Examples such as this demonstrate the benefits of shaping whole pathways and systems, rather than modulating the expression of single genes with discreet functions. Increasing knowledge of mammalian processes gained through wide ranging ‘-omic’ studies, such as transcriptomic, proteomic and next-gen sequencing studies, will provide greater knowledge of the complex interactions within cells, allowing us to highlight important targets with beneficial downstream metabolic effects whilst avoiding those with potentially negative side effects on growth or production.

1.3.7 Secondary vector elements and epigenetics

The approaches described previously demonstrate the power of regulating elements within the cell such as genes and RNA molecules to improve the cells phenotype and ultimately generate more of a protein product within a given bioprocess. These trans-acting elements function in such a manner that shapes the cells metabolism and redirects its resources to pathways that allow it to produce and secrete more efficiently.
A complementary approach to these techniques is to further engineer the vectors that deliver the product-containing gene and add secondary cis-acting elements; regulatory sequences that function to improve the expression of a target gene and ultimately allow for greater levels of productivity. These elements can have various functions, such as the reorganisation of nuclear chromatin to allow greater access by transcriptional machinery or the negation of negative epigenetic factors that repress gene expression and avoid the long term gene silencing associated with some exogenous sequences such as viral promoters.

Despite high transgene copy numbers and good initial productivity, many cell lines are found to be unstable in the absence of an inducer molecule such as methotrexate. While genetic instability and loss of gene copies have been implicated as reasons for this decrease in productivity (Weidle, Buckel and Wienberg 1988), other causes for this drop in productivity include DNA methylation and histone deactylation affecting chromatin accessibility, examples of epigenetic modifications leading to inaccessible regions of nuclear chromatin that are unable to remain transcriptionally active (Barnes, Bentley, and Dickson 2003; Razin 1998). This has been observed in CHO cells, with reduction in activity of trans-acting factors eliminated as an issue and, in some cases, productivity temporarily restored by the use of a DNA methylation inhibitor (5-Aza-2-deoxycytidine) (Yang et al. 2010) or a histone deacetylase inhibitor such as sodium butyrate (Davie 2003). However, in an attempt to circumvent the need for the persistent use of small molecules in bioprocesses, genetic factors have been investigated in order to reduce the effects of transcriptional gene silencing from epigenetic factors such as these.

1.3.7.1 Scaffold/matrix attachment regions

The use of 'scaffold/matrix attachment region' (S/MAR) sequences has been investigated in mammalian cells. These sequences are thought to serve by facilitating the binding of the nuclear matrix to DNA, organising nuclear DNA into defined functional chromatin loops, promoting position-independent transcription of transgenes and reducing negative random position effects and reducing the differences between clones with varying random inserts (Kim et al. 2004). A number of different S/MAR elements originating from different sources have been examined, with positive effects on transgene expression reported from elements such as the chicken lysozyme and human β-globin S/MARs (Wang et al. 2012; Zahn-Zabal et al. 2001). While the levels of success reported in studies utilising S/MARs seems to vary depending on the combination of cell line and elements used, their relatively small size (~3 kb) and ability to improve expression even when co-transfected on a separate plasmid (Zahn-Zabal et al. 2001) make them an potentially useful tool for generating stable, high producing clones with a reduced need for extensive screening.

1.3.7.2 Ubiquitous chromatin opening elements

A class of sequence element retaining a similar function to that of S/MARs are known as Ubiquitous Chromatin Opening Elements (UCOE). UCOEs are promoter like elements associated with ubiquitously expressed housekeeping genes. They contain extended CpG islands found to be resistant to methylation and the effects of heterochromatin formation related to transgene silencing.

A number of studies have been carried out combining the common CMV promoter with fragments derived from UCOE-like elements. One study described a vector including an 8 kb UCOE fragment, resulting in a far higher number of clones expressing high levels of a GFP control when analysed using flow cytometry (Benton et al. 2002), drastically reducing the level of screening necessary to find suitably high producers. Another study, examining a smaller 4 kb fragment isolated from a human genetic locus (driving HNRPA2B1 and CBX3 housekeeping genes' expression) found not only an increase in the number of stable clones generated, but also 2-4 fold higher average levels of recombinant protein production (Boscolo et al. 2012).

While spare vector space may be a limiting factor for some applications, UCOE fragments as small as 1.5 kb isolated from a human locus expressing housekeeping genes TBP and RNP were found to confer greater than 20 fold improvements in eGFP expression over 100 passages compared to a hCMV promoter alone (Brooks et al. 2004). Examples such as these make UCOEs, as well as S/MARs, attractive potential additions to vectors to improve phenotype in relation to productivity without the need for more potentially disruptive genetic interventions.

1.3.7.3 Use of endogenous promoter sequences

Another potential avenue for increasing specific productivity, while avoiding the problems associated with some exogenous sequences (for example, gene silencing associated with the strong CMV promoter, Brooks et al. 2004) is to use endogenous, mammalian derived promoters. One example of this is the successful use of regulatory sequences isolated from the CHO EF-1α gene (Running-Deer and Allison 2004). While mammalian promoter sequences and their associated regulatory sequences are not currently well defined, vectors containing various 5' and 3' EF-1α gene flanking sequences were found to successfully induce gene expression between 3 and 26 times greater than commonly used promoters such as CMV. This effect was also stably sustained without substantial gene silencing after 50 passages without inducer molecule (MTX). Interestingly, high expression (up to 10 fold increase) was also measured in non hamster mammalian cell lines. An obvious drawback to this example, however, is the fragment size necessary for expression, with at least 8 kb including both upstream and downstream regulatory sequences providing the greatest effect, compared to the comparatively small size of constitutive viral promoters.

Another novel use of mammalian promoter sequences is found in a study by Thaisuchat et al. (2011), in which a 1.5 kb upstream regulatory region of a CHO gene, whose expression was found to correlate strongly with temperature shift, was assayed for promoter activity. This approach led not only to successful gene expression at regular temperatures, but expression 3 times greater than an SV40 viral promoter construct when temperature was shifted to 33°C. This conditional expression confers the benefit of reducing unnecessary target gene activity during the 37°C growth

phase, and also boosting productivity in tandem with the metabolic advantages gained during temperature shift as outlined previously.

Constructs such as this containing secondary sequences that enhance transgene expression may be currently ill-suited for implementation in industrial bioprocesses due to their cumbersome size and less than distinct sequence characteristics. However, as mammalian regulatory sequences become better defined and our understanding of core sequences necessary for these elements to function increases, we can expect to see such sequences used more often to help improve vectors engineered to deliver recombinant genes for biopharma production.

1.3.8 Viral vectors

By no means a novel technology, viruses have been extensively studied as a means of introducing target sequences into mammalian cells due to their innate ability to infect cells and deliver DNA in an efficient manner. While lentiviruses and retroviruses have been studied and used successfully, adeno associated viruses (AAV) have proven a popular vector for the genetic modification of a number of mammalian cells, including mouse, hamster and human derived lines. As a gene delivery vector it has a number of qualities that make it a good candidate for such a role. It causes no known diseases in humans making it safe for laboratory use, it displays an inability to replicate independently, necessitating a helper adenovirus to reproduce, as well as having the ability to transduce both dividing and non dividing cells giving it a more versatile transduction profile than similar viruses (Schultz and Chamberlain 2008).

1.3.8.1 Adeno-associated viral vector

AAV is a small parvovirus with a single stranded DNA genome known to infect a number of host organisms. It generally exists as a latent 'provirus' due to its ability to integrate within a host cells' genome, but requiring the assistance of a secondary helper virus (such as an adenovirus) for lytic replication. These properties marked it as a good candidate for development of a safe and effective mammalian transduction vector when originally studied. Early experiments with recombinant forms proved successful at both transduction and expressing transgenes, with viral rescue and reproduction possible with subsequent infection of an appropriate helper virus (McLaughlin el al, 1988; Hermonat and Muzyczka, 1984). To date a range of AAV serotypes with varying tropisms have been discovered (Mori et al. 2004), however AAV serotype-2 remains the most extensively studied and utilised for mammalian cell engineering.

Due to the extensive study carried out on AAV, recombinant AAV generation is now relatively straightforward. The wild type AAV genome consists of two open reading frames encoding for key replication (*rep*) and packaging genes (*cap*), as well as flanking inverted terminal repeat (ITR) sequences critical for virus packaging. In recombinant vectors used for molecular engineering, these replication and packaging genes are removed, allowing for the insertion of any target transgene or DNA

sequence of interest (Adachi and Nakai 2011). For viral generation, these replication and packaging genes are delivered *in trans* on a separate plasmid to the cell line to be used for replication (human embryonic kidney cells are commonly used) along with a 'helper' virus or plasmid containing the other genes necessary for AAV reproduction.

1.3.8.2 AAV capacity

Adeno associated viruses carry a natural genome capacity of roughly 4.7 kb, of which ~0.3 kb is utilised by the mandatory ITR sequences. For recombinant vectors an optimum total of ~4.9 kb as been reported, with sharp decreases in packaging efficiency observed above this total (Dong, Fan and Frizzell 1996). Methods have been devised however, in an attempt to circumvent this limit and deliver larger constructs than would otherwise be possible using a single vector. One method was to simply co-infect with two viruses, each encoding a separate element of the total construct. It was found that separate viral genomes could form intermolecular concatemers after uptake within infected cells, linking the viral genomic elements prior to integration into the host genome. One example demonstrated a 600 fold increase in luciferase expression when the primary virus containing a minimal promoter was co-infected with a secondary virus containing extra promoter and enhancer elements, with circular concatemers confirmed within the cells (Duan et al. 2000). This demonstrates the ability to successfully transduce cells with modular sequences, utilising this concatemer-forming property to reconstruct larger complex expression cassettes from its individual components. This concatemerisation feature of dual vectors has also been used to reconstruct larger genes that would not otherwise be possible to package by introducing splice sites in between constituent exon-like elements that could be recognised and reconstructed by the targeted cell lines natural splicing machinery (Ghosh et al. 2008).

1.3.8.3 Integration site preferences

Many studies based on this method of introducing foreign DNA into host cells rely on random integration into the host genome, in a similar manner to that of any stable transfection using plasmid vectors. Wild type AAV is found to integrate preferentially into a location on chromosome 19 in human cells, however the recombinant forms of

AAV commonly used are engineered to lack the requisite Rep protein (which is instead transfected separately for viral replication). Mapping studies of its integration sites demonstrated that AAV preferentially integrated into gene sequences with a frequency of 53% (Nakai et al. 2005), with targeted genes often ones currently being expressed in the transduced tissue (Nakai et al. 2003). As there is lack of homology between recombinant AAV vector sequences and apparent integration sites, it has been suggested that random non-homologous recombination is the likely method of integration for non-targeted insertion events (Rutledge and Russell 1997). With relation to AAV2's possible use as a means of altering recombinant protein producing cells, this trait could allow for random insertion of transgenes into active genomic regions with greater frequency and possibly avoid the negative position effects associated with transcriptionally inactive heterochromatin.

1.3.8.4 Alternative viral vector systems

The versatility and high efficiency of AAV viral vectors make it a useful tool for the alteration of mammalian cells. It allows for a range of genetic alterations, coupled with relatively high efficiency and specificity when used in conjunction with homologous recombination. However, as it is the chosen vector for the gene knockout experiment carried out in this study, it is used here as an example of a successful vector, and not the sole method of transduction in mammalian cells. For example lentiviral vectors, another well known viral vector for use in mammalian cells, are generated in a similar fashion by separating genes necessary for reproduction from the functional vector genome (Trono 2000). It has the advantage of a larger capacity approaching 10 kb in size, and has been proven as a viable means of generating stably recombinant mammalian cells (Gaillet et al. 2010). Retroviral vectors, such as those based on recombinant forms of murine leukemia and Rous sarcoma viruses have also been investigated for use in mammalian cell lines (for example, Barsov and Hughes 1996), however these are more commonly associated with *in vivo* gene therapy (Hawley et al. 1994)

Differences in the transduction profiles and characteristics of various viral vectors indicate that vector choice should be most relevant for the purpose at hand. For example, *in vivo* and *in vitro* investigations of AAV and lentiviral transduction and targeting rates have indicated an advantage using AAV in a particular set of tissues

tested (de Backer et al. 2010), indicating that the viral vector to be used should be chosen carefully based on its application and cell type to be transduced.

1.3.8.5 Gene targeting

Random integration can be used for integration and expression of a given transgene. However, a common engineering application for viral vectors is their use in gene targeting via targeted homologous recombination, rather than random insertion. They have been routinely used for gene knockouts and to introduce sequence mutations in mammalian cells with efficiency many times greater than those achieved with standard plasmid based transfection methods. Studies carried out in human cells have comprehensively demonstrated this use for a number of different insertion events. Successful studies carried out to date using AAV have demonstrated their effectiveness using a variety of vector configurations. These include

- Gene repair of a mutant selection marker in order to restore function (Russell and Hirata 1998)
- Insertion into a natively expressed gene sequence to disrupt expression (Russell and Hirata 1998)
- The introduction of a single base pair mutation (Inoue et al. 2001)
- Targeted insertion of a functional transgene cassette (Hirata et al. 2002)

In all cases relatively high targeting rates were obtained (approaching 1% of cells), a vast increase over traditional transfection based methods that could achieve targeting events in the order of 10^{-6}-10^{-8} per cell (Williams et al. 1994).

1.3.8.6 Potential uses in mammalian cell line engineering

The usefulness of viral vectors in the engineering of industrially relevant cell lines becomes apparent when we consider this ability to accurately target genetic loci with such a relatively high frequency.

The most obvious beneficial use for this technology is the targeting and disruption of genes with detrimental effects on producer cell lines. As described previously, gene knockout using homologous recombination has shown to provide more effective results when compared to gene silencing technology such as siRNA (Li et al. 2010).

This ensures low level expression that may not be totally eliminated by RNA interference does not impact on phenotype, while removing the need for a constitutively expressive siRNA cassette that may be subject to stability problems in the long term. While RNAi may prove useful in screening and validation of such targets, true elimination of a genes effect on the cell is likely best suited to gene knockout facilitated by homologous recombination. As part of this project, such an investigation was carried out as to the potential use of an AAV vector for the targeting of specific loci within the CHO genome.

Another potentially useful application of gene targeting for improved recombinant protein production is the specific targeting of known transcriptionally active 'hot-spots' within the genome. Theoretically, by attempting to insert target DNA in areas of the genome known to contain transcriptionally active genes, one could hope to improve the levels of transgene expression by virtue of their location in open chromatin.

One study was carried out involving the use of a randomly integrating viral vector to identify and characterise chromosomal loci associated with high transcriptional potential (Mielke et al. 1996). Using SEAP or CAT activity to measure expression, a number of regions in embryonic mouse cells identified using inverse PCR were found to be associated with high transcriptional activity (interestingly, a number of these contained S/MAR like characteristics which, as noted previously, are associated with active chromatin). A similar experiment in CHO, investigation of existing high producing cell lines or bioinformatic analysis of the CHO genome could identify regions with similar characteristics that could be targeted to predictably improve transgene expression and reduce the lengthy screening necessary to identify clones with productivity suitable for industrial bioprocesses.

1.3.9 Engineered nucleases

While the methods of gene knockdown and knockout described previously are now commonly used and well characterised technologies, a notable and relatively novel method of gene targeting exists in the form of engineered nucleases. For example, an elegant and precise method has been described for targeted gene knockout using specifically engineered zinc finger nuclease (ZFN) enzymes (Santiago et al. 2008). This strategy utilises an engineered DNA cleavage enzyme with two functional domains; a DNA cleavage domain isolated from the FokI DNA nuclease coupled to a highly specific zinc-finger DNA binding domain. Once this enzyme has bound to its cognate sequence, the cleavage domain breaks the DNA which is then repaired via non-homologous end joining (NHEJ), a natural cellular DNA repair process. NHEJ is an inaccurate repair system that frequently introduces mutations, consequently disrupting the target gene. It has been shown that the DNA binding domain can be engineered to create novel sequence binding capabilities through the modular assembly of individual zinc finger domains that recognise specific base pair triplets (Durai et al. 2005). Zinc finger binding sites with recognition sites up to 24 bases have been described (Urnov et al. 2005), permitting the generation of ZF nucleases that can target precise DNA sequences, thus allowing for the targeted disruption or removal of highly specific DNA sequences within the genome.

This technique has been successfully demonstrated in instances of single (Santiago et al. 2008) as well as multiple (Liu et al. 2010) gene knockout. Biallelic knockout frequencies >1% were observed in each case, making this an attractive prospect for gene knockout over techniques such as plasmid transfection or viral targeting where such events may be rarer, or may introduce off-target insertion events. This technique was also used effectively to achieve an aim similar to that of an siRNA study described previously (i.e. to eliminate the effects of the pro-apoptotic genes Bax and Bak; Lim et al. 2006). Using two engineered ZFNs, researchers managed to successfully disrupt these two genes, creating double knockout cells resistant to apoptotic stimuli that could ultimately produce up to 5 fold more IgG than wild type cells (Cost et al. 2010).

Protocols for their use in the targeted integration ('knock-in') of DNA sequences have also been described using a ZFN to generate a double stranded break coupled with a targeting vector containing homologous sequences (Orlando et al. 2010). This method carries the advantages of high targeted integration rates, coupled with lower instances of off-target random insertions.

A similar class of nucleases known as TALENs (Transcription Activator-Like Nucleases) are also the subject of current research. TALENs employ a similar methodology to ZFNs, with a FokI nuclease domain coupled to a DNA binding domain isolated from the plant pathogen *Xanthomonas*. Sequence recognition is facilitated by polymorphic dual amino acid residues found in repeat sequences in the TAL binding domain, which in turn correspond to DNA base pairs. Effective methods have already been described for the construction of custom binding domain arrays to recognise novel sequences (Reyon et al. 2012; Cermak et al. 2011), and published experiments successfully utilising TALENs for gene disruption are becoming more prevalent with examples demonstrated in a range of model species such as human (Hockemeyer et al. 2011) and rat (Tesson et al. 2011). Another class of targeted nucleases known as meganucleases has also been used for genome editing in mammalian cells by inducing double stranded breaks (Arnould et al. 2010). Meganucleases recognise larger sequences (up to 45 bp), but suffer from a more difficult engineering process compared to the relative ease of their modular counterparts such as ZFNs and TALENs (Epinat et al. 2003).

Zinc finger nucleases pose a great future potential due to the specificity of the enzymes generated as well as their modular nature, allowing for the targeting of almost any genomic sequence with the generation of bespoke nuclease enzymes. However, due to the tight intellectual property rights currently exercised by Sangamo Biosciences over this technology and the cost of generating novel ZF nucleases, their use is relatively uncommon in research laboratories (Scott, 2005) as they do not currently offer a cost effective means of gene targeting for common research. However, this method of genome editing is likely to become more common and more cost effective in the future with the advent of similar competing technologies such as TALENs and engineered meganucleases.

1.4 Mutagenesis & Directed Evolution

The general method around which a portion of this project was modeled is that of mutagenesis and directed evolution. Mutagenesis is the altering of the genetic sequence of a given genetic component such (for example a gene or regulatory sequence), while directed evolution is the subsequent selection for an advantageous phenotype generated by advantageous mutations by applying an environmental pressure or selection method. It follows the basic experimental principles:

- *Diversification*: The target DNA sequence is mutated and altered in some way. This can be done using a variety of techniques, such as 'site-specific' targeted mutations or by generating a diverse random mutant library with each element containing a differing sequence. Historically, chemicals such as alkylating agents or radiation was used to introduce random mutations, however modern techniques such as error prone PCR, gene shuffling or point mutation can be specifically used to generate controlled libraries of mutant DNA sequences, as well as rational directed changes. Applying these techniques can provide a library of considerable diversity in terms of their ultimate function, which in turn can be selectively screened for advantageous changes or novel functions that provide significant benefit.
- *Selection*: Once the mutant DNA is introduced to the cell and expressed, a selection or screening process is used to select for advantageous changes. For example, an environmental pressure or negative selection method can be used to eliminate all mutants not possessing the intended phenotype, while a screening process allows for the investigation of individual mutants' or population's relative performance.

Mutagenesis and directed evolution has proven a powerful methodology in molecular biology for the improvement of a variety of biological components and products as well as their host strains. It follows the basic tenet of evolution by natural selection observed by Darwin in the 19th century; within a diverse population the fittest, according to environment factors and the pressures applied, will survive. Rather than

attempting the improvement of biological components by effecting rational changes and examining the results, we can introduce a wide variety of different changes in the form of a synthetic library and allow the chosen selection method to present us with the advantageous mutants. This high throughput becomes especially powerful when the structure/function relationship or activity of these components is poorly understood. By examining the differences between functionally superior mutants and their wild type counterparts, this also allows us to gain insight into structurally important regions that may be subject to further targeted engineering once a number of rounds of random mutations have yielded improvements.

Broadly speaking, mutagenesis techniques can be divided into random and non-random (rational) techniques. Historically, the effects of mutagenesis on a particular organism or constituent gene could be studied within the lab by inducing non-specific mutations using chemicals and radiation, often to establish basic links between gene and function. As technology has advanced in this field, techniques have been developed to allow for highly directed rational changes to be made to genomes and gene sequences whose properties are understood, as well as permitting vast mutant libraries that allow for the screening and selection of novel or improved phenotypes in situations where rational improvement may be difficult to implement. Discussed below are a range of these techniques, and how they have been successfully implemented to generate biological components and organisms with improved function.

1.5 Component Engineering

Mutation and directed evolution can be a powerful method of improving molecular components such as proteins, cis-acting sequences such as promoters as well as whole cell phenotypes. First, this section will examine the means by which researchers can generate random and targeted mutations in a given sequence in order to create diversity, and focus on examples of how these have implemented to improve on cellular components and bioprocess products such as enzymes.

1.5.1 Mutation Methods

Summarised below are a variety of methods for the introduction of variation to a natural wild type sequence, including random, semi-random and highly targeted techniques.

1.5.1.1 Random mutagenesis

Early work in the field of directed evolution was generally carried out in bacterial systems, often directed at improving the activity of biological products such as enzymes that are used as components of large scale industrial applications (for example enzymes used in food processing or as components of household detergents) Biomolecules such as enzymes were among the first biological products to be produced and used on a large scale, and attempts to improve them on a genetic basis have formed the basis for industrially relevant directed evolution techniques. A fundamental approach used for the generation of improved enzymes, and a cornerstone of artificial evolutionary techniques, is the use of low fidelity PCR to generate random mutations in a given sequence and generate a mutant library. When placed into a population of cells this library can then be screened for activity and, if successful mutants are isolated, used for subsequent mutation to further improve the phenotype.

Examples of this fundamental approach have been demonstrated, such as a study carried out in an attempt to adapt the Subtilisin E protease, an industrial biocatalyst, to maintain its activity in an organic solvent (You and Arnold 1996). A modified polymerase chain reaction was used in which reaction conditions were suitably modified to increase the frequency of mutations in the Subtilisin gene sequence. 3 rounds of random evolution were undertaken, each in which a library of the mutant genes was generated and reintroduced into *B. Subtilisin*. Clones were screened for activity between rounds, after which the clone with the highest performing enzyme was then selected for subsequent mutagenesis and screening. Ultimately, an enzyme was generated with 10 distinct mutations resulting in roughly the same catalytic efficacy in a 60% dimethylformamide solution as the wild type enzyme displayed in water. This approach built on previous similar experimental iterations carried out to improve Subtilisin activity in the solvent DMF (Chen and Arnold 1993; Chen and Arnold 1991).
Now commonplace, this technique has also been widely employed to improve the function of many enzymes, with phenotypes including activity, specificity, stability and temperature sensitivity showing improvements over wild-type variants. Examples include restriction enzymes with altered recognition sites (Samuelson el al. 2006), non-protein ribozymes with improved pH resistance (Miyamoto et al. 2005) and dehydrogenases with improved activity at low temperatures (Sasaki et al. 2008).

The examples above describes the most basic form of mutant library generation, in which a target DNA sequence is subjected to error prone PCR to generate a library, using modified PCR conditions such as increasing magnesium (Mg++) concentrations to decrease enzyme fidelity and introduce mutations. Alternative techniques have also been developed to achieve a similar goal, with modern commercial solutions commonplace. Commonly used are PCR enzymes engineered to reduce copy fidelity and remove proof reading ability (such as the one used in this thesis – Genemorph II kit, Agilent technologies) with mutant enzymes derived from *Pyrococcus furiosus* (Pfu) proving particularly effective (Biles and Connolly 2004). 'Mutator strains' of bacteria have also been used to generate mutant plasmid libraries by successive rounds of plasmid reproduction in *E. coli* strains such as XL-1 red lacking in gene repair pathways (Muteeb and Sen 2010), with increases in bacterial generations leading to larger mutation rates.

1.5.1.2 Site saturation mutagenesis

For enzymes whose structure and/or function is better understood, site saturation mutagenesis has proven a successful alternative to whole sequence random mutation. Site saturation mutagenesis involves the targeting of a narrow region of DNA (often as small as a single amino acid), and employing random mutation to generate a library of mutants localised to this previously determined location. This 'semi-rational' approach has shown that with prior knowledge and targeting of key amino acids in and around active sites and external facing structures, the library size necessary and thus the amount of screening and rounds of mutation necessary to generate a positive change in phenotype can be successfully reduced. This reduction in scale also allows for almost all variations in a small sequence space to be explored, with possibly all 20 amino acids in a given position able to be substituted and tested.

In one example, β-Fucosidase activity was enhanced in a β-Galactosidase enzyme in just one round of site saturation, as opposed to a comparable previous study requiring seven rounds of random DNA shuffling (Parikh and Matsumura 2005). Similarly, a different study was carried out in which the Cre recombinase enzyme was modified at 6 target residues known to recognise and interact with wild type loxP sequences. By targeting this active site for mutagenesis, they were able to identify a custom mutant enzyme capable of recognising a novel loxP site as well as the wild type sequence (Santoro and Schultz 2002).

1.5.1.3 Site directed (point) mutagenesis

Site directed mutagenesis, as opposed to the randomised methods above, is a useful method for tailoring specific alterations of target genes. It employs techniques in order to generate exact changes in DNA without the need for randomised libraries, generally based on known structure/function relationship data. It can be carried out to combine advantageous point mutations predetermined through random means onto a single sequence, or rationally explore the effects of highly specific mutations.

The most widely used site directed techniques generally involve the use of modified oligonucleotide primers containing the mutation of interest. This primer binds its target mutation site, and subsequent PCR cycles integrate this mutation into the sequence (An et al. 2011). This technique has been modified and refined to increase efficiency and reduce the influence of non-mutated template DNA, for example by

digesting with *DpnI* (Li et al. 2008). Rational engineering using the method described has been used successfully to synergistically combine target residues previously determined as advantageous by random means to generate a more functional laundry peroxidise enzyme (Cherry et al. 1999). In another study it was used as an intermediate tool to introduce restriction sites in order to link domains to create a fusion protein (Japrung et al. 2005). While originally limited by the ability to introduce single point mutations at a time, modified protocols have been demonstrated that allow for multiple mutations to be introduced simultaneously (Kim and Mass 2002).

1.5.1.4 Gene shuffling

Other techniques have also been used to successfully engineer stability and activity in enzymes. The above methods have proven successful, but are limited by iterative additions of small numbers of beneficial mutations. Multiple simultaneous random mutations are like to be deleterious, so progress is made in smaller steps, generally through multiple rounds of mutation after selecting for beneficial changes and/or subsequent site saturation techniques. Gene shuffling was designed as a means to overcome this limitation, by allowing the random recombination of beneficial mutations after a round of random mutagenesis in order to select for advantageous combinations, with reduced need for multiple iterative rounds of mutation on single select mutants.

First, a selection of homologous sequences are pooled and randomly fragmented (for example, a mutant library or selection of sequences with known beneficial mutations). Primerless PCR is then carried out, with homology between fragments sufficient to allow fragments to prime each other. Fragments of different sequences cause crossovers, eventually recreating the full length sequence incorporating a mixture of mutations. This library of chimeric sequences can then be screened for improved phenotype (Stemmer 1994).

This single gene technique has proven beneficial for the improvement of a number of sequences without the need for in-depth prior knowledge of its structure. Examples include a mutant GFP generated over 3 cycles of DNA shuffling with a 42-fold increase in fluorescence when expressed in CHO cells (Crameri et al. 1996). A plant

reporter gene, β-glucuronidase, was also engineered using this technique to gain thermostability even when treated to 100°C temperatures (Xiong et al. 2007).

A modified version of the gene shuffling technique known as family gene shuffling has also proven successful. Rather than recombine a gene with itself to generate new functionality, the functional diversity of homologous genes from within the same family or homologous genes from different organisms can be exploited to gain improved phenotype when these genes are recombined using shuffling techniques. The influence of natural selection to select for functional mutations over millions of years is used as the starting point, increasing diversity and the likelihood of screening for positive recombinants from a given library (Crameri et al. 1998).
A clear example of this technique involves the shuffling of yeast lipase enzymes. (Suen et al. 2004) Gene fragments are generated from homologous lipases from 3 separate yeast species. These fragments were then recombined to generate a rich library that combined the natural diversity of the organisms used, as well as the inherent heterogeneity generated in the gene shuffling technique. A number of clones were screened for that produced between 5 and 20 fold increases in activity, as well as enhanced thermostability, thereby reducing inactivation as temperatures increased up to 45°C. This same process has also been successfully replicated to generate novel cytochrome P450 enzymes for dye production (Rosic 2008) and novel promoter sequences with increased gene expression activity (Ranjan et al. 2012) amongst other examples. One study was also able to identify previously unknown homologous sequences from environmental 'metagenomic' bacterial samples encompassing a heterologous mix of organisms, overcoming a lack of genetic diversity in established databases to generate an improved bacterial lipase enzyme (Wang et al. 2010).

1.5.2 Genotype-Phenotype Linkage Methods

While enzymatic function is a relatively simple characteristic to assay, a method must be established to select for improvements via evolution of more subtle traits of proteins that may be more difficult to detect. These include activities that cannot be easily assayed on a large scale, such as DNA binding capacity and interactions with other proteins that one may want to select from a complex library.
For this reason, employing a 'genotype-phenotype' linkage method is important. As proteins are not self replicating, an improved mutant that has successfully been screened or selected for must somehow be physically linked to its original DNA or RNA sequence. At this time, a number of 'display' techniques are commonly used to link the protein of interest to its cognate nucleic acid sequence.

1.5.2.1 Phage display

Phage display is a common method of the display technique, generally used to select for novel or improved binding specificity, such that it is often used to in conjunction with mutagenesis to improve the specificity of antibodies. It is based on the filamentous bacteriophage, a virus known to infect gram-negative bacteria. A library of varying ligands is first cloned as a fusion protein together with a phage coat protein. This library is then transformed into *E. coli* and the phage generated. The target binding molecule is immobilised onto a surface, and exposed to the phage library. When the library comes into contact with the target molecule, any phage displaying a successfully bound protein will remain bound, with all others then washed away. Bound phage can then be eluted and used to generate more phage, enriching for the target proteins and their linked genetic sequences. Repeated cycles (known as panning) will eventually select for proteins or peptides with the highest binding affinity within the library, whose sequence (contained within the phage) can then be amplified.

An early example of this technique proved its efficacy in isolating antibodies with highly specific variable binding regions (McCafferty et al. 1990). This served to speed up the process through which antibodies with enhanced binding capacity could be screened, rather than assaying individual clones for improved specificity.

Modifications of this protocol have also been employed, such as one study that treated affinity tag immobilised protein-phage fusions with a protease. This served to remove unstable and poorly folded mutants on display, thus selecting for the more stably folded protein variants (Finucane et al. 1999). Examination of these stable proteins can also provide an insight into their properties, and increase our understanding of the structure-function relationship. This technique has also been effectively used for its ability to screen for novel protein-DNA interactions such as early attempts to isolate novel zinc finger proteins, a technique that would be later used to generate the novel DNA binding necessary for the powerful zinc finger nuclease technology (Rebar and Pabo 1994).

1.5.2.2 mRNA/ribosome display

Newer methods of display for selection from large libraries have also emerged, such as ribosome and mRNA displays. These methods are carried out entirely *in vitro*, removing the limits of library size imposed by transformation efficiencies for *in vivo* methods. Phage display, for example, has an upward limit of the order of ~10^9, while an mRNA library can theoretically contain upwards of 10^{13} different sequences (Gold 2001). It also avoids bias that may arise from proteins that are too toxic or difficult to produce *in vivo*, increasing variation.

For mRNA display, mRNA is first derived from gene libraries generated though mutagenesis. These sequences are then covalently tagged to puromycin, a ribosome inhibitor, at the 3' end. Translation then takes place *in vivo*, and the nascent peptide chain is left with an intact link to its mRNA due to the puromycin molecule terminating translation. The peptides are then purified and exposed to the target molecule, bound, and the unbound molecules washed away. The mRNA tagged to successfully bound proteins can then be used for amplification and further analysis. This method is similar to ribosome display, in which a spacer sequence is used in place of puromycin to terminate translation, allowing the nascent polypeptide to fold and interact with its environment while also retaining the original nucleic acid sequence in a complex with the ribosome. 'Panning' is then carried out as usual to remove non binding proteins and select for improved variants.

mRNA display has been used successfully in one instance to detect 16 novel interactors with the Jun protein by carrying out mRNA display on a mouse brain cDNA library, 10 of which were subsequently confirmed to interact *in vitro* (Horisawa et al. 2004). It has also been used to generate novel proteins with strong immunoglobulin-like binding capacity (Xu et al. 2002), as well as application in a search for previously unknown protein Bcl-xL binding partners using tissue derived mRNA libraries (Hammond et al. 2001). Ribosome display is generally used in a similar manner, with a typical study carried out Hanes et al. (2000) involving its use in the evolution of antibody fragments with improved binding capacity.

1.6 Directed Evolution of Cellular Phenotype

The methods described above have proven useful for engineering discreet protein interactions, generating increases in activity or specificity in products such as enzymes or improving the properties of sequences such as promoters. The success of such single element engineering has led research to focus on the directed evolution of entire cell lines, especially those utilised in industrial biotech processes. Applying these principles to organisms is an attractive proposition for the improvement of industrially relevant phenotypes. By helping shape overall cellular processes through directed evolutionary techniques, we can hope to develop key cellular attributes such as growth and maximum density, specific productivity, as well as resistance to apoptotic factors and reduction of waste secretion.

Rather than engineering component functions such as those outlined previously, the idea is to guide the evolution of the entire cell by facilitating the direct or indirect manipulation of a number of genes or cellular networks by introducing artificial variation through mutation or exploiting naturally occurring heterogeneity. This also takes advantage of the cells complex ability to auto-regulate the activity of a range of proteins and other regulatory factors in order to achieve a desired phenotype, cumulative effects that are hard to predict or implement through rational engineering.

1.6.1 Stress Induced Adaptive Evolution

One method of directed evolution for whole organisms is relatively simple as it requires no genetic manipulation. Adaptive evolution methods rely on the natural variation in a heterogeneous population to provide beneficial mutations that are then selected for using an environmental stress dictated by the researcher. This includes clonal derivatives that, while initially may be genetically identical, eventually develop phenotypic variation (Stockholm et al. 2007). These stresses are generally implemented gradually in order to allow for changes to build up over successive generations, eventually conferring a level of resistance to the stress, signifying a level of adaptation on a genetic level (Roth et al. 2006).

Due to their fast generation time and relative simplicity compared to mammalian cells, this approach to the improvement of industrial cell lines was first demonstrated in bacterial and yeast systems. Ethanol production in genetically engineered *E. coli*, for example, is limited by their tolerance to the product secreted, restricting maximum biofuel output of producers. One study managed to select for resistance to ethanol in *E. coli* by steadily increasing ethanol concentrations up to 50g/L, with selection of ethanol producing clones on solid medium in between increases to ensure continuing production and high growth. These evolved variants displayed high ethanol resistance without genetic intervention compared to the parent control, with no changes to other factors such as production or osmotic resistance (Yomano, York and Ingram, 1998). Further work investigated if such a phenotype could be recreated directly in parental cells without the incremental forced evolution stage. This was achieved in an experiment regarding isobutanol tolerance in *E. coli*. Initially, a similar sequential transfer method was used as above to gain tolerance and generate a phenotype that researchers would then attempt to recreate. Genome sequencing was carried out on the most successful clone. While a large number of mutations were detected, 5 were determined to have the biggest impact on the improved phenotype through gene repair. This genetic relationship to phenotype was then validated via reconstruction in a parent strain through systematic inactivation of these loci to re-establish isobutanol tolerance (Atsumi et al. 2010).

Similar methodology of stepwise adaptation has proven successful in mammalian cell lines as well. Adaption to serum free conditions is a basic adaptation method commonly used now in industrial applications to avoid the potential immunogenic and variability concerns that come with use of animal derived serums. Historically, this required no genetic intervention and could be achieved with a gradual stepwise reduction in serum concentration. Such environmental adaptations have also proven successful for other factors, demonstrating the elasticity that exists naturally in many cultured cell lines. For example, a CHO-K1 strain, previously adapted to serum free and suspension growth conditions was passaged at high densities in medium containing gradually reducing concentrations of glutamine (Bort, Stern and Borth 2010). By sorting for viable cells after the inevitable crash in viability after glutamine reduction, eventually a cell population was adapted to grow in entirely glutamine free media, with metabolism supported by an increase in glutamate and aspartate

consumption. A similar technique was applied to mouse hybridoma cell lines to select for cells exhibiting resistance to the toxic effects of ammonia accumulation by serial spiking of NH_4Cl (Matsumura et al. 1991).

Other experiments have also demonstrated a cell lines ability to evolve and adapt to withdrawal of supposedly key components, such as investigations into medium supplementation that yielded insulin independent CHO-K1 derivatives (Mendiaz et al. 1986). Another study successfully attempted to select for cells with a general improvement in phenotype related to bioreactor performance, using a method dubbed 'bioreactor evolution' (Prentice, Ehrenfels and Sisk 2007). Cells were iteratively passaged under normal bioreactor conditions, with late stage cells rescued after a decrease in viability, expanded and then used to reseed another batch. This 'training' of cells for bioreactor conditions was intended to expose cells to negative batch culture conditions such as high waste and low nutrient availability, effectively using these as selective pressures. This resulted in a stable population with a total integrated cell density 72% higher than its parental line over a 13 day batch culture. This confirms that cells can be evolved to a wide range of conditions by simply gradually exposing cells to the environment one wishes to adapt them to, effectively mimicking natural evolution *in vitro*. It is this method of exposure to industrially relevant stress conditions that helped inform the original aims that were to shape a portion of this thesis, with exposure to apoptotic late-culture conditions to select for an improved phenotype used as part of this experimental method.

1.6.3 Genetic Engineering and Directed Evolution

The methods explored above describe means by which whole organisms can be forced to adapt to environmental stresses by taking advantage of ordinary evolutionary processes and naturally occurring heterogeneity. However, limitations exist as to the complexity of phenotype that can be generated from a parental cell line without more drastic external interventions, as well as being limited by the speed at which natural mutations can accumulate with no artificial genetic modification.

This can be overcome by artificially generating diversity in manners such as those described previously such as random mutagenesis or sequence shuffling, with the methods employed as means to improving a cell lines overall phenotype as opposed to a single component and its activity or other such properties. The use of such techniques also carry the benefit of being able to produce improved phenotypes without the need to fully understand the complex relationships between genotype and phenotype, as a good selection method will eventually present such phenotypes naturally if an appropriate measure is taken, for example introducing a mutant library with a portion of beneficial variations.

Early attempts involved simpler methods such as radiation (Urlaub et al. 1986) or mutagenic chemicals (Urlaub and Chasin 1980) to induce mutations prior to screening. Modern techniques have since been developed, providing better means of increasing functional diversity or allowing us to target mutations to sequences more likely to provide beneficial results. Outlined below are examples of engineering methods undertaken to introduce variation in critical genes, pathways and even whole genomes as means of improving cellular phenotype.

1.6.3.1 Genome shuffling

As described previously, gene shuffling is a method by which a series of beneficial mutations can be randomly recombined in order to uncover complex synergistic combinations of mutations that would otherwise prove difficult to discover through repeated random or stepwise rational engineering methods. This concept has also been applied on a larger genome-wide scale in order to breed new combinations of different genotypes (Stephanopoulos 2002). Genomic shuffling methods such as these hold the benefit of being able to exploit a combination of advantageous mutations gained after

a round of mutagenesis, amplifying the diversity found within a selected population. This is in contrast to basic mutation and selection methods, in which the highest performing sequence is selected for iterative rounds of mutation, discarding other potentially beneficial mutations. In whole cell genomic shuffling, a workflow similar to that of gene shuffling is applied; first random mutagenesis is carried out to obtain a number of organisms with an improved phenotype. Then, rather than chose one specific clone with improved properties for further mutagenesis, a selection of clones with beneficial alterations are 'bred' together to accelerate the accumulation of advantageous mutations.

The key process of whole-genome recombination is based on a protocol known as 'protoplast fusion', previously shown to be successful at combining genetic markers in both bacteria (Palleroni 1983) and yeast strains (Wei et al. 2001). Protoplasts are cells with their tough outer walls degraded or removed through chemical or enzymatic means (such as the use of lysozyme or zymolase enzymes). Cell fusion can then be encouraged by incubating in a medium containing compounds such polyethylene glycol and $CaCl_2$. Homologous genetic recombination ensures a mix of different mutations in each cell, and a mixture of resulting phenotypes which can then be selected for using an appropriate environmental pressure.

A number of successful examples of this method have been demonstrated, with strains developing phenotypic improvements far quicker than with conventional mutagenesis. In one study, bacterial samples with the ability to break down an artificial pesticide (PCP) were chemically mutated using nitrosoguanidine (Dai and Copley 2004). Colonies with improved PCP degradation and growth rate were shuffled, with resulting clones displaying 10 times the activity of the parental cell line. A similar study was carried out in *Lactobacillus* using strains mutated via environmental stress as well as nitrosoguanidine. These were successfully shuffled to generate a strain resistant to low pH, ultimately able to produce threefold more lactic than its parent (Patnaik et al. 2002). The versatility of this method was also highlighted in a genome shuffling study carried out in *Saccharomyces cerevisiae*, with recombinants found to possess a number of improved traits such as resistance to elevated heat and ethanol concentrations, as well as an increase in ethanol productivity (Shi, Wang and Wang 2009).

1.6.3.2 Pathway engineering

A more directed use for random engineering methods is found in current attempts to combine mutagenesis and rational engineering of cellular pathways to generate improved phenotypes. New data is continually generated with regards to genomic and transcriptomic profiles of industrially relevant organisms, and a new frontier exists in implementing this knowledge for the engineering and fine tuning of particular metabolic pathways to improve a cell's behaviour.

Engineering methods such as non-discriminate mutagenesis and whole genome shuffling techniques outlined previously are powerful at generating phenotypes that would be difficult or otherwise impossible with predetermined rational efforts. However, it can be theorised that such efforts could be even more effective if mutagenesis is targeted to specific genomic loci. These loci can be identified using the wealth of knowledge and experience that has been generated previously on the global physiology of the cell; data on the genotype/phenotype relationship of thousands of genes based on the detailed annotations of genomes, knowledge of gene interactions and their involvement in metabolic pathways as well as transcriptomic data that details the impact of varying gene expression levels on phenotype. Pathway engineering seeks to move away from the relatively crude method of gene overexpression or knockdown, but to improve and modify pathways and their components in more subtle ways to elicit an improved or novel function.

A typical example of this approach is the rational targeting of key metabolic enzymes for improvement via mutagenesis. This can serve to remove rate limiting steps, such as the increased synthesis of key metabolites, and allow a pathway to work at an optimal level. In one instance, an enzyme known as GSP from well characterised carotenoid synthesis pathway in *E. coli* was found to be the rate limiting step (Wang, Oh and Liao 2000). Error prone PCR and staggered extension process PCR (StEP, a process similar to gene shuffling) was carried out to generate improved versions of this gene, resulting in two fold increases in lycopene product formation in an already highly optimised system. Improved activity was also generated in a study examining the effects of mutagenesis on the gene *aveC*, whose activity plays a role in the synthesis of the veterinary anti-parasitic drug doramectin (Stutzman-Engwall et al. 2005). This gene was also known to generate a fraction of undesirable analogue compounds, reducing the overall output of target product. In order to reduce this

fraction, mutagenesis and shuffling was carried out to generate a mutant with 23 fold improved production of its target metabolite.
Similar approaches have also been undertaken to generate novel enzyme specificity, rather than an improvement in existing function. In one study, a microbial enzyme (HisA) with a $(\beta\alpha)_8$-barrel structure resembling a similar enzyme (PRA) was mutated and shuffled. This yielded a new enzyme, with modified activity encompassing that of both HisA and PRA, allowing it to incorporate separate substrates into the same biological pathway (Jürgens et al. 2000). In industrial situations, this dual activity could be used potentially to increase the range of substrates available to a cell in situations where nutrient depletion may hamper growth or product formation.

A notable technique combining the benefits of pathway mutagenesis with the ability to simultaneously alter multiple gene sequences is MAGE (multiplex automated genome engineering). In this technique a number of degenerate oligonucleotide pools, each with degrees of homology and mismatch to a different corresponding target locus, are introduced into the cell. Integration of these mutant alleles is then achieved during DNA replication using a DNA binding protein isolated from the bacteriophage 'λ-Red' (Poteete 2001), effectively carrying out random mutagenesis on a number of gene sequences at once. This allows for the screening of a vast range of permutations of mutant combinations, an attractive prospect compared to the existing power of now common mutagenesis and screening techniques.
A good demonstration of this technique is found in a study examining lycopene production in *E. coli* (Wang et al. 2009). In order to optimise lycopene production, 24 targets involved in its metabolic pathway were chosen for both tuning of expression or inactivation using MAGE mutagenesis. With an average MAGE cycle time of 2-2.5 hours, up to 15 billion variants were generated after 35 cycles. This resulted in strains with up to 5 fold greater lycopene production than parental strains. Factors such as oligo sequence diversity and cycle number variation were also demonstrated to allow a good degree of control over mutational rates. This technique, if developed to operate efficiently in mammalian cells, could prove a powerful tool for the modification of metabolic pathways and improvement of host strains.

1.6.3.3 Global Transcriptional Machinery Engineering

A successful method known as 'Global transcription machinery engineering', or gTME, has been successfully employed to generate complex phenotypes in prokaryotic cells. It is an applied use of mutagenesis in industrially relevant cell lines, however, instead of the objective being improvement in function of a given cellular component, gTME seeks to improve the overall fitness of the cell by generating functional diversity at the highest levels of a cell's transcriptional machinery.
By introducing a variety of subtle perturbations in the mechanisms that control the expression of all other genes within the cell via mutant gene library, we can hope to fundamentally alter the transcriptomic landscape of the cell and generate a wider range of variations in phenotype. This is preferable to the targeting of genes with less central roles in cellular metabolism, whose limited range of downstream effects is less likely to elicit the phenotypic variation necessary to allow selection of an improved phenotype.

A typical example of this approach to cell line engineering is found in a mutagenesis experiment carried out in *E. coli* (Alper, Stephanopoulos 2007). The rpoD gene encoding the sigma factor σ^{70} was first subjected to random mutagenesis and then reinserted into cells prior to screening. This gene was chosen due to its role as a key transcription factor for a wide range of other genes, with the theory that mutations could shift the promoter preference of the native RNA polymerase, thus generating a wide range of perturbations in the transcriptional profiles of the transformed cell population. Once transformed, cells were subjected to a high concentration of ethanol overnight, after which surviving colonies were picked and analysed. The relationship of the phenotype to the mutant gene was verified by retransforming into a parental host population and testing for tolerance, and then subjected to subsequent rounds of mutation and increased ethanol concentrations.

A similar study in *E. coli* documented tolerance to butanol and other alcohols via mutation of cellular polymerase (Klein-Marcuschamer et al. 2009). This approach was also used successfully in an earlier study by Alper et al. (2006) in *S. cerevisiae*, a eukaryote naturally containing more complex metabolic machinery than bacteria. Random mutagenesis was carried out on the *SPT15* gene encoding the TATA-Binding protein (TBP) in yeast, as well as *TAF25*, a TBP associated factor. Once again, ethanol tolerant strains with improved production characteristics were generated, even with starting concentrations of 20% v/v EtOH.

More recent studies have replicated this beneficial effect with the mutagenesis of the transcription factor CRP (cAMP receptor protein) by error prone PCR and gene shuffling. Implementing a variety of different stresses, strains have been developed with phenotypes such as improved osmotolerance to levels of NaCl greater than 1M (Zhang et al. 2012a) as well as an increased resistance to the negative effects of 1-butanol (Zhang et al. 2012b).
A study examining the effects of sigma factor mutagenesis in *Lactobacillus plantarum* demonstrated the effect of various mutation rates on the levels of diversity in phenotype gained and the chances of isolating an improved phenotype using resistance to low pH as their example. A number of conclusions were reached regarding the effects of gTME-like mutagenesis (Klein-Marcuschamer and Stephanopoulos 2008). They found that such alterations to globally acting genes increases phenotypic diversity (more effectively than chromosomal mutagenesis), improving the chances of selecting for beneficial mutants. Using the wild type gene as a control, it was also found that phenotypic divergence increases as higher mutation rates are employed. However, as a greater number of misfolded and non-functional proteins accumulate, this may ultimately serve to reduce diversity as negative phenotypes begin to dominate, suggesting a level of optimisation is necessary. It was also concluded that such mutations are pleiotropic, suggesting that such an approach is versatile as populations can then be screened under multiple conditions to search for benefits with regard to a number of different phenotypic traits. These conclusions underline the results from the successful studies highlighted earlier, marking this approach as an attractive method of cell line engineering.

1.6.3.4 gTME in CHO

It is the above techniques involving mutation of a key transcriptional regulatory gene to enhance directed evolution that we hope to successfully apply in a mammalian cell line, namely Chinese Hamster Ovary cells. We have selected the CHO TATA-Binding protein (TBP) as our target for mutagenesis due to its analogous function in mammalian cells to that of the sigma factor and other transcription factors examined in previous mutational studies in bacteria and yeast. It holds a wide range of interactions with a host of other transcription factors and plays a key role as a subunit in Transcription factor IID, a general transcription factor responsible for the recruitment of RNA polymerase II to a large portion of genes in mammalian cells (Cler et al. 2009), among other transcriptional roles. It is theorised that, in a similar fashion to the studies outlined above, one can introduce a wide range of perturbations into the transcriptional machinery of a mammalian cell population therefore causing a high level of heterogeneity. From there, one can then apply an environmental pressure and hopefully select for advantageous mutants that provide a suitably 'evolved' phenotype, better suited to perform in a bioprocess.

1.7 TBP (TATA-binding protein)

TATA-binding protein (TBP) is a general transcription factor expressed in mammalian cells, and plays an important role in the transcription of virtually all expressed genes due to its key function in the initiation of the three RNA polymerases (Chasman et al. 1993). It functions as a component of initiation factors for both RNA Polymerases I and III. However, it is most commonly associated with its involvement in the facilitation of RNA Polymerase II (Pol II), the polymerase involved in the expression of protein coding genes. TBP, along with a number of TBP associated factors, form a subunit of transcription factor II-D (TFII-D). TFII-D then, along with general transcription factors TFII-A, -B, -E, -F and -H form the main structure of the Polymerase II pre-initiation complex. This complex, coordinated primarily through the direct binding of TBP to a core promoter DNA sequence, allows for the definition of the transcriptional start site and the recruitment of RNA polymerase II to an expressed gene for its subsequent transcription (Smale and Kadonaga 2003). This high level involvement in the transcriptional machinery that makes TBP an attractive target for random mutagenesis due to the wide range of potential effects it could have on the cells metabolism, and was chosen for this study as the prime candidate

1.7.1 TBP Structure

The TBP orthologue found in CHO consists of 315 amino acids, compared to the mouse (316 aa), rat (318 aa) and human (339 aa) sequences. Due to the critical transcriptional function of this gene, it is a highly conserved gene in all eukaryotes. While many species may contain orthologous sequences shorter than the more highly conserved mammalian version, the key TATA binding domain generally remains unchanged.

Structurally, two features are noted as important in the function of this protein, the more variable N-terminus sequence and the highly conserved C-terminal binding domain.

The N-terminus' function is not widely understood, and difficulty in understanding its function is compounded by the variety found in different species, with divergence in

length and sequence common. A common feature of mammalian TBP N-terminus sequences is a polyglutamine domain. Mutation and deletion studies in yeast have found it to be important in modulating the effect of TBP, indicating interactions with other transcription factors as well as the core of the TBP protein itself. (Friedman et al. 2007; Lee and Struhl 2001)
At the TBP C-terminus is the TATA box binding domain, comprising of ~180 amino acids. This portion accounts for the majority of the true functionality of this gene, with truncated 'c-terminal only' versions functioning sufficiently for cell growth in experiments (Cormack et al. 1991). It is also the most highly conserved portion, with sequence similarity to the human sequence of over 75% found across almost all species (Hampsey 1998). Once correctly folded, this domain takes on a saddle shaped structure due to its intramolecular symmetry. Acting as a monomer, this saddle shape sits within the minor groove of the target TATA box sequence, unwinding the double helix through 120° and compressing the major groove by 80°. This sharp bending of the DNA template causes a strain on the DNA base pair bonding, allowing separation of the strands and subsequent access to the sequence by RNA polymerase for transcription.

1.7.2 TATA box and other core promoter elements

The TATA box is a core promoter element found 25-30 bp upstream of the transcriptional start site of TATA-containing genes, and the sequence most associated with TBP DNA binding capacity. It is this element that assists DNA unwinding due to the concentration of thymine-adenine (T-A) bonds found in its sequence. It may be impossible to define an exact consensus for all TATA boxes, however a binding site analysis identified the sequence 5′-TATATAAG-3′ as preferential for TBP recognition (Hampsey 1998). The relaxed specificity of TATA binding is thought not to be a result of an exact base order, but is permitted by the relatively weaker interactions of T-A bases containing only two hydrogen bonds as well as avoiding the C_2-NH_2 group protruding from G-C bonds, thus facilitating easier unwinding of DNA (Juo et al. 1996).

TATA dependent promoters are a common feature of transcription initiation and their method of action is relatively well understood, however they are by no means the only method. For example, in humans the TATA box or TATA-like elements are found in approximately 24% of all genes (Yang et al. 2007), ~30% of genes in *Drosophila* (Ohler et al. 2002) and ~19% in yeast (Basehoar, Zanton and Pugh 2004). This means that a large portion of genes are regulated by TATA-independent promoter elements. Nevertheless, TBP (as a key subunit of the TFIID transcription factor) is still required for successful transcription, likely mediated through other DNA-binding transcription factors and co-activators (Pugh and Tjian 1991). Other core promoter elements have been identified that facilitate the interaction of the pre-initiation complex with DNA. Well defined elements with proven function include:

- The Initiator (Inr) element, a sequence that encompasses the transcriptional start site and interacts with TFIID subunits TAFI and TAFII (Chalkley and Verrijzer 1999)
- The downstream promoter element (DPE) found ~30 bp downstream from the transcriptional start site that is found to interact with TFIID subunits TAF6 and TAF9. (Yang et al. 2007).
- The TFIIB recognition element (BRE). Consisting of 7 nucleotides and located immediately upstream of TATA-like elements, it is notable for being one of the more well known elements not interacting directly with TFIID.

In these cases, the role of TBP is shifted from direct DNA binding to one facilitating the interactions of other transcription factors. Support for this function was described in a study in which the DNA binding site of TBP was mutated to remove binding activity. Transcription of TATA driven genes was diminished, however genes containing the Inr element were largely unaffected. Studies have shown that TATA-less promoters function via a combination of common elements such as these, as well as other more gene specific promoter elements (Anish et al. 2009; Xi et al. 2007) that are not as well characterised at this time. From this we can infer that the TFIID complex and the subsequent polymerase II holoenzyme is a heterogeneous mix that may differ from gene to gene in accordance with the combination of different transcription factors and TBP associated factors that interact with cis- regulatory elements.

Interestingly, there seems to be an apparent difference between genes with and without TATA-based transcription in terms of their function. A study of human genes using the Gene Ontology database identified that lists of TATA responsive genes generally include more highly regulated genes such as those involved in stress response or developmental cues, while those responsive to Inr elements and other promoters tended to be associated with basic constitutive biological functions such as intracellular transport and protein metabolism (Yang et al. 2007). A similar study in yeast provided equally interesting results (Basehoar, Zanton, and Pugh 2004). It was demonstrated that when subjected to an environmental stress such as a low glucose environment or heat shock, genes whose regulation was altered the most to accommodate the changes were generally those with TATA boxes. These were also genes that tended to have the highest or lowest expression at that time, indicating the involvement of TATA containing genes in highly regulated responses to stress activated pathways.

1.7.3 TBP Interactions

While TBP is implicated in the direct binding of DNA, such as in the category of genes described above, it holds an extensive number of interactions with various other proteins. A large category of known and predicted interactions exists, listed below are examples of known TBP interactors and the different activities that are mediated by TBP

- *TFIIA* – Subunit of the Pol II holoenzyme, recognises the TBP-DNA interaction and is recruited by TBP. Known to stabilise this protein-DNA interaction (Weideman et al. 1997)
- *TFIIB* – Functions as a molecular bridge between the TFIID complex and RNA polymerase II. Can also function in DNA recognition at sites around the TATA box, such as the BRE (Tang et al. 1996)
- *TBP associated factors (TAFs)* – Along with TBP, TAFs make up the core of TFIID. These vary in function, from interaction with other general transcription factors and co-activators to modulate transcription, to DNA recognition function as noted above (Hampsey 1998)

- *p53* – A tumour suppressor protein also involved the cell cycle. Interaction with TBP serves to repress transcription of a category of genes, acting as a link between TBP activity and the cell cycle (Seto et al. 1992)
- *UBF* – Upstream binding factor (UBF) is shown to interact with SL1 (a complex of TBP and 3 associated TAFs), ultimately implicating TBP in the machinery necessary for rRNA transcription by RNA Pol I (Tuan, Zhai and Comai 1999)
- *TFIIIB* – TBP is also a component of Transcription factor IIIB, a protein complex involved in the transcription of tRNA and other small RNAs (Hernandez 1993)

While by no means comprehensive, the examples above give an indication of the variety of proteins found to interact with TBP, hinting at the potential for a range of different downstream perturbations in CHO metabolism that may arise from interactions with a mutant TBP library.

1.7.4 TBP Mutagenesis

We can see from the examples above that TBP is a versatile protein, playing a vital role at the core of practically all cellular transcriptional mechanisms. By being involved in direct DNA binding, as well as recruiting and interacting with a wide range of other proteins, we can begin to view this particular transcription factor as an attractive candidate for mutagenesis due to the wide range of possible altered interactions a mutated TBP molecule may have.

As discussed previously, random mutagenesis of transcription factors and metabolic targets involved at a high level in the cells metabolic functions can have a greatly beneficial effect on the cell once a suitable screen has been applied to eliminate cells with detrimental function. Small alterations in the action of these regulatory molecules can have a tremendous knock-on effect to the rest of the cells metabolism. With a large enough mutant library to generate a wide number of different phenotypes, we can hope to select for clones whose properties allow them to outgrow their counterparts in culture, naturally outcompeting less successful mutants and gradually filling the environmental space with phenotypically superior cells.

1.8 Expression Profiling

As part of this thesis, a gene expression profiling experiment was carried out on a number of successful clones derived from the directed evolution project. Gene expression profiling using microarrays offer a means of evaluating the relative expression levels of thousands of genes across a number of cell lines and samples. This provides a powerful, unbiased tool for evaluating the underlying mechanisms that give rise to the various phenotypes that can be found amongst various strains of genetically identical cell lines such as CHO. By comparing an interesting cell line such as a particularly high producer or apoptotic resistant clone to an appropriate control, we can hope to uncover the specific genes that are responsible for these phenotypic differences. These genes then become attractive targets for future rational engineering of mammalian producer cell lines.

1.8.1 Principles of microarray profiling

A DNA microarray consists of large numbers of short DNA probe sequences, each bearing homology to a different gene (for example, the Affymetrix arrays used in the course of this experiment contain 25 bp length probes, with at least 11 different probes corresponding to each gene to used increase statistical accuracy when measuring expression). Reverse transcription is used to generate complimentary nucleic acid sequences from an extracted RNA sample. Each sample sequence will then bind to its spotted probe and produce a fluorescent signal in a manner proportional to the abundance of copies in the original sample. This signal can then be compared to equivalent spots on separate chips for experimental and control samples. The collection of signals from all these gene spots gives a detailed account of the transcriptomic fingerprint of the sample analysed, and can indicate the relative up or downregulation of genes when compared to an appropriate control cell line.

1.8.2 *Expression profiling in CHO*

This technique has been standard for some years, providing a high throughput means of assessing large numbers of samples for transcriptomic profiles. Early iterations of this technology were costly, and arrays allowed for detection of tens of hundreds of transcripts (Shalon, Smith and Brown 1996). Spot selection would generally be hypothesis driven in order to target genes relevant to particular pathways or applications such as toxicology or cancer screens (DeRisi et al. 1996). Modern chips allow for the spotting of tens of thousands of probes spanning entire genomes. An example of a current high density chip, the Affymetrix Human Genome U133+ 2.0, provides analysis of 47,000 individual transcripts. This is based on extensive sequencing, annotation and understanding of the human genome and its expressed transcripts. Unfortunately, knowledge such as this relating to CHO cells has been unavailable to the public for many years, possibly due to industrial competition and an unwillingness to share proprietary data with competitors due to CHOs prolific use in industrial biopharma production. Homology with related species such as mouse and rat allowed for transcriptomic work to progress despite a lack of CHO specific data, however analysis demonstrated that only subsets of cross species probes gave a consistently reliable result, with many of the 25 bp probes investigated displaying a poorer ability to accurately quantify differential expression than CHO specific probes based on known sequences (Yee et al. 2008).

Rapid progress in generating CHO specific data has been made since the foundation of the Consortium on Chinese Hamster Ovary Cell Genomics in 2006 (. Recent efforts have led to the publication of the CHO genome (Xu et al. 2011), an important milestone and invaluable resource for large scale '-omic' experiments such as proteomic or transcriptomic microarray experiments. Sequencing, analysis and annotation of thousands of CHO expressed sequence tags (ESTs) has allowed for the current generation of accurate, CHO-specific microarrays capable of quantifying a wide range of thousands of expressed genes (Kantardjieff et al. 2009).
This new generation of CHO-specific microarrays has been put to use by a number of labs, including a number of successful experiments carried out in the NICB.

1.8.3 Possibilities for experimental design

Traditionally, experiments involve comparison of a given cell line possessing an advantageous trait or one placed under a specific treatment to a relevant control. A typical example carried out by Doolan et al. (2012) involved comparing cell lines with sustained productivity to those whose productivity is shown to drop after day 7 of culture. This experimental design and cell lines chosen allowed for a hypothesis based on comparison of cell lines differing in a specific phenotypic trait, thus allowing conclusions to be drawn relating these differentially regulated gene targets to that particular phenotypic trait. A similar approach was taken in a study investigating clones of fast and slow growth rates (Doolan et al. 2010). In this instance a proteomic analysis of all differentially expressed proteins also carried out in parallel was cross referenced with the lists of differentially regulated genes generated from micro array experimental data. This study identified 21 genes common to both approaches, strong contenders for further functional validation and possible engineering experiments. This approach addresses one of the drawbacks to transcriptomic profiling, in so far as transcription levels do not always correspond to expressed functional protein levels. By eliminating genes that are not differentially expressed at the protein level, we can disregard genes that may not have a true impact on cell metabolism due to any of the post translational regulatory processes (such as RNAi) that prevent mRNA from becoming a functional protein and truly impacting a cells metabolism.
Reactions to environmental pressures or treatments in identical cells can also be quantified on the transcriptomic level. For example, CHO cells undergoing sodium butyrate (NaBu) treatment and temperature shift were found to improve in productivity from 40pg/cell/day to over 100pg/cell/day (Kantardjieff et al. 2010). The causes of this effect were assessed, among other techniques, by transcriptional profiling. By evaluating the differential expression of a wide range of genes, a number of different functional classes were identified as important to the improved protein production and secretory abilities such as genes relating to cell cycle and the golgi apparatus.

Highlighting the advantages of larger data sets and the advantages of increased statistical confidence, a broader profiling experiment was carried out in contrast to the narrower trait/treatment-specific studies outlined above. It incorporated 295 microarrays from over a hundred individual clones, each with a range of relevant

characteristics analyzed including growth, viability, specific productivity and waste secretion (Clarke et al. 2011). Coupling this large data set with suitable statistical analysis, transcriptional patterns for groups of genes (known as modules) rather than single genes could be discovered. These modules were found to maintain consistent correlation of expression across multiple cell lines and environmental variables, with subsequent analysis of these gene groups providing us with a global, holistic overview of the CHO metabolism and how groups of genes work in tandem to generate a phenotype. This 'guilt by association' approach also allows us to predict the function of novel genes, by assigning them to functional classes based on their similarly co-expressed partners, an approach that has also been shown to work successfully in yeast (Hughes et al. 2000). Knowledge of these gene networks and functional classes provide us with a better opportunity to rationally modify the processes within industrially relevant cells. By targeting classes of genes and the regulatory switches that control them, we may begin to improve upon the older, single gene oriented engineering approaches.

1.8.4 Potential disadvantages

Of course, despite the power of this technique, there are inherit weaknesses and drawbacks to this methodology. Statistical confidence in results values is limited by sample number, which may limit the numbers of genes that can be confidently identified by smaller scale experiments when an appropriate threshold for fold change expression is set. Due to the inherent noise of data generated in such experiments, higher statistical limits must be set. This might exclude genes from analysis that have profound impacts despite small changes in their expression levels and, conversely, highlight genes with little effect despite large fold changes in expression. Also, as discussed previously, the effects of some genes may be regulated at the protein level rather than the RNA level. This makes the comprehensive proteomic/transcriptomic approach a more costly but highly accurate depiction of the cell at a given point in time, with both techniques complementing each other by covering the potential drawbacks of each.

1.8.5 TBP Mutant Profiling

For the purposes of this thesis, expression profiling was used to investigate the genetic basis for the differences in phenotype witnessed in the directed evolution experiment. As a number of phenotypically advantageous clones were isolated, we wanted to dissect the transcriptomes of these clones and identify gene activity potentially responsible for improvements in growth and viability. Samples were selected to give a broad indication of the effects of the various stages of the experiment, and how they might interact in a synergistic manner to generate an advantageous phenotype. These included examining the effects of TBP overexpression and the effects of the stress procedure on transfected and control cells. High performing clones were also included to gain an insight into their transcriptomic profile, hopefully elucidating some of the genetic bases responsible for their advantageous phenotypes and provide us with further insight to rationally generate such phenotypes in future.

Section 2.0

Materials and Methods

2.1 Routine Management of cell lines

2.1.1 Safety precautions

All routine cell culture work was carried out in a class II down flow re-circulating laminar flow cabinet (Nuiaire Biological Cabinet), while any work involving toxic drug compounds was carried out in a cytoguard safety cabinet (Gelman). Strict aseptic techniques were adhered to at all times (see NICB SOP No. 000-01). Laminar flow cabinets were swabbed with 70% industrial methylated spirits (IMS) prior to and following all work, as well as all equipment used during experiments. Only one cell line was worked with at a time in a cabinet, with 15 minutes clearance time given between work with individual cell lines. Each week cell culture cabinets and any incubators used were cleaned with industrial detergents (Virkon) and IMS. A separate lab coat was worn during aseptic work and gloves were worn at all times during cell culture work.

2.1.2 Subculture of adherent cell lines

Adherent cell lines were grown in $25cm^2$ or $75cm^2$ tissue culture flasks and typically fed every 2-3 days. These were maintained at an incubation temperature of 37°C, with 5% atmospheric CO_2. During subculturing or harvesting of adherent cell lines for experimentation, cells were removed from their vessels by enzymatic detachment.

Cell culture flasks were emptied of waste medium and rinsed with pre-warmed (37°C) trypsin/EDTA solution (0.25% trypsin (Gibco, cat. 15090), 0.01% EDTA (Sigma, cat. E4884) solution in PBS) in order to remove any naturally occurring trypsin inhibitors present in residual serum. Fresh TV was applied to the cells (i.e. $1ml/25cm^2$ flask, $2ml/75cm^2$ flask) and then incubated at 37°C until cells were seen to have detached fully (5-10 mins). The trypsin was then inactivated by the addition of growth medium (i.e. containing 5% serum). This solution was then transferred to a 20ml sterile container (Greiner, cat. 201151) and centrifuged at 1000 rpm (170 x *g*) for 5 mins. The resulting cell pellet was then resuspended in fresh growth medium and counted

(see 2.1.4) allowing for the required cell number to be re-seeded in culture or for a future experiment.

2.1.3 Subculture of suspension cells

Cell lines adapted to suspension growth were generally grown in either sterile disposable 250 ml flasks (Corning, cat. 431144) with a 50ml working media volume or a disposable 50 ml spin-tube (Sartorius, cat. DF-050MB-SSH) with a 5ml working media volume. These conditions were used as they closely mimic the responses of large scale cultures on a smaller magnitude that is manageable in the lab.

First, a 100µl was removed from the flask or tube and counted using trypan blue (see 2.1.4). The cell suspension was then removed to a 20ml sterile container (Greiner, cat. 201151) and centrifuged at 1000 rpm (170 x *g*) for 5 mins. The resulting pellet was resuspended in fresh warm growth medium, and an appropriate volume used to seed fresh flasks/tubes for further subculture or to seed for planned experiments.

2.1.4 Cell counting

Cell counting and viability estimations were carried out using a trypan blue dye exclusion technique.
First a sample was taken from a cell suspension and mixed 1:1 with trypan blue (i.e. 100µl cell sample mixed with 100µl trypan blue, 0.2% solution in PBS). This was incubated for 3 mins at room temperature, after which a sample was applied to a haemocytometer over which a glass coverslip has been placed. Cells in the outer four corners of the chamber were then counted microscopically using Nikon TS100 microscope and 10x objective lens. Non-viable cells were stained blue, while viable cells remained white. An average number of viable cells in each corner was calculated with the dilution factor taken into account and multiplied by 10^4 to determine the cell per ml concentration in the original sample. The volume occupied by the sample chamber is 0.1cm x 0.1cm x 0.01cm i.e. 0.0001cm^3, therefore average cell number multiplied by 10^4 is equivalent to cells per ml.

2.1.5 Cell freezing

To allow for long term storage of cell stocks, cells were cryo-preserved in liquid nitrogen at temperatures below -180°C, allowing the indefinite storage and preservation of cell samples. Dimethyl sulphoxide (DMSO) (Sigma, cat. D8418) was used as a cryoprotectant.

1. Cells to be frozen down were grown until the exponential phase, harvested and counted in single cell suspension as per 2.1.3.
2. Cells were then pelleted by centrifugation at 1000 rpm (170 x *g*) for 5 mins and resuspended in medium to give a concentration of at least 5 x 10^6 cells/ml.
3. An equal volume of 2X concentrated freezing medium was then added dropwise. Due to the toxicity of the DMSO used this is added slowly to prevent shock to the cells, allowing them to adapt to its presence. Two distinct freezing media were used; one for cells cultured in the presence of serum (DMSO-10%, serum-20%, serum free medium-70%) and one for cells cultured in serum free medium (DMSO-15%, serum-40%, serum free medium-45%)
4. This suspension, containing minimum 2.5 x 10^6 in freezing medium, was aliquoted into cryovials (Greiner, cat. 12279) and quickly placed into a freezer at -20°C for 1-2 hours. This was then transferred to a freezer at -80°C overnight before finally being placed into storage in liquid nitrogen.

2.1.6 Cell thawing

1. Prior to the removal of a cryovial from liquid nitrogen storage for thawing, a sterile universal tube containing pre-warmed (37°C) growth medium was prepared for the rapid transfer and dilution of thawed cells to reduce their exposure to the toxic DMSO present in the freeze medium.
2. Once removed from liquid nitrogen the cryovial was thawed in warm water.
3. Once slightly thawed the contents of the cryovial was transferred to the pre-warmed medium.
4. This suspension was centrifuged at 1000 rpm (170 x *g*) for 5 mins. The supernatant was then removed and the pellet re-suspended in fresh warm growth medium.

5. This thawed cell suspension was then placed in an appropriate flask (i.e. 75cm2 tissue culture flask for attached growth cells or a 5ml spin tube for suspension growth)
6. After 24 hours, the medium was then replaced with fresh growth medium to remove any potential traces of DMSO and the cells subcultured if necessary.

2.1.7 Sterility checks

Sterility checks were routinely carried out on all media, supplements (serum etc.) and trypsin used for cell culture (see NICB SOP 002-01). A 1ml sample of the material being examined was inoculated into 9mls of both tryptone soya broth (TSB) and thioglycollate broth (Thio) and incubated at 37°C for 7 days. Both samples were then checked for turbidity and change in colour, indicating contamination. TSB supports the growth of yeasts and aerobes, while Thio mainly allows for the growth of both aerobic and anaerobic bacteria.

2.1.8 *Mycoplasma* analysis

Routine *Mycoplasma* examinations were carried out on all cell lines used in this study by a trained technician.

2.2 Preparation for cell culture

2.2.1 Water

Ultrapure water was used in the preparation of all solutions used for cell culture. Pre-treatment, involving activated carbon, pre-filtration and anti-scaling was first carried out. This water was then purified by a reverse osmosis system (Millipore Milli-RO 10 Plus, Elgastat UHP), which is low in organic salts, organic matter, colloids and bacteria with a standard of 12 – 18 MΩ/cm resistance.

2.2.2 Glassware

All solutions for use in cell culture and maintenance were prepared and stored in sterile glass bottles. Bottles, lids ad all other glassware used for any cell related work were prepared as follows: all glassware and lids were soaked in a 2% (v/v) solution of RBS-25 (AGB scientific, cat. 83460) for at least 1 hour. This acts as a deproteinising agent, which removes proteinous materials from the bottles. Glassware was scrubbed and rinsed several times in tap water; the bottles were then washed by machine using Neodisher detergent, an organic, phosphate-based acid detergent. The bottles were then rinsed twice with distilled water, once with UHP water and sterilised by autoclaving.

2.2.3 Sterilisation

Water, glassware and all thermolabile solutions were sterilised by autoclaving at 121°C for 20 mins under 15 p.s.i. pressure. Thermolabile solutions were filtered through a 0.22μm sterile filter (Millipore, cat. SL6V033RB), with low protein binding filters used for all protein-containing solutions.

2.3 DNA Manipulation and analysis

2.3.1 Production of unique plasmid DNA samples

In order to generate the unique plasmids necessary for the subsequent experiments detailed in this study, plasmids containing target DNA sequences were generated by digesting the target DNA sequence and plasmid with restriction enzymes (see 2.3.5) to create the 'sticky' ends necessary for a standard ligation reaction (see 2.3.9). These target sequences were either created via polymerase chain reaction (PCR) using a genomic DNA template (see 2.3.7) or removed by restriction digestion from another plasmid containing the fragment and purified by means of gel electrophoresis (see 2.3.8).
Once successfully cloned and verified using restriction digest analysis or PCR screening (see 2.3.10), plasmid samples were then transformed into bacteria cells (2.3.2) and large-scale prepped to create the amounts necessary for further experiments.

2.3.2 Transformation of bacterial cells

1. 1 vial (50µl) of competent cells (DH5α, Invitrogen, cat. 18263-012) was removed from storage at -80°C and thawed on ice. Transformation tubes Falcon, cat. 352059) were also chilled on ice during this time.
2. Once thawed the bacteria was added to a transformation tube along with 2.5µl (5% total volume) of plasmid sample or ligation reaction mixture.
3. This mixture was left on ice for 10-15 mins, heat shocked in a 42°C waterbath for 30 seconds then placed back on ice for a further 1.5 mins.
4. 200µl of SOC medium (Invitrogen, cat. 15544) was then added, and this mixture incubated at 37°C for 1 hour at 200 rpm agitation speed.
5. The sample was then transferred to a fresh 1.5ml tube (Costar, cat. 3620) and spun at 3000 rpm in a benchtop microfuge (Hettich Mikro 120) for 2 mins to pellet the bacterial cells.

6. 160μl of the supernatant was removed and the pellet resuspended in the remaining 100μl SOC. This was then spread on LB agar plates (10g Tryptone (Sigma, cat. T7293), 5g Yeast Extract (Oxoid, cat. LP0021), 10g NaCl (Sigma, cat. S7653), 20g agar select (Sigma, cat. A5054), to 1L with UHP water, autoclaved before use) containing Ampicillin (100ng/ml working concentration) and incubated overnight at 37°C.

2.3.3 Small scale preparation of plasmid DNA (Miniprep)

This technique was employed in order to generate small amounts (10-30μg) of plasmid DNA for use in restriction digest and ligation reactions.

1. After successful generation of transformed bacterial colonies (see 2.3.2), a single colony was picked and grown overnight at 37°C, 130 rpm in a 10ml aliquot of LB broth (10g Tryptone (Sigma, cat. T7293), 5g Yeast Extract (Oxoid, cat. LP0021), 10g NaCl (Sigma, cat. S7653), to 1L with UHP water, autoclaved before use) containing Ampicillin (100ng/ml working concentration).
2. This culture was then processed using a Qiagen QIAprep Spin Miniprep kit (Cat. No. 27106, see manufacturers protocol for details), with a final DNA elution step carried out using 50μl of TE buffer.
3. The concentration of this DNA prep was determined using a NanoDrop 1000 spectrophotometer. This could then be used in subsequent DNA manipulation reactions or stored at -20°C.

2.3.4 Large scale preparation of plasmid DNA (Maxiprep)

This technique was employed in order to generate large amounts (<200µg) of plasmid DNA for use in restriction digest and ligation reactions.

1. After successful generation of transformed bacterial colonies (see 2.3.2), a single colony was picked and grown at 37°C for 7-8 hours, 130 rpm in a 10ml aliquot of LB broth (10g Tryptone (Sigma, cat. T7293), 5g Yeast Extract (Oxoid, cat. LP0021), 10g NaCl (Sigma, cat. S7653), to 1L with UHP water, autoclaved before use) containing Ampicillin (100ng/ml working concentration) to generate a starter culture.
2. This starter culture was then added to a further 250ml of LB broth containing Ampicillin and incubated overnight at 37°C, 130 rpm.
3. After incubation this culture was then spun at 6000g for 15 mins, 4°C, and the supernatant discarded.
4. The resulting cell pellet was then processed using a Qiagen Plasmid Maxi kit (Cat. No. 12362, see manufacturers protocol for details), with a final DNA resuspension step carried out using 500µl of TE buffer.
5. The concentration of this DNA prep was determined using a NanoDrop 1000 spectrophotometer. This could then be used in subsequent DNA manipulation reactions or stored at -20°C.

2.3.5 Restriction digest of DNA samples

Restriction digest is a key technique involved in the manipulation of DNA sequences. Once plasmid DNA or PCR sequences have been generated, restriction digest can be employed to linearise circular DNA, generate “sticky ends” for cloning reactions or confirm sequences and sequence orientation within plasmids based on key restriction sites within.

A standard restriction digest reaction is detailed below;

Component	Volume
DNA	1µg
NEB Buffer (10x)	2µl
Bovine Serum Albumin	0.2µl
Restriction Enzyme*	1µl (5%)
$H_2$0	to 20µl
Total volume	20µl

* If multiple enzymes are used, they are kept below a final concentration of 5%

This reaction was incubated at 37°C for at least 3-4 hours to ensure complete digestion.

The volumes of these reagents could be up-scaled in proportion to generate volumes larger than 20µl of digested DNA. This was sometimes necessary to create large amounts of insert DNA for ligation reactions (see 2.3.9)
Also, multiple restriction enzymes could be used in single reactions provided a suitable NEB buffer was available, while keeping the volume of enzyme below 5% of the total reaction volume.

2.3.6 Alkaline Phosphatase treatment of digested plasmid samples

To prevent the re-circularisation of digested plasmid backbone samples and to improve the efficiency of ligation reactions, purified backbone samples were treated with alkaline phosphatase (AP)

1. After digestion, the reaction was run on a low melting agarose gel (Sigma, A9414) and excised using a clean scalpel.
2. This gel fragment was purified using a Gel Extraction Kit (Qiagen, cat. 20021) and eluted in 20µl TE buffer.
3. To this solution 2.2µl of phosphatase buffer was added, along with 0.8µl (16U) alkaline phosphatase (Roche, cat. 11097075001) and incubated for 1hr at 37°C.
4. To remove any traces of enzyme that may damage DNA or inhibit further reactions, this solution was then purified using a MinElute PCR Purification Kit (Qiagen, cat. 28006).

This purified and AP treated plasmid DNA could then used in subsequent ligation reactions (see 2.3.9)

2.3.7 Polymerase Chain Reaction to generate fragments for cloning

In order to construct plasmids for future use in cell engineering or virus propagation, PCR was carried out on a suitable template to generate fragments that we're subsequently used for ligation reactions. For this purpose, a high fidelity enzyme (KOD Hi-fi) was used to minimise possible mutations or errors during amplification.

Component	Volume (μl)
10x Polymerase buffer	5
2mM dNTP	5
KOD Polymerase[1]	0.4
Forward Primer[2] (10nM)	1
Reverse Primer[2] (10nM)	1
Template DNA[3]	1
H_20	9.5
Total Volume	50

1 - KOD Hi-fi Polymerase (Merck, cat. 71085)

2 - Primer sets designed to generate relevant fragments, usually incorporating restriction sites for generation of 'sticky' ends prior to ligation

3 - Template DNA incorporating target sequence (e.g. plasmid, genomic, cDNA etc.)

These reactions were generally run in a 0.2ml thin walled PCR tube in a thermocycler (Thermo Scientific Hybaid Px2) under the following conditions;

Stage	Temperature	Time	Cycle Number
Initial Denaturation	95°C	2 mins	
Denaturation	94°C	30 secs	
Annealing	(primer specific)	30 secs	25 cycles
Elongation	72°C	1 min/kb	
Final elongation	72°C	10 mins	

A 5μl sample of each reaction was then run on an agarose gel to confirm the presence of the target band, and determine if any nonspecific contaminating bands are present.

2.3.8 Analysis of DNA samples using agarose gel electrophoresis

Agarose gel electrophoresis was used to visualise and isolate specific fragments of any DNA sequences used in this study.

1. First, a stock of 50x TAE buffer was created (242g Tris Base, 57.1ml Glacial Acetic Acid, 100ml 0.5M pH8.0 EDTA, to 1L with UHP water). This was diluted to 1x stock with UHP water and Agarose (Sigma, cat. A9539) added to a final concentration of 1% w/v. This percentage was chosen to visualise most fragments, however this percentage could be increased of decreased to achieve better resolution on smaller or larger fragments respectively.
2. This solution was then heated in a microwave until molten, Ethidium Bromide was then added to a final concentration of 0.5 µg/ml and poured into the appropriate mould with a comb to allow for sample wells.
3. Once solidified the gel was placed in an electrophoresis tank filled with 1x TAE buffer. DNA samples, mixed with a suitable loading buffer, were then added to the wells and current applied to the gel. 90-100V was generally used for visualisation; however greater resolution could be achieved at a lower voltage applied for a longer period.
4. A reference DNA ladder sample was also generally run alongside samples to measure visualised band sizes.
5. Once sufficiently separated, bands could be visualised in a UV light box at a 254nm wavelength.

2.3.9 Ligation of DNA samples

In order to insert target DNA sequences into a plasmid, both samples (insert and plasmid vector) were first restriction digested to generate ‘sticky’ ends (see 2.3.5) and treated with alkaline phosphatase (see 2.3.6). These samples could then be ligated using a T4 DNA ligase enzyme (see 2.3.9).

1. The default vector:insert ratio chosen was 1:10. First, the correct amount of insert was calculated using the formula below:

$$\text{Insert mass (ng)} = \frac{\text{Insert length (bp)}}{\text{Vector length (bp)}} \times \text{Vector Mass (ng)} \times 10 \text{ (ratio)}$$

2. A standard ligation reaction was then set up on ice as detailed below, with control reactions used to estimate the efficiency of insertions in comparison to plasmid self ligation.

	1:10 Insert Ratio	Control (No insert)	Control (No ligase)
Ligase Buffer (10x)	1.5	1.5	1.5
Plasmid Backbone	1	1	1
Insert DNA	variable*	0	0
Ligase Enzyme	0.5	0.5	0
H2O	to 15µl	to 15µl	to 15µl
Total Volume	15	15	15

*Note: The insert DNA volume was variable depending on the sample concentration. Volume used was sufficient in each case for a minimum of 1:10 vector:insert ratio.

3. This reaction mixture was incubated at 14°C overnight.
4. The enzyme was inactivated by incubation for 10 mins at 65°C. This mixture was then used for bacterial transformation (see 2.3.2).

2.3.10 Screening of cloned plasmid samples using PCR

After ligation and transformation, screening of colonies was necessary to ensure the insertion of the correct sequence into the plasmid backbone before plasmid preparation. This was achieved by running a high throughput PCR screen on individual colonies, using a small culture of each colony as a source of DNA.

1. After transformation of a ligation mixture (see 2.3.2), colonies were picked using a sterile pipette tip into 100μl of LB medium in a 96 well plate with Ampicillin and grown overnight at 37°C.
2. A standard PCR reaction was set up on ice as below. A master mix was created with excess volume to allow for pipetting errors.

Component	Volume (μl)
Platinum Supermix[1]	23
Forward Primer (10nM)[2]	0.5
Reverse Primer (10nM) [2]	0.5
Bacterial culture	1
Total Volume	25

1 – Platinum supermix (Invitrogen, cat. 11306)
2 – Primers selected to amplify any selected region within the ligated insert sequence that would not be otherwise present in the original plasmid backbone.

This reaction was performed under standard PCR conditions in a 96 well PCR plate;

Stage	Temperature	Time	Cycle Number
Initial Denaturation	95°C	2 mins	1 cycle
Denaturation	94°C	30 secs	
Annealing	(primer specific)	30 secs	20 cycles
Elongation	72°C	45 secs	
Final elongation	72°C	10 mins	1 cycle

3. A 10µl sample of each PCR reaction was then run on a 1% gel (see 2.3.8). A band of the expected size would indicate a positive result, i.e. a plasmid that has been successfully ligated with the target insert.
4. A sample from the 100µl bacterial culture could then be used to generate a 5ml starter culture when incubated at 37°C, which in turn could be used for small scale or large scale plasmid preparation (see 2.3.3/2.3.4)

2.3.11 cDNA generation from RNA for qRT-PCR

The protocol outlined below allows for the isolation of RNA and generation of a cDNA library. This can then be used in a subsequent qRT-PCR to measure the relative expression of a target gene between cell samples.

1. A flask of cells for each cell line to be analysed via qRT-PCR was cultured til 50-60% confluence, trypsinised and pelleted. This pellet was then processed using the RNeasy Mini kit (QIAgen, cat. 74106) and finally eluted in 40µl of H2O.
2. A standard RT reaction was then set up as below on ice (Applied Biosystems, cat. 4387406), with an RT negative control reaction also run for each sample.

Component	Volume (µl)	
	RT+	RT-
2x RT Buffer	10	10
20x RT Enzyme Mix	1	-
Nuclease-free H_20	-	1
RNA sample	9	9
Total Volume	20	20

The following thermocycler conditions were programmed for this reaction:

Stage	Temperature	Time
1	37°C	60 mins
2	95°C	5 mins
3 (storage)	4°C	-

3. This reaction was then diluted 1:10 with nuclease free H2O and stored at -20°C or used immediately in a qRT-PCR reaction.

2.3.12 Quantitative PCR (qPCR)

In order to determine whether a gene is currently over expressed or knocked down in a cell line, as well as comparing its relative expression between clones, qPCR is used to measure this expression level.

These levels are measured by first isolating the RNA content of the cell, then generating a corresponding cDNA library via reverse transcription. qPCR is then carried out on this cDNA sample. The differences in expression between cell lines can be determined using the expression of a known housekeeping gene as a control, while a standard curve of known concentration can be used to measure sequence copy number

1. The qPCR reaction detailed below was set up on ice in a MicroAmp optical 96-well plate (Applied Biosystems, cat. 4346906) using a qPCR mastermix containing SYBR green to quantitate the levels of double stranded DNA generated (Fast SYBR Green Master Mix, Applied Biosystems, cat 4385612). The table outlines the components for this reaction. Samples were run in triplicate, including the RT+ and RT- samples.
   This complete sample set was also run using a second set of control primers based on a housekeeping gene know to have a relatively constant expression level.

Component	Volume (µl)
2x qPCR Mix	10
Forward Primer (10nM)	1
Reverse Primer (10nM)	1
cDNA	1
H_2O	7
Total Volume	20

2. This reaction is then carried out on an Applied Biosystems 7500 Fast Real-Time PCR System under the following cycling conditions;

Stage	Temperature	Time	Cycle Number
Enzyme Activation	95°C	20 secs	1 cycle
Denaturation	95°C	3 secs	40 cycles
Annealing/Elongation	60°C	30 secs	

3. Results are then analysed using Applied Biosystems 7500 software.

If gene expression is measured in comparison to an internal housekeeping gene (qRT-PCR), the "$2^{(-\Delta\Delta Ct)}$" method is used to calculate relative expression, while standard curves are used to determine absolute copy number.

2.4 Virus Generation and Purification

2.4.1 Propagation and transfection of HEK-293 cells

For this study a helper free adeno-associated viral system was utilised (Agilent Technologies, cat. 240071). This system consists of a number of components including:

- HEK-293 cells used to generate the viral particles
- Plasmids (pRC and pHelper) coding the genes necessary for viral production
- pAAV plasmid modified to carry sequences both used for viral packaging as well as the homologous targeting cassettes used in this study
- A pAAV-GFP control plasmid was also used in each transfection to calculate transfection and transduction efficiencies.

HEK cells were used in this study as means of generating adeno-associated viruses (AAV), which were themselves used in all subsequent gene knockout experiments. This protocol describes the method used to grow and transfect HEK-293 cells with the viral construct prior to their lysis and harvesting of complete viral particles (see harvest/purification).

1. The HEK-293 cells used in this study were cultured in DMEM medium with 10% FCS in T-75 flasks, with minimal passaging between revival and transfection to ensure no change in phenotype.
2. 24 hours before transfection cells were trypsinised and plated on 96mm tissue culture dishes at a density of 2 x 10^6 cells per plate. For high concentration virus preparations (for example knockout virus constructs) 10 plates were seeded; 9 plates for virus generation and 1 plate to act as a transfection control.
3. Immediately prior to transfection the medium was replaced with 8ml of fresh DMEM (10% FCS) per plate.
4. DNA solutions were made by mixing 10µg of each plasmid (pHelper, pRC and pAAV) in a 1.5ml tube (including 1 mixture containing pAAV-GFP for a control transfection).

5. 30µl FuGene 6 transfection reagent (Roche, cat. 11 814 443 001) was then mixed with 500µl OptiMEM (Invitrogen, cat. 11058-021) medium for each plate to be transfected. This solution was left to sit at room temperature for 5 mins.
6. Each DNA mix was then added to a FuGene/OptiMEM solution and mixed well. This solution was left to set at room temperature for 20 mins.
7. Each DNA/FuGene/OptiMEM mixture was then slowly added to each plate of HEK-293 cells dropwise. These plates were then returned to the incubator under normal growing conditions for 72 hours until harvesting (37°C, 5% CO_2).
8. After 48 hours, the AAV-GFP control plate was also viewed under a fluorescent microscope (Leica) to ensure fluorescence of the cells, indicating a successful transfection.

2.4.2 Harvest and purification of viral particles

After successful transfection, HEK cells were lysed using a freeze/thaw method and purified using a commercial viral purification kit (ViraBind AAV Purification kit, Cell Biolabs, cat. VPK-141)

1. Plates containing successfully transfected virus producing HEK-293 cells were first scraped using a sterile scraper and the cells and supernatant collected.
2. For multiple plates (such as the 9 generated for the targeting construct viruses), these were combined and centrifuged at 1500 rpm (380 x *g*) and the resulting cell pellets resuspended in 25ml of supernatant (a prerequisite volume for the purification kit used in this experiment)
3. This cell suspension was then subjected to 4 rounds of freeze thaw lysis. This was achieved by submerging the tubes in liquid nitrogen and thawing in a 37°C waterbath.
4. After lysis, this suspension was then spun at 10,0000 x *g* for 10 mins and the supernatant containing viral particles was removed and stored at -20°C prior to purification (single GFP virus plates were not subject to purification but used neat to estimate transduction efficiency).
5. Supernatants were subsequently purified as per the protocol from the Virabind purification kit. This resulted in a final volume of ~130µl of purified virus.

2.4.3 Transduction of CHO-K1 cells

Once purified, AAV viral particles were applied to CHO-K1 cells to allow for delivery of the targeting cassette sequence into the nucleus of the cell.

1. CHO-K1 cells in attached culture were trypsinised and seeded in 6 well plate at a density of 5 x 10^4 cell per well with 2ml of fresh medium. These were incubated for 24 hours before proceeding. (Assuming a doubling time of roughly 24 hours, this would generate 1 x 10^5 cells per well overnight.)
2. After 24 hours, the old medium was replaced with fresh medium. In one well, the entire stock of purified virus was added (~130µl). To other wells 200µl of control GFP virus was added in order to confirm the presence of viable viral particles and estimate transduction efficiency.
3. After 48 hours cells transduced with targeting viruses were trypsinised and seeded into a T-75 flask. Appropriate drug selection was also applied to remove the background of untransduced cells.
4. AAV-GFP transduced cells were also trypsinised and removed for analysis of fluorescent cell numbers via flow cytometry.

2.4.4 Single cell cloning

The protocol detailed below was used to isolate single cell clones from a mixed cell population.

1. A T-75 flask of cells was cultured till 50-60% confluence. These were initially re-fed with fresh warm medium 3-4 hours prior to single cell cloning.
2. The flask of cells was then trypsinised, and resuspended in 5mls of medium. This was pipetted up and down to ensure a single cell suspension.
3. Another 15ml was added to this suspension and the solution mixed thoroughly. At this point a number of separate samples (at least 3) were taken for cell counting.
4. Once an accurate count had been obtained, serial dilutions were then carried out to bring the concentration to a target of 5 cells/ml.

For example, an initial dilution in 20mls of medium would bring the concentration to 2 x 10^4 cells/ml. Further dilution steps ranging from 1:50 to 1:100 fold dilutions would then bring the concentration to its target of 5 cells/ml, with care taken to ensure homogenous mixing in between.

5. The final dilution step was carried out in fresh medium containing 15% conditioned medium (conditioned for 48-72 hours on CHO-K1 cells) to increase the survival rate of single cells in isolated wells.
6. 100µl of this suspension was then added to each well of a 96 well plate. This gives a theoretical rate of 0.5 cells per well; high enough to ensure a practical number of clones per plate while reducing the number of wells with multiple cells.
7. Plates of clones were then monitored for growth over the next 8-10 days and single cell colonies identified (disregarding wells containing more than one colony), re-feeding with fresh medium as necessary.

Once cells had reached near confluence, selected single cell clones were then trypsinised and split in duplicate. One plate was then used to isolate genomic DNA for PCR screening (see 2.4.6), while its corresponding identical plate was frozen down for future revival.

2.4.5 Cell freezing (96 well plate format)

Once single cell clones had been isolated, a sample of these cells was usually frozen down to enable further study or characterisation to be carried out on any DNA or RNA extracted from these cells. The protocol below was used to freeze large numbers of these clones in a 96 well format, which were then stored at -80°C.

1. Plates were initially fed with fresh medium a number of hours prior to freezing.
2. All medium was then removed and wells rinsed twice with sterile DPBS –Cl –Mg (Gibco, cat. 14190)
3. 30µl of trypsin was applied to each well, an incubated for 10 mins at 37°C.
4. 70µl of chilled medium with 10% serum was then added to inactivate trypsin.
5. 100µl of chilled 2x freeze medium (DMSO-15%, serum-40%, cell culture medium-45%) was then added.

6. A 50µl overlay of sterile mineral oil was then added to each well to prevent evaporation while frozen, and the plates sealed with parafilm.
7. Plates were placed in a sealed Styrofoam container and placed in a -20°C freezer initially for 2 hours.
8. This sealed container was then placed in storage at -80°C for longer term storage.

Revival of these clones when necessary was carried out in a similar manner to that of regular vials (see 2.1.6), with revived cells being placed in 48 well plates due to their reduced cell density.

2.4.6 Isolation of genomic DNA via ethanol precipitation

Once a large number of single cell clones had been isolated after viral transduction, an extraction of genomic DNA was first carried out to allow for the characterisation of homologous recombination events via PCR. The protocol below outlines the high throughput method used to isolate genomic DNA from single cell clones propagated in a 96 well plate format.

1. First, cells were grown to reach confluence in a 96 well plate.
2. All medium was removed and each well rinsed twice with PBS.
3. To each well, 50µl of lysis buffer (10mM Tris, 10mM EDTA salt, 10mM NaCl, 0.5% w/v SDS, with 1mg/ml Proteinase K (Sigma, cat P-2308) added immediately prior to use) was added.
4. Plates were then incubated at 60°C overnight in a humid atmosphere (achieved by placing on top of tissue soaked in water, all in a sealed container)
5. After incubation, 100µl of an NaCl and cold ethanol mix was added to each well (150µl 5M NaCl mixed with 10ml ice cold ethanol, with constant mixing needed to ensure a homogenous solution)
6. Plates were allowed to stand undisturbed at room temperature for 30 mins to allow for the precipitation of genomic DNA.
7. After precipitation, plates were carefully inverted and the salt/ethanol mixture discarded. Excess liquid was blotted on tissue.

8. 150μl of 70% ethanol solution was slowly dripped down in order to wash each well. This wash was repeated for a total of 3 washes.
9. Each plate was then blotted and allowed to dry at room temperature, with care taken not to over dry (to prevent the difficult resuspension of DNA)
10. 50μl of TE buffer was then added to each well and allowed to sit for 2 hours to promote resuspension. These resuspended samples could then be stored at -20°C.

2.4.7 PCR screening of genomic DNA

In order to screen large numbers of clones for recombination events subsequent to viral transduction, a PCR method was chosen for its ability to characterise different insertion events on a large scale using a small number of primer sets. The protocol outlines here is the final method optimised to give clear results when used to amplify bands from the genomic DNA samples obtained previously (see 2.4.6)

1. First, a standard PCR reaction is set up on ice as detailed below. For each plate these volumes were multiplied by 100 to generate a master mix that would allow for all samples as well as pipetting error

Component	Volume (µl)
DreamTaq Master Mix[1]	12.5
Forward Primer[2] (10nM)	0.5
Reverse Primer[2] (10nM)	0.5
Genomic DNA	2
H_20	9.5
Total Volume	25

(1) - DreamTaq PCR Master Mix (Fermentas, cat. K1072)

(2) - Primer sets chosen to screen for relevant insertion events

2. These reactions were carried out in a 96 well PCR plate (Applied Biosystems, cat. 4349606) under the following conditions (denaturation times and cycle numbers were increased during original optimisation to improve product yield from genomic DNA)

Stage	Temperature	Time	Cycle Number
Initial Denaturation	95°C	10 mins	
Denaturation	94°C	1 min	
Annealing	(primer specific)	30 secs	35 cycles
Elongation	72°C	1 min/kb	
Final elongation	72°C	10 mins	

3. A 10µl sample of each reaction was then run on an agarose gel to determine what samples were positive for the criteria being examined (for example the presence or absence of a particular band or a difference in band size based on a homologous recombination event)

2.4.8 qPCR to quantify viral insertion events

In order to estimate the numbers of off-target insertion events in cells having undergone viral transduction and integration, qPCR using genomic DNA was carried out on targeted samples.

For this reaction genomic DNA samples were isolated and diluted to a suitable concentration. An untransduced CHO-K1 sample was used to control for variance in concentration due to the difficulty in obtaining absolutely equal concentrations using the Nanodrop instrument. Instead, β-actin is used as a reference gene, assuming 2 intact alleles in each genome assayed. The neomycin resistance gene within the targeting cassette is then used as the target to measure insert frequency. Levels of this gene were measured against a standard curve generated using serial dilutions of a *neo*-containing plasmid. These reference samples were also spiked with an appropriate level of control genomic DNA to account for differences in amplification efficiency that may arise from the use of a plasmid template alone.

1. The RT-PCR reaction detailed below was set up on ice in a MicroAmp optical 96-well plate (Applied Biosystems, cat. 4346906) using a qPCR mastermix containing SYBR green (Fast SYBR Green Master Mix, Applied Biosystems, cat 4385612). The table outlines the components for this reaction. Samples were run in triplicate. For standard curve samples, a plasmid template spiked with CHO-K1 genomic DNA was used.

Component	Volume (μl)
2x qPCR Mix	10
Forward Primer (10nM)	1
Reverse Primer (10nM)	1
DNA sample	2μl (30ng)
H_2O	6
Total Volume	20

2. This reaction is then carried out on an Applied Biosystems 7500 Fast Real-Time PCR System under the following cycling conditions;

Stage	Temperature	Time	Cycle Number
Enzyme Activation	95°C	20 secs	1 cycle
Denaturation	95°C	3 secs	40 cycles
Annealing/Elongation	60°C	30 secs	

3. Results are then analysed using Applied Biosystems 7500 and Microsoft Excel software.

2.5 DNA Mutagenesis

2.5.1 Mutagenesis PCR (Megaprimer synthesis)

The first step in the process of generating the library of mutant gene sequences used in this study involved a kit based low fidelity PCR enzyme, used to amplify and incorporate mutations into the target sequence. The protocol below details the optimised protocol used for the generation and isolation of the gene sequences used to generate the mutant library.

1. After successful generation of the plasmid containing the target gene sequence, a mutagenesis PCR was carried out using the lo-fi enzyme contained within the GeneMorph II EZClone Mutagenesis kit (Agilent Technologies, cat. 200552).

   In order to examine a number of mutation rates, three reactions (high, medium and low mutation rates) were set up by differing the plasmid concentrations therein. Also, a control reaction was set up using a control plasmid and primers contained within the kit. These reaction mixtures are detailed below.

The reactions below were set up on ice in PCR tubes.

Component	Volume (µl)			
	Low	Medium	High	Control
10x Buffer	5	5	5	5
Forward Primer[1]	0.5	0.5	0.5	1
Reverse Primer[1]	0.5	0.5	0.5	
Plasmid DNA[2]	100ng	20ng	0.1ng	1ng
dNTP (40mM)	1	1	1	1
Polymerase (2.5U/µl)	1	1	1	1
H_2O	to 50µl	to 50µl	to 50µl	to 50µl
Total Volume	50			

1 – Primers selected to amplify region containing target gene sequence. 125ng of each target primer used (1µl of control primers used contains primer mix)

2 – Plasmid DNA amounts added was based on target sequence concentration, not total plasmid concentration

The above reactions were run using the cycle program below:

Stage	Temperature	Time	Cycle Number
Initial Denaturation	95°C	2 mins	
Denaturation	95°C	30 secs	
Annealing	(primer specific)	30 secs	35 cycles
Elongation	72°C	1 min/kb	
Final elongation	72°C	10 mins	

2. A 5µl sample of this reaction was visualised via gel electrophoresis (see 2.3.8) to verify that the PCR had worked.
3. The remainder was electrophoresed on a low melting agarose gel at a low voltage (50V) to ensure good resolution, then excised and purified using a Gel Extraction Kit (Qiagen, cat. 20021) and eluted in 10µl TE buffer.

2.5.2 EZ-Clone reaction

Once the mutant gene sequences had been generated, these were then used as "megaprimers" in a secondary PCR reaction used to amplify the target plasmid while incorporating the mutant DNA sequences into the plasmid itself. A high fidelity enzyme was utilised to prevent any further mutations being incorporated into the plasmid sequence.

1. A reaction was prepared on ice as below for each of the high, medium and low mutation megaprimer samples generated previously.

Component	Volume
2x EZClone Enzyme Mix	25µl
Template Plasmid	50ng
Megaprimer sample	500ng
EZClone Solution	3µl
H_20	to 50µl
Total Volume	50µl

The above reactions were run using the cycle program below:

Stage	Temperature	Time	Cycle Number
1	95°C	1 min	1 cycle
2	95°C	50 secs	25 cycles
	60°C	50 secs	
	68°C	2 min/kb of plasmid length	

2. Following temperature cycling, these reactions were placed on ice for 2 mins to cool the reaction to ≤37°C.
3. 1µl (10U) of *Dpn* enzyme was then added to the reaction and gently mixed by pipetting. This reaction was then incubated for 2 hours at 37°C in order to digest any remaining parental (non-mutated) plasmid DNA. This treated plasmid sample could then be used to transform bacterial cells.

2.5.3 Estimation of potential library complexity

The experiment below was carried out in order to estimate the number of individual plasmids in a sample of transformed bacteria and, in turn, an indication of the potential complexity of the mutant library.

1. First, varying amounts of plasmid sample were transformed into 50µl of DH5α as per the protocol (see 2.3.2). This transformation was then spread on LB agar plates containing Ampicillin.
2. After 24 hours incubation at 37°C the number of colonies per plate was then counted. The transformation that yielded the most colonies was determined to be the optimal volume for transformation.
3. This transformation was then repeated using the optimum volume established above.
4. 10µl (4%) and 40µl (16%) of this transformation was then spread on separate LB agar plates containing ampicillin.
5. After 24 hours incubation at 37°C the number of colonies per plate was then counted. This number of colonies, taken as a portion of the entire transformation reaction, was used to calculate the number of individual colony forming units generated per transformation. This number also indicated a rough indication of library complexity, assuming a high efficiency of transformation.

2.5.4 Large scale preparation of mutant library plasmid

This protocol outlined below was followed in order to generate a large stock of mutant plasmid DNA that could be used to generate stably transfected mammalian cells. To prevent the introduction of bias in the library due to differences in bacterial growth after transformation, a rich medium (Terrific Broth) was used as opposed to the LB broth used in standard DNA preps.

Terrific Broth	10x Phosphate Salts
12g Tryptone	11.56g $(KH_2PO_4)2H_2O$
24g Yeast Extract	62.7g $(K_2HPO_4)2H_2O$
4ml Glycerol	-
to 1L with UHP water	to 500ml with UHP water

Both solutions are autoclaved before use. 30ml of 10x phosphate salt solution was then added to 270ml terrific broth to generate the working medium solution.

1. Ten individual transformation reactions were carried out as per the standard transformation protocol using the optimum DNA volume determined previously.
2. After the addition of SOC medium and incubation for 1 hour, 2ml of Terrific Broth containing Ampicillin was added to each transformation and these cultures incubated for 1 hour at 230 rpm.
3. All 10 cultures were then added to 280ml of Terrific Broth containing Ampicillin and grown overnight at 37°C at 230 rpm.
4. After incubation this culture was then spun at 6000 x *g* for 15 mins, 4°C, and the supernatant discarded.
5. The resulting cell pellet was then processed using a Qiagen Plasmid Maxi kit (Qiagen, cat. 12362, see manufacturers protocol for details), with a final DNA resuspension step carried out using 500µl of TE buffer.
6. The concentration of this DNA prep was determined using a spectrophotometer (NanoDrop 1000).

2.5.5 Stable cell line generation

A transfection followed by selection for cells stably integrating the transfected plasmid to generate a mixed population was carried out as detailed below. A lipid based transfection reagent (Lipofectamine 2000, Invitrogen cat. 11668) was used for this method.

1. Healthy CHO-K1 cells were seeded in 6-well plates at a density of 5×10^5 cells per well and incubated overnight in 2mls of fresh medium. A control well was also seeded that would not be transfected with plasmid.
2. Prior to transfection the medium was replaced with fresh medium.
3. DNA and lipofectamine mixes were created. 2µg of plasmid DNA and 4µl of lipofectamine were added to separate 50µl aliquots of serum free medium and left to stand for 5 mins.
4. The DNA and lipofectamine mixes were then combined and left to stand for a further 15 mins.
5. This DNA/lipofectamine solution was then slowly applied dropwise to a well of cells.
6. After 48 hours medium was replaced and the appropriate selective drug applied to kill cells that have not successfully taken up the plasmid.
7. After 4 days cells were then trypsinised and moved to a T-75 flask with appropriate medium and selective pressure. Cells were continually monitored and maintained until all cells in the control sample were dead, indicating the background of untransfected cells was eliminated.
8. Once a population of stably transfected cells was achieved a stock was frozen down and this population then used for further experiments.

2.6 Gene Expression Profiling

Gene expression profiling is a powerful technique that allows for the measurement of expression levels across a large number of genes. By comparing samples under different experimental conditions to a suitable control, one can deduce from the differentially expressed genes the genetic basis from which a particular phenotype may have arisen and the specific genes responsible for the differences. As with any experiment, statistical confidence is increased with a greater number of biological replicates, however due to the economic costs triplicate samples are usually run as standard.

The protocol followed for this experiment was developed in-house by Dr. Padraig Doolan and Dr. Sinead Aherne, a modified version of the original protocol designed for use with the commercial GeneChip 3' IVT Express Kit (Affymetrix, 901229). The specific gene chips used for this experiment were 'Hamster 3a 520384' chips (Lot no. 4115243, ref no. 520384), manufactured by Affymetrix with the ability to measure the expression of roughly 19,000 CHO gene transcripts generated to measure ~8,000 genes. Due to the large volume of data generated, suitably powerful statistical analysis is then needed to identify gene targets substantially up or downregulated.

Experimental conditions for comparing samples may vary depending on the application, for example a single cell line may be subjected to varying conditions such as temperature shift or have various genes artificially up or downregulated. In this instance a number of cell lines resulting from the TBP mutagenesis project including suitable controls were grown under standard growth conditions, with 1×10^6 cells pelleted and their RNA extracted for profiling. To understand the genetic mechanisms that differentiate the various clones and help discover the differences in gene expression that may be responsible for the changes in phenotype, this RNA is then processed and analysed on a gene profiling chip.

The protocol described below follows the basic workflow of a standard profiling experiment such as this:

- Cell lines to be compared are seeded and grown under standard growth conditions
- Total RNA is extracted from the experimental cell samples at a suitable time point and quantified
- Quality control assesses sample integrity to prevent problems with downstream processing.
- Double stranded cDNA is synthesised from extracted RNA. Exogenous spiked prokaryotic RNA is included as an experimental control
- In Vitro Transcription (IVT) is carried out to generate biotin labelled antisense RNA (cRNA). These samples are then purified and QC carried out on these samples.
- cRNA samples are fragmented and hybridised to the gene chip. Suitable wash and staining steps are carried out
- The chips are then placed in the GeneChip Scanner to be scanned and the signal detected. The resulting data is then analysed, with suitable statistical limits set to select for gene targets with substantial changes in expression in relation to a control sample.

All buffers and enzymes are contained within the GeneChip 3' IVT Express Kit (Affymetrix, 901229) unless otherwise stated.

2.6.1 RNA extraction

Cell lines were seeded at 2 x 10^5 in triplicate and allowed to grow under standard growth conditions. Cells were measured for density and viability from day 2 onwards, with samples taken daily for future processing.
Samples consisted of 1 x 10^6 viable cells pelleted, washed in PBS and resuspended in 0.5ml TRI reagent (Invitrogen, cat. 10296-010). RNA was purified and extracted as per the manufacturer's protocol and resuspended in 20µl nuclease-free H_20. The concentration of this RNA prep was determined using a NanoDrop 1000 spectrophotometer, diluted to 100ng/µl and stored at -80°C.

Due to the precious nature of the primary RNA samples, particular care was taken during this experiment when handling these samples in order to prevent degradation from RNAses. This included precautions such as

- Through cleaning of all work surfaces and tools (pipettes etc.)
- The use of RNaseZap (Ambion, cat. AM9780) to reduce RNAse contamination on surfaces and tools
- The use of fresh nuclease-free H_20 for any required applications
- The use of filtered pipette tips to prevent any possible cross contamination

2.6.2 RNA Quality control

RNA quality control was carried out to ensure sample integrity prior to embarking on the profiling protocol. This was carried out using an Agilent 2100 Bioanalyser.

A 1µl aliquot of each sample was diluted 1:1 with nuclease free H_20 and run using the RNA 6000 Nano Kit (Agilent, cat. 5067-1511) according to the manufacturers protocol.

These RNA chips function in a similar fashion to an electrophetic gel, albeit on a microscopic scale. The sample is added to a gel matrix, which then passes through a number of connected microchannels when a current is applied. This allows for the

analysis of RNA fragment sizes and their concentrations. Degradation, if present, is measured by the software and sample integrity designated with a RIN (RNA integrity number) score between 1 and 10, with RINs approaching 10 proving the highest quality. A simulated visual depiction is also generated, similar to that of a traditional electrophoretic gel, in which band 'smearing' (indicative of sample degradation can be easily identified.

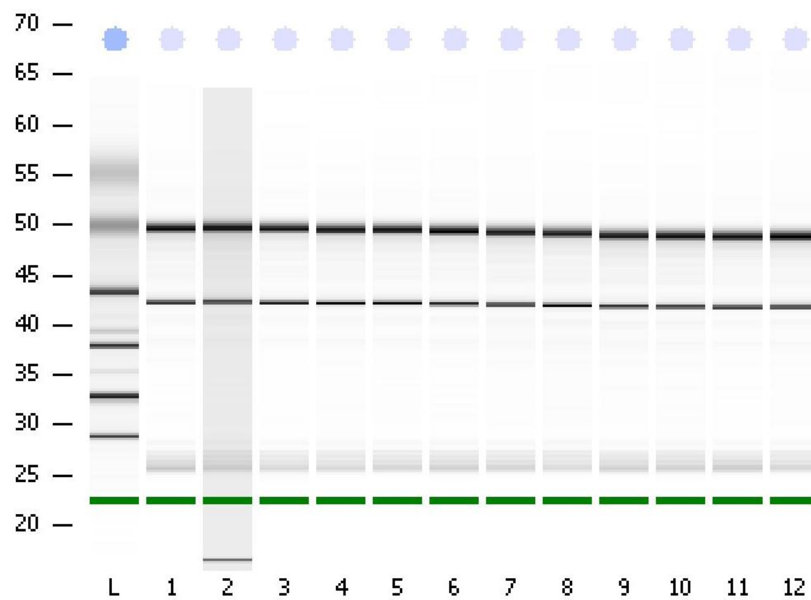

Figure 2.6.1.1: A simulated visual result from a selection of samples used in this study generated using the RNA 6000 Nano Kit. A 1µl aliquot of each RNA sample was diluted 1:1 with nuclease free H_20, and analysed using the Agilent RNA 6000 Nano Kit. All samples in this instance provided RIN scores >9, indicating a sample quality suitable for further profiling analysis.

2.6.3 cDNA synthesis

First strand synthesis is carried out as the first stage of cDNA generation. Poly A primers bind to the RNA transcripts, with a DNA polymerase generating the first and second strands of cDNA.

1. 2μl of exogenous control RNA, diluted according to the manufacturer's instructions, is added to 3μl of the experimental RNA sample. This is used as a basis to create the first strand synthesis reaction mix

Component	Volume (μl)
RNA sample (100ng/μl)	3
Diluted RNA controls	2
First-Strand Buffer Mix	4
First-Strand Enzyme Mix	1
Total Volume	10

2. This is incubated for 2 hours at 42°C in a thermocycler.
3. After incubation, the reaction is placed on ice. The mix is then made up for second strand synthesis, adding in the 10μl first strand synthesis reaction immediately before incubation

Component	Volume (μl)
Second-Strand Buffer Mix	5
Second-Strand Enzyme Mix	2
Nuclease-free Water	13
First-Strand Reaction Mix	10
Total Volume	30

4. This second strand mix was incubated for 1 hour at 16 °C followed by 10 minutes at 65 °C in a thermal cycler. After incubation, the reaction is placed on ice, and used directly for the next in vitro transcription reaction.

2.6.4 In Vitro Transcription (IVT) to synthesize labelled cRNA

The following reaction is carried out to convert the double stranded cDNA to its complementary RNA labelled with biotin. It is this cRNA that is then used for subsequent binding to the gene chip.

1. The following reaction mix is made up at room temperature, adding the previous 30µl of synthesised cDNA immediately prior to incubation.

Component	Volume (µl)
IVT Biotin Label	4
IVT Labeling Buffer	20
IVT Enzyme Mix	6
cDNA Reaction Mix	30
Total Volume	60

2. This reaction is then incubated overnight for 16 hours at 40 °C in a thermal cycler. Once the reaction is complete, the product was used purified and fragmented directly in the next step.

2.6.5 cRNA Purification and fragmentation

Before using the cRNA directly on the chip, it is first purified to remove reaction components that would otherwise contaminate the chip. It is then fragmented to break up any secondary structures and reduce RNA fragments to an optimal size for binding to the gene chip probes (with an average of ~100-120 bp)

1. Purification of the RNA samples was carried out using Affymetrix GeneChip Sample Cleanup Spin Columns. The protocol was followed as per the manufacturer's instructions, following a basic binding/wash/elution protocol.
2. RNA was eluted in 15µl. This eluent was then placed back on the cleanup column and spun again at ≤ 25,000 x g for 1 minute to recover as much RNA as possible.
3. The concentration of this RNA was determined using a NanoDrop 1000 spectrophotometer, and a sample evaluated via Agilent 2100 Bioanalyser running a RNA 6000 Nano Kit as above to ensure sample integrity.
4. The following fragmentation mixture was then assembled using a suitable volume of purified cRNA from the previous labelling reaction.

Component	Volume (µl)
cRNA	Variable (12.5 µg)
5X Fragmentation Buffer	7.5
Nuclease-free Water	Variable
Total Volume	37.5

5. This reaction is incubated at 94°C for 35 minutes in a in a thermal cycler.
6. After incubation, samples are put on ice and used directly in the following hybridisation step on the gene chip, or stored at -20°C.

2.6.6 Target hybridization and staining

The final stages prior to scanning the chip involve hybridisation of the samples to the chip and washing of any unbound or loosely bound non-specific targets that may provide a false positive signal. After hybridization, the chip is then stained with streptavidin-phycoerythrin (SA-PE), a molecule that binds to the biotin labelled probe targets. A subsequent step then employs an anti-streptavidin antibody and biotin labelled goat antibody to provide signal amplification via a bound fluorescent molecule. A confocal laser is used to excite this fluorophore and provide the ultimate signal which is then scanned and interpreted by the analysis software

1. The gene chip array is first equilibrated to room temperature prior to use.
2. The probe is filled with 200µl pre-hybridization mix and incubated at 45°C for 10 minutes with 60 rpm rotation in a hybridisation oven (Affymetrix Hyb Oven 640)
3. A hybridisation cocktail for each sample is made up using the 37.5µl of successfully fragmented RNA from the previous step.

Component	Volume (µl)
Fragmented cRNA	37.5
Control Oligo B2	4.2
20X Hybridisation Control mix (*bioB, bioC bioD, cre*),	12.5
2X Hybridization Mix	125
DMSO	25
Nuclease-Free Water	45.8
Total Volume	250

4. This hybridisation cocktail is heated to 99°C for 5 minutes in a heat block, transferred to a 45 °C heat block for 5 minutes then spun down for 5 minutes at maximum speed in a bench top centrifuge.
5. The pre-hybridisation mix is removed from gene chip array and 200µl of the hybridisation cocktail is then added.

6. PVC ‘Tough-spot’ label stickers are placed over the septa on the array to prevent sample leakage.
7. The array is then placed in the hybridization oven) at 45°C, 60 rpm for 16 hours overnight to hybridise the sample.
8. Arrays are then placed in the Affymetrix GeneChip Fluidics Station 450. the following staining reagents are then placed in the instrument and the appropriate fluidics protocol run:
   - 600µl of stain cocktail 1 in an amber microcentrifuge tube (light sensitive solution, exposure to light should be minimised)
   - 600µl of stain cocktail 2 in a 1.5 ml (clear) microcentrifuge tube
   - 800µl of array holding buffer in a 1.5 ml (clear) microcentrifuge tube
9. This staining procedure takes ~70 minutes to process. Processed array chips are stored at 4°C until ready for scanning.

2.6.7 Scanning of gene chips and data collection

Once all gene chip arrays have been processed and successfully stained, all samples are placed simultaneously in the Affymetrix GeneChip Scanner 3000 7G. An autoloader and barcode scanner system ensures samples are read sequentially, with a single chip being scanned in roughly 20 mins using Affymetrix Operating Software, version 1.4)

Data was subsequently collected and processed with suitable statistical analysis to identify statistically relevant up or down-regulated genes.

2.7 Protein Quantification Assays

2.7.1 Western Blot

Western blotting is a standard technique that was used in the course of this project. It is carried out to identify a target protein within a sample by means of an immunogenic reaction between the protein of interest and a specific antibody. This binding can then be detected and visualised with an enzyme linked secondary antibody via a chemiluminescent reaction.

Protein Extraction

1. Experimental cells are grown and harvested at a suitable time. Cells are pelleted and stored at -80°C or used directly for protein extraction.
2. Lysis buffer is used for cell lysis and sample dilution (Lysis Buffer: 7M Urea, 2M Thiourea, 4% CHAPS, 30mM Tris, pH to 8.5 with HCl). An appropriate volume of lysis buffer is added to cells (200µl per 10 x 10^7 cells).
3. Add Nuclease solution to a final concentration of 1x (100x solution, GE, cat. 80-6501-42). This is then left at RT for 30 mins, with occasional vortexing.
4. Spin samples at full speed (13,000 rpm) in a benchtop centrifuge at 4°C. The supernatant is the protein fraction, the pellet is discarded.

Bradford assay to determine concentration

Note: This assay has an accurate linear range up to 1mg/ml of protein. Samples should be diluted in this range to gain accurate quantification

5. A set of sample dilutions of bovine serum albumin (BSA) is generated to create a standard curve by which experimental samples can be read (i.e. 1, 0.75, 0.5, 0.25, 0.125 and 0 mg/ml)
6. In a flat bottom 96 well plate, 5µl of each standard concentration and experimental samples are added to wells along with 250µl 1x Bradford dye reagent (Biorad, cat. 500-0205) and mixed well. Samples should be measured in minimum of triplicate.

7. Absorbance is then read in a microplate reader at a wavelength of 595nm. Sample concentrations can then be calculated using the standard curve generated from reference samples.

One Dimensional Gel Electrophoresis

In this experiment, a Bis-Tris gel (Invitrogen, cat. NP0322BOX) was used to separate proteins.

8. 10µg of protein per sample is diluted in 5µl lysis buffer. 5µl Laemmli buffer is added to this sample, and heated to 95°C for 3 mins to denature protein.
9. The gel apparatus is constructed. MOPS with added antioxidant is used as a cathode buffer, with MOPS used as an anode buffer.
10. 9µl of each sample is added to separate wells, along with 1 well of a suitable molecular size marker. The gel is then run at 200V (constant voltage)

Transfer of protein to blot

11. A suitably sized membrane is cut (GE, cat. RPM303F) and immersed in methanol for 20secs.
12. This membrane is then equilibrated in Transfer Buffer (20% Methanol, 10% Tris-Glycine, 70% H_20) for 15 mins.
13. The gel is removed rinsed in H_20 and equilibrated in transfer buffer for 5 mins.
14. The gel 'sandwich' is then constructed in an electro-blot apparatus in the following configuration (filter paper is pre-equilibrated in transfer buffer) ;
    (+)Anode – 6x filter paper – Membrane – Gel – 6x filter paper – Cathode(-)
15. The transfer is run at 0.24A (constant amps) for 35 mins.

Blocking and application of Primary Antibody

16. After transferring, the blot is covered in blocking solution (5% Milk Marvel (w/v) in Tris Buffered Saline (TBS) with 0.5% Tween) and incubated with gentle shaking for 2 hours.
17. The blot is rinsed twice with TBS + 0.1% Tween, before adding the primary antibody solution (TBP + 0.1% Tween, with 1µg/ml antibody). Antibody concentration may be varied, depending on the strength of the target band and/or other non-specific bands that may be detected.
18. This is incubated with gentle shaking for overnight at 4°C.

Detection of signal

19. The blot is then rinsed 3 times in TBS + 0.5% Tween for 15 mins at RT with gentle shaking.
20. The secondary enzyme linked detection antibody is then added. This is selected based on the primary antibody used (i.e. primary mouse antibody requires a secondary anti-mouse antibody). 15mls TBS + 0.1% Tween is used containing a secondary antibody at a 1:2000 dilution, incubated for 1.5 hours at RT with gentle shaking.
21. The blot is then rinsed 3 times in TBS + 0.5% Tween for 15 mins at RT with gentle shaking.
22. The wash buffer is removed and 2ml ECL substrate added (Thermo, cat. 34075)
23. After 1 min of incubation the blot is removed from the substrate and the signal detected by exposure to a suitable x-ray film (Thermo, cat. 34090)

2.7.2 SEAP Assay

SEAP is a commonly used marker protein used to measure expression levels in transfection assays. The protocol outlined below is used to detect SEAP levels in the supernatant of CHO cells transfected with a SEAP containing plasmid.

1. 2x SEAP buffer is first prepared (10.50g diethanolamine, 50 µl of 1M $MgCl_2$, 226 mg L-homoarginine brought to 50ml with distilled water)
2. Samples are collected from SEAP producing cells. These are spun at 1000 rpm (170 x *g*) for 5 mins to pellet cells, and the supernatant removed for analysis.
3. 50µl of each sample is added to a flat bottom 96 well assay plate along with 50µl 2x SEAP buffer. This is then pre-warmed to 37°C
4. Phosphatase substrate solution, p-Nitrophenyl Phosphate (pNPP) (Sigma, cat. P4744) is made immediately before use (0.158mg pNPP in 5mls 1x SEAP buffer)
5. 10µl of this substrate solution is then added to each well
6. Change in absorbance per minute is then read in a microplate reader at a wavelength of 405nm, with the plate ideally incubated at 37°C

2.7.3 ELISA assay

An ELISA (Enzyme-linked immunosorbent assay) is a common technique used to detect an antigen of interest via an enzyme linked antibody specific to the analyte of interest. When non-specific unbound antigens are washed away, the linked enzyme is used to generate a colorimetric signal whose signal is proportional to the concentration of target analyte in the original sample.
Buffers used in this protocol:

- Coating Buffer – 0.05M Carbonate Bicarbonate, pH 9.6
- Wash Solution – 50mM Tris, 0.14M NaCl, 0.05% Tween20, pH 8.0
- Blocking Solution – 50mM Tris, 0.14M NaCl, 1% BSA, pH 8.0
- Sample Diluent – 50mM Tris, 0.14M NaCl, 1% BSA, 0.05% Tween20
- Stop Solution – 0.18M H_2SO_4

1. Dilute 1µl coating antibody in 100µl coating buffer for each sample, and add to each well
2. Incubate coated wells at 4°C overnight
3. After incubation, remove the coating buffer and wash plate 5 times with wash buffer
4. Add 200µl blocking solution to each well and incubate for 1 hour at RT
5. Remove this solution and wash plate 5 times with wash buffer
6. Prepare samples and relevant standard curve in sample diluent
7. Add 100µl sample/standard to their assigned wells and incubate for 1 hour at RT
8. After incubation, remove the samples and standards and wash plate 5 times with wash buffer
9. Dilute the enzyme (HRP) linked detection antibody in sample diluent at a dilution of 1:100,000 and add 100µl to each well and incubate for 1 hour at RT
10. After incubation, remove the coating buffer and wash plate 5 times with wash buffer
11. Add 100µl HRP substrate solution (TMB - 3,3',5,5'-Tetramethylbenzidine) to each well and incubate in darkness for 15 mins
12. Add 100µl stop solution to each well and tap plate gently to mix
13. Absorbance can then be read in a microplate reader at a wavelength of 450nm

2.8 Southern Blot

In order to detect genomic insertion events, a non-radioactive southern blot detection kit was used. This kit was based on a DIG (Digoxigenin) labelled nucleotide base that is incorporated into the target probe via PCR. When this probe is bound to its target sequence, an enzyme linked anti-DIG antibody is then used to detect this probe, with alkaline phosphatase fluorescence used as the signal detection method. The Roche DIG-labelled southern blot system was used for this experiment, including reagents for labelled probe generation and blot hybridisation (Roche, cat. 11585550910, cat. 11603558)

Probe labelling

1. The first step is to carry out the probe generating PCR reaction in which the DIG labelled dUTP base is incorporated into the sequence. This is carried out alongside an unlabelled control reaction. When visualised on an agarose gel, a slight increase in size for the labelled probe band compared to the control is indicative of successful integration of the DIG labelled base.

Component	Volume (µl)	
	Probe	*Control*
PCR Buffer	5	5
DIG Nucleotide Mix (10x)	5	-
dNTP (10x)	-	5
Primers (F/R) (10pmol/µl)	2	2
Enzyme Mix	0.75	0.75
Template DNA (10pg/ul)	2	2
H_20	35.25	35.25
Total Volume	50	

2. The following cycling conditions is used for the PCR labelling reaction;

Stage	Temperature	Time	Cycle Number
Initial Denaturation	95°C	2 mins	
Denaturation	95°C	30 secs	
Annealing	52°C	30 secs	30 cycles
Elongation	72°C	40 secs	
Final elongation	72°C	7 mins	

DNA digestion and separation

3. The following reaction is set up to digest genomic DNA prior to separation on an agarose gel.

Component	Volume (µl)
DNA	variable
NEB Buffer 3 (10x)	8
BSA	0.8
BamH1	1.6
Bgl II	1.6
Spermidine (0.1M)	2
H_20	to 80µl
Total volume	80µl

4. These digested samples are then run on a 0.7% agarose gel, run at ~40V to ensure clean separation of bands.

Membrane transfer

5. To depurinate DNA, the gel is submerged in 250mM Hcl for 20 mins and rinsed in double distilled H_20.
6. To denature DNA, the gel is then submerged in denaturation solution (0.5 NaOH, 1.5M NaCl) for 30 mins and rinsed in double distilled H_20.
7. The gel is then equilibrated for 10 mins in 20 x SSC (3M NaCl, 300mM sodium citrate, pH 7)
8. A standard blot transfer is then carried out to transfer DNA onto a nylon membrane. A piece of Whatman 3MM paper is placed on a bridge above a reservoir of 20xSSC, with its ends used to wick fluid upwards. A blot 'sandwich' is then constructed on top of this bridge in the following order; agarose gel – nylon membrane – Whatman 3MM paper (soaked) – Stack of paper towels – Flat weight (~400g).
9. DNA is allowed to transfer onto the nylon blot overnight, then the apparatus disassembled
10. DNA is then cross linked to the membrane by exposure to 120mJ UV for 1 min.

Hybridisation

11. The blot is placed in a suitably sized roller bottle, and 15ml (or enough to cover the blot) pre-warmed hybridisation solution added (*pre-hybridisation step*).
12. This is incubated for 30 mins in a rotating incubator at the appropriate hybridisation temperature. This temperature is calculated using the following formula, taking into account the sequence GC content (%G+C) and length (L) in base pairs:

$$T_{hyb} = [49.82 + 0.41\ (\%\ G{+}C) - 600/L] - (20°C\ {-}25°C)$$

13. 20µl of the labelled probe is added to 5ml pre warmed hybridisation solution.
14. The pre-hybridisation buffer is removed, and the probe/hybriisation buffer added.
15. This is then incubated overnight

16. After incubation, the blot is rinsed twice for 5 mins at room temperature with shaking in fresh low stringency buffer (2 x SSC, 0.1% SDS)
17. The blot is then rinsed twice for 15 mins in high stringency buffer (0.5 x SSC, 0.1% SDS) preheated to 65°C

Detection

18. The blot is then transferred to a new container and rinsed for 2 mins in washing buffer (0.1M maleic acid, 0.15M NaCl, pH 7.5, 0.3% Tween 20) with shaking.
19. After removing the washing buffer, the blot is incubated for 30 mins in 100ml blocking solution (Roche, cat. 11585762001) with shaking.
20. The blocking solution is then discarded, and replaced with 20ml anti-DIG antibody solution (Roche, cat. 11585614910) and incubated for 30 mins
21. The membrane is then washed twice in 100ml washing solution with shaking.
22. The blot is then equilibrated for 3 mins in 20ml detection buffer (0.1M Tris-Hcl, 0.1M NaCl, pH 9.5)
23. Chemiluminescent substrate (CSPD – Roche, cat. 11858614910) is then added dropwise to the blot which is then sealed in a clear envelope and incubated for 5 mins.
24. The signal is then detected by exposure to a suitable x-ray film (Thermo, cat. 34090).

2.9 Statistical analysis

Throughout the course of this study the appropriate statistical analysis was carried out on the data generated in order to successfully interpret its significance in relation to the experiment being undertaken. This was done to ensure that reliable conclusions could be drawn from the results.

The most common calculations and methods of analysis used to evaluate data throughout this thesis include;

- The standard deviation and coefficient of variance (C.V.) of experimental replicates to ensure accuracy of results
- Student's t-tests to investigate if significant statistical differences exist between groups of experimental results to confirm or deny a given hypothesis
- Linear regression to generate standard curves of known analyte concentrations from which to evaluate experimental samples

Section 3.0

Results

3.1 Viral Gene Targeting

3.1.1 Introduction

One of the main techniques employed in the course of this study was an investigation into the use of a viral vector for gene targeting in a CHO cell line.
This study was originally conceived as a method of gene targeting in which an adeno-associated viral vector would be used to deliver a basic expression cassette to a defined genomic locus. This cassette would be flanked by two ‘targeting arms’; sequences identical to a specific region of genomic DNA that was to be targeted. This would allow for homologous recombination to take place, inserting the expression cassette contained within the viral vector into the target homologous region of genomic DNA (Rutledge and Russell 1997).

Gene targeting, the insertion of exogenous DNA into a specific site within the genome, has the potential to be used successfully for a number of aims within industrially relevant mammalian cells such as CHO. Its main obvious potential use in industrial CHO cell lines is that of targeted gene knockout, the initial aim of this study when it was carried out. In this configuration, a viral vector is constructed containing homologous targeting arms specific to a gene of interest whose effect we wish to remove from the cell (Russell and Hirata 1998). Insertional disruption of these genes (for example, pro-apoptotic genes) then provides a benefit to the cellular phenotype when their effect is removed.
Methods such as RNA interference technology currently exist for controlled knockdown of genes, and this method can quickly generate an altered phenotype by reducing the levels of a target gene RNA. This can be useful for screening and validation of large numbers of potential gene targets, and stable siRNA producers can be generated to produce a cell line with persistent altered properties. However, due to possible genetic instability from the maintenance of a transcriptionally active shRNA expression cassette, as well as the potential for ‘leaky’ low level expression of the targeted gene, total gene knockout has been shown to be preferable in instances where complete elimination of a gene is the aim (Li et al. 2010).

This experiment was carried out as a ‘proof of concept’ study exploring the possibility of using homologous recombination via a viral vector to successfully target and disrupt a pair of model genes in CHO cells (namely *DHFR* and *HPRT*). This was attempted by utilising a viral vector containing a cassette containing elements necessary for homologous recombination, a common strategy for gene targeting. Key to this strategy are two sequences homologous to a section of the gene to be inactivated, known as the ‘targeting arms’. These flank the central cassette elements in the expression vector, in this case a selectable marker to allow for the selection of successful integrants. Once the vector is introduced into the cell, these ‘arm’ sequences allow for homologous recombination to occur, integrating the targeting cassette into the targeted region (Vasileva, Linden and Jessberger 2006). This insertion leads to a disruption of the genomic gene sequence, preventing the target gene’s transcription and effectively removing its effect from the cell.

Homologous recombination is a key process in eukaryotic cells for the repair of double stranded breaks in DNA, as well as the recombination of parental genetic material prior to meiosis. For AAV mediated gene targeting via homologous recombination, a pre-existing break in the sequence at the point of homology is exploited to introduce the exogenous DNA. This break is processed by nucleolytic enzymes, and the resulting single strand is bound by the protein RAD51. This protein then searches for homology (for example on a sister chromatid or, in this case, is found on the homologous arm of the recombinant viral vector), and ‘invades’ this sequence. The homologous vector DNA then serves as the template for repair, with a polymerase using the invading strand as a primer and the vector sequence as a template for new DNA synthesis. This reconstruction at the break site incorporates the vector sequence into the genome at this locus, and successful recombination at both up and downstream targeting arms permits the integration of the interstitial cassette sequence into the target site (Porteus 2007).

The homologous method of DNA repair exploited for AAV recombination is in contrast to the other main double stranded break repair pathway known as non-homologous end joining (NHEJ). This method repairs DNA via ligation of blunt ends, and is known to mediate viral integration into random locations in the genome as well as eliminating viral genomes through concatemerisation, leading to their subsequent

degradation. As such, the knockdown of this pathway is known to increase homologous AAV targeting rates and reduce off-target insertions (Bertolini et al. 2009; Fattah et al. 2008).

The adeno-associated virus serotype 2 (AAV-2) was chosen as a suitable vector for this study. It has previously been shown to achieve high targeting rates approaching 1% (Russell and Hirata 1998), and its broad tropism has allowed it to be used successfully to target specific gene sequences in a wide range of mammalian cell lines such as mouse, rat, human and other primates. Its genome size of ~4.7 kb is also suitable for this purpose, permitting the inclusion of a suitable selection marker (in this case neomycin) alongside the targeting arms necessary for homologous recombination. Manipulation and generation of AAV is also relatively straightforward; due to its popularity as a vector a number of kit-based solutions exist for creating custom vectors such as the one used in this experiment.

Described below are the details and results of the experiment, including the generation of the recombinant AAV vectors used to target the two model genes, the application of the virus and multiplicity of infection (MOI) used to infect the targeted CHO, PCR screening carried out to detect correctly targeted inserts as well as experiments carried out to investigate the numbers of undesirable non-homologous, off-target insertion events.

3.1.2 AAV Helper-free system

The strategy used to generate viral particles in this study was a ‘helper-free’ AAV kit-based system from Agilent Technologies (cat. 240071). In this system a number of separate plasmids are transfected into a suitable cell line (in this case human embryonic kidney (HEK-293) cells) to generate the final stock of viral particles. Wild-type AAV require a secondary ‘helper’ virus for lytic replication. To remove the necessity for this secondary virus, the adenoviral-derived genes necessary for lytic replication as well as packaging and capsid genes are provided *in trans* by the plasmids pAAV-RC and pHelper. When co-transfected with the plasmid encoding the recombinant AAV vector sequence itself into modified HEK-293 cells, this brings together all the components necessary for correct viral production and packaging. This modularisation of components ensures the integrity of the targeted cell line by preventing future spontaneous viral replication, as well as maintaining safety of the user.

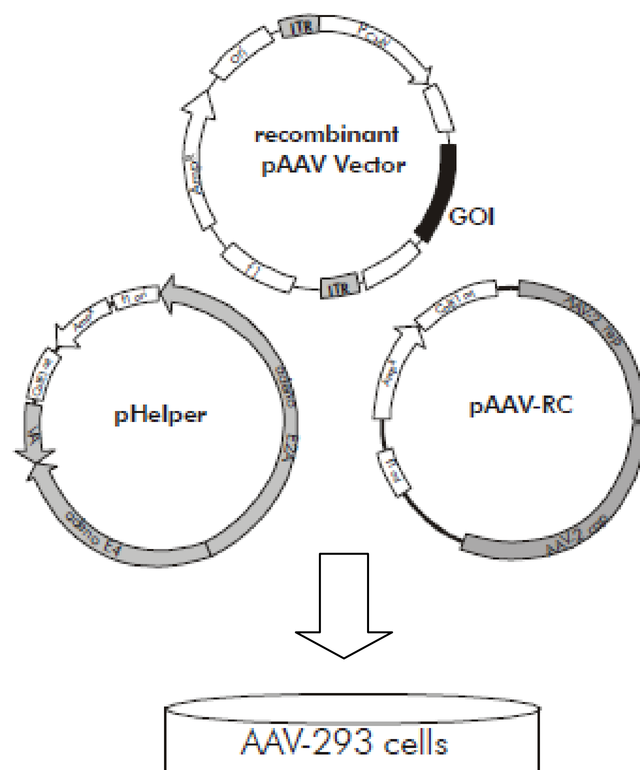

Figure 3.1.2.1: Components necessary for successful AAV production and packaging are delivered on separate plasmids. pAAV-RC contains AAV *rep* and *cap* genes, while pHelper encodes adenoviral-derived genes necessary for lytic viral replication. The recombinant vector plasmid encodes for the viral sequence and the targeting cassette.

3.1.3 Selection of genes for targeting

The first step prior to the generation of the targeting viral vectors was to select suitable genes. Ideally, the genes selected would provide a phenotype that could be easily screened for in the event of a homozygous gene knockout, without having a detrimental effect on the cell's ability to survive. Also, as these vectors were constructed prior to the publication of the CHO draft genome (Xu et al. 2011), a degree of sequence information regarding these genes would be necessary for their design. To these ends, two model genes were chosen for targeting; *DHFR* and *HPRT*.

\- *DHFR*

The *DHFR* gene encodes for the Dihydrofolate reductase protein, an enzyme involved in the generation of tetrahydrofolate, an important precursor necessary for the generation of nucleic acids. Cells lacking in this enzyme can survive using alternative metabolic pathways however, with the addition of hypoxanthine and thymidine (HT) to its growth medium (Urlaub and Chasin 1980). This provides us with a means to easily screen for *DHFR* -/- knockouts in a targeted CHO cell line (i.e. by placing clones in HT deficient medium and screening for cells that do not survive in such an environment).

Another advantage to using this gene in CHO was the sequence data available to us when generating the *DHFR* targeting construct. A paper relating to DNA replication in this locus (Sasaki et al. 2006) provided a sequenced region of over 67 kb worth of data (GenBank accession: AB239764.1), allowing us to confidently identify intron and exon regions, as well as generate the primers necessary to amplify homologous arm sequences and carry out post-transduction screening of infected cells to identify correctly targeted recombinants.

- *HPRT*

The second model gene chosen for targeting was the *HPRT* gene encoding the Hypoxanthine-guanine phosphoribosyltransferase enzyme. This enzyme is involved in a purine salvage pathway, however this pathway is not strictly necessary for growth in a supplemented growth medium, therefore knockout of this gene would have no detrimental effects on CHO cell growth if targeted.

One advantage for the use of this gene for knockout screening is the sensitivity it confers to the drug 6-Thioguanine (6-TG). If an active version of the *HPRT* gene exists within the cell, this catalyses the transformation of 6-TG into 6-thioguanine monophosphate (TGMP). This compound is cytotoxic due to its ability to prevent the production of guanine based nucleotides, as well as deleterious effects when further TGMP metabolites are incorporated into DNA and RNA. As such, it has been used as a selective marker in previous *HPRT* knockout studies (Russell and Hirata 1998) to easily screen for clones that are homozygous for disrupted *HPRT* alleles.

Unlike *DHFR*, an ideal amount of sequence data was not initially available for the CHO *HPRT* genomic locus when this targeting vector was constructed. To circumvent this problem and generate sufficient sequence data to generate primers and allow us to create the necessary targeting arms, existing sequence data from a similar organism (*Cricetulus longicaudatus*, Long-tailed Hamster) was used as a basis to generate primers for sequencing experiments that could then be carried out on the CHO genome. Using this CHO specific sequence data, we could then create the homologous targeting arms necessary to create the *HPRT* targeting vector.

3.1.4 Targeting vector design

The first step in creating the plasmid constructs necessary to generate viral stocks used for gene targeting was the creation of the targeted knockout cassette. The targeting cassette used in this study was based on a cassette generated by Dr. Olga Piskareva for use in a similar experiment targeting LINE1 elements, a common repetitive sequence within the genome. This cassette (comprising the elements between the homologous arm sequences) was copied and used as the basis for the *DHFR* and *HPRT* targeting vectors used in this study. The configuration of these elements can be seen in figure 3.1.4.1. These targeting vectors are comprised of the following key functional elements:

- *Homologous targeting arms:*

The targeting arms are a key component of the targeting cassette. These sequences are generated via PCR directly from CHO genomic DNA, ensuring their sequence is identical to the targeted region within the genome. This homology encourages recombination at the chosen region, allowing the expression cassette to become inserted within this target sequence.

Although the exact mechanism behind this method of recombination is unknown, evidence suggests that double stranded break (DSB) homologous recombination is a likely method for its action, with one study demonstrating AAV targeting frequencies 60-100 fold greater in conjunction with induced DSBs (Miller, Petek and Russell 2003). Elimination of genes whose activity is involved in homologous recombination (such as RAD54) has also been demonstrated to reduce or eliminate site specific targeted insertions, furthering the evidence implicating this method of action (Vasileva, Linden and Jessberger 2006; Essers et al. 1997).

- *Neomycin resistance gene:*

A key feature of the targeting cassette, conferring resistance to the antibiotic G418 and allowing us to select for cells that have undergone any form of recombination having incorporated the viral vector into its genome.

This variant of the *neo* resistance is broken into two exons (*neo* and Δ*neo*), separated by a single 146 bp intron.

- *Intron and LoxP sites:*

A 146 bp intron separates the two exons that constitute the *neo* resistance gene, with LoxP sites contained within this intron and downstream at the 3' end of the Δ*neo* exon (see figure 3.1.4.1). This was designed to allow for inactivation of the *neo* gene using Cre recombinase to excise the Δ*neo* portion of the gene, thus avoiding the negative effects of selectable markers on recombinant gene expression (Pham et al. 1996; Artelt et al. 1991).

The single LoxP site and *neo* fragment that would remain after Cre treatment would also facilitate future work, such as an investigation into the transcriptional potential of targeted sites by introducing a gene of interest via recombinase-mediated cassette exchange (RMCE) (Turan et al. 2011). A correctly pre-targeted cell line would ultimately allow for a product gene to be inserted and neo resistance restored at this target site and, eliminating the variability in expression inherent in populations with random insertions.

- *Polyadenylation signal:*

A Polyadenylation (polyA) signal is included at the end of the neo sequence. Functioning in a similar method to a 'gene trap', the polyA signal works in tandem with the splice site at the start of the Δ*neo* exon to prevent correct splicing and expression of the target gene transcript (Carlson and Largaespada 2005). This generates a truncated, non-functional version of the target gene, removing its effect from the cell and generating a 'knockout' phenotype.

- *CMV Promoter:*

A standard cytomegalovirus promoter (pCMV), a common strong viral promoter, is used to drive *Neo* expression.

- *pAAV-MCS Backbone:*

The features described above, once correctly assembled, were in turn ligated into the backbone of the pAAV-MCS plasmid. Using the NotI restriction sites provided (see figure 3.1.5.1.4), the generic expression cassette is removed from this plasmid and replaced with the custom targeting cassettes. A notable feature of this backbone is the Inverted Terminal Repeat (ITR) sequences flanking the cassette. These 145 bp sequences contain the viral origin of replication, and are critical for viral packaging (Zhou and Muzyczka 1998).

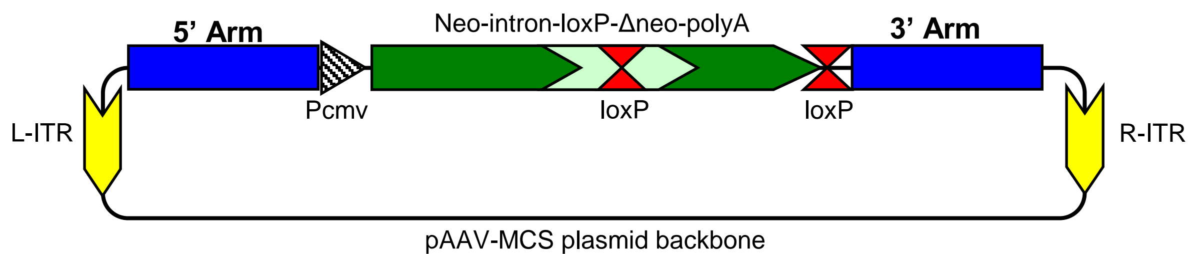

Figure 3.1.4.1: This diagram demonstrates the ultimate planned design of the targeting vectors pAAV-DHFR and pAAV-HPRT. The key components are highlighted below

- *5’/3’ targeting arms*: These sequence fragments flank the other cassette components and are homologous to a portion of the DHFR gene to be targeted, generated by PCR on target genomic DNA. If recombination occurs, these recombine with the target site, also integrating the sequence information between the arms and ultimately disrupt the target gene
- *Neomycin resistance gene*: Allows the selection of successful integrants by application of G418 after transduction. Also includes a single intron, which contains one of the vectors two loxP sites. A polyadenylation signal at its 3’ end also serves as a termination signal for targeted gene transcription
- *L/R-ITR*: Inverted terminal repeat sequences necessary for the replication and packaging of viral particles
- *LoxP sites*: Allows inactivation of Neomycin resistance via Cre recombinase once a successful gene knockout cell line has been established, as well as the future integration of a target gene at this defined locus
- *CMV promoter*: Drives the expression of the resistance gene
- *pAAV-MCS Backbone:* The plasmid backbone taken from a generic AAV expression vector used as the basis for the targeting vectors used in this study

These components were generated through subcloning from existing vectors, or generated via PCR of native CHO sequences using primers generated from CHO sequence data.

3.1.5 Targeting vector construction

In order to create the final targeting vector, the targeting cassette was first assembled in a pBluescript cloning vector. Components such as the *neo* resistance gene and loxP sites were subcloned from a similar targeting vector generated previously, with targeting arms generated specifically to the model genes examined in this experiment.

3.1.5.1 Targeting arm generation

DHFR

The DHFR gene itself consists of 6 exons, with variable amounts of intronic DNA between each. A large intron (2331 bp) between exons 2 and 3 was chosen as the integration site for the targeting cassette. Primers were designed to amplify regions of this intron to generate the 5' and 3' targeting arms. These were 920 bp (5' arm, contained within the intron) and 1413 bp (3' arm, contained within the intron and overlapping exon 3 by 64 bp) in size respectively (see figure 3.1.5.1.1). These were then ligated into the pBluescript vector containing the other cassette elements. This completed cassette sequence was then removed using the flanking NotI restriction sites and successfully ligated into the pAAV backbone to generate the pAAV-DHFR targeting vector (see figure 3.1.5.1.5).

Arm	F/R	Sequence (5' – 3')	Arm Size (bp)
5' Arm	*Forward*	*aaaaaagatctgcggccgc*GCAGACGTGGAAGTGCG	920
	Reverse	*aaaaagctagc*TGTGTCCCACCAGGCC	
3' Arm	*Forward*	*aaaaaactcgag*GGAGGCAGAGACAGAAAGATC	1413
	Reverse	*aaaaaaggtaccgcggccgc*GGTCGATTCTTCTCAGGAATG	

Figure 3.1.5.1.1: Details of the primers used to amplify the regions of CHO genomic DNA comprising the *DHFR* targeting arms. Bases in uppercase denote those used in direct sequence binding, while lowercase italicised characters indicate overhangs containing restriction sites used for subsequent digestion and ligation of PCR fragments.

HPRT

A similar rationale to that used to design the *DFHR* vector was followed for design of the *HPRT* targeting arms. However, due to the lack of reliable sequencing data, work was initially based on mRNA sequence data from the long-tailed hamster (*Cricetulus longicaudatus*, GenBank accession: x59652).

From this sequence, primers were designed and used to sequence intronic sections, providing further data which was ultimately used to create the primers necessary to amplify the *HPRT* targeting arms (see figure 3.1.5.1.2). These were 935 bp (5' arm) and 922 bp (3' arm) in size respectively (see figure 3.1.5.1.3). As with the DHFR targeting cassette, these were then ligated into the pBluescript vector containing the other cassette elements. This completed cassette sequence was then removed using the flanking NotI restriction sites and successfully ligated into the pAAV backbone to generate the pAAV-HPRT targeting vector (see figure 3.1.5.1.6).

HPRT locus in genome

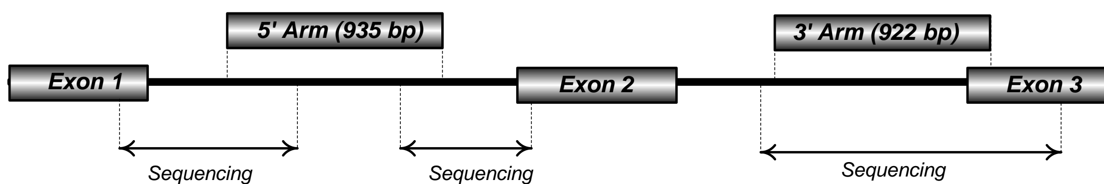

Figure 3.1.5.1.2: Schematic detailing the sequencing experiment carried out on the CHO HPRT locus. Initially, sequencing primers were generated using a long-tailed hamster mRNA sequence. Using the resulting sequence data, primers were then generated to allow the amplification of the regions necessary to create the targeting (5' and 3') arms.

Arm	F/R	Sequence (5' – 3')	Arm Size (bp)
5' Arm	*Forward*	*aaaaagcggccgc*TTCCTCATGGAGTGATTATGGACAG	935
	Reverse	*aaaaagctagc*GATGGGAGTGAGAGTG	
3' Arm	*Forward*	*aaaaaactcgag*GTAGGGACCATGCTTTAT	922
	Reverse	*aaaaaaggtaccgcggccgc*GTCCCCTGTTGACTGATCAT	

Figure 3.1.5.1.3: Details of the primers used to amplify the regions of CHO genomic DNA comprising the *HPRT* targeting arms. Bases in uppercase denote those used in direct sequence binding, while lowercase italicised characters indicate overhangs containing restriction sites used for subsequent digestion and ligation of PCR fragments.

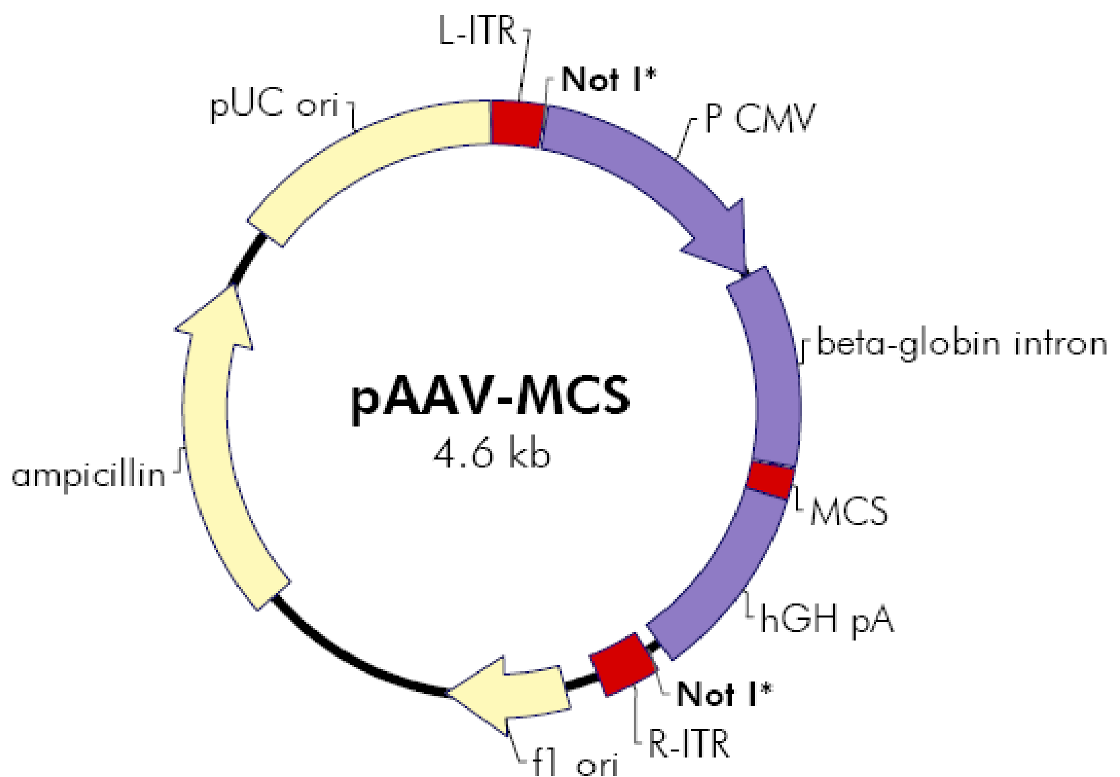

Figure 3.1.5.1.4: A schematic diagram of the pAAV-MCS vector that provides the backbone for the fully constructed targeting cassette. Highlighted in red are the left and right inverted terminal repeats (L/R-ITR), sequences critical for the successful packaging of viral particles.
The highlighted NotI restriction sites were used to remove the existing expression cassette and insert the completed targeting cassettes once they were excised from the pBluescript vector used as a scaffold for their construction.

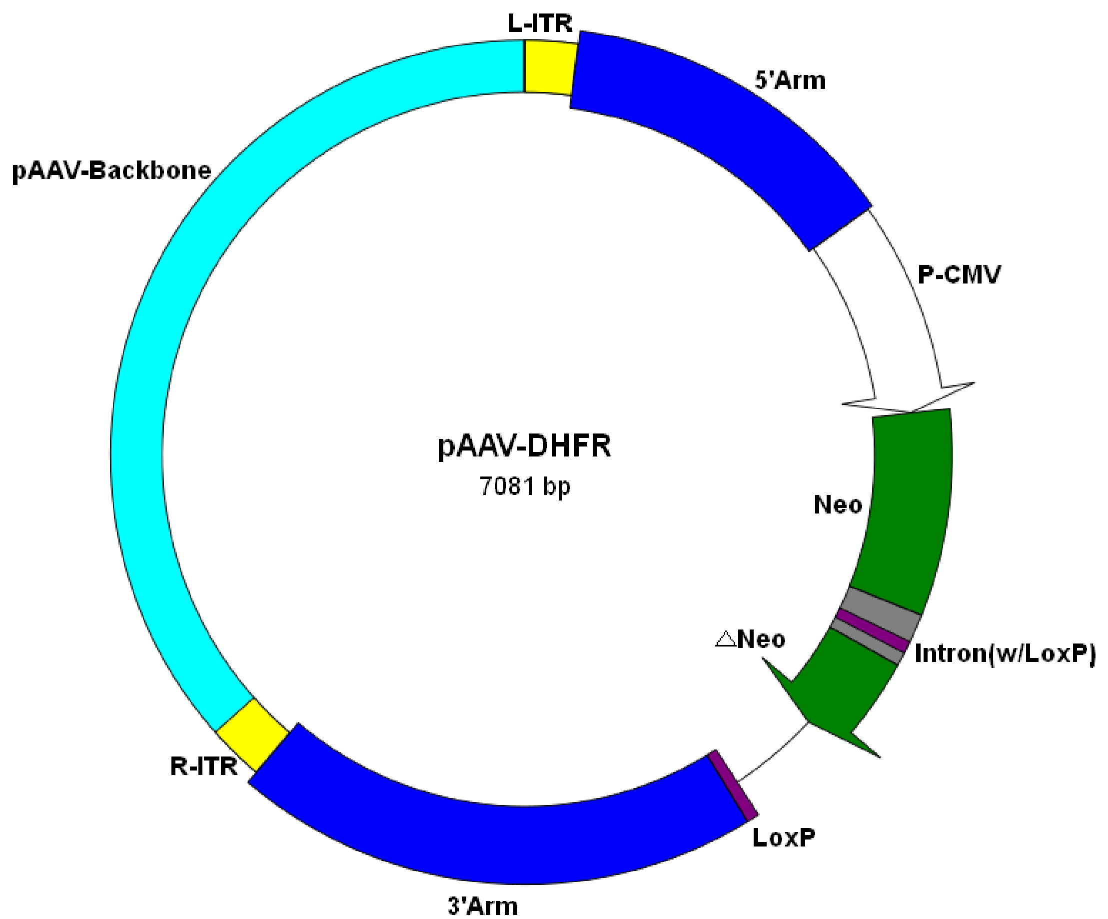

Figure 3.1.5.1.5: A scaled representation of the fully constructed pAAV-DHFR plasmid. While the total plasmid size is over 7 kb, the size of the viral genome between and including the ITR sequences is ~4.5 kb, below the upper limit of ~4.7 kb for efficient AAV2 viral packaging. The complete annotated sequence of this plasmid can be found in appendix 5.2.1. NotI restriction sites outside of the LTR sequences (see figure 3.1.5.1.4) were used to join the pAAV backbone to the targeting vector sequence once fully constructed in a temporary parent vector (pBluescript).

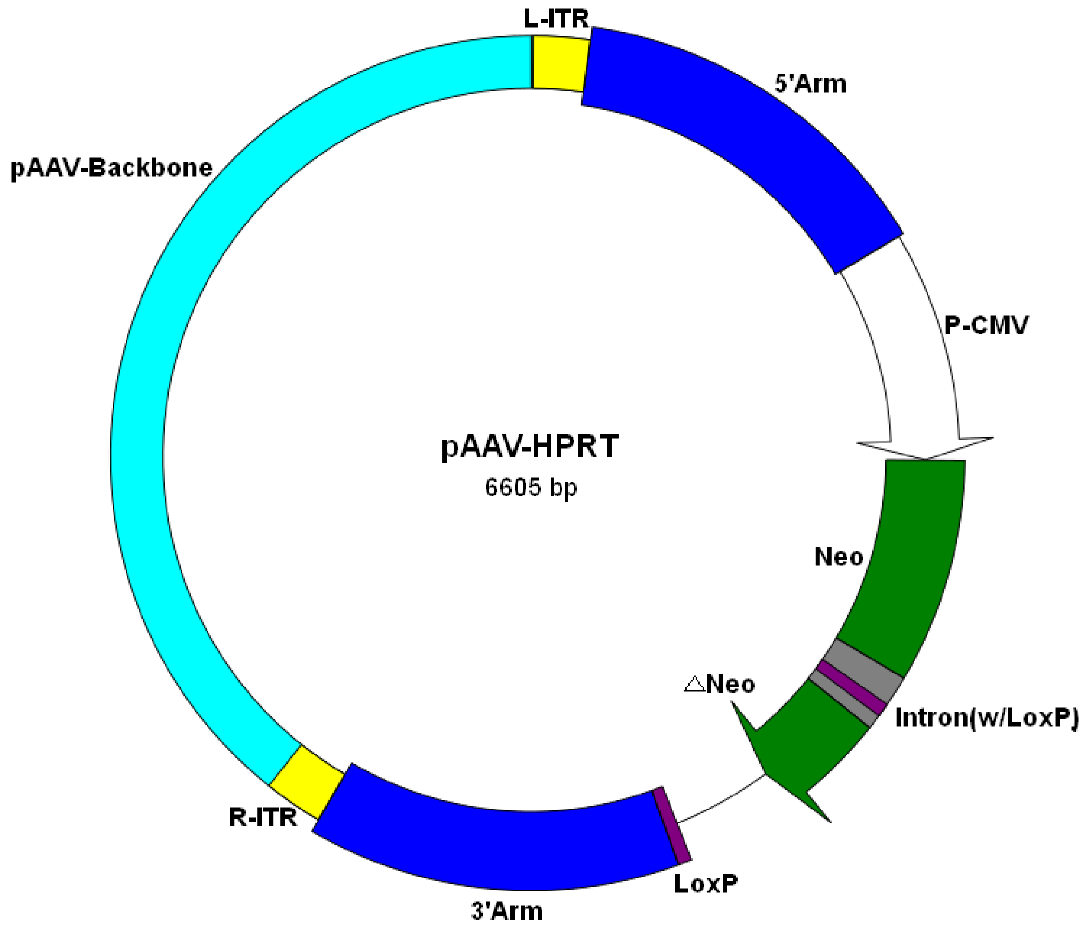

Figure 3.1.5.1.6: A scaled representation of the fully constructed pAAV-HPRT plasmid. While the total plasmid size is over 6.6 kb, the size of the viral genome between and including the ITR sequences is ~4 kb, below the upper limit of ~4.7 kb for efficient AAV2 viral packaging. The complete annotated sequence of this plasmid can be found in appendix 5.2.2. NotI restriction sites outside of the LTR sequences (see figure 3.1.5.1.4) were used to join the pAAV backbone to the targeting vector sequence once fully constructed in a temporary parent vector (pBluescript).

3.1.6 Virus generation and purification

Once these targeting constructs had been successfully generated, the protocols for virus generation and purification (as detailed in materials and methods section 2.4) were followed. For both targeting constructs (*DHFR* and *HPRT*), 9 x 96mm plates of HEK-293 cells were transfected with the plasmids pHelper, pAAV-RC and its respective targeting plasmid (pAAV-DHFR and pAAV-HPRT) to generate the targeting viral stocks.

A single plate of a GFP-expressing control virus was also generated in each case to determine successful generation of viral particles. This control virus was used subsequently to ensure the infectivity of the viruses produced and examine their potential for infecting CHO cells. Transduction efficiency of CHO with this virus could be estimated using flow cytometry to quantify GFP positive cells.

The protocol used for this purpose (section 2.4.1) was adapted to utilise a commercial transfection reagent (FuGene, Roche, cat. 11 814 443 001). Optimisation of this process was found to provide superior efficiency compared to the default calcium chloride transfection protocol suggested by the AAV kit manufacturer.
Originally, 10µg of each plasmid (pRC, pHelper and pAAV-GFP) was mixed with 0.3M $CaCl_2$ and 2 x HEPES Buffer solution as per the manufacturers protocol (Agilent, cat. 240071). However, after harvest a 200µl sample of viral supernatant was found to only transduce 6% of CHO-K1 when applied to a sample of 1 x 10^5 cells (see 2.4.3). Increases in HEK-293 cell concentration and plasmid concentration did not improve this result, and a protocol previously successful in a similar viral transfection experiment utilising the FuGene reagent was adapted and employed with greater initial success.

Initially, lower concentrations of plasmid (2µg pRC and pHelper, 4µg pAAV-GFP) were mixed with 18µl of transfection reagent, resulting in a viral supernatant with 33.5% transduction rate when harvested. Further increases in plasmid DNA and FuGene concentration were found to achieve greater success, with a maximum concentration of 10µg of each plasmid mixed with 30µl FuGene found to produce the greatest level of viral production. A 200µl sample of this unpurified virus, produced with the final optimised protocol, was found to transduce over 70% of cells (see 3.1.7).

Using this method, 9 plates of both targeting viruses were then produced and concentrated. These viral stocks were then concentrated and purified using the ViraBind AAV Purification kit (Cell Biolabs, cat. VPK-141). These final concentrated viral stocks were eluted in a final volume of ~130µl, which was then subsequently used for targeting experiments by applying to an adherent monolayer culture of CHO-K1 cells.

3.1.7 Estimation of viral titre

MOI (multiplicity of infection) is a term that quantifies the relative number of viral particles applied, per cell, to a population (for example, an MOI of 10 would describe 10 viral particles per cell). For the purposes of this experiment, we aimed to achieve an MOI as high as possible, as evidence has shown that increased targeting efficiencies relate to an increase in MOI (Russell and Hirata 1998, Porteus et al. 2003). For this reason, a larger number of transfected HEK-293 plates were combined and concentrated, and applied to a relatively small number of cells.

In order to determine an estimate of the MOI, qPCR (as detailed in section 2.4.12) was carried out on the control GFP sample to determine the number of viral particles/ml of viral stock that were produced per single plate under the transfection conditions used for production.

The GFP control AAV plasmid was serially diluted and analysed with samples of the GFP virus. Knowing the number of plasmid copies within each control sample then allowed us to generate a reference curve. This was achieved using the following formula:

$$\textit{Plasmid Copy Number (per } \mu l) = [\textit{amount (ng) x } 6.022\times10^{23}] / [\textit{plasmid length x } 1\times10^{9} \textit{ x } 660]$$

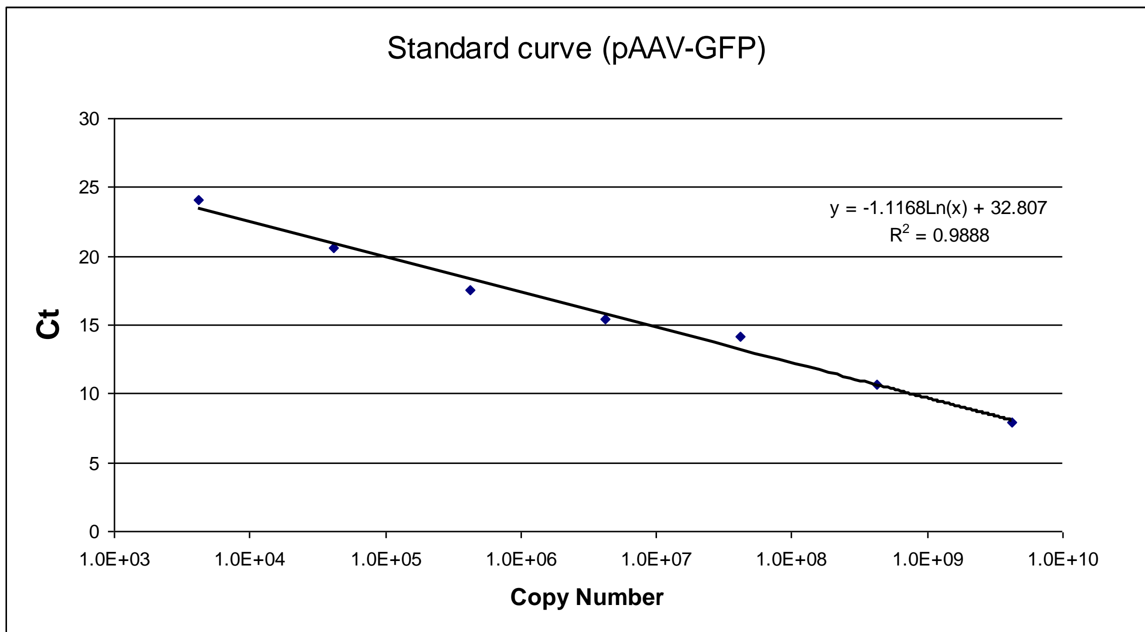

Figure 3.1.7.1: The standard curve used to calculate the number of viral particles present per ml of viral stock, generated via qPCR on a plasmid with a pre-determined copy number. Samples were measured in triplicate within the linear range between 4.2×10^9 and 4.2×10^3 plasmid copies.

From the GFP viral stock that was generated, a minimum of 4.82×10^8 viral particles per ml was calculated using the above reference curve.

To calculate the final MOI that would be used when the stock of concentrated DHFR virus was applied to cells, the following factors were taken into account;

- 1 plate = ~8mls
- 9 plates of targeting vector were generated
- These were concentrated with a >60% recovery rate from the purification kit
- This concentrated stock was applied to 1×10^5 cells (see transduction protocol, section 2.4.3)

This leads to a minimum calculated MOI of 2.08×10^5 (~200,000) viral particles per cell.

To ensure the functionality of the virus and confirm the AAV-2 tropism extended to CHO cells, 50μl and 200μl sample volumes of the unpurified GFP control virus supernatant were applied to 1 x 10^5 cells in a 6 well plate. After 48 hours fluorescence was measured on a Guava flow cytometer and, by gating with an untransduced control population, the percentage of successfully transduced cells was determined.

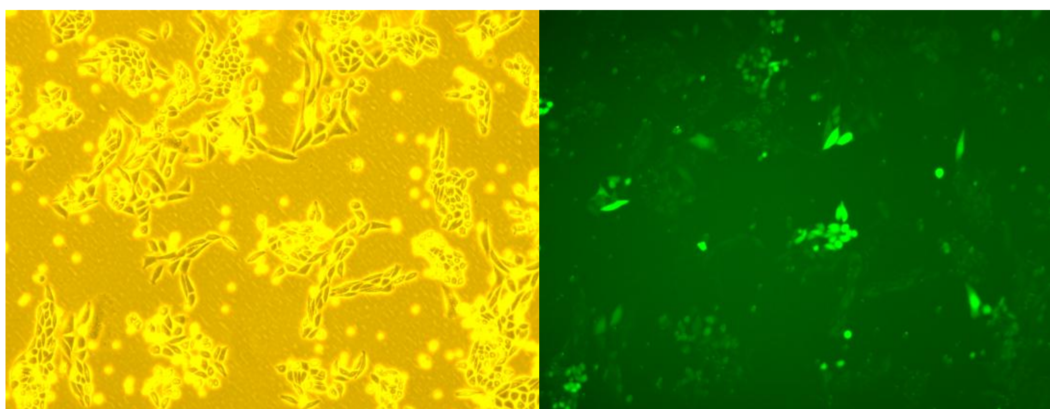

Figure 3.1.7.2: An image of CHO-K1 cells 48 hours post-transduction with 200μl unpurified AAV-GFP virus (left), with the same field viewed with a fluorescent filter (right).

When fluorescence was measured on the flow cytometer, the following percentage of fluorescent cells was measured from application of the 50μl and 200μl GFP samples

Volume AAV-GFP	% fluorescent cells	Estimated MOI
200μl	74.8%	9.64 x 10^2
50μl	31.5%	2.41 x 10^2

This data suggested the viruses generated were viable and even a relatively small MOI (less than 1000 particles per cell) from an unconcentrated sample could successfully infect a large number of CHO cells.

3.1.8 Transduction and selection of targeted cells

Once the viral stocks had been generated and purified, the entire concentrated volume (~130µl) was applied to 1 x 10^5 cells (an estimated MOI of 2.08 x 10^5 viral particles per cell).
A 200µl sample of the unconcentrated GFP control virus was also applied to a separate cell sample as above. This control transduction provided 74.25% GFP positive cells, indicating successful transduction (see figure 3.1.7.2).

After 48 hours, these cells were placed under Neomycin selection at a concentration of 0.5mg/ml, a concentration previously determined to successfully eliminate non *neo* resistant cell background. Once this untransduced background population was cleared, a stock of the transduced population was frozen for liquid nitrogen storage. Further investigations were then carried out to examine efficiency of targeting, as well as selective screening for homozygous knockouts.

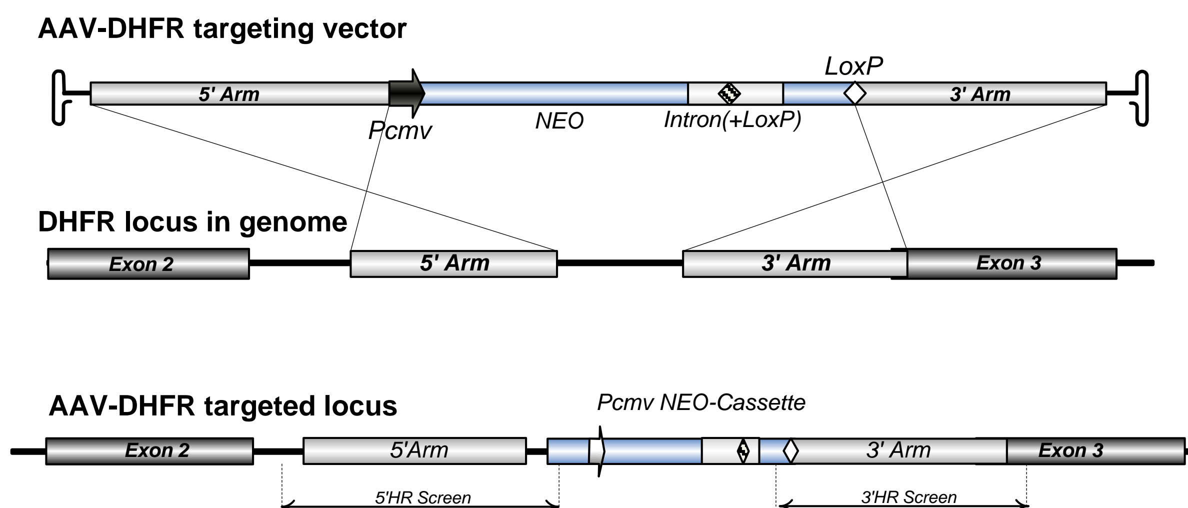

Figure 3.1.8.1: This diagram describes the homologous recombination event expected to occur post-transduction once the viral DHFR knockout genome containing the homologous arms recombines with the CHO genome sequence. The arm sequences themselves are homologous to the region between exon 2 and 3 of the CHO DHFR gene, with a slight overlap (64 bp) into exon 3 at the 3’ arm. Once homologous recombination takes place, we can predict the genomic sequence to resemble the lower diagram, having incorporated the targeting vector at the designated locus and disrupted the target gene. Regions targeted for PCR screening of targeted recombinants are also highlighted.

3.1.9 PCR Screening to estimate targeting efficiency

In order to estimate the efficiency of successful gene targeting in comparison to cells with randomly inserted cassettes, a number of single cell clones were isolated from the *DHFR* targeted cell population. These were then screened using a series of PCR reactions designed to indicate the successful integration of the *neo* expression cassette, as well as targeted recombination events at both the 5' and 3' targeting arm locations.

To achieve this, 3 reactions with individual sets of primers were used.

- *Neo:* Designed to amplify a portion of the neomycin resistance marker. This would indicate the presence of the targeting cassette at any position in the genome
- *5'/3' arms:* To detect correctly targeted HR events, primer pairs were designed featuring one anchored within the targeting cassette, and the other binding just outside the arm sequence in genomic DNA. A correctly sized band from this reaction would indicate a successfully integrated targeting arm.

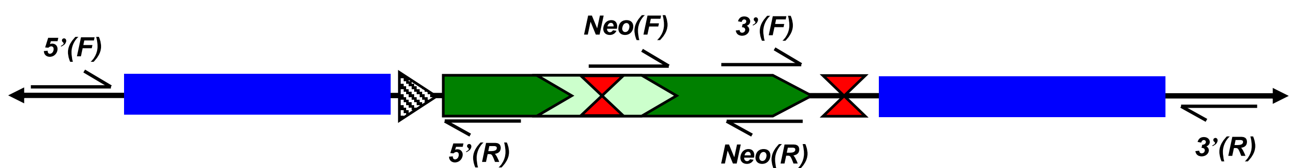

Figure 3.1.9.1: The binding positions of primer sets used for screening of insertion events. Initially, a set binding internally to the cassette was used [Neo F/R] to screen for all clones with any insertion events. These *neo* positive clones were then screened with primers to determine targeted events [5'/3' F/R]. By placing one primer within the cassette and one outside the arm sequence, a positive band of the predicted size would indicate a successful insertion.

Initially, over 300 single cell clones were isolated from a G418 selected population of *DHFR* targeted cells. Genomic DNA was extracted using the ethanol precipitation method detailed in section 2.4.6.
PCR was then optimised to run in a high throughput plate format with a genomic DNA template. This optimisation included increases in denaturation times and cycle numbers which were found to have a positive effect on amplifying the target sequences from genomic DNA.

3 sets of primers were successfully used; an internal set to detect the *Neo* gene and two to detect the 5' and 3' arm recombination events using an internal and external primer.

Screen	F/R	Sequence	Product Size (bp)
Neo	Forward	5' – AAGATGGATTGCACGCAGG – 3'	300
	Reverse	5' – GAGATGACAGGAGATCCTG – 3'	
5' Arm	*Forward*	5' – TGAATGACCACCACCTCC – 3'	1000
	Reverse	5' – TAACGCGGAACTCCATATATG – 3'	
3' Arm	*Forward*	5' – AGCGCATCGCCTTCTATCG – 3'	1880
	Reverse	5' – GTGCGCGTGCGTGCAC – 3'	

Table 3.1.9.2: Details of the primer sets used for the PCR screening designed to estimate targeting efficiency. Sequence and expected product size are noted.

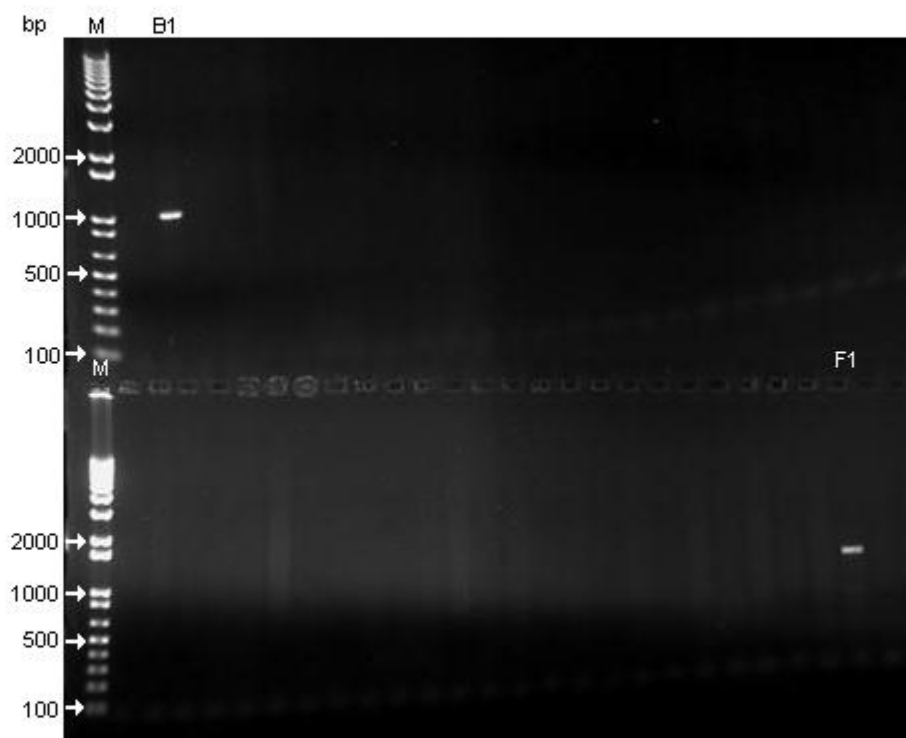

Figure 3.1.9.3: This image displays a typical result from a high throughput (96-well) PCR screen when visualised via gel electrophoresis. The above image, for example, shows the result from a 5' arm insertion screen, with 2 visible positive results (1000 bp in lanes B1 and F1).

Screen	Number of positive clones	Targeting Efficiency
Neo	195	-
5' Arm	11	5.6%
3' Arm	6	3.1%

Table 3.1.9.4: Results of PCR screening for targeted insertions. Targeting efficiency is measured as a percentage of all confirmed insertion events (i.e. all *neo* positive clones).

Of the ~300 clones isolated, 195 genomic samples gave *neo* positive results. The remaining 100+ *neo* negative clones were likely a result of poor quality DNA extracted. Due to the high-throughput nature of the ethanol extraction method employed, not all wells provided adequate concentrations of DNA for PCR.
While all clones examined were G418 resistant, a lack of an integrated *neo* gene may also be a result of AAVs ability to exist episomally within the cell, thus maintaining resistance without the need for genomic integration (Bertran et al. 1996; Penaud-Budloo et al. 2008).

Result:
11 (5.6%) of these *neo* positive clones proved positive for the 5' arm insertion event. Only 6 (3.1%) proved positive for successful 3' arm recombination. Interestingly, these 6 clones were a subset of the 5' positive group, meaning no 3' arm insertion events happened independently of 5' arm recombination events.
This result compares favourably to the commonly reported targeting frequency of ~1% using AAV (Hirata et al. 2002; Russell and Hirata 1998) and a vast increase over plasmid based methods (Williams et al. 1994), however this figure is also enhanced through the use of drug selection to remove untransduced cells.

3.1.10 Screening for homozygous (-/-) knockouts

With targeting efficiency established at over 3%, a variety of screens, including selective and PCR screens, were carried out to determine if the successfully targeted *DHFR* clones were heterozygous for the disrupted *DHFR* allele, or true null (-/-) mutants.

PCR screening was necessary to indentify *DHFR* targeted cells due to the lack of a suitable negative selective pressure. However, due to the sensitivity of *HPRT*-containing cells to the guanine analog 6-TG, this compound could be used directly to screen for knockout cells potentially generated by the *HPRT* targeting vector. Having calculated a suitable concentration to eliminate untargeted cells, a negative selection screen was carried out on *HPRT* targeted cells using 6-TG to select for cells having potentially undergone a double allele knockout.

3.1.10.1 Selective DHFR *(-/-) screen*

DHFR is necessary for growth under HT deficient conditions to generate critical folate derivatives, and as such the inability to survive in HT deficient medium would indicate these clones have lost both alleles and are null mutants.
This was achieved by seeding each of the 6 clones previously identified for correctly targeted insertions in hypoxanthine and thymidine (HT) deficient CHO-S-SFM medium (Invitrogen, cat. 31033-020), including dialysed serum to ensure no trace amounts of these vital compounds.

Result:
Unfortunately, all 6 cell lines tested retained the ability to grow in this medium, suggesting there was still a functional allele of this gene within the cell.

3.1.10.2 PCR screen for intact DHFR *allele*

A PCR screen was also carried out using primers to detect the intact version of the *DHFR* gene (see figure 3.1.10.2.2). Primers were designed to flank the targeting cassette insertion point within targeted cells. A predicted band size of 371 bp would indicate the presence of an intact *DHFR* allele.

Forward 5' – GAAACAGGTCAGCAGAGCAGATGTTG – 3'
Reverse 5' – CACTGTGCACCTGTTTGCACAGG– 3'

Figure 3.1.10.2.1: PCR primers used to detect the intact DHFR allele

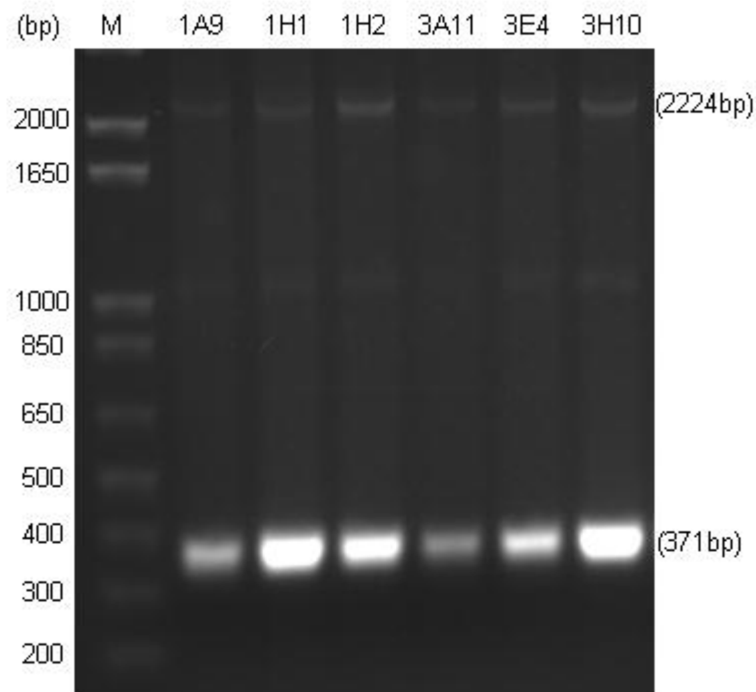

Figure 3.1.10.2.2: The result of the PCR screen to detect intact *DHRF* alleles in the targeted clones. The bands visible at 371 bp in each lane indicate an intact copy of the DHFR gene in the CHO genome, while the fainter band at 2224 bp signifies the targeted allele containing the inserted targeting cassette.

Result:

For all 6 correctly targeted clones analysed, an intact copy of the DHFR allele was found to be present in each case (i.e. *DHFR* +/-), confirming the results of the selective –HT medium screen.

3.1.10.3 qPCR screen for DHFR *expression*

Finally, qPCR was carried out to detect the levels of DHFR expression in the insertion positive cell lines isolated. Primers were designed to generate a 142 bp *DHFR* fragment suitable for qPCR, with PabpnI used as a reference gene for normalisation. The protocol for qPCR was followed as per methods section 2.3.12.

Forward	5' – GAAGCCATGAATCAGCCAGGCC– 3'
Reverse	5' – GGACTTCAGAAAGGACCCCTGG– 3'

Figure 3.1.10.3.1: qPCR primers used to detect functional DHFR expression

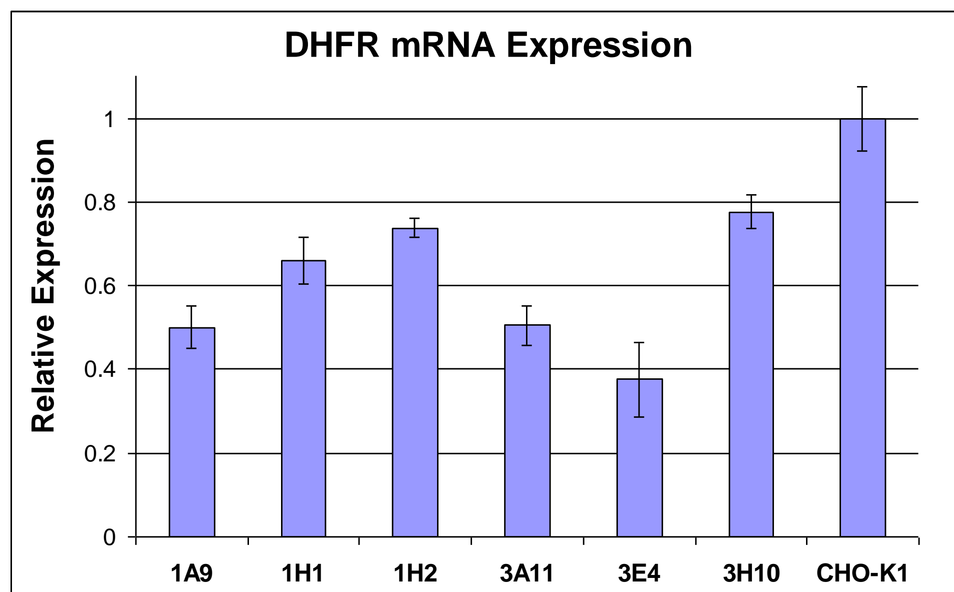

Figure 3.1.10.3.2: DHFR mRNA expression from *DHFR* +/- targeted clones. RNA was isolated from each cell line and analysed in triplicate via qPCR, error is reported as the standard deviation of these technical replicates. Expression is reported relative to an untransduced control cell line.

Result:

Continued expression is noted at a reduced level (average 59%) in all clones. While expression levels were generally lower than an untargeted negative control as expected in a single allele knockout cell line, all clones still showed positive DHFR expression**.**

3.1.10.4 6-TG negative selection screen for HPRT *knockout cells*

In order to screen for *HPRT* targeted cells that had potentially undergone homologous recombination at both alleles, a negative selection screen was carried out. While *HPRT* positive cells are sensitive to 6-TG due to its deleterious effects on nucleotide metabolism, HPRT negative (-/-) cells are able to survive its effects. This feature can be exploited to eliminate untargeted and functionally heterozygotic targeted cells, as well as bypassing the need to carry out a PCR screen to identify successful knockout cells.

Before applying to virally targeted cells, a kill curve was carried out by seeding 1 x 10^5 untargeted CHO cells in 6 well plates containing various concentrations of the selective agent. From this experiment, it was determined 3µg/ml 6-TG was sufficient to eliminate wild type CHO-K1 cells in 7 days (with intermittent media changes).

Result:
Unfortunately, no *HPRT* targeted cells survived this selective process. This suggests that, as with the *DHFR* targeted cells, no null mutant knockout cells were generated in this process.

3.1.11 Alternative techniques for null mutant selection

The PCR screening carried out on *DHFR* targeted cells confirmed a relatively high number of correctly targeted cells (>3%). However, after subsequent analysis of these cell lines they were found to contain a remaining intact copy of the *DHFR* allele. A number of alternative techniques were attempted in an effort to generate null mutants. These included exposing homozygous targeted cell lines to a high pulse of selective drug in order to stimulate gene conversion at the untargeted allele, as well the development of a secondary viral vector containing an alternative selective marker in order to disrupt the untargeted allele.

3.1.11.1 Increased G418 concentration to select for null mutants

Due to the failure of the initial DHFR targeting virus to successfully generate null mutants, we investigated the use of a method in which a high pulse of the selective drug used to generate the initial heterozygous mutants is applied. This causes loss of heterozygosity, with mechanisms such as chromosomal loss (and subsequent duplication), gene conversion as well as mitotic recombination to be responsible for this phenomenon. This technique has been demonstrated effectively in mammalian embryonic stem cells (Mortensen et al. 1992), as well protozoan eukaryotes such as *Leishmania* (Hwang et al. 1996).

In this attempt a selection of correctly targeted (5'/3' arm insertion), heterozygotic clones isolated from the previous *DHFR* experiment were seeded at 1 x 10^5 cells per well of a 6 well plate. The 3 clones used were designated *1A9*, *1H1* and *3H10*, as well as a control cell line maintaining G418 resistance but with no targeted *DHFR* recombination.

Increasing concentrations of G418 were applied to these 3 clones. This was carried out to find a suitable concentration of selective marker that would inhibit growth and stimulate a genetic response such as the chromosomal recombination detailed in previous studies resulting in null mutants. Data from a previous example of this technique (Mortensen et al. 1992) would suggest a benefit to efficiency by using higher concentrations, as well as reducing the numbers of resulting clones to be screened. Due to the unknown number of *neo* insertions in these clones, this concentration would also have to overcome the G418 resistance conferred by a potentially high number of *neo* gene copies.

Plates were tested under a range of G418 concentrations. 0.5mg/ml is generally used to eliminate background levels of untransfected cells, as well as maintain resistance in cell lines using *neo* as a selective marker. In this case, 0.5, 1.5, 2.5, 3 and 5mg/ml G418 were tested.

Concentrations as high as 1.5mg/ml were found to have little or no impact on the growth of the cell lines tested, suggesting a high level of resistance to the drug. As expected, the extremely high concentration of 5mg/ml was found to impact cell growth the greatest. This was lethal to two of the cell lines tested, with less than 1 x 10^4 viable cells remaining after 7 days selection in the population derived from clone *1H1*.

Result:

The cell line *1H1*, after exposure to this high concentration treatment, was expanded and a population of single cell clones isolated (n=192). These were subsequently seeded in HT deficient medium with dialysed serum for a number of passages, however all clones maintained their ability to grow in this environment suggesting none of these cells had undergone the desired mutation.

3.1.11.2 Secondary viral knockout construct

A second approach explored a technique more commonly used to generate second allele knockouts. This method involves the use of a secondary knockout vector. This vector is generally identical to the initial targeting vector, however the selectable marker is replaced with a different resistance gene to allow for the specific selection of secondary vector integration events.
This has been successfully employed in CHO cells (albeit using plasmid vectors as opposed to the recombinant viral vectors used in this study) to sequentially target both alleles of a gene, resulting in homozygous knockout cell lines (Yamane-Ohnuki et al. 2004; Yamane-Ohnuki, Yamano & Satoh 2008).

To generate this secondary *DHFR* targeting vector, the *DHFR* vector plasmid (pAAV-DHFR) was modified. Using the unique restriction sites BamHI and XhoI flanking the *neo* gene, this resistance marker was removed and replaced with a Zeocin (*zeo*) selectable marker. This secondary marker was amplified from pcDNA3.1/Zeo plasmid, resulting in a 669 bp fragment which was subsequently ligated into the pAAV-DHFR/Zeo plasmid (the sequence of this plasmid can be found in appendix 5.2.3).

Arm	F/R	Sequence (5' – 3')	Fragment size
5' Arm	*Forward*	*aaaaaggatcc*CAAGGTGAGGAACTAAACCATGGC	669 bp
	Reverse	*aaaaactcgag*GAGGTCGACGGTATACAGACATG	

Figure 3.1.11.2.1: Details of the primers used to amplify the regions of plasmid DNA comprising the Zeocin resistance gene. Italicised sequences contain restriction sites for digestion and ligation purposes.

This secondary DHFR/Zeo targeting virus was created as before, with 9 plates transfected, purified and concentrated.

For the purposes of transduction the heterozygotic clone *1A9*, previously determined to contain both a functional and disrupted DHFR allele, was chosen for re-targeting.

Transduction and selection was carried out as detailed previously, using 200µg/ml Zeocin (Invitrogen, cat. R250-01).
After the background of untransduced cells was removed (those not expressing zeocin resistance), 4 x 96-well plates of single cell clones were isolated and placed under selection in HT deficient medium with dialysed serum for a number of passages.

Result:
All clones isolated maintained their ability to grow in this environment suggesting this virus was ineffective at disrupting the second *DHFR* allele.

3.1.12 Determination of off-target insert frequency

While viral targeting may efficiently generate targeted cells due to the effects of homologous recombination, it also suffers from the unintended consequences of random off-target insertions. These insertions may be mutagenic, or otherwise place an undue transcriptional burden on the cell due to the expression of multiple selective marker integrations.

Initially, a southern blot technique was investigated for its use in detecting off-target insertions. For this purpose, the Roche DIG (Digoxigenin) system was purchased and tested with a number of virally targeted samples (see methods section 2.8). This system based on a non-radioactive DIG-labelled probe, with an anti-DIG enzyme linked antibody used to produce a chemiluminescent signal. The following primers were first used to successfully generate a suitable 306 bp DIG-labelled probe designed to bind to the *neo* selection marker contained within the viral targeting cassette;

Forward	5’ – AAGATGGATTGCACGCAGG– 3’
Reverse	5’ – GAGATGACAGGAGATCCTG– 3’

Following the protocol described in section 2.8, 5µg of genomic DNA from each sample was digested and transferred onto a nylon blot. However, after hybridisation and signal detection no bands were visible in any of the experimental samples. A sample of digested plasmid DNA containing the *neo* target locus at the correct size was visible, suggesting the probe was suitable.

A number of optimisations were made to the protocol in order try and achieve a positive result;

- Successively lower hybridisation temperatures were tested
- Wash stringency was lowered by reducing the wash temperature and increasing SSC concentrations
- Increased and decreased concentrations of target genomic DNA were used
- Alternative size probes (including a smaller 165 bp and larger 757 bp probe) were tested
- Increases in probe concentration (double and triple concentration) was attempted

Unfortunately, none of these changes in protocol, either alone or in combination, were found to generate a positive band signal in any of the targeted cell lines tested. A limit of detection experiment using the standard prescribed conditions was tested using dilutions of the control plasmid, the only sample shown to generate a signal. Despite the manufacturers claims that 0.1 pg DNA is detectable, our results showed that 25 pg of plasmid DNA was the lowest detectable signal (3.3 x 10^6 plasmid copies). A rough estimate would suggest ~1.8 x 10^6 genome copies in the 5µg of genomic DNA suggested, placing our samples out of this range. Increases in DNA were not successful in capturing any signal, suggesting a possible limit to the capacity of the membrane or a reduction in capillary transfer efficiency. Ultimately, this technique was not successful, so an alternative method was devised.

In order to quantify the frequency of total insertion events, including non-homologous off-target insertions, qPCR was performed on a number of correctly targeted *DHFR* clones. This technique was carried out as an alternative to the southern blot analysis described above, which was found to be not sensitive enough for this experiment. Each sample was assayed using a total of 50ng genomic DNA from each targeted cell line, with a primer set targeting the *neo* transgene within inserted cassettes used to indicate insertion event copy number.
The vector plasmid containing this cassette was used to generate a standard curve for quantification. The samples used to generate this standard curve were also spiked with 50ng of background CHO-K1 genomic DNA to reduce bias in amplification efficiency that may arise from using plasmid DNA alone.

Due to a difficulty in obtaining precise dilutions of genomic DNA across a range of different samples, a β-actin reference gene was also measured to control for slight variances in genomic DNA concentration. Assuming 2 intact copies of this gene, this control could also then be used to calculate the number of CHO genomes in a given sample, and ultimately calculate the total number of inserts per genome for each sample.

Samples assayed included 6 correctly targeted (5'/3' arm HR events) clones, as well as a partially targeted (5' arm HR event) clone and a randomly integrated (no HR) clone. Insertion numbers per genome were calculated based on a number of calculated factors including the number of insertion events measured via qPCR, sample concentration, genome copy number measured using a β-actin reference as well as an estimated haploid CHO genome size of 2.6 gb (Xu et al. 2011).

Result:

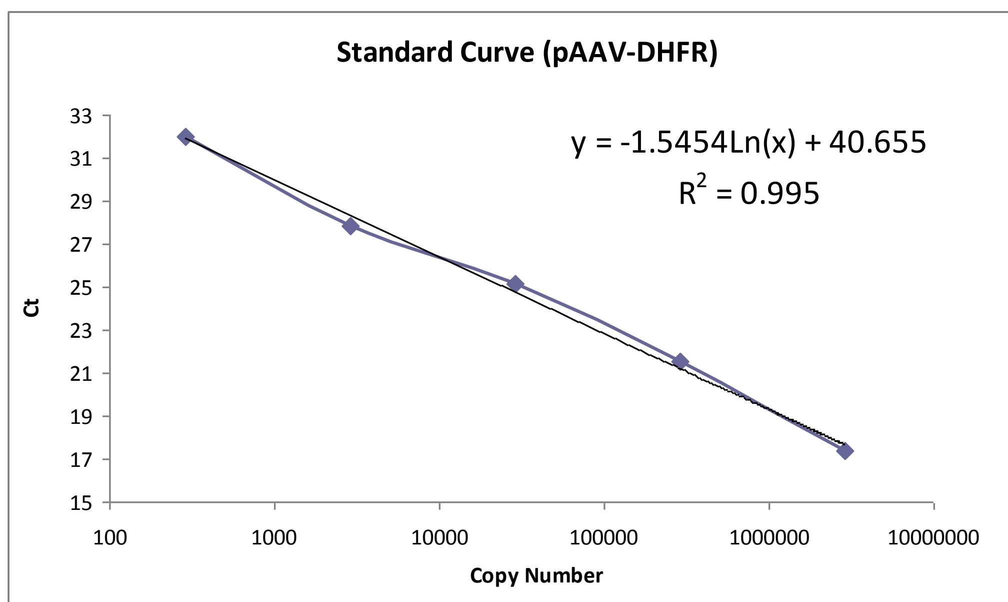

Figure 3.1.12.1: The qPCR standard curve generated from the pAAV-DHFR plasmid using primers designed to target the *neo* resistance gene. These samples were also spiked with an appropriate concentration of genomic DNA to better mimic amplification conditions and efficiencies resulting from the use of genomic DNA alone. Samples were measured in triplicate within the linear range between 2.86 x 10^6 and 2.86 x 10^2 plasmid copies.

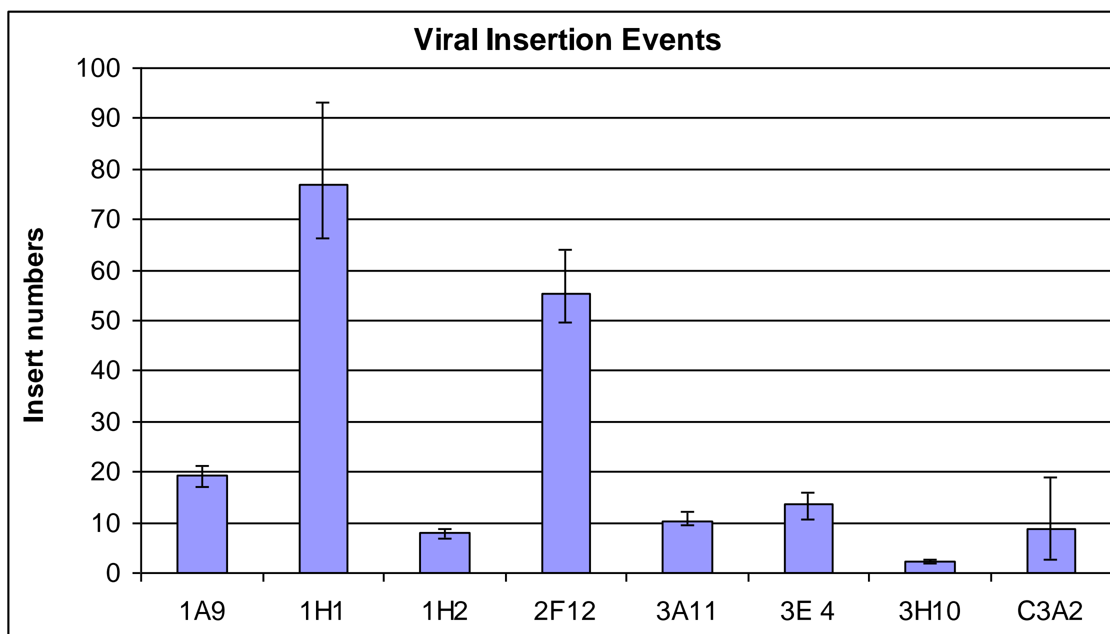

Figure 3.1.12.2: Calculated insertion events from the individual clones assayed. qPCR was carried out on genomic DNA isolated from targeted clones, with the *neo* transgene used as the quantified target and a β-actin reference used to control for differences in DNA concentration. In order to factor in inaccuracy resulting from the nature of the experiment, error (denoted above as a minimum and maximum range of inserts) was also calculated by combining the highest and lowest estimated *neo* and β-actin replicate results to generate the range of possible calculated insert number values.

From the clones assayed we see an average insert frequency of ~24 instances. This average result is increased by the high insert frequency clones *1H1* and *2F12*, with a calculated 77 and 55 inserts respectively. A targeted clone (*3H10*) was also isolated containing only 2 measured inserts, likely the targeted allele as well as one other single random insertion.

A summary and further discussion of the results generated in this study is presented in section 4.1 of this thesis.

3.2 Mutagenesis and Directed Evolution Results

3.2.1 Introduction

This experiment was originally devised to investigate if mutagenesis and directed evolution techniques previously carried out to improve the properties of bacterial and yeast culture systems could also be successfully applied in CHO cells. These 'whole cell evolution' techniques have proven successful in lower order species, but have yet to be applied to mammalian cells. For this reason, this novel approach was attempted in CHO to investigate whether such techniques could be successful in eliciting an improved phenotype with regards to biopharmaceutical production.

Two elements were combined with the goal of improving relevant phenotypic characteristics in a standard CHO-K1 cell line. Firstly, a gene was chosen for random mutagenesis to generate a mutant library with sufficient diversity. Tata binding protein (TBP) was chosen for this purpose. This choice was informed by similar studies using a technique described as 'global transcription machinery engineering' (gTME) in yeast (Alper 2006) and *E. coli* (Alper and Stephanopoulos 2007) in which high-level transcription factors directly or indirectly involved in the transcription of a wide range of genes was mutated and expressed to alter the cellular phenotype. TBP's selection was supported by its role as a key mammalian transcriptional factor and essential component of the pre-initiation transcription complex that binds RNA polymerase II, the enzyme responsible for the transcription of a large portion of RNA in mammalian cells. It was theorised that any alteration to its sequence could modify this proteins binding affinities with its wide range of interactors (including DNA and a range of other transcription factors), therefore leading to a wide range of perturbations in the transcriptomic profile of a transfected cell. By producing a mixed population of transfected cells, we can hope to generate a wide heterogeneous pool of cells from which potentially advantageous mutants can be selected.

Once a heterogeneous population expressing various mutant TBP sequences is generated, the second element of this technique is the application of an environmental stress in order to select for cells that may have an improved phenotype resulting from their altered transcriptome. This element is based on a number of similar successful experiments in other mammalian cell lines in which a persistent environmental stress is used as a selective factor, for example the adaptation of CHO cells to grow in glutamine free medium (Bort, Stern and Borth 2010) or resistance to high ammonia concentrations in mouse hybridomas (Matsumura et al. 1991), as well as the gTME technique upon which this experiment was based.

For the purposes of this study, two advantageous phenotypes were investigated; high speed growth and extended viability, with a suitable stress applied to a population of mutant library transfected cells in order to select for each particular phenotype. In order to select for high speed growers cells were passaged at a particularly low density, under the theory that faster growers would eventually overtake the remaining cell population after repeated passages and become the dominant phenotype. Cells with extended viability were selected for by allowing a standard culture to drop to a low viability before re-passaging, effectively selecting for cells that can better withstand the cumulative negative effects of late stage culture conditions such as nutrient depletion and waste build-up.

Once the cell lines had undergone a number of passages under stress, both mixed populations and single cell clones were then isolated and their properties examined. This characterisation stage involved analysing properties such as growth and viability over the course of a standard culture and productivity levels. Analysis was also carried out on the mutant TBP sequences contained within successful clones, as well as a transcriptomic profiling carried out in order to determine the changes in gene expression that may contribute to an improved phenotype.

3.2.2 Generation of a wild type TBP plasmid vector

The first step taken in the generation of a mutant TBP library was to first clone the CHO TBP sequence into a suitable expression vector. For this purpose, the pcDNA3.1 plasmid (Invitrogen, cat V860-20) utilising a selective marker conferring resistance to zeocin was chosen.

First, PCR using a high fidelity enzyme (Merck, cat. 71085-3) was successfully carried out on a CHO cDNA library using primers to amplify the TBP cDNA, a 978 bp fragment that was subsequently visualised and purified.

Forward 5' – *catcggatcc*ATGGACCAGAACAACAGC – 3'
Reverse 5' – *aatagcggcgc*CTATGTGTCTTCCTGA – 3'

Figure 3.2.2.1: Details of the primers used to amplify the TBP cDNA sequence. Bases in uppercase denote those used in direct sequence binding, while lowercase italicised characters indicate overhangs containing restriction sites (BamHI/NotI) used for digestion and ligation of PCR fragments

This was then cloned into the pcDNA3.1 multiple cloning site plasmid by digesting both PCR fragment and vector with BamHI and NotI, then ligating together to generate the wild type TBP plasmid. TBP expression is driven by a constitutive CMV promoter upstream of its position, with Zeocin (Invitrogen, cat. R250-01) used a selectable marker for transfected cells. The sequence of this plasmid can be found in appendix 5.2.4.

3.2.3 Generation of the mutant TBP library

In order to generate a mutant library of TBP sequences, a kit based mutagenesis protocol was followed utilising the GeneMorph II EZClone Mutagenesis system (Agilent Technologies, cat. 200552) as detailed in the materials and methods section 2.5. The workflow follows the basic principles;

- Low fidelity PCR is carried out on the TBP sequence under controlled conditions to generate a particular frequency of mutations
- These mutant sequences are used to anneal to the original donor plasmid as 'megaprimers' in a subsequent PCR step which extends and recreates the original plasmid, incorporating the mutant sequences.
- *DpnI* is used to digest the original unmutated (methylated) donor plasmid, leaving a mixture of PCR-generated pcDNA plasmids incorporating the mutant TBP sequences.

Following this protocol as detailed, 3 mutant libraries (low, medium and high frequency) were generated using reaction conditions specified by the kit to alter the levels of mutation within the target sequence.
Once these libraries were generated and transformed, single cloned sequences were isolated and sent for sequencing. However, the first instance of this procedure revealed a lower than expected mutation rate. Mutagenesis was repeated with lower starting DNA concentrations in an attempt to increase these rates, however the resulting mutation rate was still lower than expected.

To achieve the desired mutation rates, two elements were optimised for the 3rd attempt at mutation. Firstly, the primers used in the mutagenesis reaction were extended to increase their annealing specificity and raise their melting temperature by 10°C. This brought the reaction into an optimal temperature range suggested by the kit, resulting in a greater amplification of mutant TBP. Secondly, the amount of template DNA was lowered to increase the rate of mutation in each reaction. After this 3rd round of mutation, 5 plasmid clones from each library were sent for sequence analysis in order to estimate mutation frequency (see figure 3.2.3.1.1).

Forward	5' – *AT*TAATACGACTCACTATAGGG*AGACCCA* – 3'
Reverse	5' – *AC*TAGAAGGCACAGTCGAGG*CTG* – 3'

Figure 3.2.3.1: This figure shows the final mutagenesis primers used, flanking the TBP sequence. Italicised letters indicate the extensions added to increase the T_m and improve PCR efficiency in the final attempt to generate suitable mutant TBP libraries.

3.2.3.1 Mutant TBP library mutation rates

Initial Target DNA (ng)

Mutation rate	Recommended	**Used**
Low	500-1000	**100**
Medium	100-500	**20**
High	0.1-100	**0.1**

Mutation rates (per kb)

Mutation rate	Expected	**Average Achieved**	Average number of Base changes/insertions/deletions
Low	0 - 4.5	**6.2**	4.5 / 0.5 / 1.2
Medium	4.5 – 9	**9.5**	7.25 / 0 / 2.25
High	9 - 16	**13.5**	9.75 / 1.75 / 2

Figure 3.2.3.1.1: Details of the 3 final mutant libraries that were generated. Various concentrations of target plasmid DNA (noted above) were used in a low fidelity PCR reaction to achieve the measured mutation rates. A selection of 5 individual plasmids from each library was sequenced in order to estimate the average number of mutations in each library.

Having generated libraries with acceptable mutation rates, it was decided that work would continue using the medium TBP mutant library with an average rate of ~9 mutations per 957 bp gene sequence. This was chosen to avoid the likelihood of large amounts of deleterious or non-functional mutations associated with the highest mutation rate, while introducing a larger amount of heterogeneity than the low library in order to distinguish its effects from unmutated wild type TBP.

3.2.4 Estimation of library complexity and large scale preparation

In order to maximise the possible library complexity of a large scale mutant plasmid preparation, work was carried out to optimise the transformation efficiency of the library.

To optimise this process varying volumes of the library were transformed in DH5α competent bacterial cells, along with varying incubation times on ice. An optimum was found at 3μl volume of library plasmid, with an extended 30 min incubation time found to increase efficiency. In order to generate the large scale plasmid preparation, 10 separate transformation reactions were pooled to increase overall complexity and subsequently grown in terrific broth medium (see section 2.5.4). This was then prepared using the plasmid maxi prep protocol (see section 2.3.4), producing a concentrated mutant TBP plasmid library.

Result:

An efficiency of at least 10^4 colony forming units was noted in single transformation tube. A combination of 10 individual transformations resulted in a large scale plasmid preparation containing an estimated complexity of ~10^5 individual mutant sequences.

3.2.5 Generation of stably transfected cells

Stable cell lines were then generated using the protocol detailed in section 2.5.5. The following plasmids were transfected to generate individual cell lines;

- Mutant TBP (Library)
- Wild Type TBP (WT-TBP)
- Empty vector control (pcDNA)

Transfections were carried out using 2µg plasmid DNA on 1 x 10^6 adherent cells (~70% confluent) using 4µl Lipofectamine 2000 (Invitrogen, cat. 11668) according to the manufacturers instructions. A 'cells only' mock transfection was also carried out, as well as a GFP plasmid positive transfection control. After 72 hours, this GFP control was measured as ~55% of GFP positive cells, indicating a successful transfection.

After 48 hours, cells were placed under selective pressure to select for stably transfected cells using the Zeocin selection marker contained on the pcDNA plasmid backbone. The concentration for effectively clearing the background of untransfected cells was previously determined to be 200µg/ml of Zeocin. This concentration was also used subsequently as a selective agent to maintain TBP expression. Once the background of untransfected cells was eliminated, these stable populations were adapted to suspension growth and serum free conditions in CHO-S-SFM II medium (Invitrogen, cat 31033-020) for use in subsequent experiments.

Result:

3 individually transfected mixed populations were generated for use in stress selection experiments.

The mutant library transfected cells (designated 'Library') serve as an experimental population, while the wild-type TBP and control empty vector transfected cells (designated 'TBP' and 'pcDNA' respectively) serve as controls to measure the impact our mutant TBP library may have on CHO compared to constitutive over expression or absence of recombinant TBP.

3.2.6 Application of stress to select for advantageous phenotypes

Once a stable population was generated that was adapted to typical culture conditions, an environmental stress was applied for a number of passages. This was carried out in order to allow cells with possibly beneficial or advantageous mutations to survive or otherwise outgrow their counterparts and eventually dominate the population. For the purposes of this experiment two selective pressures were applied in order to select for two distinct phenotypes; resistance to apoptotic conditions and high speed growth.

- *Extended Viability*:

This stress was applied in order to select for cells resistant to late stage (apoptotic) environmental culture conditions. Such a property would be advantageous in any industrial culture, increasing the possible productive cell time available. Cells were passaged at a standard concentration (2×10^5 cells per ml) and left in culture for an extended period of time until viability began to drop below a given level. The remaining cells were then passaged, resuspended at a standard concentration of viable cells and re-stressed for a number of rounds in culture. Cell density and viability were the key metrics measured at the point of each passage. Initially, 50% viability was decided upon as a guideline level at which the cell lines would be passaged into fresh medium.

- *High speed growth*:

In order to investigate an alternative possible phenotype that may arise from the library transfected cells, an alternate contrasting stress was applied. While an extended viability phenotype would benefit cells in the later stages of culture, cells displaying an improved ability to grow to high density would reduce the time necessary to get a culture into a productive phase. Cells were passaged at a low concentration and allowed to grow back to high density. It was theorised that any cells with the ability to grow quickly would soon outgrow the population. Initially cells were passaged at 5×10^4 cells per ml. After a number of rounds, this was then lowered to 1×10^4 cells per ml in the presence of 10% conditioned medium to promote growth.

In each case 3 replicate suspension culture tubes (5ml working volume) were seeded for each of the empty vector control (pcDNA), wild type TBP (TBP) and mutant TBP (Library) transfected populations. In between passages cells were monitored transiently by count on a haemocytometer.

Viability and cell density measurements were then taken on the day of passage using the Guava Easycyte flow cytometer and Viacount reagent (Millipore, cat. 4000-0041), measured in triplicate to ensure accuracy.

3.2.6.1 Results of 'viability' stressed cultures

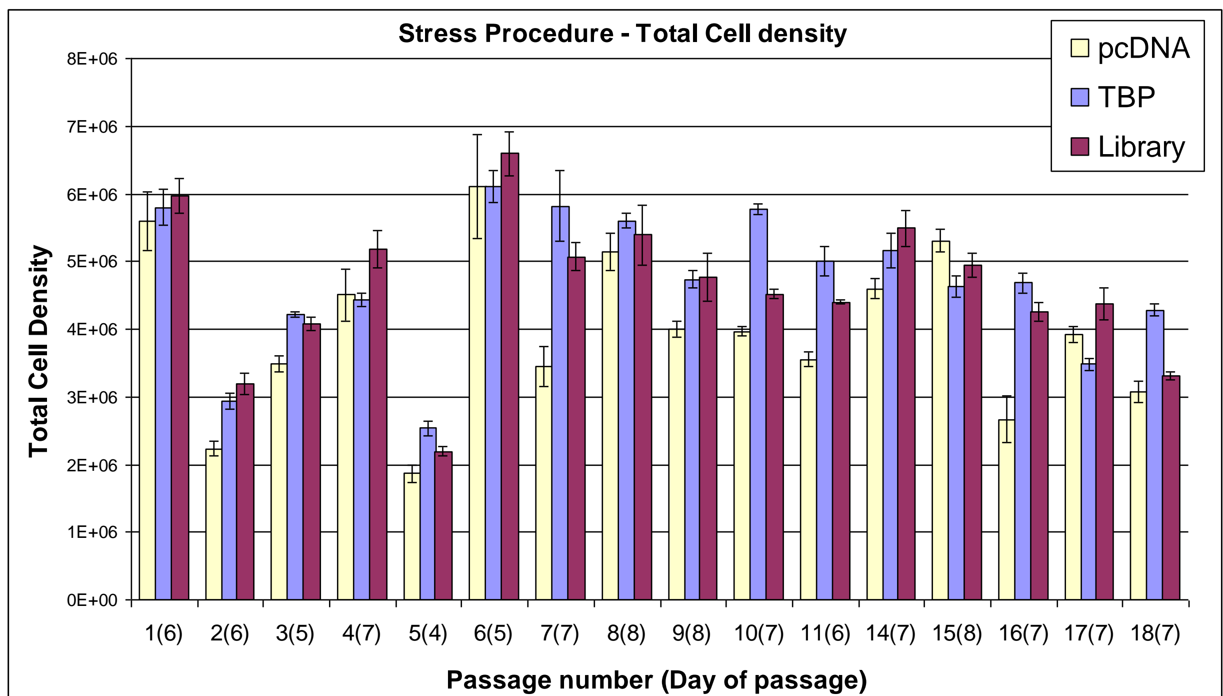

Figure 3.2.6.1.1: Details of the total cell densities (including both living and dead populations) of each cell line as measured on the day of passage throughout the stress procedure designed to select for an extended viability phenotype. X axis values represent the passage number, with culture duration noted in parentheses. Each cell line was passaged in biological triplicate to a density of $2x10^5$ viable cells/ml, with each sample's cell density and viability measured in triplicate.

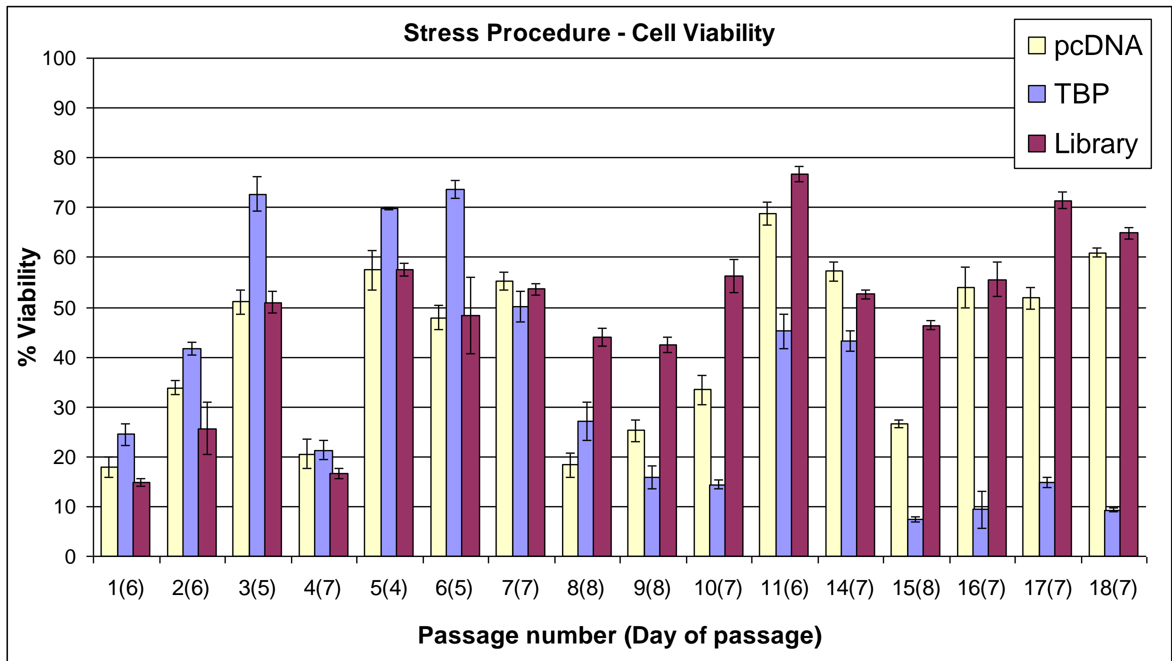

Figure 3.2.6.1.2: Percentage viability of each cell line as measured on the day of passage throughout the stress procedure. X axis values represent the passage number, with culture duration noted in parentheses. Each cell line was passaged in biological triplicate to a density of $2x10^5$ viable cells/ml, with each sample's cell density and viability measured in triplicate.

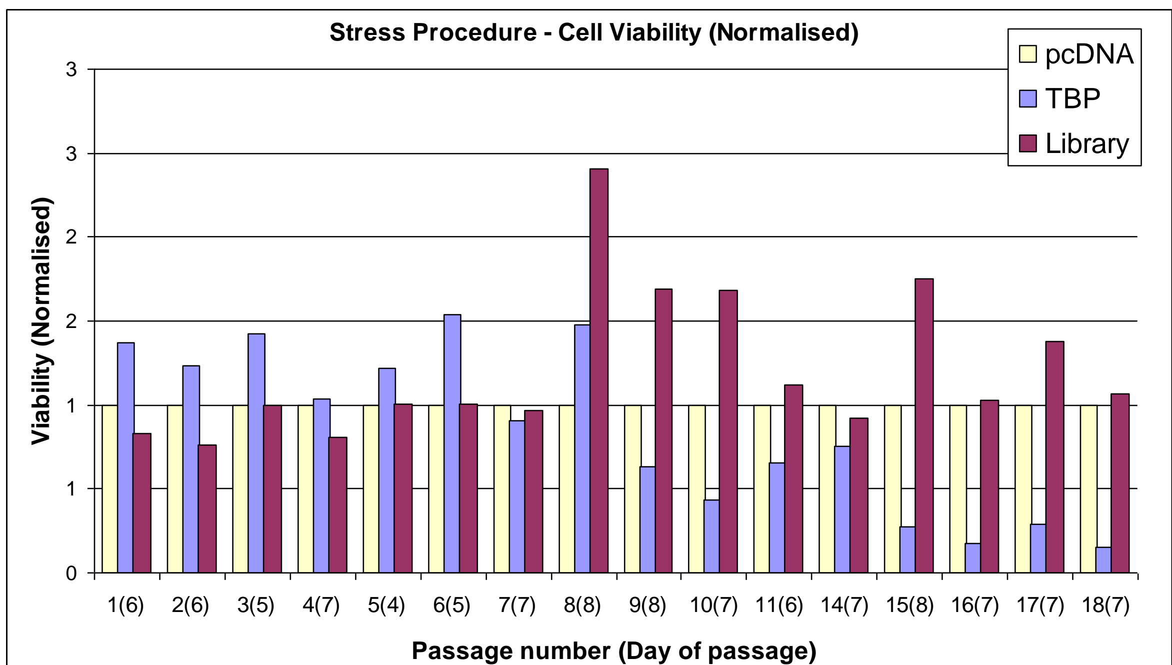

Figure 3.2.6.1.3: The data represented previously in 3.2.6.1.2, with values normalised to the pcDNA result. This representation allows us to examine the distinct effects the TBP and Library transfection procedures have in comparison to the control cell line.

3.2.6.2 'Viability' stressed cultures discussion

This key experimental step was designed to function as a formative and selective stress. The effect of this stress on a mostly homogenous population would be seen in both control and TBP populations, with only the natural variation found in their populations contributing to any adaptation that may take place. This effect would perform in a similar manner to other experiments utilising directed evolution to generate cell lines with improved properties (as discussed in section 1.6.1).
However, the experimental Library transfected cell line incorporates both natural cellular variation as well as the artificial heterogeneity generated by the different mutant TBP sequences expressed in each cell, and would hopefully have a greater ability to respond to this pressure due to this variation. This selective effect is more reminiscent of similar experiments involving mutagenesis as well as a selective pressure, such as the gTME experiments described in 1.6.3.3.
By comparing these different populations, we hoped to gain an insight into the benefit of both TBP and Library overexpression compared to the technical control, as well as the differences between them that may arise as a result of the selective stress.

The figures in this section display the total cell density and viability values measured on the day of passage for each of the control, TBP and Library transfected cell lines. Figure 3.2.6.1.3 also represents the normalised percentage viability data, displayed as a fold difference of the control pcDNA cell line.
While 50% viability was initially decided upon as a guideline level at which the cell lines would be passaged into fresh medium, strict adherence to this limit was difficult to maintain due to the unpredictability of decline in viable cell numbers, and the differences in survival rates that arose between cell populations. A separate culture was seeded intermittently and preserved in liquid nitrogen as a precaution in case of a catastrophic loss in viability.

In total 16 passages of selection were carried out. Passages 12 and 13 (not displayed in results) were carried out with no stress applied. Due to a fatal crash in viability in which the cultures were lost, these passages were used to revive frozen samples and re-continue the experiment from this point.

Due to the nature of the process and the resulting data, it is difficult to make any clear conclusions regarding the effectiveness of the stress whilst cells are continually within the stress process, as seen above. This is in part due to the changing nature of the cells being used to re-seed each passage, with differing results at each point making the identification of trends difficult.
As a calculated volume of viable cells was used to re-seed the next culture, this invariably includes a large number of dead cells, possibly affecting both the environment of viable, reproducing cells, as well as the total cell counts of successive cultures. This approach is in contrast to a similar experiment in which cells are stressed and allowed to recover before re-seeding (Prentice, Ehrenfels and Sisk 2007), eliminating the large amount of dead cells forwarded in this process. Another beneficial modification to this procedure may be the use of FACS to allow for a strict selection of healthy cells, with this process being used successfully to sort for viable cells gradually adapted to grow in a glutamine free medium (Bort, Stern and Borth 2010). However, FACS sorting was unavailable at the time of this experiment.

Despite the inherent noise in this data, some features are notable or at least suggestive of the changes accumulating over the course of this experiment. Looking at total cell densities (figure 3.2.6.1.1) we see a trend of TBP and Library transfected cells possessing a maximum cell density generally higher than the pcDNA control, suggesting that the overexpression of this protein (or its mutant variations) has a beneficial effect on cell growth and maximum cell density. This is also suggested by overexpression of TBP in human disease tissue, with increased TBP concentrations linked to changes in cell growth and proliferation due to its associations with proto-oncogenic cellular components such as p53 and Ras signalling proteins (Johnson et al. 2003a; 2003b)
Tracking the Library culture viability, the total and normalised percentage viability suggests that this stress is indeed selecting out an advantageous subpopulation, with many results from the halfway point (passage 8) demonstrating a proportion of viable cells greater or equal than the control equivalent, improving from an initial trend (passages 1-7) of only lower or equal viability.

This trend is somewhat reversed in the TBP culture. Persistently high total cell densities are measured on the passage days and, looking at the normalised data, an initial boost to late stage viability is apparent in early passages. These cultures soon appear to deteriorate at a faster pace (passage 9 onwards). The reason for this is not immediately obvious, with mycoplasma being eliminated as a possible factor through a standard detection procedure carried out on all in-house cell lines. Morphologically, TBP cells also began to clump on a macroscopic scale faster than their experimental and control counterparts, also affecting the accuracy of cell counts.

As all cultures were measured and passaged at the same time to simplify the experiment and hopefully make comparisons easier, TBP cells' premature deterioration also had the effect of reducing the possible incubation time length of cultures maintaining relatively high viability. For example, in the latter stage of the experiment rescue of TBP cultures, necessitated by viabilities of <20%, came at the expense of Library cells, whose viability suggested an ability to last longer and thus potentially have a greater stress placed on this population to further select for cells more resistant to later stage culture conditions.

After this artificial stress procedure was carried out, these cultures were maintained under normal growth conditions with regular passaging at 3-4 days to allow for recovery, before single cell cloning and further characterisation was carried out on both mixed and clonally derived populations.

3.2.6.3 Results of 'low density' stressed cultures

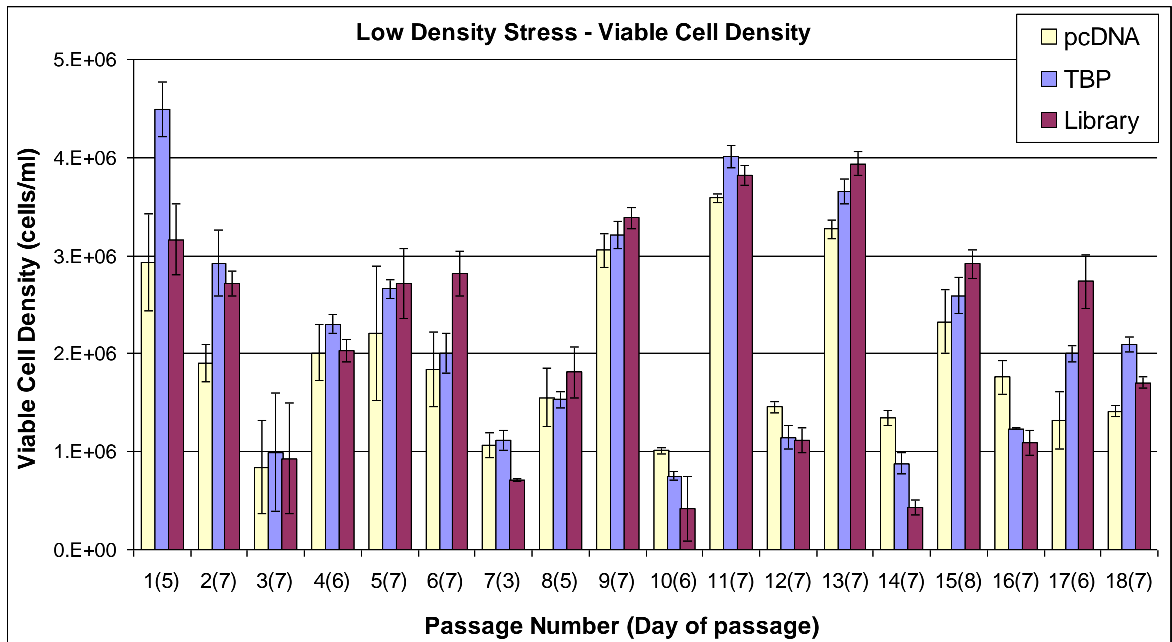

Figure 3.2.6.3.1: Details of the viable cell densities of each cell line as measured on the day of passage throughout the stress procedure designed to select for an accelerated growth phenotype. X axis values represent the passage number, with culture duration noted in parentheses. Each cell line was initially passaged in biological triplicate to a density of $5x10^4$ cells/ml, further reduced to $1x10^4$ upon passage 10.

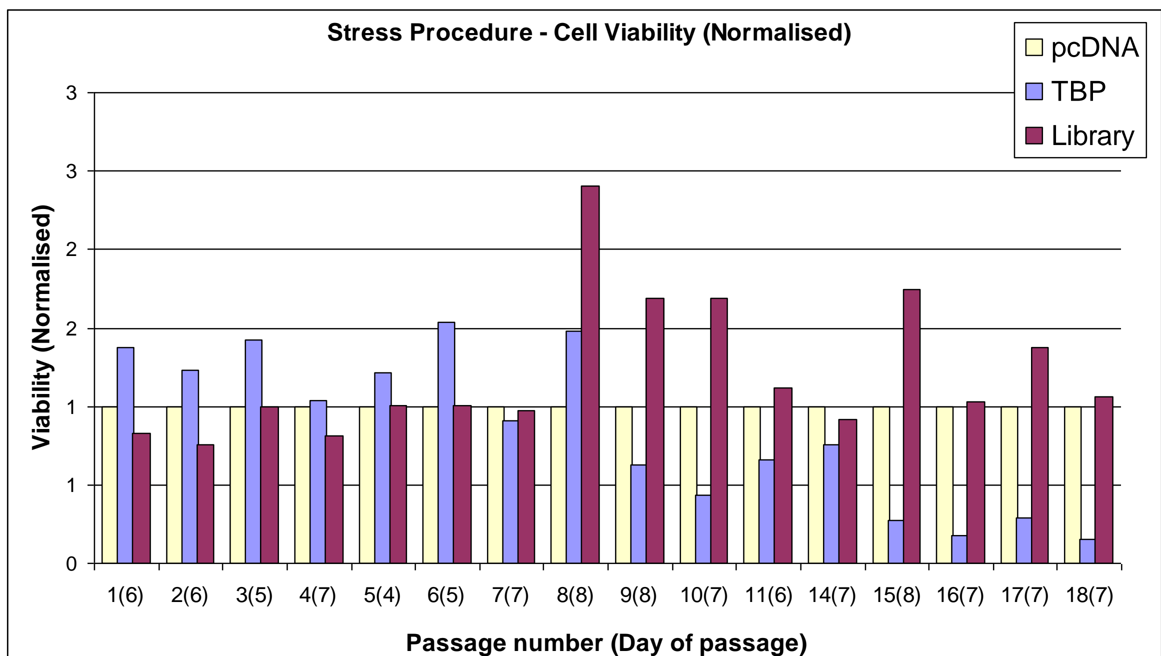

Figure 3.2.6.3.2: The data represented previously in 3.2.6.3.1, with values normalised to the pcDNA result. This representation allows us to examine the distinct effects the TBP and Library transfection procedures have in comparison to the control cell line.

3.2.6.4 'Low density' stressed culture discussion

As with the 'viability' stress designed to select for a specific change in phenotype, the conditions of this experiment were designed to allow cells with a potential increase in growth rate to flourish and outgrow slower cells. Any improvement in exponential growth would allow for advantageous clones to quickly fill the available environmental space, with subsequent rounds of selection allowing the fastest growing cells to prevail. Initially cells were seeded at 5 x 10^4 cells/ml, a four-fold lower concentration compared to a normal passage. Beginning at passage 10, this was reduced to 1 x 10^4 cells/ml with 10% conditioned medium (a twenty-fold reduction in viable cells) as a further selective pressure.
Figure 3.2.6.3.1 above represents the viable cell density values measured on the day of passage for each of the control, TBP and Library transfected cell lines. Figure 3.2.6.3.2 also represents the normalised viable cell density data, displayed as a fold difference of the control pcDNA cell line.

Most of the passages carried out on these cell lines were conducted once the cells had reached the stationary phase, and as such cannot tell us much about early stage growth patterns (this would be examined in subsequent single cell clones derived from these populations). However, the maximum viable cell concentrations of stationary phase cultures again hints at the growth benefit of TBP overexpression, with some data points indicating an increase in maximum cell density in both TBP and Library transfected lines. However, normalised to the control cell line, we can see this effect is not particularly strong.
This is in contrast to a selection of passages carried out on lower density cells still in an exponential growth phase (i.e. passages 3, 6, 12 and 14). While the control cell line may not reach maximum densities as high as the experimental lines due to its lack of TBP overexpression, in these instances it appears to be able to grow at a faster exponential rate.

This data would suggest no clear benefits gained from this stress procedure aside from the initial advantage gained in maximum cell density generated by TBP overexpression.

3.2.7 Pre-stress transfected clone analysis

Prior to examining the characteristics of the stressed populations, a selection of pre-stress clones were examined under standard culture conditions to assess the sole effect of TBP and Library transfection (and empty vector control transfection) on the parental CHO-K1 cells used in this experiment.

Originally, the wild-type TBP gene was transfected alongside its mutant library counterpart as well as the empty vector control. By measuring a mixed population, this would return an average result of cell growth and viability made up of each population's constituent single cells. However, as the mutant library transfected population introduces an increased level of heterogeneity due to the different mutant TBP sequences contained within each of the constituent cells, this 'average' result would be generated from cells expressing both advantageous and deleterious sequences.

In order to look closer at the effects of each transfection a number of single cell clones from each population were compared to investigate, on a clonal level, the effect each transfection may have. By comparing a selection of clones, this allows us to see the range of possible phenotypic effects different mutant TBP sequences may have on individual cells, while an averaged result from this selection would also give an indication as to the cumulative effect that the expression of mutant and wild type TBP sequences would have in a mixed population

In this experiment 12 clones from each of the empty vector, TBP and Library transfected cells were examined alongside clones isolated from a parental CHO-K1 population. These were grown in serum free medium under standard suspension conditions using multi-well suspension culture plates (working volume of 1ml) agitated at 170 rpm. Each clone was seeded at 2×10^5 viable cells in triplicate and measured using the Guava Easycyte flow cytometer.

3.2.7.1 Pre-stress transfected clone results

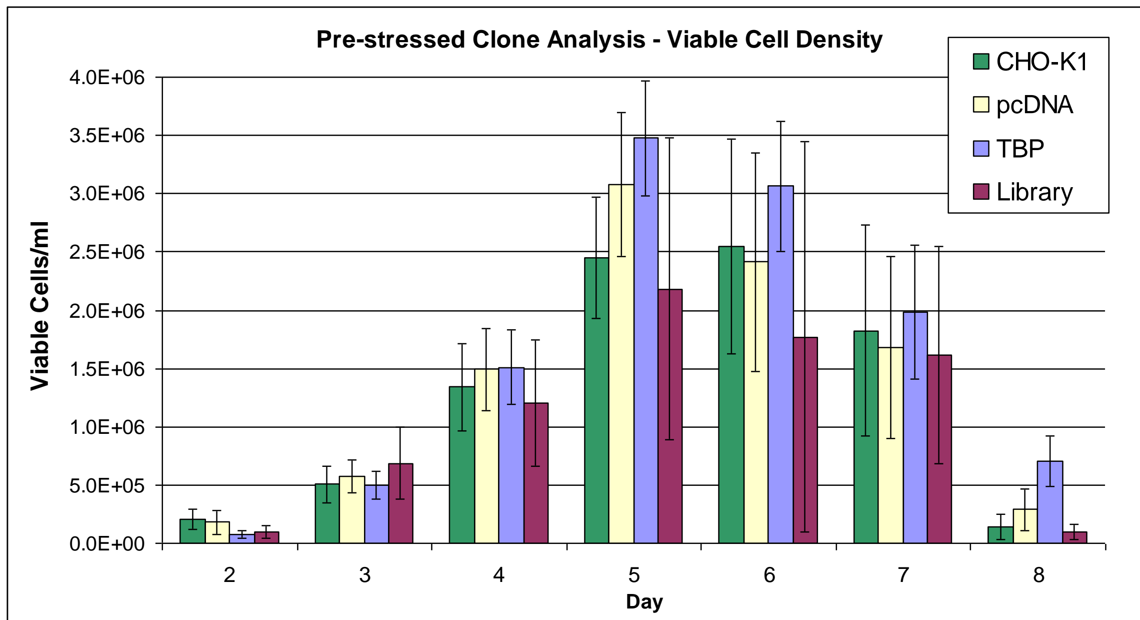

Figure 3.2.7.1.1: Average viable cell densities from groups of 12 clones isolated from each pre-stress mixed population, with error reported as the standard deviation of inter-clonal variability within each group. Clones were grown in triplicate (3x1ml), with single samples from each replicate analysed daily.

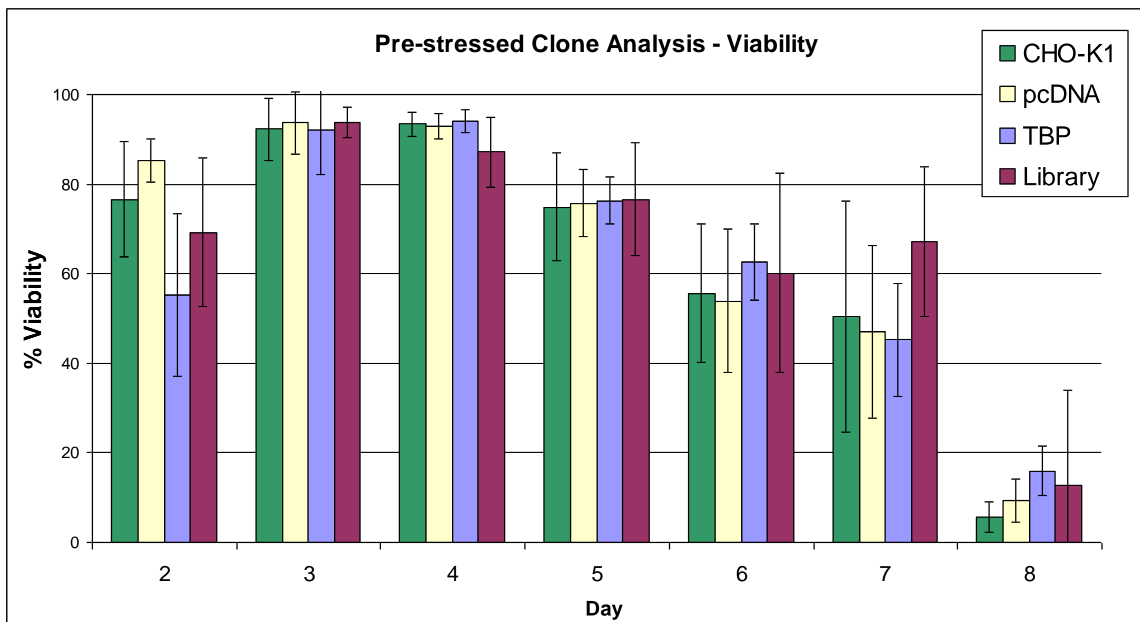

Figure 3.2.7.1.2: Average percentage viabilities from groups of 12 clones isolated from each pre-stress mixed population, with error reported as the standard deviation of inter-clonal variability within each group. Clones were grown in triplicate (3x1ml), with single samples from each replicate analysed daily.

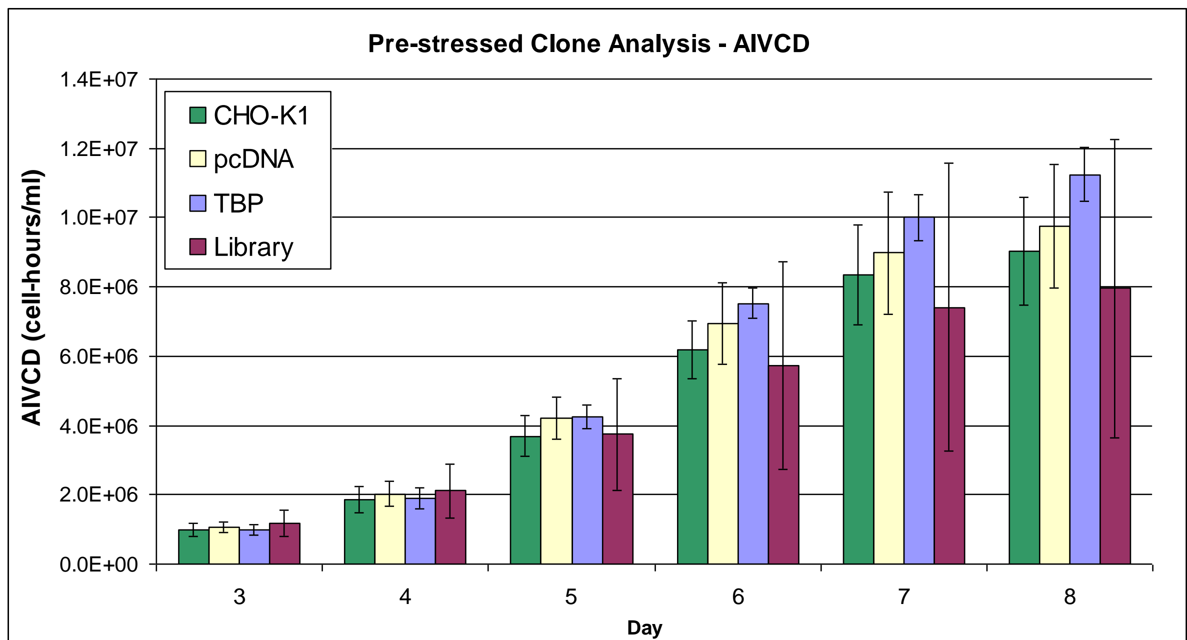

Figure 3.2.7.1.3: Accumulated integrated viable cell density (AIVCD) of each group of 12 clones analysed, with error reported as the standard deviation of inter-clonal variability within each group. AIVCD describes the cumulative result of the integrated area below the cell curve (e.g. figure 3.2.7.1.), allowing us to compare the total ‘cell-time’ of each cell curve when total cell density and viability varies between samples.

3.2.7.2 Pre-stress transfected clone discussion

The results detailed above are displayed as an average result from each group of 12 clones, with error bars displaying the standard deviation arising from the clonal differences across each group. This data was presented in such a manner to allow us to assess the impact of each treatment (or lack thereof) in a general sense, while enabling us to investigate individual clones and the maximum potential of successful individuals from each population.

Looking at each variable individually, we can begin to understand the differences between the groups and the impact each treatment has had.

Examining the viable cell density graph, we first see the differences in maximum viable cell density reached on day 5. As predicted from the stress selection procedure, wild type TBP over expression appears to have a positive effect on cell growth, with a 41.9% increase in maximum cell density reached over the untransfected K1 control. However, this average increase is only 13% over the more similar empty vector control.

Despite the apparent advantage in maximum growth gained from the transfection procedure, viable cell density remains similar in both control groups in the growth (days 2-4) and decline phases (days 6-8). Increased viable cell density is notable in the TBP group in the latter part of the growth curve, maintaining the highest levels from day 5 onwards.
As expected, Library transfected cells display the highest amount of variability with almost a twofold range of densities on day 5 within its particular group (from 1.6 x 10^6 viable cells to 3.11 x 10^6 viable cells per ml). This is likely due to their inherent heterogeneity stemming from the variety of mutant sequences expressed within, compared to the relatively heterogeneous TBP clones (3.38 x 10^6 - 3.52 x 10^6 viable cells per ml). Prior to the application of any stress or selection the overall effect of the TBP library seems to be deleterious, with Library clones exhibiting an average 37% decrease in maximum viable cell numbers reached compared to its wild type equivalent indicating that, in the absence of a selective pressure, mutant TBP sequences on average do not pose a benefit.

When percentage viability (figure 3.2.7.1.2) is looked at independent of cell density, we see mostly similar results across all groups for the greatest proportion of the growth curve, with the only standout result being the actual increase in this percentage for Library viable cells on day 7. This may be a result of reduced waste and increased availability of nutrients due to lower cell densities throughout the culture, allowing for greater viability at this later stage.

Figure 3.2.7.1.3 displays the accumulated integrated viable cell density (AIVCD) of each clonal group. This metric represents the accumulated hours of viable 'cell time' units existing in a culture up to a given point, with this figure increasing as cells persist (this figure may still grow as cells begin to become apoptotic, however then rate of growth will increase or decrease in relation to changes in cell density).
This metric was chosen for use in this study due to the inherent difficulties in comparing cell lines and growth curves with varying levels of viability and cell density, and makes direct comparisons easier. For example, a temperature shifted CHO culture typically experiences a reduction in cell growth in tandem with delayed onset of apoptosis (Moore et al. 1997). This later stage cell survival brings an overall

benefit to the longevity of culture, with an improved AIVCD possible despite its lower cell density in comparison to an identical culture incubated at a higher temperature.

This figure is calculated using the following formulae. Growth rate is first determined and then used in turn to calculate the daily integrated viable cell density (IVCD, i.e. the area encompassed by the growth curve). The cumulative sum of these IVCD figures is then used to calculate AIVCD, with units denoted as [cell-hours/ml]

- *Growth Rate* = [Ln.Density (*b*) – Ln.Density (*a*)] / [Hours elapsed]
- *Daily IVCD* = [Density (*b*) – Density (*a*)] / [Growth rate*24]
- ***AIVCD*** = Day 1 IVCD + Day 2 IVCD…. + Day (x) IVCD

Looking at the AIVCD curve generated for the samples used in this experiment (fig 3.2.7.1.3), we can see the overall potential of each cell line in terms of growth and sustained viability. Comparing the control populations (K1 and pcDNA), we see a difference of 8.5% in final day AIVCD for the control transfected population, with a similar co-efficient of variation (C.V.) for both (17.5% and 18.5% respectively). However, a students t-test comparing the day 8 AIVCD totals for each selection of 12 clones indicates that despite the small increase in average performance there is no significant statistical difference between these groups (p=0.28)
TBP overexpression does appear to provide a beneficial impact on overall performance, with a 25.9% and 16.1% increase in day 8 AIVCD over the K1 and pcDNA control populations respectively. TBP clones also display less variety in their overall performance, with a day 8 AIVCD clonal C.V. of 7.0%, with a notable statistical difference between the TBP population and the empty vector controls (p=0.01).
Library transfected cells also behave as predicted, with a wide variety in performance in all metrics measured. However, despite a lack of selection, we can see the potential of individual clones even in the relatively small sample measured here. For example, while the day 8 AIVCD for TBP clones range from 1.0 - 1.25 x 10^7 cells, Library clones present a more extreme range of values from 3.69 x 10^5 for the poorest clone isolated up to 1.6 x 10^7 for the highest performing clone. These ranges are visualised below as a box plot in figure 3.2.7.2.1, with asterisks representing the extreme outlying values.

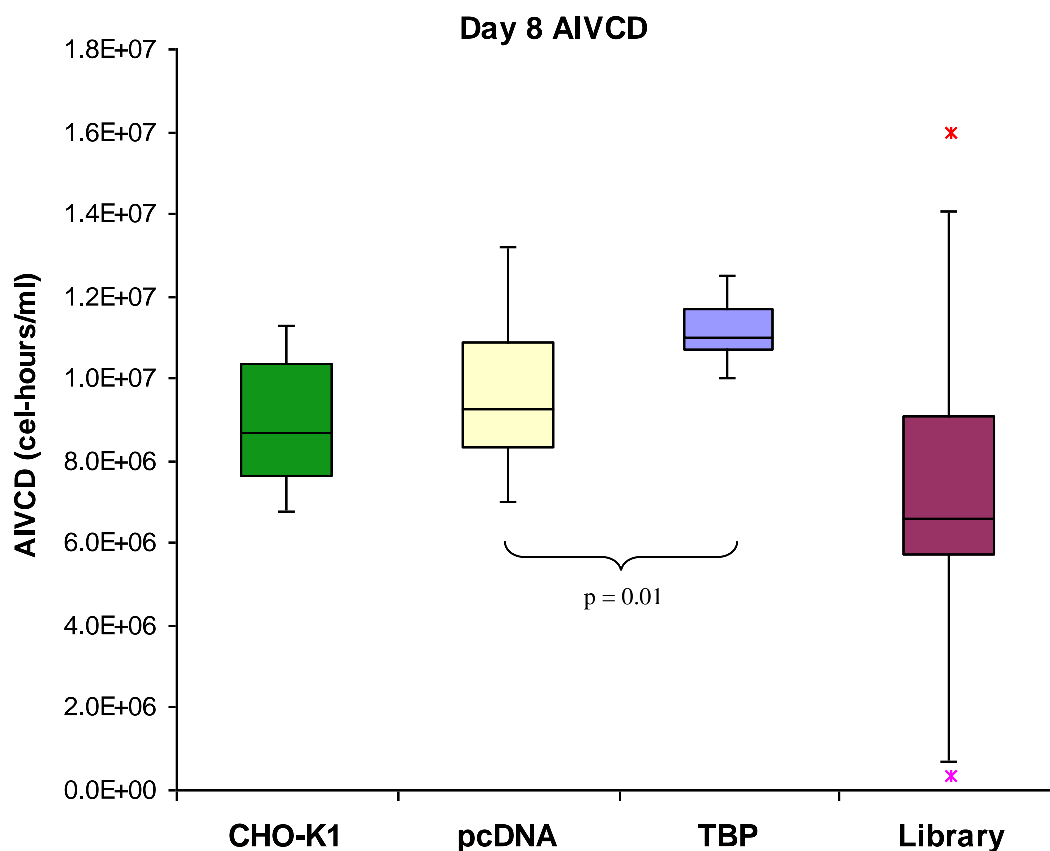

Figure 3.2.7.2.1**:** Box-plot representation of the spread of resulting final day accumulated cell integrals (AIVCD) from the data displayed in fig 3.2.7.1.3. Median values are represented, flanked by coloured boxes representing upper and lower quartiles. A t-test comparing clonal groups indicates the consistent phenotypic benefit of TBP overexpression compared to the pcDNA control (p=0.01), while unselected Library expression provides a wide range of clonal behaviour, with examples of both positive and negative extremes represented by asterisks.

From the data generated above, we can conclude that TBP overexpression alone provides a consistent benefit to cell growth and the ultimate performance of a culture as measured by its accumulated IVCD in comparison to a similarly treated control cell line. We can also see that the mutant TBP library generates both positive and negative results, likely as a result of the different sequences expressed in each clone. This is in the absence of selection, and while clones with beneficial properties in excess of those provided by the wild type gene were isolated in this instance, we would expect the proportion of advantageous clones to increase once a selective pressure has been applied.

3.2.8 qPCR to determine TBP expression in mixed populations

Prior to the examination or characterisation of post-stress clones, qRT-PCR was carried out on stressed TBP and Library mixed populations to investigate expression levels.

To determine the levels of TBP expression within these populations, reverse transcription was first carried out on mRNA isolated from actively growing TBP and Library transfected cell cultures, as well as a pcDNA empty vector control to generate cDNA (see section 2.3.11). qRT-PCR was then carried out using primers designed to bind to the 3' end of the cDNA molecule using the protocol detailed in section 2.3.12. PABPNI was used a housekeeping control gene to normalise expression levels across these samples.

Forward	5' – TTCTCCTTATTTTTGTTTCTGG – 3'
Reverse	5' – TATGTGGTCTTCCTGAATCC– 3'

Figure 3.2.8.1: Primer sequences used in the qPCR reaction. These were designed to bind the 3' end of the TBP cDNA sequence, generating a 119 bp product.

3.2.8.1 TBP qRT-PCR (mixed population) results

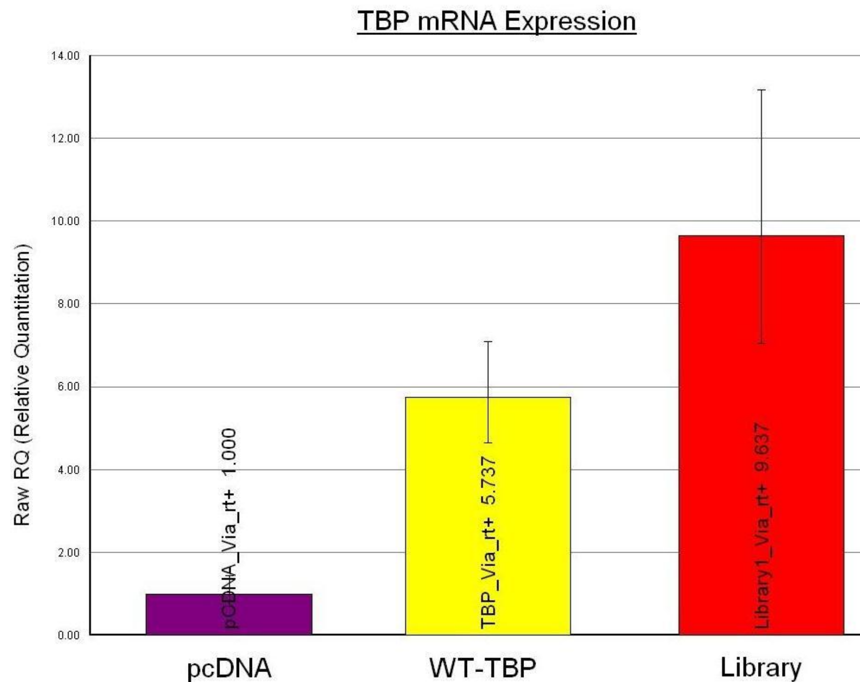

Figure 3.2.8.1.1: Graph detailing relative gene expression levels of TBP within the stressed cell TBP and Library transfected populations. Each cell line was grown in biological triplicate, with technical triplicate cDNAs analysed via qPCR from each sample using the primers described in figure 3.2.8.1. Error is represented as the standard deviation of these multiple samples. TBP and Library samples are normalised to pcDNA (control) expression.

Wild type and mutant library TBP were found to be over 5.7 and 9.6 times higher respectively in mixed populations than the empty vector transfected reference sample. This was carried out on post-stress mixed pools to ensure that possible improvements and variation were related to TBP and Library overexpression. If cells with TBP and Library expression were ultimately deleterious overall, we would expect overexpression to be selected out by an evolutionary pressure, with differences simply a result of natural clonal variation. This verification experiment was carried out before further investigative work into post-stress clones was considered.

3.2.9 Post-stress transfected clone analysis

From the previous experiments carried out and the resulting data generated, we initially concluded that TBP overexpression bestowed a consistent benefit in terms of cell growth. This extra growth did not come at the cost of a drop in viability that one might expect from the increased consumption of nutrients etc. associated with a higher cell density, leading to an overall increase in useful cell-time as measured by an increase in the calculated AIVCD. This effect was also noted in a portion of high performing Library transfected clones, however the overall result from this clonal group was poor due to the contribution of deleterious clones to the average result.

An experiment was carried out similar to that detailed above on a selection of TBP and Library clones isolated from both of the post-stress populations. This was done in order to investigate whether the stress procedure would, as hypothesised, select for Library cells with beneficial mutations as well as acting as a formative stress, causing changes in gene regulation and expression as a response to the unfavourable environmental conditions these cultures were challenged to grow in. By focusing on a selection of Library and TBP clones, this experiment would determine whether the selective process was capable of eliminating the large number of deleterious mutant TBP sequences from the Library transfected population and improve the overall fitness of this group.

This experiment was carried out using the same conditions as those described previously in 3.2.7, with clones seeded at 2×10^5 viable cells in triplicate and grown in a 1ml working volume of serum free medium. 12 clones were isolated from Library and TBP cultures resulting from both ‘viability’ and ‘low density’ stress procedures, and their relevant phenotypes examined. Each clone was measured daily using the Guava Easycyte flow cytometer.

3.2.9.1 Post-stress clone results ('Viability' stress)

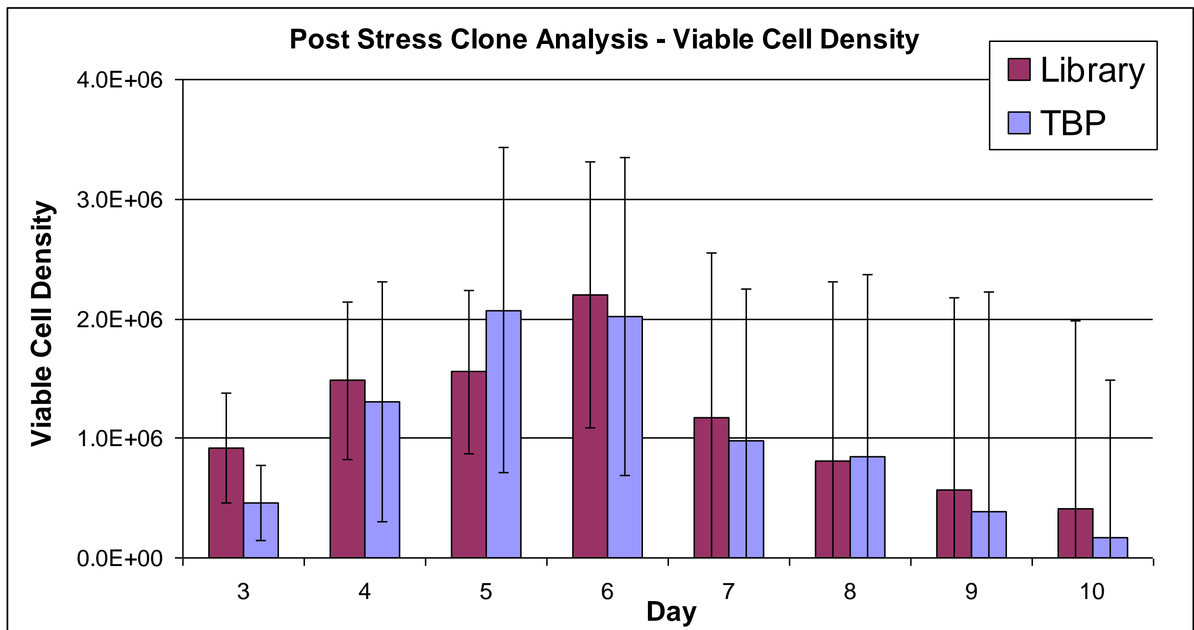

Figure 3.2.9.1.1: Average viable cell densities from groups of 12 clones isolated from each post-stress mixed population, with error reported as the standard deviation of inter-clonal variability within each group. Clones were grown in triplicate (3x1ml), with single samples from each replicate analysed daily.

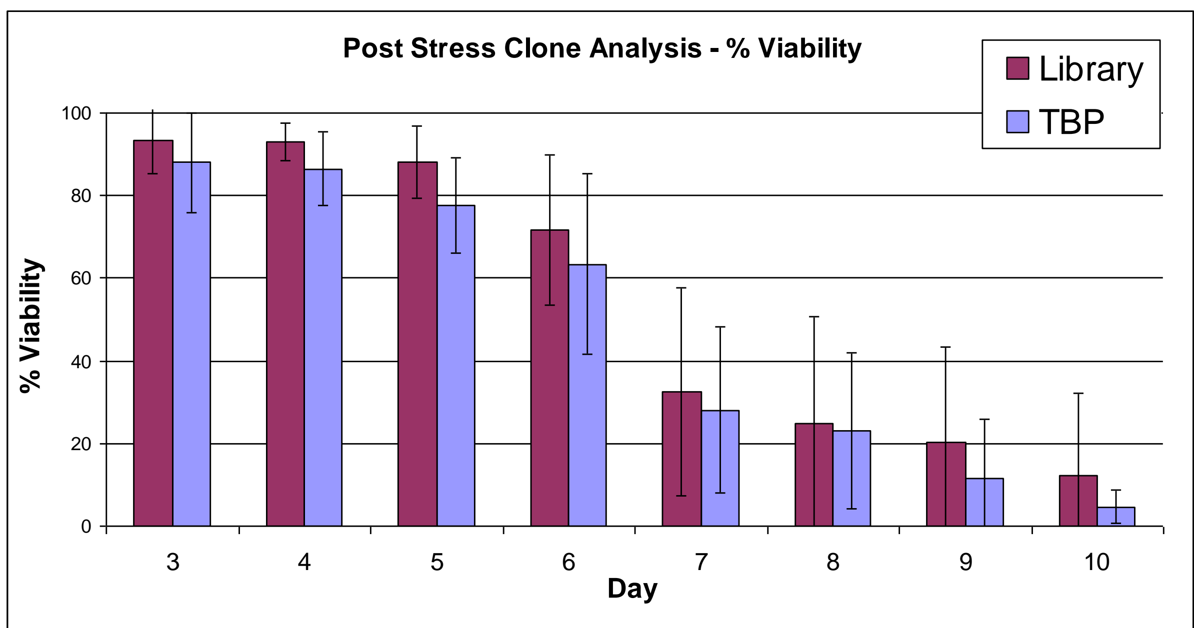

Figure 3.2.9.1.2: Average percentage viabilities from groups of 12 clones isolated from each post-stress mixed population, with error reported as the standard deviation of inter-clonal variability within each group. Clones were grown in triplicate (3x1ml), with single samples from each replicate analysed daily.

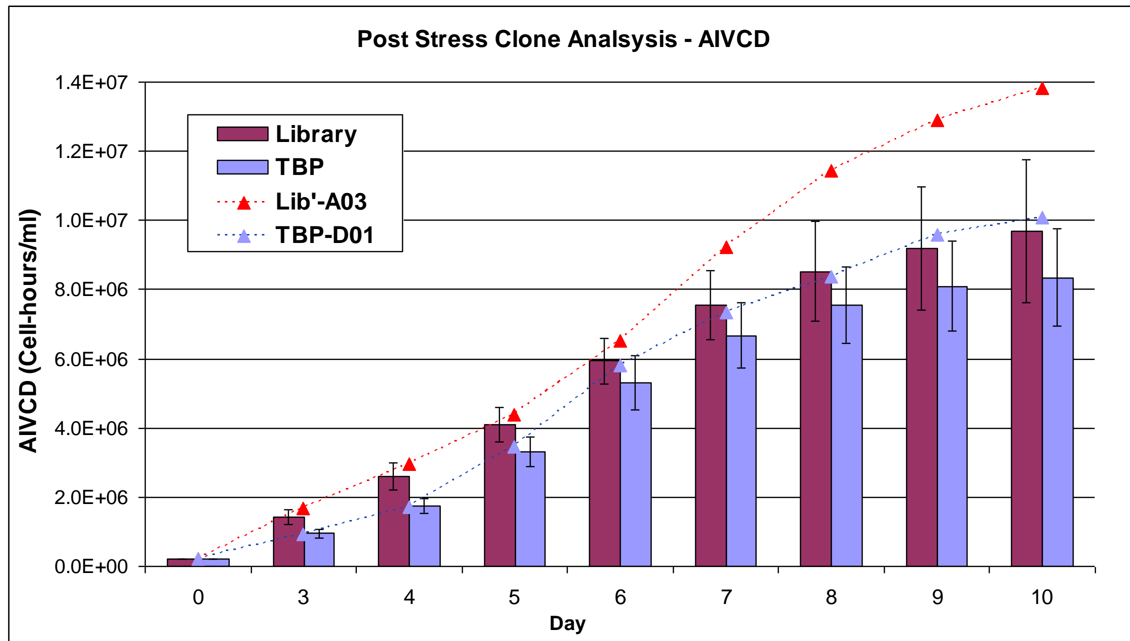

Figure 3.2.9.1.3: Accumulated integrated viable cell density (AIVCD) of each group of clones analysed, with error reported as the standard deviation of inter-clonal variability within each group. The highest performing clones from each group are also represented by the dotted lines (A03 & D01).

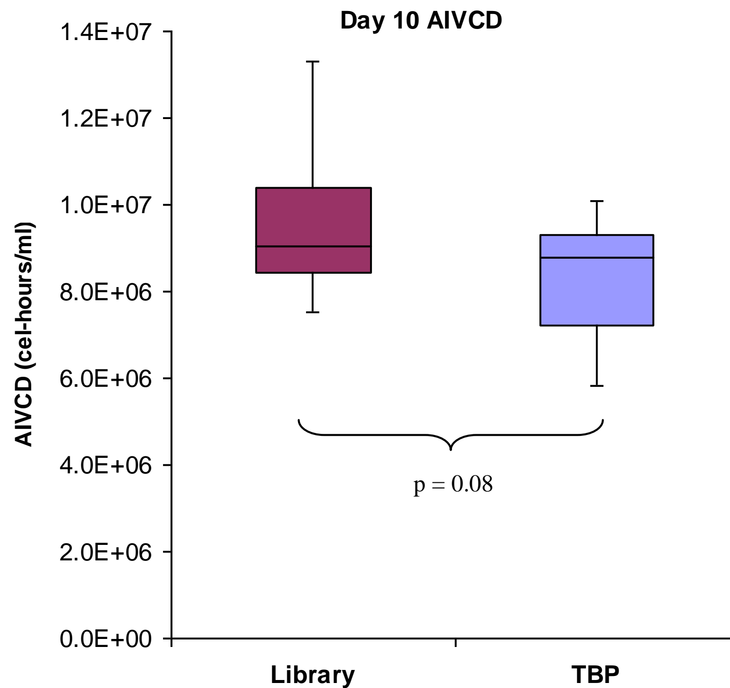

Figure 3.2.9.1.4: Box-plot representation of the spread of resulting final day accumulated cell integrals (AIVCD) from the data displayed in fig 3.2.9.1.3. Median values are represented, flanked by coloured boxes representing upper and lower quartiles. A t-test comparing the statistical difference between groups returns the value p=0.08.

3.2.9.2 Post-stress clone discussion ('Viability' stress)

The results detailed above are displayed as an average result from each group of clones, with error bars displaying the variability arising from the clonal differences across each group. It should be noted that while 12 clones from each group were assayed, due to persistent evaporation in a corner well of the suspension culture plates used leading to unreliable results, the results of 1 TBP clone was omitted from the final analysis.

This experiment was carried out to examine the difference between the stressed TBP and Library populations on a clonal level. The previous experiment had established the beneficial effect of TBP overexpression, however prior to any selective pressure the Library transfected cells displayed a poor average performance. Despite the inclusion of a number of advantageous clones in the Library sample set, the median AIVCD result was found to be poorer than the empty vector control, indicating the negative effect that deleterious mutant TBP sequences were having on the unselected Library population as a whole. We would predict the stress procedure to remove these deleterious mutants an ultimately improve the overall fitness of the Library transfected pool.

When we examine these results we see a marked increase in Library clone performance compared to its wild type TBP counterpart, suggesting the stress procedure was successful in eliminating a large proportion of Library cells expressing detrimental TBP mutants. In general, average Library clone results exceed those of TBP in terms of viability, while the viable cell concentrations suggest a difference between the growth curves, with Library cells continuing to grow until their maximum on day 6 (2.2×10^6 viable cells per ml, see figure 3.2.9.1.1) compared to the day 5 TBP maximum (2.07×10^6 viable cells per ml), with a difference of 6.3%. This improvement in both maximum cell density and overall viability contribute to a 16.1% increase in final day AIVCD (figure 3.2.9.1.3).

In this experiment, both populations display a high degree of clonal variability in their overall performance. For TPB this is in contrast to its pre-stress performance, with an increase in final day AIVCD variance from 7.0% to 16.8% in this instance. However, this level of heterogeneity in Library clones is lower in comparison to their pre-stress counterparts, with AIVCD variance reduced from 55.2% to 21.4% in this experiment. A student's t-test comparing the final day AIVCD values of both populations provides a p-value of 0.08, suggesting the differences between these populations is not wholly significant with only 2.7% difference in median values (compared to the 16.1% mean difference, see figure 3.2.9.1.4). However, the improvement of Library transfected cells from their unstressed counterparts in unmistakeable, and due to the inherent variance of this population a number of high performing clones worthy of further characterisation were isolated in the outlying portion of AIVCD performance (see clone A03 in figure 3.2.9.1.3)

Notably, both TBP and Library populations drop below 40% viability between days 6 and 7, while surviving at low percentages until day 10 (see figure 3.2.9.1.2). This in contrast to the pre-stressed populations, which did not drop below this level until day 8. However this crash in viability was more pronounced, with no cells surviving beyond this day. This may be the result of an evolutionary response to the stress procedure, selecting for cells with a slower decline in the latter stages of culture. It also may be a result of nutrient depletion due to a higher maximum cell density reached by stressed cells, (suggested by the improvement in maximum Library viable cell density) causing an earlier decline below this threshold.

Unfortunately, the comparison of inter-experimental absolute cell totals was hampered by the cell counting method used in the course of this study, and direct comparisons between the viable cell concentrations of different studies was unreliable. Due to a number of mechanical errors, part replacements and changes in operational procedures for the cytometer used throughout this thesis, drifts in calibration over time do not allow us to make accurate direct comparisons. While the data generated here indicates an improvement in Library cells resulting from the stress procedure compared to wild type TBP, further direct comparisons of clones from both pre- and post- stress populations allowed us to make more quantifiable conclusions regarding the benefit of the stress procedure overall, particularly with regard to cell density.

3.2.9.3 Post-stress clone results ('Low density' stress)

This experiment was carried out as a follow up to the preliminary results gained throughout the course of the stress procedure designed to select for cells with an improved growth rate. While these initial results indicated no apparent benefit to the initial growth rate of TBP and Library transfected and selected cells (section 3.2.6.3), a selection of 12 clones from both Library and TBP cultures resulting the 'low density' stress procedure were isolated and grown under standard culture conditions.

For this experiment, viable cell density was measured for the first 4 days of growth. In order to examine the exponential growth of these cultures both a basic growth rate calculation ([Ln.Density (*b*) – Ln.Density (*a*)] / [Hours elapsed]) was used as well as a more sophisticated online model using least square fit of exponential data (Roth 2006) to calculate the average days per doubling for this data.

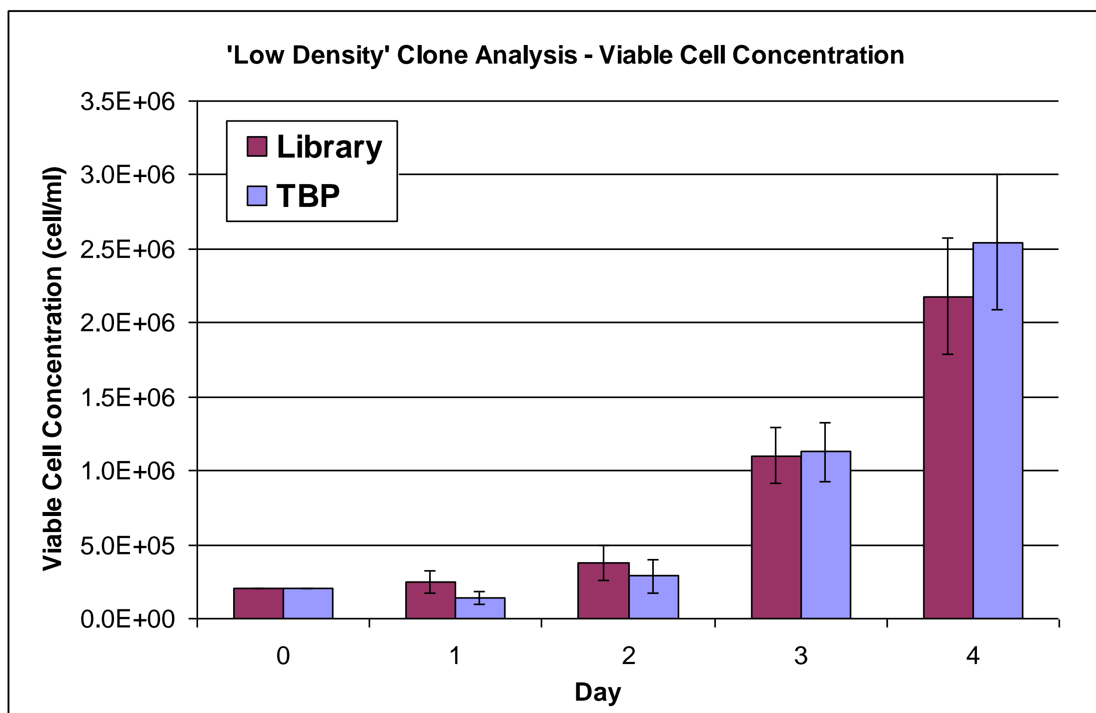

Figure 3.2.9.3.1: Viable cell density from each group of 12 clones analysed, with error reported as the measure of clonal variability within each group. Cell lines were seeded in triplicate at 2 x 10^5 viable cells in 5ml suspension culture, and sampled every 24 hours until day 4 (96 hours).

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	Growth Rate (hr $^{-1}$)	CV	**Days per doubling**	CV
Library	0.0258	7.1%	1.04	11.8%
TBP	0.0278	6.3%	0.86	7.5%

Figure 3.2.9.3.2: The average hourly growth rate and 'days per doubling' metrics as a measured average of the 12 clones isolated from the 'low density' stress mixed populations. Each clone was analysed separately using a basic growth rate calculation (growth rate/hour) and least square fit analysis tool (days per doubling), and the resulting figures analysed as a group to generate the results above. Differences between Library and TBP appear significant, with t-tests comparing both growth rate and 'days per doubling' of clonal groups returning values $p<0.01$.

3.2.9.4 Post-stress clone discussion ('Low density' stress)

The results detailed above are displayed as an average result from each group of clones, with error and CV detailing the levels of clonal variability within each group. Ideally, we would see an increased growth rate and reduction in days per doubling in the Library cells to indicate that the stress procedure had indeed selected for advantageous clones. However, the reverse was observed in the data generated.

Looking at the growth curve generated (day 0-4), we see a slight reduction in viable cell density for TPB transfected cells for the first 48 hours compared to the stressed Library clones. This is similar to the initial dip in viability witnessed in unstressed TBP clones, suggesting a slight negative impact of TBP overexpression in early (i.e. 24-48 hours post seeding) culture periods. This growth advantage in Library transfected cells is lost by day 3 however, and the average hourly growth rate across the four days is on average 7.3% less in Library clones ($p<0.01$ when comparing these two groups). The least square fit analysis of this data also confirms this difference, with an average doubling time of over 24 hours (1.04 days) for Library cells, compared to less than 22 hours (0.86 days) for TBP clones.

From this data we can conclude that the transfection of the mutant library provides no additional benefit to initial cell growth speed after the application of the 'low density' stress procedure in comparison to the use of the wild type TBP gene. This may be indicative of the relative usefulness of the stress applied, with no strong negative selective pressure endured by these cultures (compared to the harsher conditions of the 'viability' stress for instance). It may also indicate that TBP or its mutant derivatives may be of no use with regards to improving initial growth speed, with a previous study identifying TBP responsive genes as being traditionally linked to cellular stress responses, as opposed to basic metabolic functions that may have a greater impact on initial growth (Yang et al. 2007).

Ultimately, due to the lack of any noticeable improvement in the Library transfected cells, these clones were discarded and further characterisation carried out on the more promising advantageous clones isolated from the 'viability' stressed cultures.

3.2.10 qRT-PCR to determine TBP expression in selected clones

After the characterisation of the clones isolated from stressed mixed populations, qPCR was also carried out on selected clones using the same process as detailed in section 3.2.8.

To determine the levels of TBP expression within these populations, reverse transcription was first carried out on mRNA isolated from actively growing TBP and Library transfected clones, using a previously isolated pcDNA control mRNA sample as a control. qRT-PCR was then carried out using primers designed to bind to the 3' end of the cDNA molecule using the protocol detailed in . PabpnI was used a housekeeping control to normalise gene expression levels across these samples.

Forward	5' – TTCTCCTTATTTTTGTTTCTGG – 3'
Reverse	5' – TATGTGGTCTTCCTGAATCC– 3'

Figure 3.2.10.1: Primer sequences used in the qPCR reaction. These were designed to bind the 3' end of the TBP mRNA sequence, generating a 119 bp product.

3.2.10.1 TBP qRT-PCR (selected clone) results

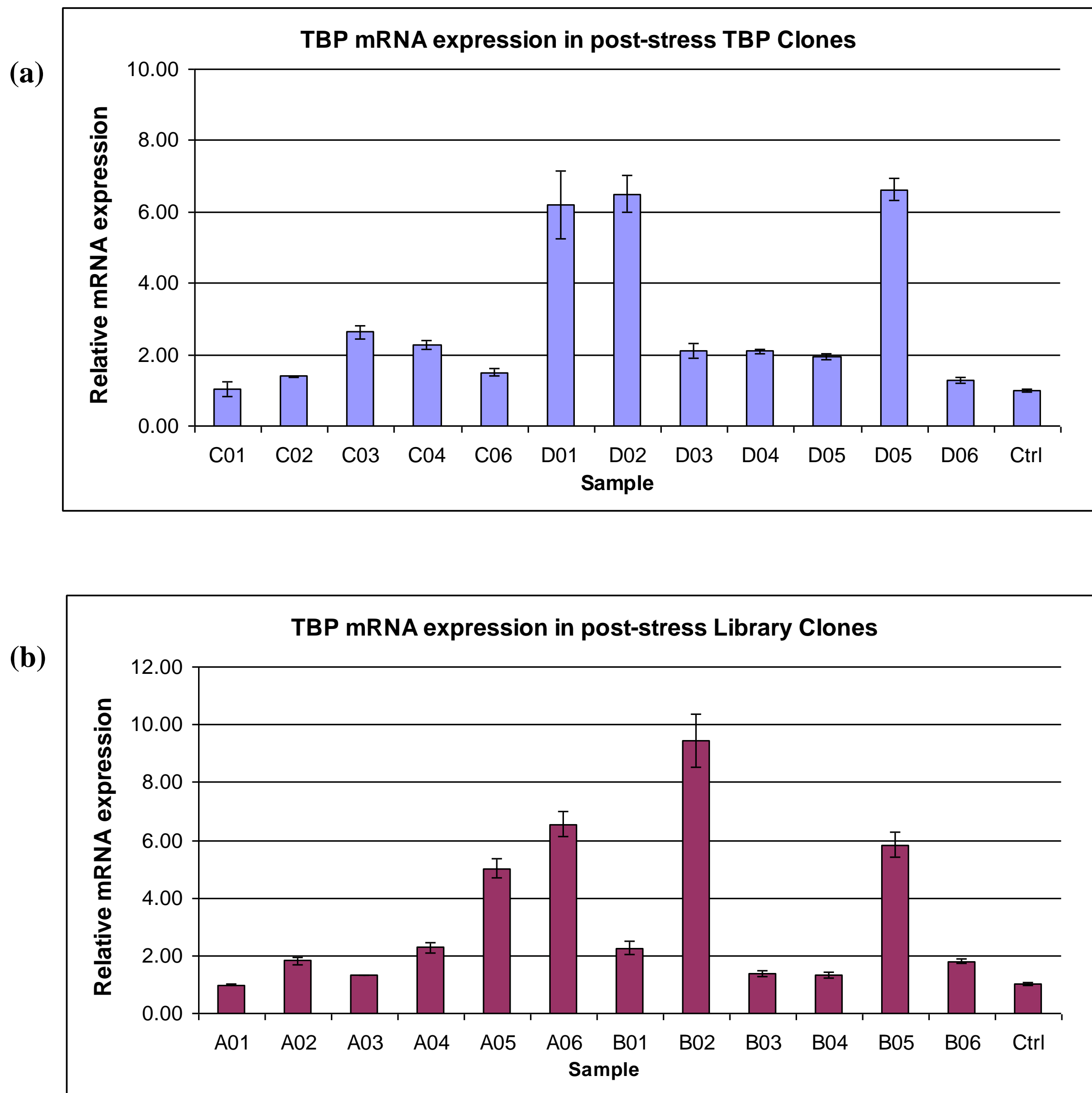

Figure 3.2.10.1.1: Graphs detailing relative gene expression levels of (a) TBP and (b) mutant library within the stressed cell TBP and Library transfected populations**.** The "$2^{(-\Delta\Delta Ct)}$" method was used to calculate expression relative to the PabpnI housekeeping gene, with fold change normalised to an untransfected CHO control. RNA Samples were isolated from actively growing cultures, and analysed via qPCR using triplicate technical cDNA replicates (error is represented as the standard deviation of these replicates).

Figure 3.2.10.1.1 describes the relative expression of TBP in the 12 clones isolated from the ‘viability’ stress TBP and Library transfected populations analysed in the previous experiment. Interestingly, many of these clones, including some found to exhibit advantageous properties, did not express TBP (or its mutant derivatives) at the higher levels indicated by the mixed population results. This may be a result of each individual clone’s particular configuration, with gene copy number, position effects and associated epigenetic regulation affecting expression levels.

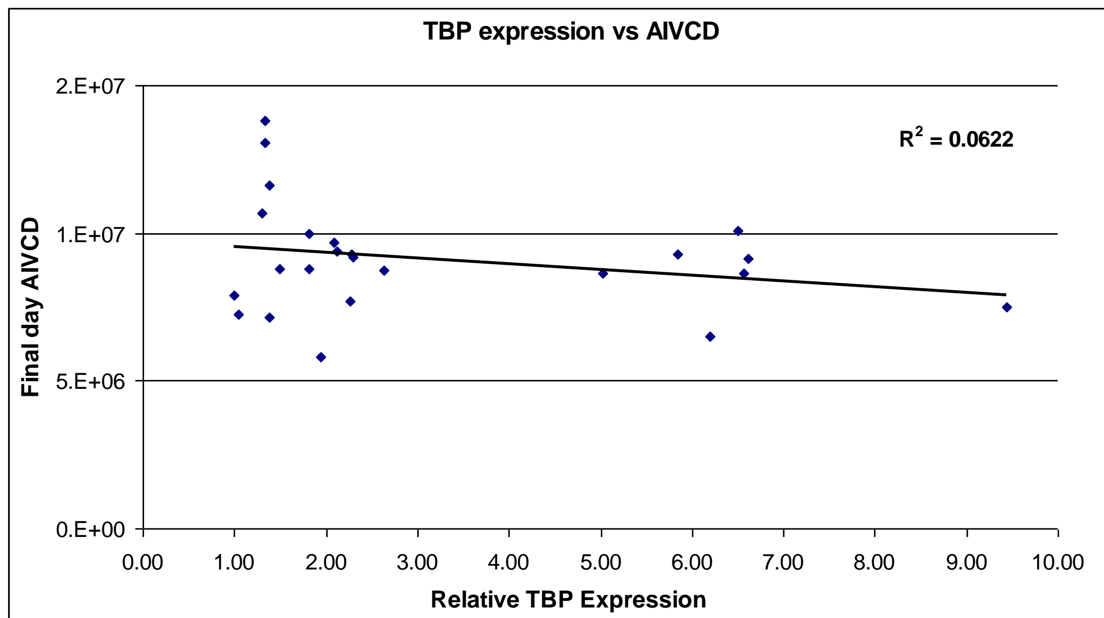

Figure 3.2.10.1.2: Scatter plot comparing relative TBP expression (data generated in this study) to final day AIVCD values generated in section 3.2.9. This data suggests no statistical correlation (R^2=0.06) between these two variables, however the relatively small sample size (n=24) may inhibit the confidence of this conclusion.

These figures were not found to correlate specifically to metrics such as maximum cell density or final day AIVCD (such as the example comparison displayed in figure 3.2.10.1.2), suggesting that TBP and library expression levels are not the direct cause of any changes in phenotype and would not serve as a predictive marker for an improvement in performance. Rather, phenotype is likely a result of more complex changes in overall gene expression patterns mediated in some way by the overexpression of TBP and, in the case of Library transfected cells, the various effects of different mutant sequences, with the forced evolutionary stress selecting for advantageous differences.

3.2.11 Pre-stress versus post-stress direct comparison

At this point, the data generated with regard to the pre- and post-stress clones (selected under deleterious late stage culture conditions) indicated a benefit to overall cell performance (AIVCD) from TBP overexpression, as well as a likely additional benefit arising from selected Library expression after prolonged exposure to the stressed environment. However, in the absence of a direct comparison (made difficult due to disparities in inter-experimental absolute cell numbers arising from technical complications), it was impossible to rule out a number of potential explanations for these apparent differences. For instance, it could be argued that while Library clone performance is improved in comparison to the wild type TBP transfected clones, this apparent improvement may be due to an actual drop in post-stress TBP culture performance (this theory would be supported by the gradual drop in TBP culture viability over the course of the stress procedure, see figure 3.2.6.1.3).

In order to verify that post-stress TBP and Library clones had in fact produced a superior phenotype to their pre-stress counterparts, an experiment was carried out in order to directly compare a number of pre- and post-stress TBP and library clones within the same experiment. This would eliminate experimental variation as a factor in comparative calculations and provide direct evidence of the impact provided by the applied stress.

For this experiment the top 3 performing clones were chosen from previously isolated Library and TBP clone panels, both stressed and unstressed. Samples were assayed in triplicate in 1ml suspension format and seeded at 2×10^5 viable cells per ml, with samples removed for analysis daily. Arising from the variability of clonal performance, we have demonstrated the ability to extract clones with phenotypic characteristics greater than the average performance of their original respective panels, with this effect being more pronounced in the heterogeneous Library populations as would be predicted. Comparing these 'strong' clones from each group would demonstrate that, with even a modest amount of screening, advantageous clones with significantly improved phenotypes could be isolated from these mixed populations.

3.2.11.1 Direct comparison results

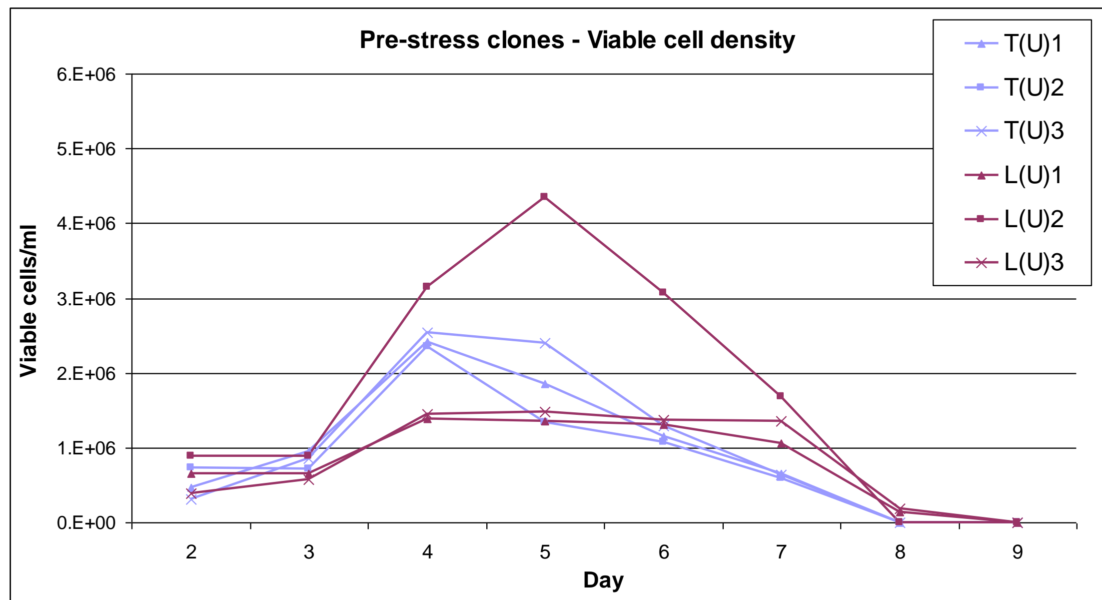

Figure 3.2.11.1.1: Viable cell density curves from individual pre-stress clones characterised in this study. Each cell line was grown in triplicate (3x1ml), with single samples from each replicate analysed daily. Unstressed TBP clones denoted [T(U)1-3] are marked in red, with unstressed Library clones [L(U)1-3] are marked in red to highlight the differences between treatments.

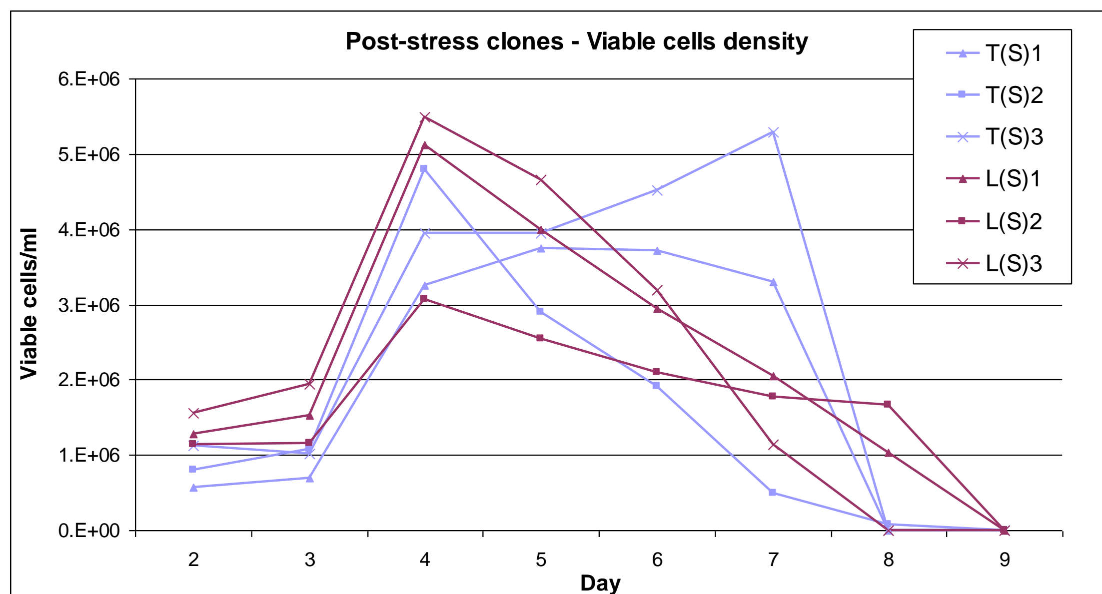

Figure 3.2.11.1.2: Viable cell density curves from individual post-stress clones characterised in this study. Each cell line was grown in triplicate (3x1ml), with single samples from each replicate analysed daily. Stressed TBP clones denoted [T(S)1-3] are marked in red, with stressed Library clones [L(S)1-3] are marked in red to highlight the differences between treatments.

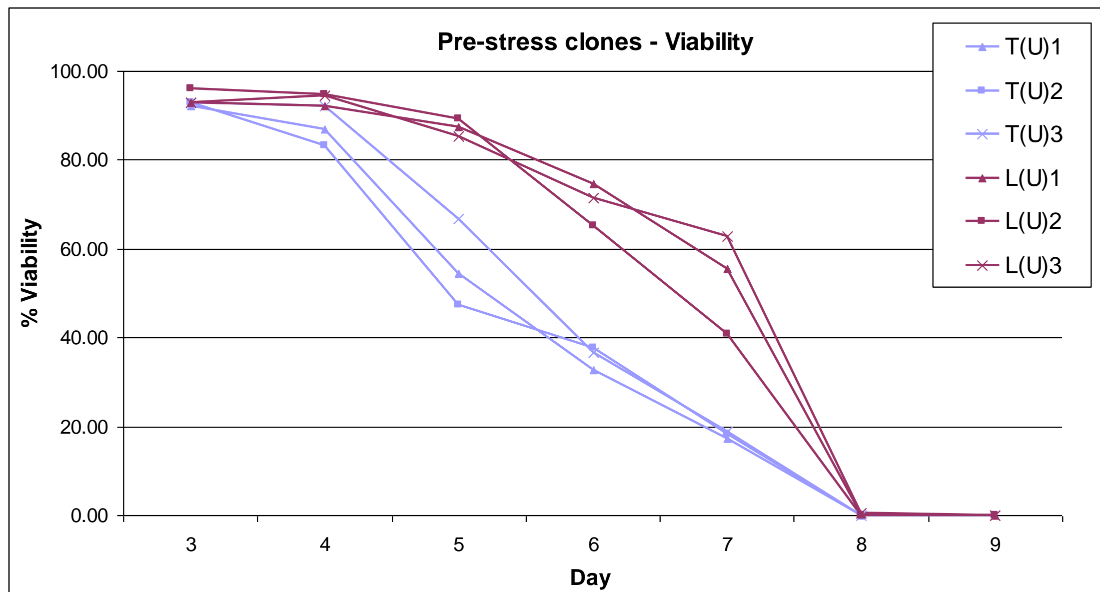

Figure 3.2.11.1.3: Percentage viability curves from individual pre-stress clones characterised in this study. Each cell line was grown in triplicate (3x1ml), with single samples from each replicate analysed daily. Unstressed TBP clones denoted [T(U)1-3] are marked in red, with unstressed Library clones [L(U)1-3] are marked in red to highlight the differences between treatments.

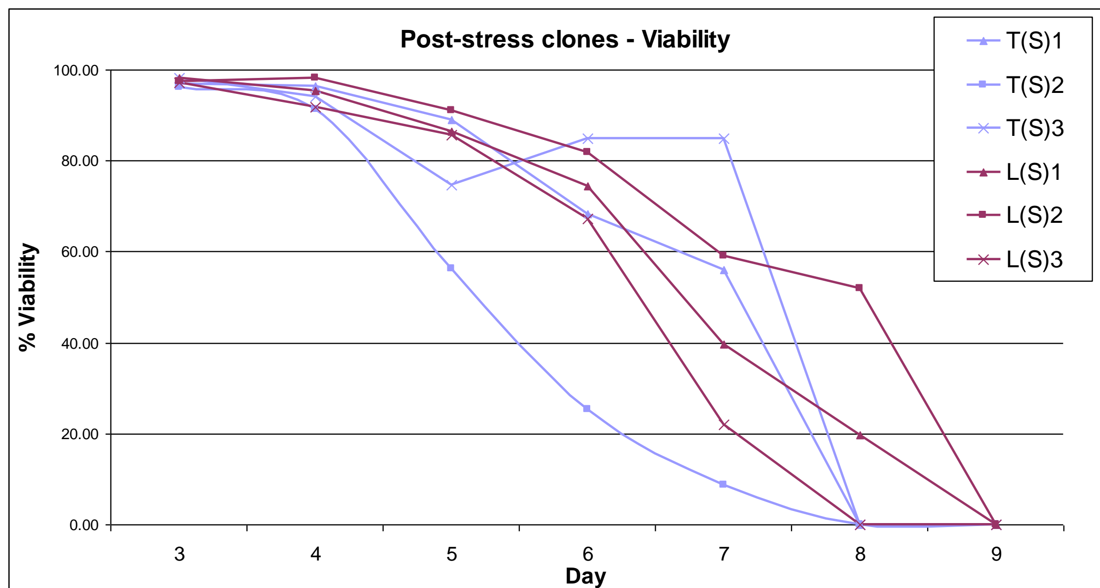

Figure 3.2.11.1.4: Percentage viability curves from individual post-stress clones characterised in this study. Each cell line was grown in triplicate (3x1ml), with single samples from each replicate analysed daily. Stressed TBP clones denoted [T(S)1-3] are marked in red, with stressed Library clones [L(S)1-3] are marked in red to highlight the differences between treatments.

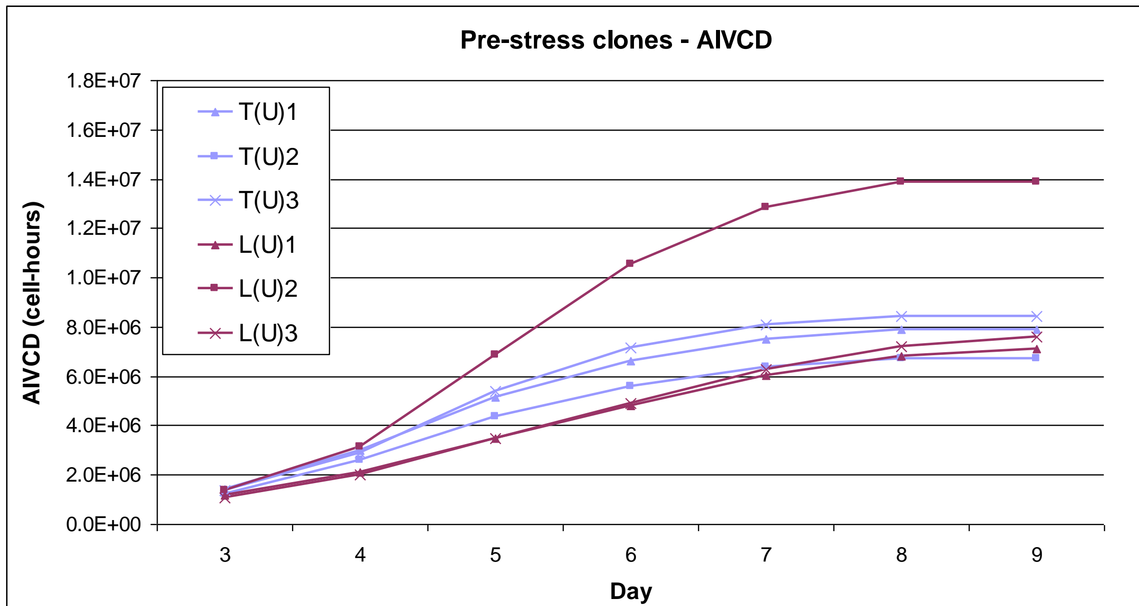

Figure 3.2.11.1.5: AIVCD results from individual pre-stress clones characterised in this study, an alternative method of analysis for the data presented in figure 3.2.11.1.1 represented as cumulative "cell-hours". Unstressed TBP clones [denoted T(U)1-3] are marked in red, with unstressed Library clones [L(U)1-3] are marked in red to highlight the differences between treatments.

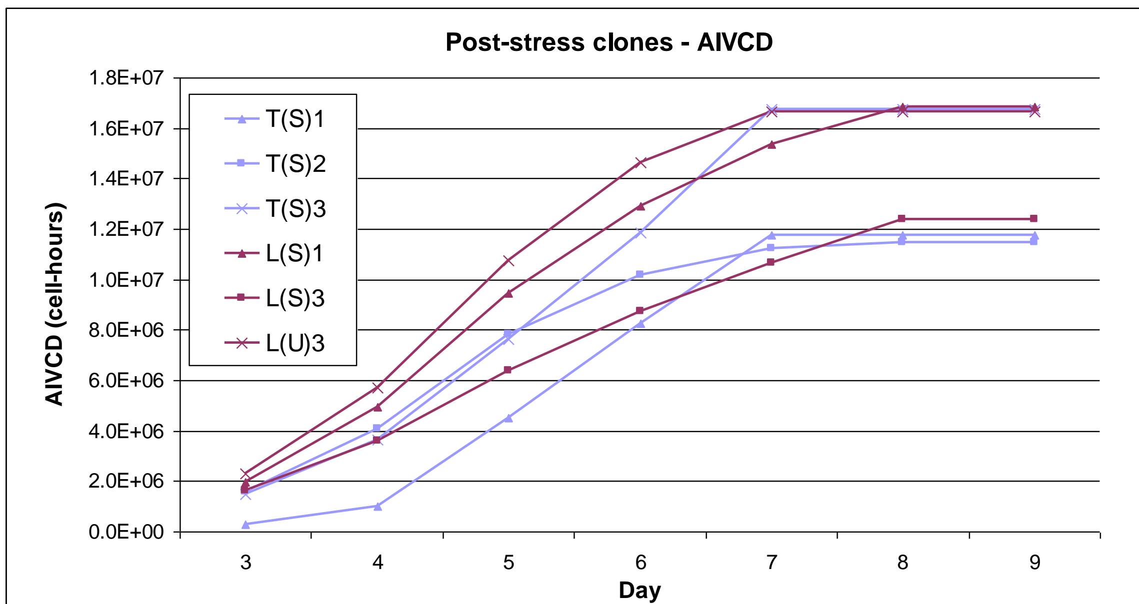

Figure 3.2.11.1.6: AIVCD results from individual pre-stress clones characterised in this study, an alternative method of analysis for the data presented in figure 3.2.11.1.2 represented as cumulative "cell-hours". Stressed TBP clones [denoted T(S)1-3] are marked in red, with stressed Library clones [L(S)1-3] are marked in red to highlight the differences between treatments.

3.2.11.2 Direct comparison discussion

The figures detailed above describe the viable cell densities, percentage viabilities and AIVCD values for individual clones as measured over the course of this study. While previous clonal results were usually grouped and discussed with respect to the average performance of a particular panel of clones, the reduced sample sizes of these groups and relative variation in performances for these clones make average comparisons difficult to interpret over the course of the majority of the graphs generated, and as such are displayed individually.

Looking initially at the performance of the unstressed TBP group, we see a relatively homogenous set of results from the 3 clones analysed. Maximum cell density peaks on day 4 at ~2.4×10^6 viable cells per ml, with viability steadily decreasing at this point till complete death on day 8. This homogeneity is reflected in their final day AIVCD, with the 3 clones clustering tightly around an average of 7.7×10^6 viable cell hours per ml.

As noted in the post-stress clone analysis carried out previously, the stress procedure appears to increase variability in the TBP transfected clones, and the 3 characterised in this instance provide 3 very different growth patterns; T(S)1 maintaining a steady pattern of growth and decline with a mid curve cell density peak between days 5 and 6, T(S)2 achieving a higher maximum viable cell density (4.8×10^6) on day 4 with a sharper decline to below 30% viability by day 6, and T(S)3 managing to achieve steady growth until a day 7 maximum above 5.2×10^6 viable cells on day 7, before a total crash in viability was measured 24 hours later. This peculiar growth pattern contributes to an impressive final AIVCD of 1.67×10^7, greater than double the average of their unstressed counterparts, while T(S)1 & 2 average ~1.16×10^6 viable cell hours per ml AIVCD.

Despite the heterogeneity introduced into the TBP transfected population by the stress procedure as noted in both examinations of post-stress clones, this stress does indeed appear to benefit the overall performance of these clones, with the average performance increased from 7.69×10^6 viable cell hours per ml (pre-stress) to 1.33×10^7 viable cell hours per ml (an increase of 73.3%).

An examination of the pre-stress Library transfected clones reveals two clones with almost identical behaviour (L(U)1&3), with another particularly potent clone (L(U)2). While 1&3 maintain a relatively flat curve once reaching a maximum of ~1.4×10^6 viable cells per ml, L(U)2 manages to reach over 4.3×10^6 on day 5. Each clone maintains a similar steady drop in viability until day 8, although this is a less pronounced decline than the wild type TBP pre-stress clones with all 3 sustaining viability >60% by day 6. This could be related to the comparatively smaller maximum cell density reached for L(U)1&3, with a reduction in initial nutrient depletion permitting a longer survival. However the surprising maximum density of L(U)2 suggests this clone has incorporated beneficial mutant TBP sequences allowing it to retain its later stage (days 5-7) viability percentages in combination with its high cell densities, and has likely been isolated by chance in this case in the absence of any selective pressure or selection.

The post-stress Library clones exhibit more uniform behaviour than the post-stress TBP clones, all reaching a maximum cell density on day 4 and steadily dropping in percentage viability and viable cell numbers until day 8 or 9. The rate of change in viability does not appear to be significantly different in post-stress Library clones compared to their pre-stress counterparts. However the drastic increases in cell densities seen through out the stressed library clone growth curves contribute to the greater final overall results gained from the clones analysed here (a 60.6% improvement in average day 9 AIVCD from 9.52×10^6 to 1.53×10^6 viable cell hours per ml).

The data generated here appears to confirm that the stress procedure has a positive effect on both TBP and Library transfected clones. This experiment was performed on a relatively small sample set of only 3 clones from each treatment. However, as these were the top performing clones from their original panels, any apparent improvements in performance when these groups are directly compared prove significant when considering the effect of both mutagenesis and stress as these clones represent the most successful fraction of each group and their respective limits of potential performance.

While the original stress was carried out to select for cells with resistance to apoptotic culture conditions, the comparative data generated here suggests increases in viable cell density rather than percentage viability to be more of a contributing factor to the overall improvements in performance noted previously. In this instance, no significant cell survival was demonstrated beyond day 9 in any cell line tested. However due to the average increased viable cell density in post-stress clones, the likelihood of an apoptotic environment and nutrient depletion affecting these clones at earlier time points may be suggestive of an actual resistance to such conditions. In any case, in a given industrial cell culture situation, an increased maximum cell density (reflected in useful AIVCD cell hours) is likely to be beneficial in generating a greater proportion of product and is ultimately a useful characteristic to improve upon.

Environmental nutrients are finite, imposing a limit to the level of growth and survival possible in a given batch culture set up (for example, no clone assayed in this instance manages to overcome a limit of 1×10^7 viable cell hours, while a number of successful clones converge on this limit). However, improvements to late stage culture viability could possibly be improved upon with the supplementation of additional nutrients. This would hopefully provide further noticeable performance gains in conjunction with the artificially engineered cell growth measured here, a theory that is explored in later experiments.

3.2.12 Scale-up phenotype investigation (selected clones)

Previous experimentation up until this point had largely been carried out to determine the differences between the various treatments used in this study, and the advantages in growth and viability gained from each. These differences were determined through screening investigations of clones isolated from each group, with the effect of each treatment (i.e. TBP/Library overexpression and the application of a selective stress) measured by looking at the average difference between these groups of clones. For practical reasons these comparison were generally carried out in small scale suspension cultures using 1ml multiwell plates to allow the comparison of larger numbers of clones. Experiments carried out on these clones suggested benefits to cellular phenotype after the application of a suitable selective stress, as well as a heightened ability to isolate advantageous clones from a population expressing mutant copies of a beneficial gene due to the artificial heterogeneity contained within.

Having isolated a number of seemingly advantageous clones with improved phenotypic growth and viability properties, this experiment was carried out in scaled up 50ml suspension cultures. While small scale cultures had proven useful for screening of larger numbers of individual clones, larger scale vessels were then used to ensure that these phenotypes could be replicated in environments more closely resembling those of large scale industrial reactors in which such cell lines might prove useful, as well as reducing some of the problems associated with multiwell plates such as evaporation.
This comparison was carried out using the three best performing TBP and Library clones (used in the previous comparison to evaluate the effect of the stress procedure). As a controlled comparison, two common in-house cell lines were used; an untransfected CHO-K1 line derived from the original parental cells used in this study (representing basal cell line performance), as well as a SEAP expressing cell line shown to proliferate rapidly to high densities (CHO-SEAP, representing a known high performing cell line).

3.2.12.1 Scale-up investigation (selected clones) results

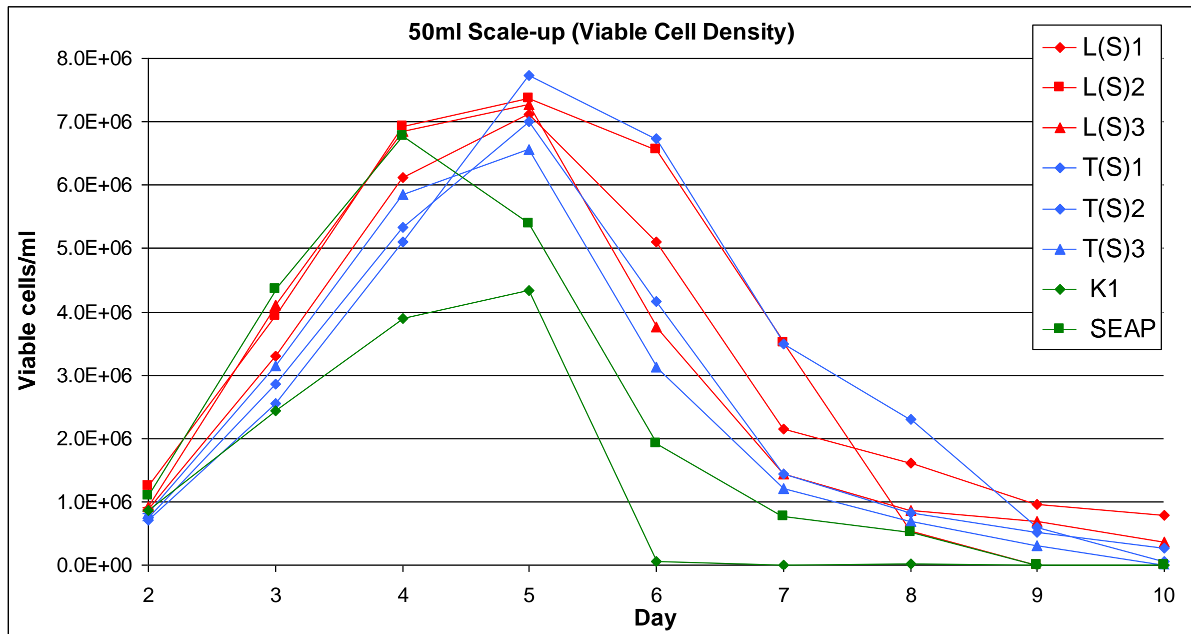

Figure 3.2.12.1.1: Viable cell densities of the 6 isolated advantageous clones over a 10 day batch culture in 50ml volume seeded at $2x10^5$ viable cells per ml. Also included are 2 control CHO cell lines (K1 and SEAP). Cell lines were seeded in individual 50ml suspension culture flasks, with triplicate samples taken daily for analysis.

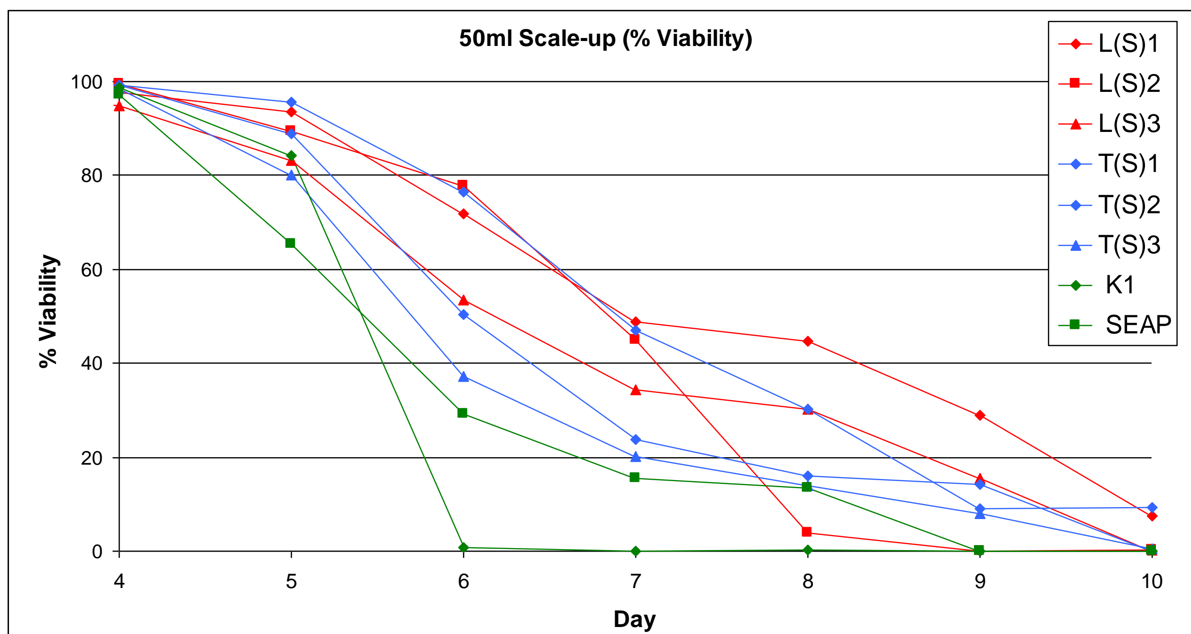

Figure 3.2.12.1.2: Percentage viability of the 6 isolated advantageous clones over a 10 day batch culture in 50ml volume seeded at $2x10^5$ viable cells per ml. Also included are 2 control CHO cell lines (K1 and SEAP). Cell lines were seeded in individual 50ml suspension culture flasks, with triplicate samples taken daily for analysis.

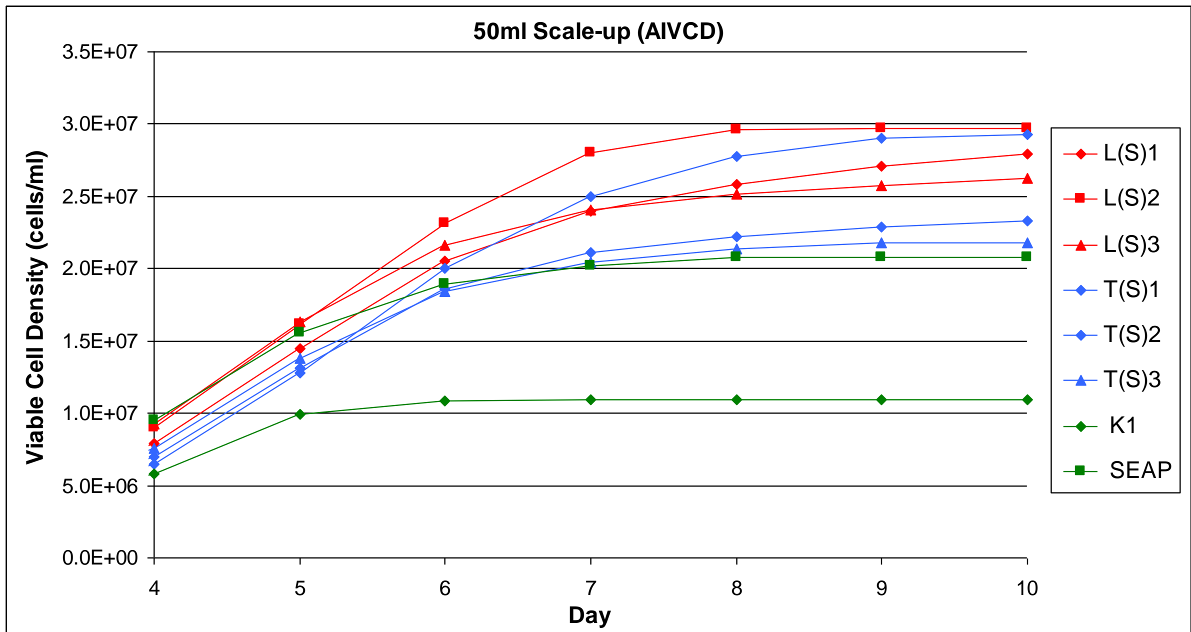

Figure 3.2.12.1.3: AIVCD analysis of the data generated by the 6 isolated advantageous clones. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml volume and measured over a 10 day batch culture. Also included are 2 comparative control CHO cell lines (K1 and SEAP).

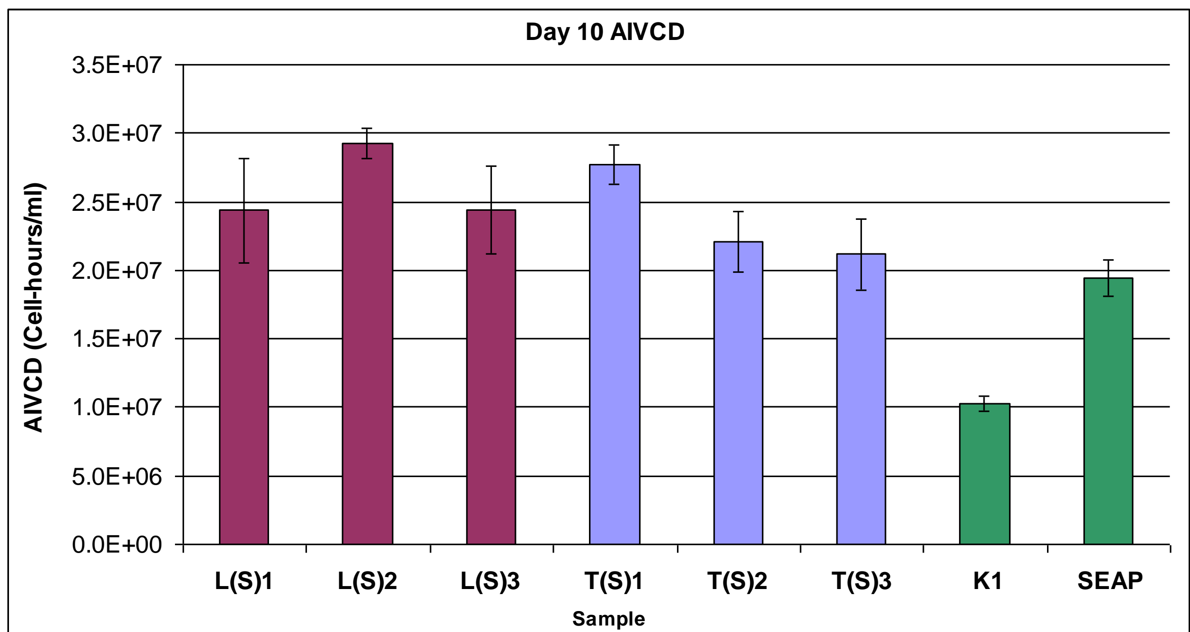

Figure 3.2.12.1.4: Day 10 AIVCD data of the 6 isolated advantageous clones and 2 comparative control cell lines. To ensure reproducibility, this experiment was also repeated in both flask (50ml working volume) and triplicate suspension culture tube (5ml working volume) growth formats. Error bars in this figure represent standard deviation measured between all replicate experiments carried out in both formats.

3.2.12.2 Scale-up investigation (selected clones) discussion

This data illustrated in section 3.2.12.1 details the high growth potential of all the advantageous TBP and Library clones isolated in this study.
All clones manage to reach peak densities >7 x 10^6 viable cells per ml on day 5 (with the exception of T(S)3, measuring 6.56 x 10^6 viable cells per ml peak density). These results compares favourably to CHO-SEAP with a 6.77 x 10^6 viable cell maximum, considering this is one of the most proliferative cell lines commonly used in this institute. This also marks a 65% average increase in maximum density over the parental K1 cell line originally used to generate the transfected cell lines used in this study.

Despite the high concentrations of cells reached by day 5, viability was maintained at higher levels in most experimental cell lines than both controls for the majority of culture. CHO-SEAP dropped to below 30% viability by day 6, with experimental clones averaging above 60% at this time point. K1 viability dropped precipitously after day 5 to complete death.

These heightened levels of both cell density and viability allowed all experimental clones to outperform both controls in terms of day 10 AIVCD, with selected Library and TBP clones exhibiting an average of 2.48 x 10^7 and 2.80 x 10^7 viable cell hours per ml respectively. This relates to an average of 33.9% higher AIVCD for Library clones and 21.7% for TBP clones when compared to the high density SEAP cells.

To ensure reproducibility, this experiment was also repeated in both flask (50ml working volume) and triplicate suspension culture tube (5ml working volume) growth formats. Similar results were returned in these experiments, and the data presented here in figures 3.2.12.1.1-3.2.12.1.3 displaying the results generated from the first comparative experiment is representative of the true phenotypic behaviour of these cell lines.

3.2.13 Scale-up phenotype investigation (mixed populations)

While the data from the previous section details the results gained from the selected clones isolated from mixed populations, this scale-up experiment was also repeated on TBP and pcDNA stressed and unstressed mixed populations.
This was originally carried out to provide cell samples from which RNA could be extracted for future profiling experiments, however the results prove useful in their own right by demonstrating the growth patterns of these mixed populations as well as indicating the relative effects of the stress procedure and TBP overexpression in comparison to the control cell lines K1 and SEAP.

In the resulting data, unstressed populations are denoted pcDNA-U and TBP-U, while stressed populations are denoted pcDNA-S and TBP-S.

3.2.13.1 Scale-up investigation (mixed populations) results

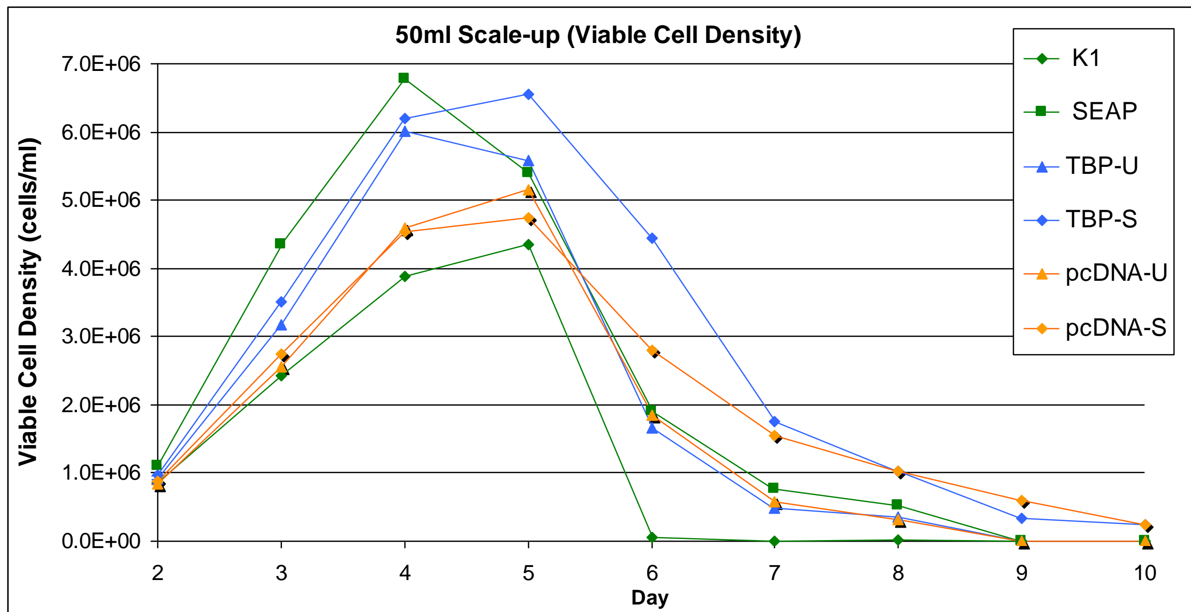

Figure 3.2.13.1.1: Viable cell densities of the pre-/post-stress TBP and pcDNA mixed populations in 50ml volume seeded at $2x10^5$ viable cells per ml. Also included are 2 control CHO cell lines (K1 and SEAP). Cell lines were seeded in individual 50ml suspension culture flasks, with triplicate samples taken daily for analysis.

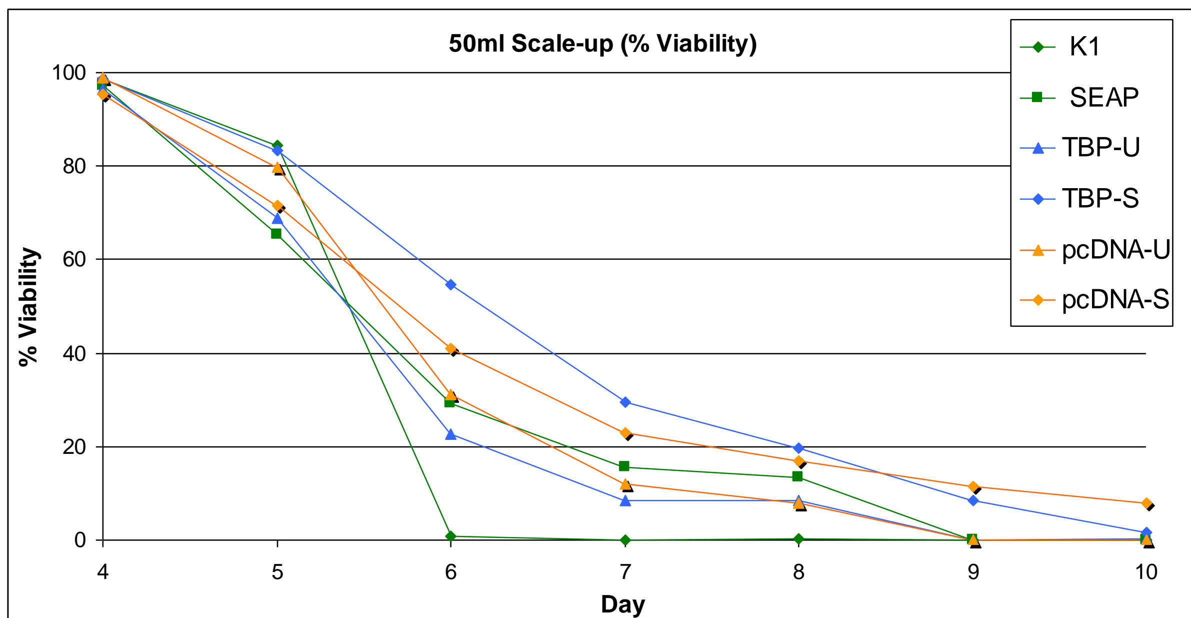

Figure 3.2.13.1.2: Percentage viability of the pre-/post-stress TBP and pcDNA mixed populations in 50ml volume seeded at $2x10^5$ viable cells per ml. Also included are 2 control CHO cell lines (K1 and SEAP). Cell lines were seeded in individual 50ml suspension culture flasks, with triplicate samples taken daily for analysis.

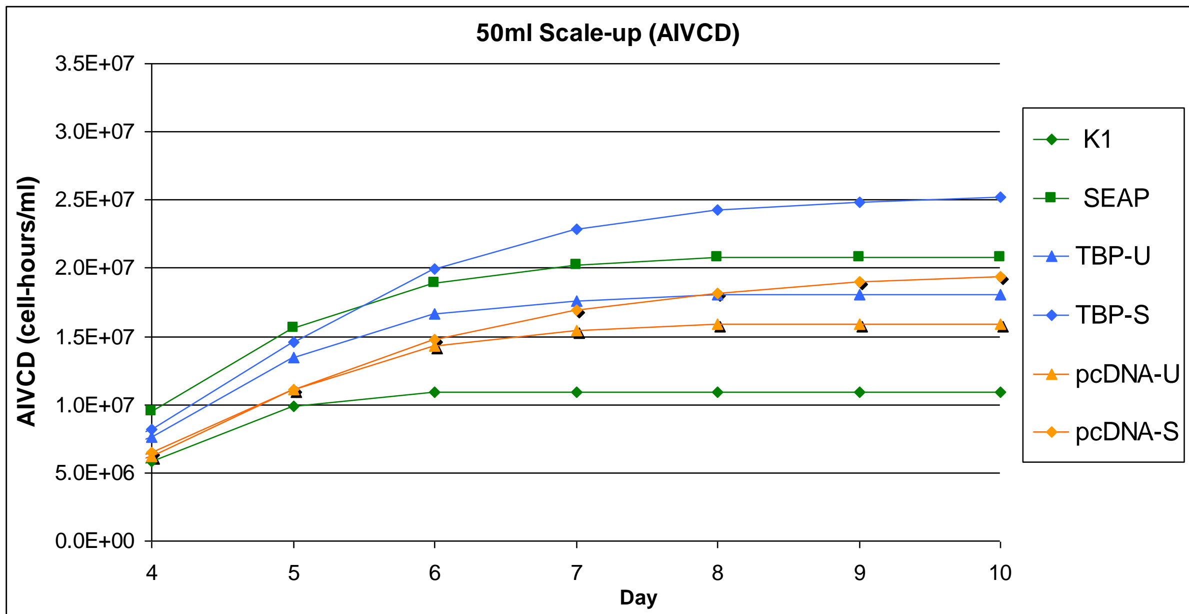

Figure 3.2.13.1.3: AIVCD analysis of the data generated by pre-/post-stress TBP and pcDNA mixed populations. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml volume and measured over a 10 day batch culture. Also included are 2 comparative control CHO cell lines (K1 and SEAP).

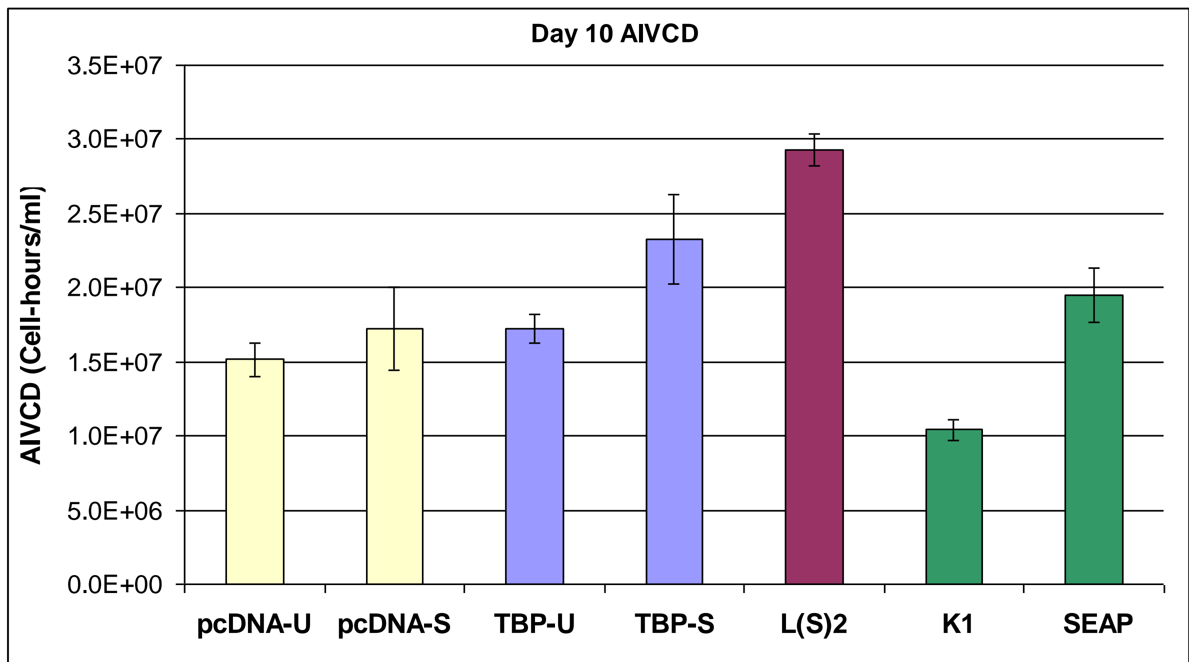

Figure 3.2.13.1.4: Day 10 AIVCD data of the pre- and post-stress TBP and pcDNA mixed populations. Also included are the comparative control cell lines K1 and SEAP, as well as the performance of the highest performing clone (L(S)2) as measured in the previous set of results. Error bars represent the standard deviation of results between replicate experiments (carried out in both flask and tube formats).

3.2.13.2 *Scale-up investigation (mixed populations) discussion*

The results gained from the mixed population growth curves are generally indicative of growth and viability patterns confirmed in previous clonal screen studies, but serve as a useful confirmation of the differences between treatments. Library transfected mixed populations were originally ruled out of any potential profiling experiment due to the likelihood of a noisy heterogeneous gene profile mixture, allowing us to focus on clones with proven advantageous phenotypes. As such, these samples were omitted from this study, whose original purpose was to provide samples for such a profiling experiment.

Once again we see the benefit to growth potential of TBP overexpression compared to the empty vector control, with a 16.8% increase in maximum cell density between unstressed empty vector and TBP transfected populations, and a 38.2% increase between post-stress empty vector and TBP populations. These percentages are mirrored in the improvements for final day AIVCD, with TBP providing a 13.8% improvement (pre-stress) and a 30.2% improvement (post-stress) when compared to the empty vector control.

In this instance the stress procedure appears to have had the least effect on empty vector control populations compared to its experimental gene-expressing counterparts. Post-stress pcDNA populations exhibit a benefit in viability in the later stages of culture, contributing to an increased AIVCD despite a slightly reduced maximum cell density. However, without the increased growth potential granted by TBP overexpression, the benefit to post-stress AIVCD is less pronounced. This result may also be contributed to by the lack of variation in gene expression in these cells, a factor demonstrated in this study as being advantageous for directed evolution by TBP and Library cells.

3.2.14 Investigation of selected clone productivity

The data generated to this point has demonstrated the advantages gained from stressed Library and TBP cultures with respect to growth and viability, and the positive impact this treatment has in terms of overall viable cell time (AIVCD). However, such gains would ultimately be without merit if these clones were aversely affected in terms of their ability to produce and secret product, a key characteristic of any cell line utilised in a productive industrial cell culture environment.

A series of experiments were carried out to investigate whether the genetic engineering and directed evolution techniques applied have affected these cells' ability to remain productive. A series of transient transfections were carried out using plasmids encoding a variety of products commonly expressed in industrial mammalian cell lines. These products included;

- *SEAP (Secreted Alkaline Phosphatase)*

A commonly used reporter gene used to estimate productivity levels, measured in cellular supernatant using a standard colorimetric assay

- *EPO (Erythropoietin)*

Recombinant form of human EPO, a well known biopharmaceutical product. Intracellular levels of this protein were measured using a western blot.

- *IgG (Immunoglobulin)*

A recombinant mouse IgG, another common industrial product. Secreted levels were estimated using an ELISA assay on a sample of supernatant.

In each case, 1 x 10^6 suspension cells were transfected with 2.5µg plasmid DNA in 2.5mls serum free medium in triplicate. This was done using 7.5µl TransIT-2020 Transfection Reagent (Mirus, cat. MIR 5400) and carried out in accordance with the manufacturers instructions. Transfection efficiency was estimated using a separate GFP plasmid transfection, with the percentage of GFP positive cells measured after 24 hours. Due to the high initial density of transfected cells, these samples were diluted in fresh medium 48 hours after transfection.

3.2.14.1 SEAP productivity results

Cell Line	L(S)1	L(S)2	L(S)3	T(S)1	T(S)2	T(S)3
% GFP	66.4	61.6	70.0	60.5	54.4	51.5

Figure 3.2.14.1.1: Percentage of GFP positive cells in each cell line after transfection. One separate control sample of cells from each cell line was transfected with a plasmid expressing GFP in parallel to the experimental samples. GFP positive cells (indicating transfection efficiency) were then measured after 24 hours.

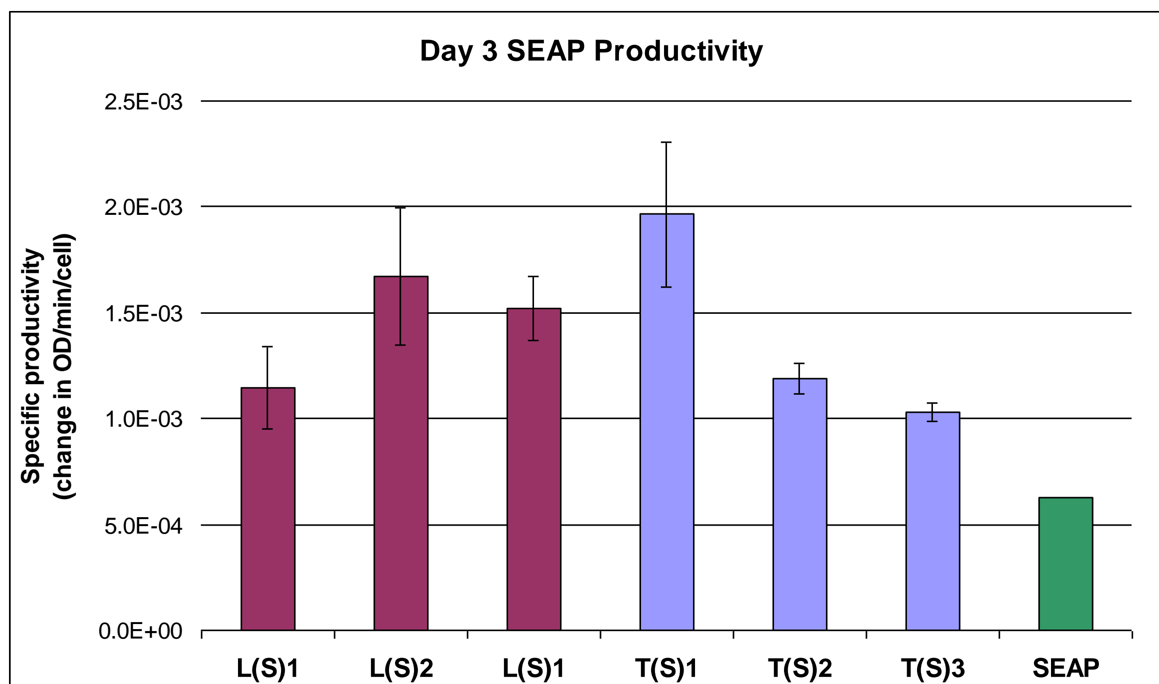

Figure 3.2.14.1.2: Specific productivity of each cell line as measured on day 3. SEAP productivity was estimated via kinetic assay in which the rate of change of SEAP product is measured. Units are reported as [change in OD/min], or mean velocity. Triplicate tubes were transfected, with technical triplicate samples from each tube analysed via SEAP assay (error bars represent the resulting standard deviation). A day 3 sample was also removed from a standard culture of CHO-SEAP (constitutively expressing SEAP), and the result included here as a reference.

3.2.14.2 EPO productivity results

Cell Line	L(S)1	L(S)2	L(S)3	T(S)1	T(S)2	T(S)3	K1
% GFP	49.7	57.1	53.9	41.4	45.0	44.5	53.9

Figure 3.2.14.2.1: Percentage of GFP positive cells in each cell line after transfection. One separate control sample of cells from each cell line was transfected with a plasmid expressing GFP in parallel to the experimental samples. GFP positive cells (indicating transfection efficiency) were then measured after 24 hours. A parental K1 control was also included in this experiment alongside the 6 selected clones.

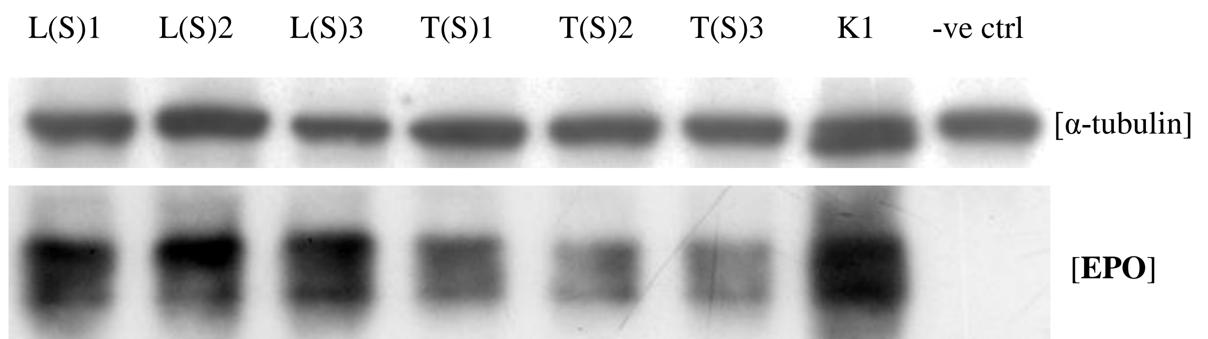

Figure 3.2.14.2.2: Western blot of intracellular EPO isolated from transfected TBP and Library clones alongside an EPO transfected K1 control. 10µg protein isolated from cell pellets was added per well, as measured by a Bradford assay. The α-tubulin housekeeping gene was used as a loading control.

3.2.14.3 IgG productivity results

Cell Line	L(S)1	L(S)2	L(S)3	T(S)1	T(S)2	T(S)3	K1
% GFP	57.1	59.8	62.3	47.6	43.1	45.3	55.4

Figure 3.2.14.3.1: Percentage of GFP positive cells in each cell line after transfection. One separate control sample of cells from each cell line was transfected with a plasmid expressing GFP in parallel to the experimental samples. GFP positive cells (indicating transfection efficiency) were then measured after 24 hours. A parental K1 control was also included in this experiment alongside the 6 selected clones.

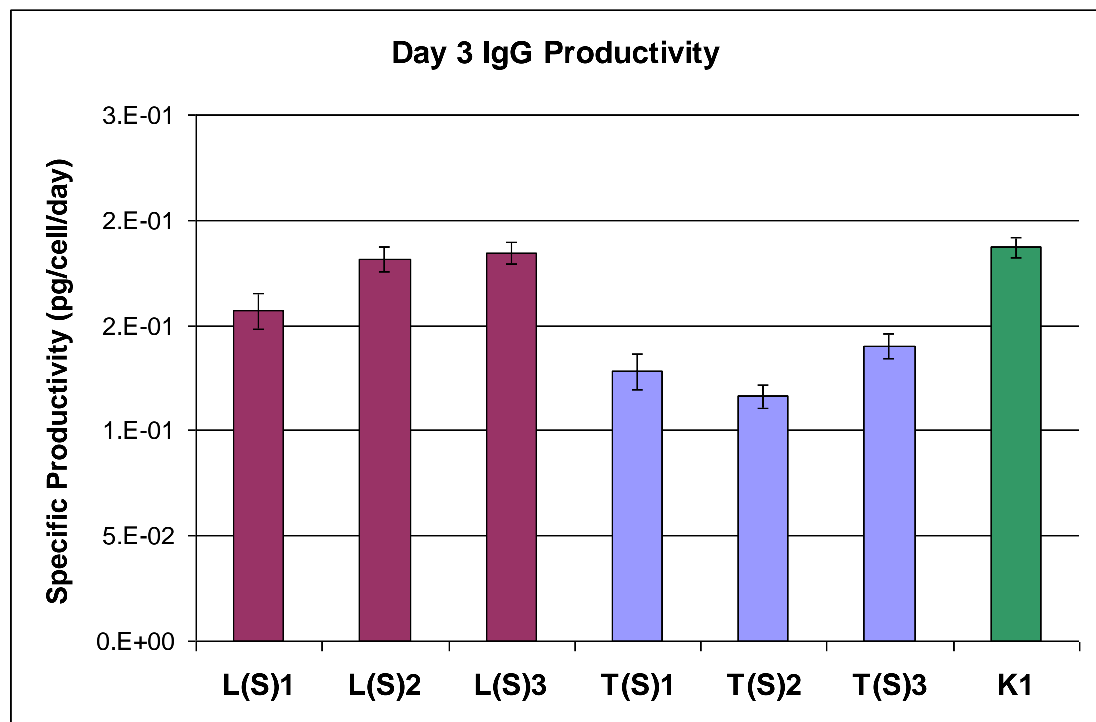

Figure 3.2.14.3.2: Specific productivity of each IgG transfected cell line as measured on day 3. IgG productivity was estimated via ELISA assay (see 2.7.3). Triplicate tubes were transfected, with technical triplicate samples from each tube assayed for IgG content (error bars represent the resulting standard deviation between samples).

3.2.14.4 Discussion of productivity results

Prior to the use of actual product encoding plasmids, a number of optimisation experiments were first carried out to determine the ideal concentrations of DNA/plasmid/cell numbers etc. to maximise transfection efficiency without adversely affecting cell viability. Experience has shown that some cell lines are naturally more resistant to transfection, demonstrated by the differences in transfection efficiency of some cell lines commonly used in-house and indeed by the differences noted in this study. The conditions described above were found to produce the highest percentage of GFP fluorescent cells without drastically impacting cell growth and viability after transfection, however the 3 selected Library clones exhibit generally higher average rates of efficiency under these conditions.

The data generated from the SEAP transfection assay indicates some level of variation in productivity between clones, with almost a 2-fold difference in productivity between the highest and lowest producer. There appears to be little appreciable difference between Library and TBP clones, with 1.45×10^{-3} and 1.39×10^{-3} average changes in OD/min/cell respectively. While only a small number of clones from both Library and TBP populations are investigated in this instance, we cannot determine with any confidence whether either treatment has had a significant impact on productivity. However, no clone appears to exhibit any particular difficulty in producing and secreting SEAP, with variation likely to be a result of clonal differences (Pilbrough, Munro and Gray 2009) and factors such as transfection efficiency. The specific productivity of the CHO-SEAP sample included in this experiment does not appear as high, however this is expected considering the effect of high levels of transfected plasmid DNA producing transiently compared to a stable constitutive producer.

The EPO transient transfection produces results also indicating successful EPO productivity in all samples tested, including a parental CHO-K1 transfected population in this instance. While there is a measure of variability between samples (for instance, T(S)1-3 appear to have fainter EPO concentrations than the Library and K1 cell lines), once again this may be influenced by clonal variation and transfection efficiencies. We can infer from this result that the ability of all clones to produce an industrially relevant product (in this case an un-secreted recombinant product) has not been compromised by the genetic manipulation and directed evolution employed in this study, as evidenced by their similarity to the K1 control sample.

The IgG transient transfection provides a similar result to previous transfections. Productivity compared to a wild type parental control does not appear to be significantly diminished. While variation in selected clones is present (including an apparent reduction in productivity in TBP selected clones) variation up and down appears to correlate with transfection efficiencies suggesting that this may be the cause of differences in overall IgG secretion.

Without further in-depth analysis of a larger number of clones, we cannot conclude with certainty the relative impacts on productivity the TBP and Library transfections may have had in conjunction with the stress procedure. However, the data presented above does indicate that this impact is minimal (either positive or negative), with little observable difference between either treatments except with regard to transfection efficiencies, or when compared to a parental K1 control.

An industrial processes' total output is governed by both specific productivity (per cell) and volumetric productivity (per unit volume). While a simultaneous improvement in specific productivity would be ideal, the techniques applied in this study were designed to elicit improvements in growth and viability. Ultimately the valuable phenotypic improvements demonstrated by the selected clones isolated in this study do not appear to compromise the cells' ability to produce a target product, and in most given industrial batch or fed batch processes this would in theory contribute to a greater total output of target product.

3.2.15 Nutrient feed and temperature shift

Temperature shift is an established method of increasing overall productivity. After an initial growth period, a reduction in temperature causes a decrease in cell cycle activity. This channels cellular resources towards generation of the target product, leading to an increase in specific productivity as well as an associated reduction in waste products (Kaufmann et al. 1999).

In order to investigate whether this technique could be applied to the clones isolated in this study to further prolong their productive culture time, a number of experiments were carried out in which temperature was shifted from 37°C to 31°C following an initial growth phase (typically after 72 hours of growth). Cells were also re-fed at selected time points in order to simulate a typical 'fed batch' culture. Initially this was crudely accomplished by replacing the total growth medium with fresh medium, however in subsequent iterations of this experiment a concentrated nutrient feed was used to provide more realistic results (Gibco CHO CD EfficientFeed A, cat. A10234-01).

Cells were seeded in 50ml shake flasks at 2 x 10^5 cells as usual, and temperature shifted to 31°C on day 3 (72 hours). Feeding regimens varied, with an initial experiment fully replacing the medium once on day 3. Further experiments were carried out to maximise late stage cell survival, in which a concentrated nutrient feed was used instead at various time points.

Batch-1:

Batch-1 was carried out initially to determine if viable cell densities could be preserved beyond the limits of a standard culture using temperature shifted method. A pair of 50ml shake flasks were seeded and grown for each selected Library and TBP cell line, with one remaining at 37°C for the entire culture and its 'twin' temperature-shifted to 31°C at day 3 after exponential growth had begun.

Prior to the use of a concentrated nutrient feed, a cruder system was used in this instance in which the entire serum free growth medium was replaced with fresh medium. This was only done after an initial phase of growth (day 3).

Batch-2:

Once the effect of temperature shift was established in the previous experiment, a commercial feed medium, supplemented at 10% of the total volume at each feed, was substituted for entire medium replacement. This was carried out to allow us to deduce if this more realistic method of fed-batch culture would provide similar results when applied to temperature shifted cells. In this experiment, each cell line was seeded in 50mls, and temperature shifted at day 3. Feeding time points at day 3 (after exponential growth) and day 6 (maximum density) were used. A parental CHO-K1 and high density SEAP control were also included in this instance.

Batch-3:

Having established the effects of both the temperature shift and a minimal number of re-feeds, a third experiment was carried out using a larger number of feed time-points to see if viable cells numbers could be sustained for a further length of time. This included a day 0 supplementation of feed medium (in accordance with the manufacturers recommendations, 10% of total volume), as well as subsequent days 3, 5, 8 and 11 supplementations of 10% additional volume each of feed medium.

3.2.15.1 Nutrient feed and temperature shift results

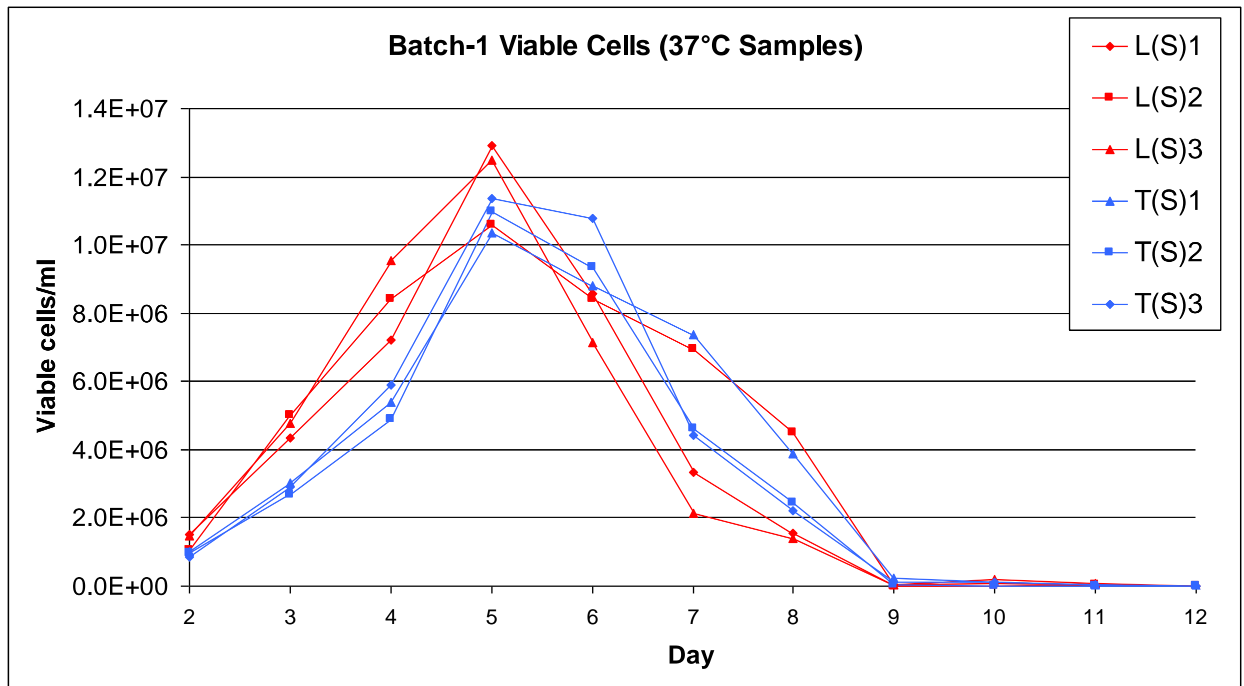

Figure 3.2.15.1.1: Viable cell densities of Library and TBP clones from experiment 'Batch-1', grown persistently at 37°C. Cell lines were seeded at 2×10^5 cells per ml in a 50ml culture, with all cells pelleted and resuspended in fresh medium on day 3.

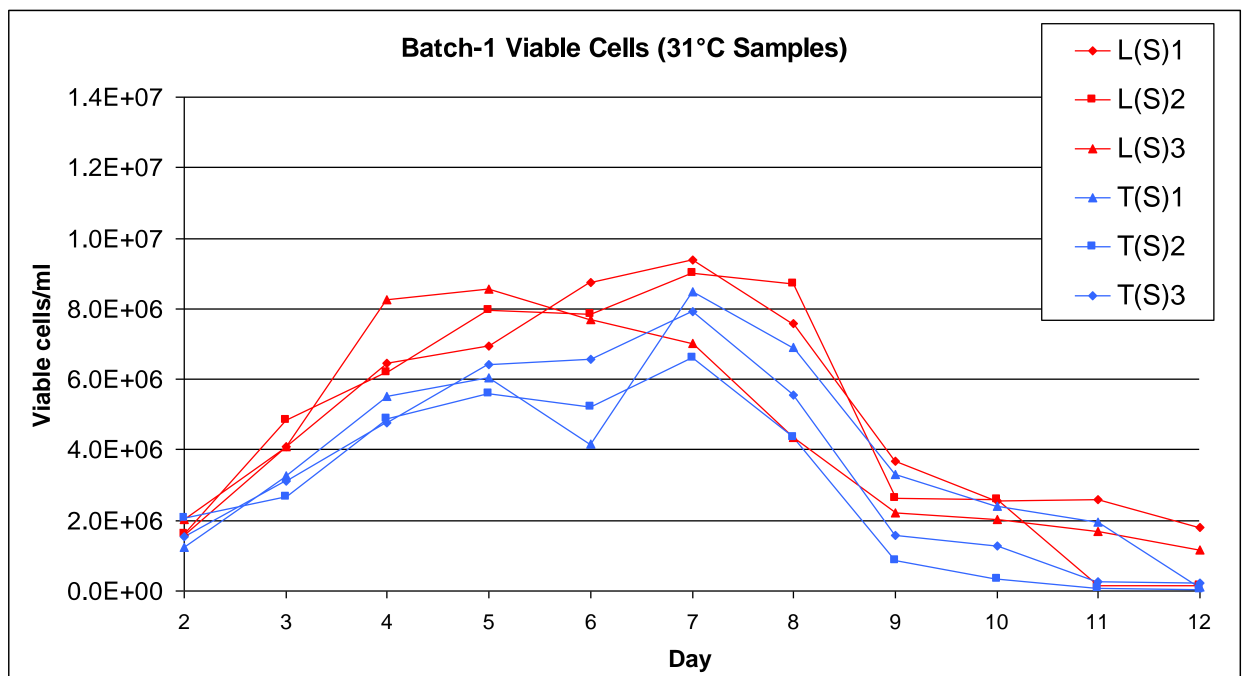

Figure 3.2.15.1.2: Viable cell densities of Library and TBP clones from experiment 'Batch-1', temperature shifted at day 3 to 31°C. Cell lines were seeded at 2×10^5 cells per ml in 50ml culture, with all cells pelleted and resuspended in fresh medium on day 3.

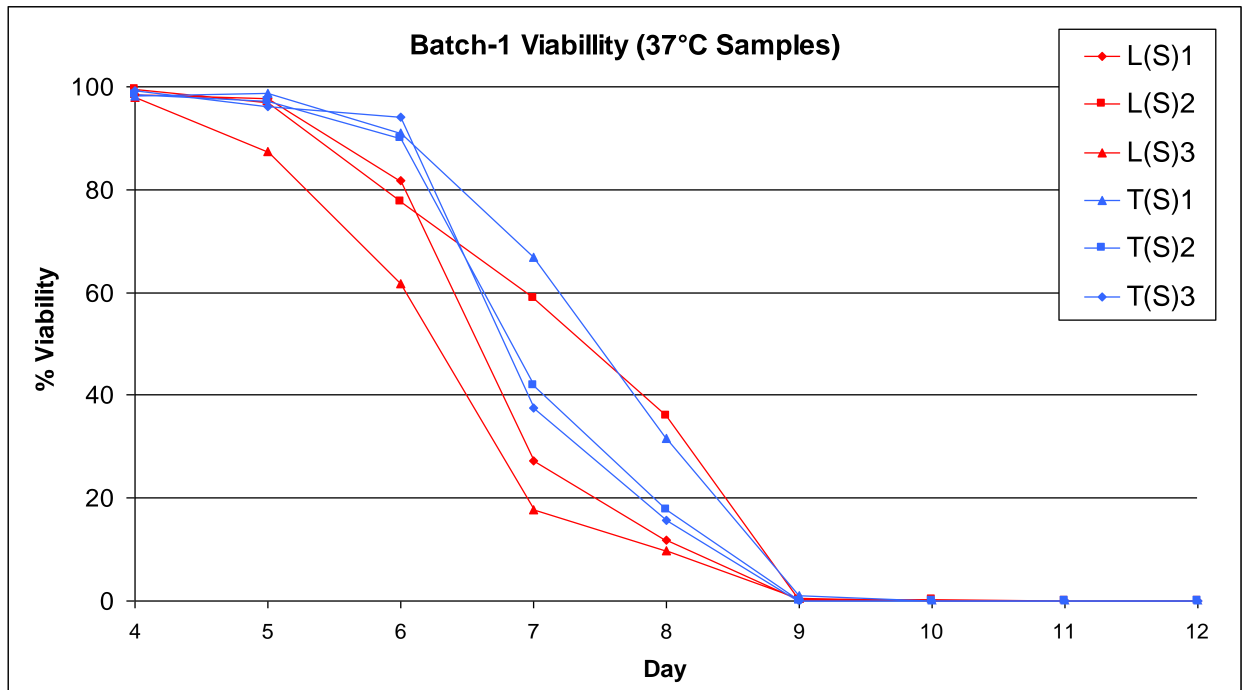

Figure 3.2.15.1.3: Percentage of viable cells of Library and TBP clones from experiment 'Batch-1', grown persistently at 37°C. Cell lines were seeded at $2x10^5$ viable cells per ml in a 50ml culture, with all cells pelleted and resuspended in fresh medium on day 3.

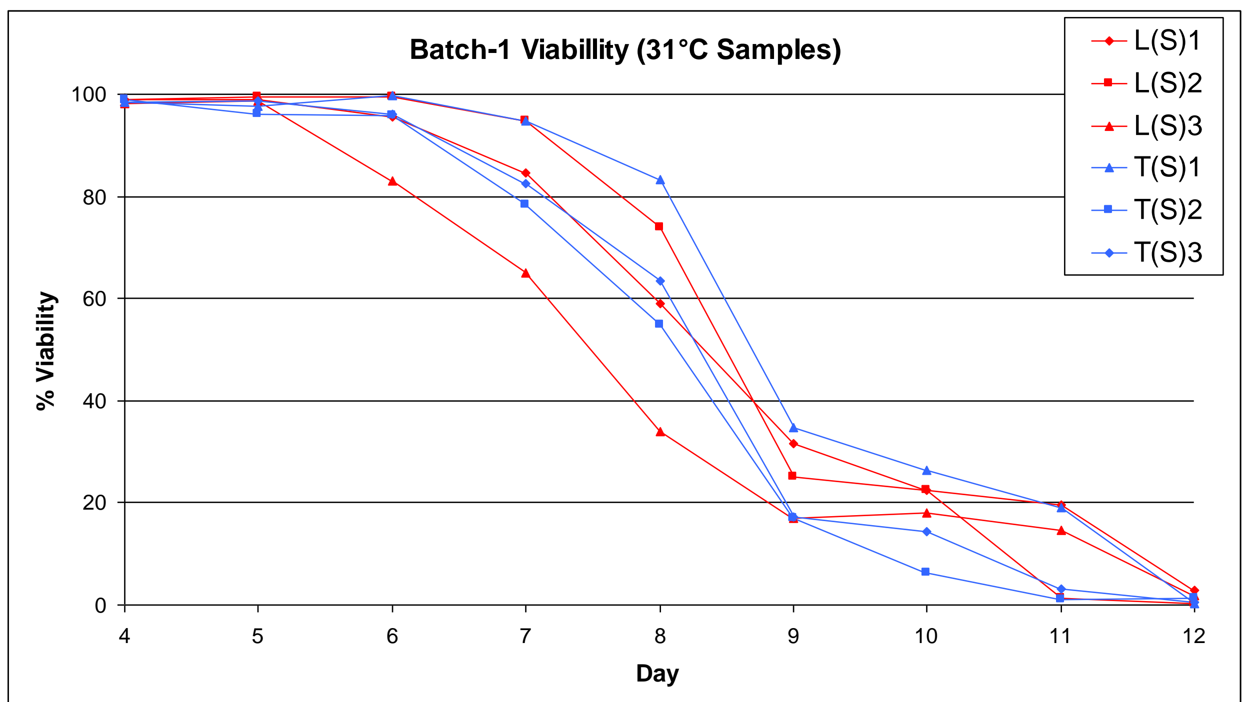

Figure 3.2.15.1.4: Percentage of viable cells of Library and TBP clones from experiment 'Batch-1', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in a 50ml culture, with all cells pelleted and resuspended in fresh medium on day 3.

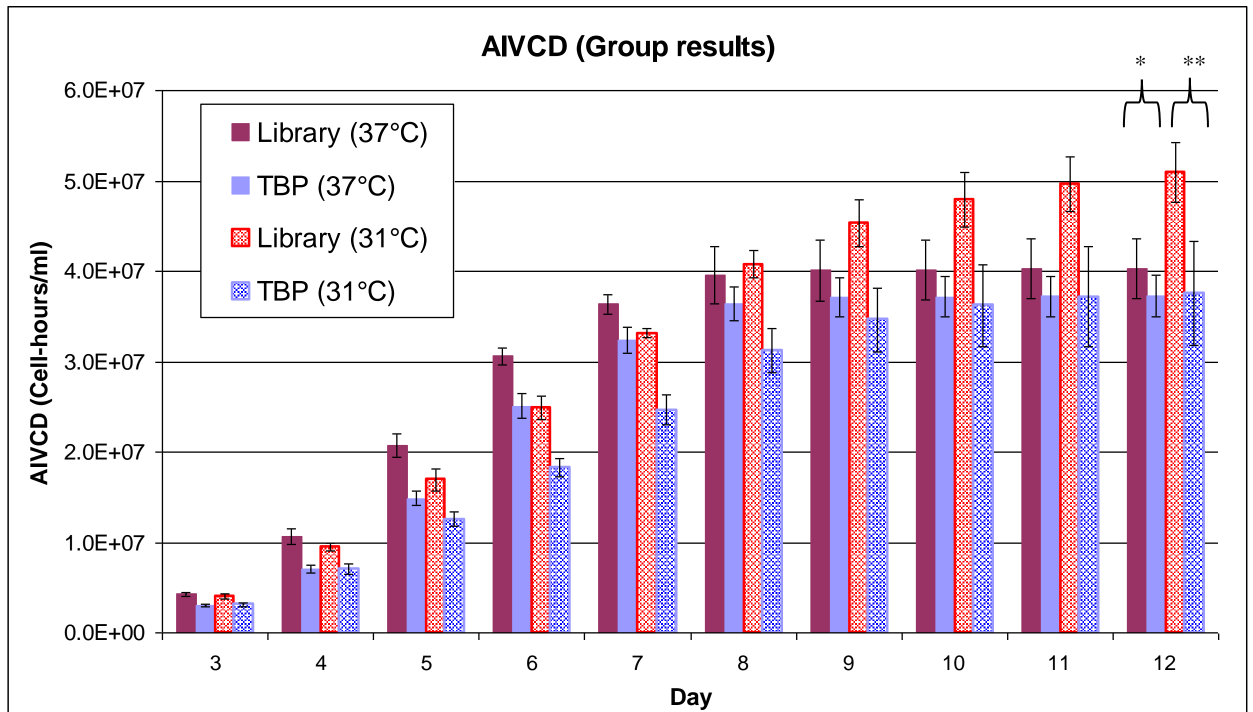

Figure 3.2.15.1.5: AIVCD analysis of the data generated from the 'Batch-1' experiment. Each bar represents the average accumulated daily AIVCD figure for each group of 3 clones (Library and TBP clones at both temperatures). With the addition of fresh nutrients in all cultures, performance between TBP and Library clones is minimal (*p=0.26), while the same conditions under temperature shift provide a more distinguished improvement to Library clone performance when compared to TBP (**p=0.04).

Final Day AIVCD (cell-hours/ml)

Clone	37°C	31°C	**% Difference**
L(S)1	3.85×10^7	5.43×10^7	**41%**
L(S)2	4.41×10^7	5.07×10^7	**15%**
L(S)3	3.84×10^7	4.77×10^7	**24%**
T(S)1	3.92×10^7	4.28×10^7	**9%**
T(S)2	3.48×10^7	3.13×10^7	**-10%**
T(S)3	3.77×10^7	3.84×10^7	**2%**

Figure 3.2.15.1.6: Final day AIVCD figures for each selected clone for both 37°C and 31°C cultures, and the percentage difference between them.

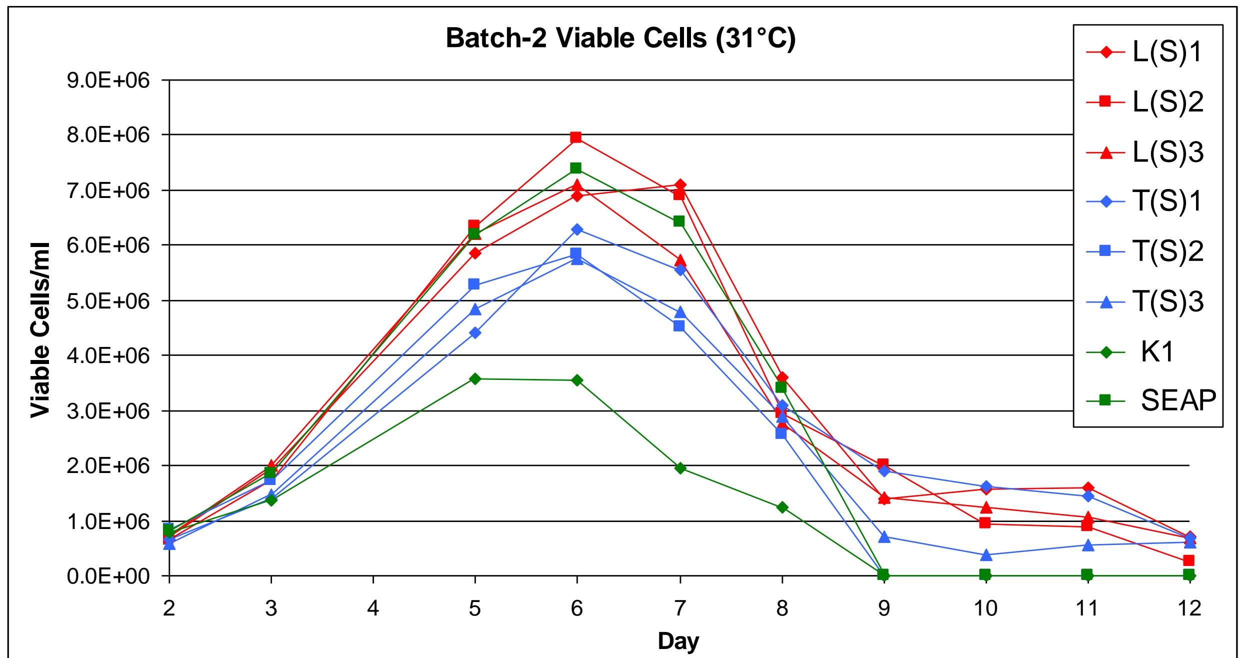

Figure 3.2.15.1.7: Viable cell densities of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-2', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 3 and 6 (10% of total volume).

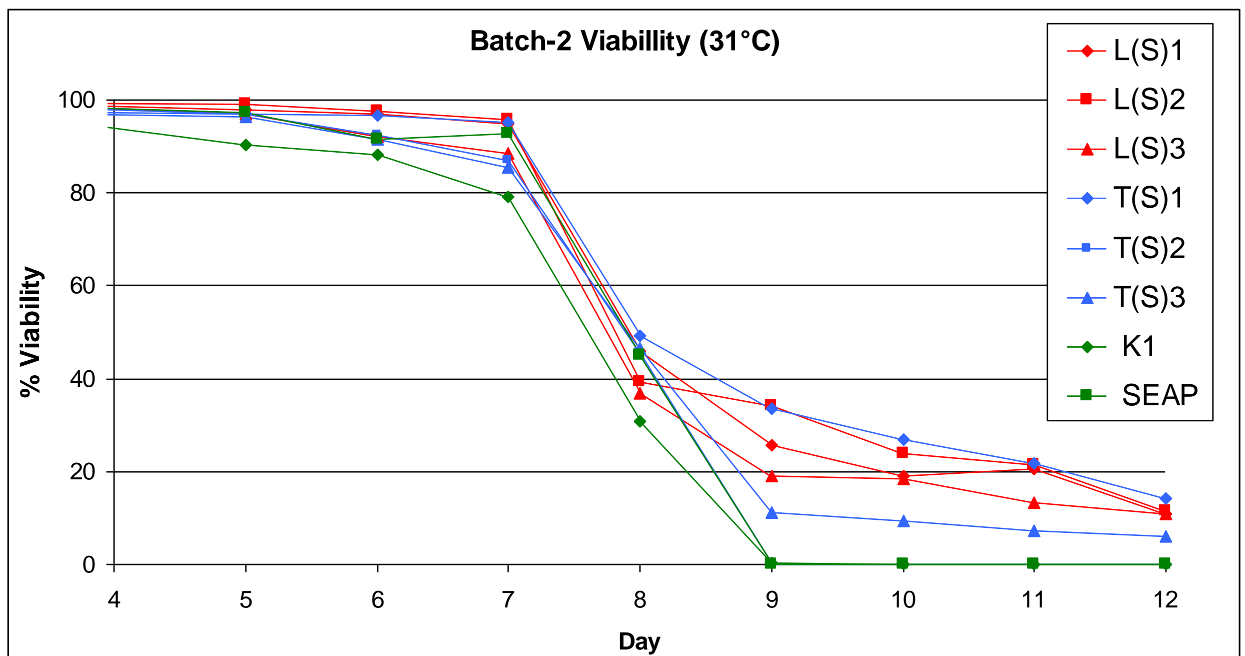

Figure 3.2.15.1.8: Percentage of viable cells of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-2', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 3 and 6 (10% of total volume).

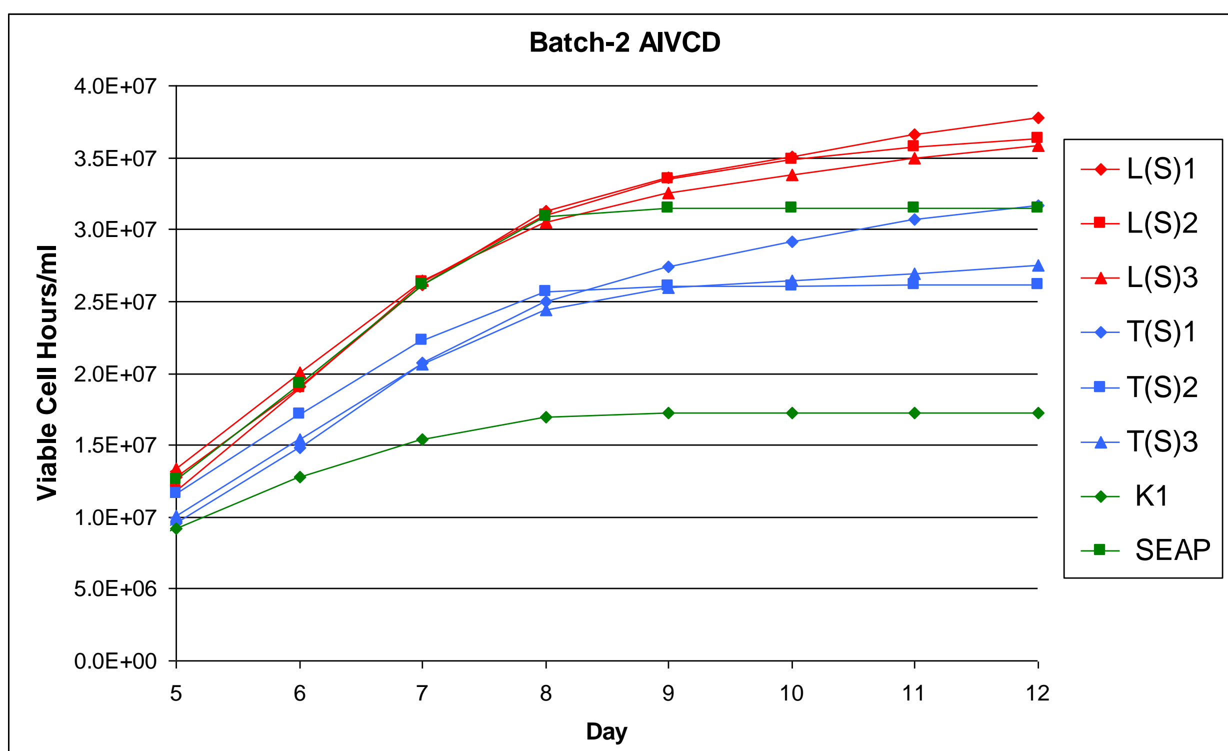

Figure 3.2.15.1.9: AIVCD data of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-2', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 3 and 6 (10% of total volume).

Final Day AIVCD (cell-hours/ml)

Clone	AIVCD	vs. K1	vs. SEAP
L(S)1	3.77E+07	119%	20%
L(S)2	3.63E+07	111%	15%
L(S)3	3.58E+07	108%	14%
T(S)1	3.17E+07	84%	1%
T(S)2	2.61E+07	52%	-17%
T(S)3	2.75E+07	60%	-13%
K1	1.72E+07	-	-45%
SEAP	3.15E+07	83%	-

Figure 3.2.15.1.10: Final day AIVCD figures for each selected clone and control cell line from the Batch-2 experiment. Also included are the relative percentage differences between each sample line and the two control cell lines measured (K1 and SEAP).

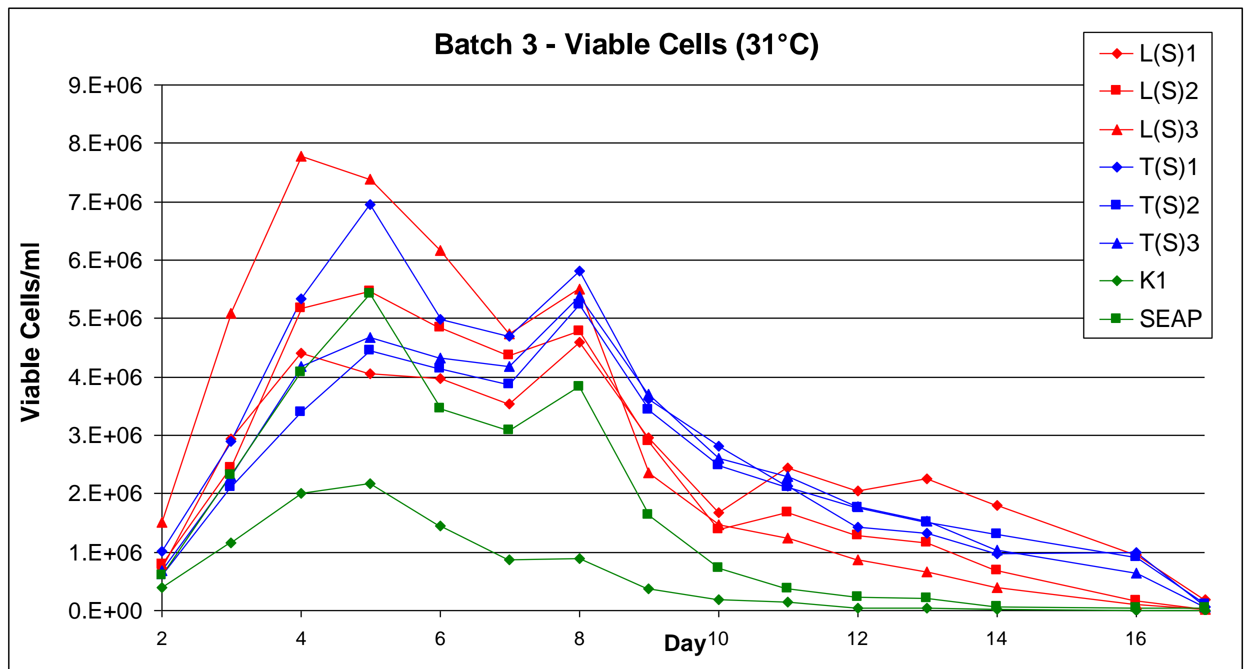

Figure 3.2.15.1.11: Viable cell densities of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-3', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 0, 3, 5, 8 and 11 (10% of total volume).

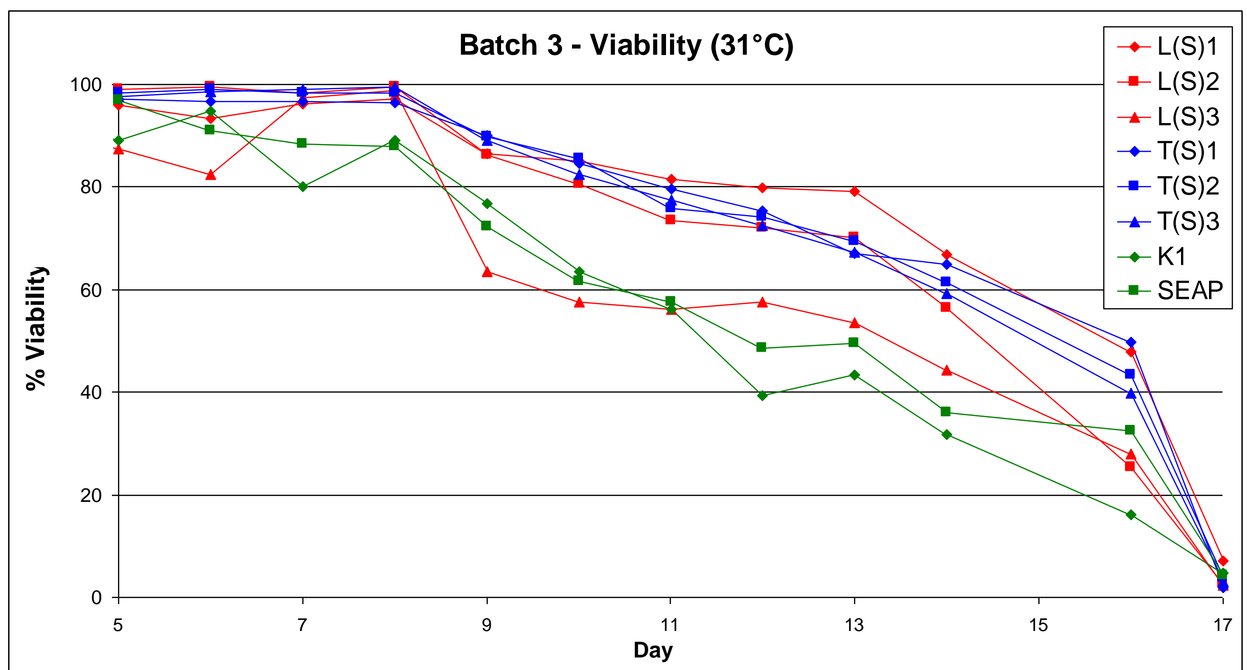

Figure 3.2.15.1.12: Percentage of viable cells of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-3', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 0, 3, 5, 8 and 11 (10% of total volume).

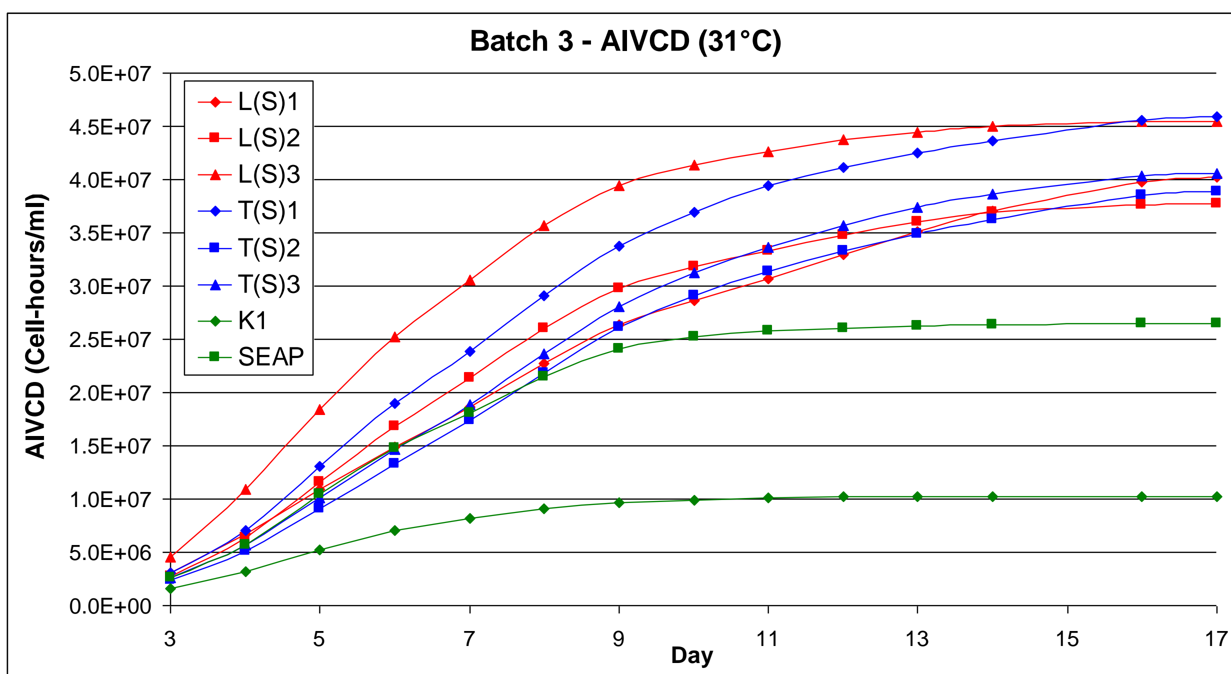

Figure 3.2.15.1.13: AIVCD data of Library and TBP clones (including K1 and SEAP control lines) from experiment 'Batch-3', temperature shifted at day 3 to 31°C. Cell lines were seeded at $2x10^5$ viable cells per ml in 50ml, with a concentrated nutrient feed added on days 0, 3, 5, 8 and 11 (10% of total volume).

Final Day AIVCD (cell-hours/ml)

Clone	AIVCD	vs. K1	vs. SEAP
L(S)1	4.02E+07	291%	52%
L(S)2	3.77E+07	267%	42%
L(S)3	4.55E+07	343%	71%
T(S)1	4.60E+07	347%	73%
T(S)2	3.89E+07	278%	47%
T(S)3	4.06E+07	295%	53%
K1	1.03E+07	-	-61%
SEAP	2.65E+07	158%	-

Figure 3.2.15.1.14: Final day AIVCD figures for each selected clone and control cell line from the Batch-3 experiment. Also included are the relative percentage differences between each sample line and the two control cell lines measured (K1 and SEAP).

3.2.15.2 Nutrient feed and temperature shift discussion

Batch 1:

Batch 1 was originally intended to investigate the effect of temperature shift, with the only extra nutrients provided on day 3 in the form of a media change. This injection of extra nutrients has stimulated the 37°C cultures to continue exponential growth reaching densities above 1 x 10^7 on day 5 (figure 3.2.15.1.1). This is in contrast with the temperature shifted cultures, reaching a lower average peak density (~8.3 x 10^6, figure 3.2.15.1.2), generally at the later stage of day 7 (with the exception of L(S)3, peaking on day 4). The 31°C shifted growth cultures bear the hallmarks of a hypothermic temperature shifted culture, with flatter, wider growth curves, slower decline in viability coupled with survival of viable cells beyond day 9 (figure 3.2.15.1.4), by which the 37°C culture has fully collapsed (figure 3.2.15.1.3).

In figure 3.2.15.1.5 (AIVCD data), we see that this flatter growth curve permits the 37°C Library clones on average to outperform their 37°C counterparts by overtaking their average number of viable cell-hours/ml by day 8 (with this 31°C AIVCD figure continuing to increase due to the continued presence of viable cells towards day 12). TBP clones do not perform as well under temperature shift, generally maintaining lower viable cell densities than the similarly treated Library clones, contributing to mixed final day AIVCD results. However, as with Library cells, viability is seen to decrease less drastically than 37°C cultures.

While changes in AIVCD may provide an estimate to changes in overall productivity based on the cumulative number of viable cell-hours, this does not take into account the change in specific productivity (Qp) associated with temperature shifted cultures. Despite lower cell densities, a common characteristic of hypothermic CHO cultures is improved ‘per cell’ Qp contributing to an overall improvement in total productivity (for examples see Fox et al. 2006; Chen et al. 2004 etc). While productivity is not measured here due to the lack of a constitutively expressed product, we could speculate that the predictions regarding growth and viability demonstrated experimentally as holding true would also apply to the prediction regarding an increase in the productivity of these cells when placed in a hypothermic environment.

Batch 2:

Having witnessed the typical phenotypic effects of temperature shift in the first instance, the hypothermic cultures were once again seeded and grown under the same conditions, however in this instance the entire medium replacement at day 3 was substituted for a commercial nutrient feed, added at 10% of the total volume at days 3 and 6. Both K1 and SEAP controls were also investigated in this instance.

In this experiment we see more consistent growth patterns across all clones compared, with peaks in cell density coinciding on day 6 (figure 3.2.15.1.7). The varying growth rates in the previous 31°C batches may have been a reaction to the removal of conditioned medium and the cells re-adaption to entirely fresh nutrients on day 3. The feed regimen applied in this instance is more akin to that found in a true fed-batch culture, and the delayed decline in viability (figure 3.2.15.1.8) is similar to the previous hypothermic culture, with no significant decline in viability until day 8 (compared to previous standard 37°C 50ml batch cultures in this and previous experiments (see figure 3.2.12.1), where this decline would tend to set in ~48 hours earlier, with no real viable cell presence beyond day 9 or 10).

In this experiment we also see both low and high density controls (K1 and SEAP) leading to complete cell death day 9, highlighting the advantageous phenotype that has been isolated in the selected clones in this study.

Due to the inclusion of only 2 feed points at relatively early time points, this batch did not generate late stage survival significantly different to that of the previous culture. In order to investigate if this viable culture length could be lengthened through the use of continual nutrient feeds in tandem with the temperature shift, this experiment was repeated a final time with an increased number of feeding time points.

Batch 3:

Having established the beneficial effects of both temperature shift and supplementary nutrient feeds on overall viability in an extended batch culture, the final fed-batch experiment was carried out to investigate how long viable cell densities could be maintained with a further number of feed time points in order to prolong the time span of useful cell culture. Cells were seeded as usual at 2 x 10^5 cells/ml in 50mls (45 CHO-SFM serum free medium and 5mls concentrated feed, as per manufacturers recommendations) and supplemented on days 3, 5, 8 and 11.

In general, maximum cell densities were reached on day 6 as previously (an exception to this is cell line L(S)3, whose early and high peak density may also be responsible for its apparent lower viable cell percentage due to an earlier build up of dead cells and debris, see figures 3.2.15.1.11-12). In this instance cell viability did not begin to drop substantially in any cell line till day 9, by which precipitous drops in culture health were seen in previous fed-batch experiments with fewer or no medium supplements. This is likely due to the additional nutrients and pH buffering provided by the media feeds, delaying the onset of apoptotic conditions. This also results in a slower decline in viability, with most selected clones maintaining viability >60% even by day 13. Despite the benefits of additional nutrients, both control lines' viability drop sooner, with an average benefit of 15-20% to cell viability in selected clones. This demonstrates that, even with the feed medium mitigating negative environmental effects, stress selected cells maintain a benefit in viability as a result of their heritage. However, despite the relatively high percentage viabilities of the selected clone lines, suspended cell densities are seen to drop consistently from days 8-9 onwards. A number of physical factors are likely to have contributed to this, such as the macroscopic clumping of cells reducing the numbers of actual suspended cells (especially prevalent in control cell lines, however in a proper reactor environment this problem could be reduced with impeller mixing combined with the use of an anti-clumping agents). This is combined with the gradual diluting effect of the nutrient feeds with total cell densities diluted almost 50% by day 12 after 4 x 10% total volume supplements. The increased osmolarity caused by the increased concentrations of nutrients may also have served to accelerate cell lysis, as evidenced by the increasing levels of cell debris detected by the flow cytometer when samples were measured in later culture stages, eventually limiting the effectiveness of continued feeds.

3.2.16 Determination of TBP copy number

In order to fully characterise the advantageous TBP and Library clones isolated in this study, the qPCR technique originally used to quantify the numbers of viral inserts in section 3.1.12 of this thesis was applied to these cell lines to estimate the number of TBP gene copies within the genome following transfection.

A protocol was followed identical to that carried out in section 3.1.12, utilising primers targeting the zeocin selection marker found beside transgenic TBP on the original pcDNA/TBP expression vector. These were chosen over primers directly binding TBP as to avoid interference from amplification of the wild type genomic sequence.

As before, the original transfected plasmid was used to generate a standard curve for quantification, with the samples used to generate this curve spiked with control CHO-K1 genomic DNA to reduce bias in amplification efficiency that may arise from using plasmid DNA alone. Measurement of a β-actin reference gene was also used to control for any variance in genomic DNA concentration.

Forward	5’ – TTCTGGACCGACCGGCTCG– 3’
Reverse	5’ – ATCTCGGTCATGGCCGGCC– 3’

Figure 3.2.16.1: Primers used to target the zeocin sequence within the TBP expression vector, resulting in a 145 bp fragment suitable for qPCR.

3.2.16.1 Results of TBP copy number qPCR

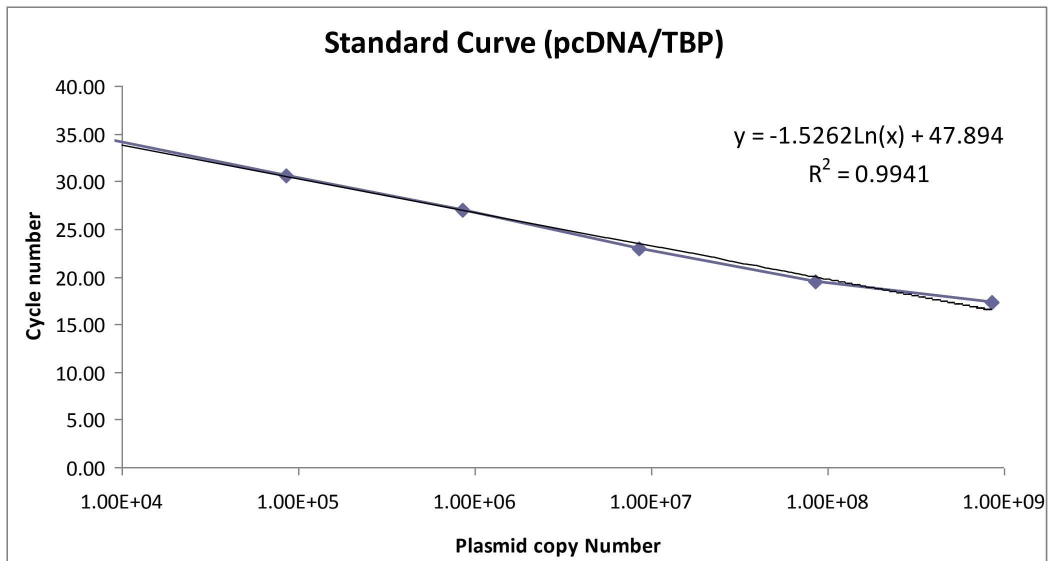

Figure 3.2.16.1.1: The standard curve generated from the pcDNA/TBP plasmid. Samples were also spiked with an appropriate concentration of genomic DNA to better mimic amplification conditions and efficiencies resulting from the use of genomic DNA alone. Samples were measured in triplicate within the linear range between 8.4 x 10^9 and 8.4 x 10^3 plasmid copies

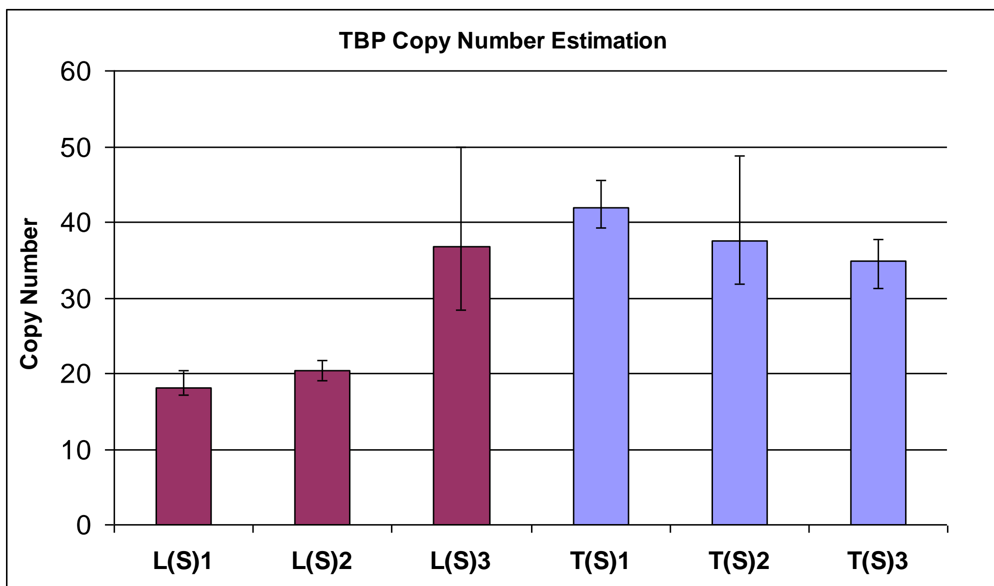

Figure 3.2.16.1.2: Calculated TBP copy numbers from the transfected cell lines assayed. Genomic DNA isolated from each cell line was measured in triplicate for both TBP and β-actin concentrations .In order to factor in inaccuracy resulting from the nature of the experiment, error (minimum and maximum insert numbers) was also calculated by combining the highest and lowest estimated TBP and β-actin replicate results to generate a range of possible TBP copy numbers.

3.2.17 Estimation of intracellular TBP protein concentration

A western blot to detect intracellular TBP concentrations was carried out on selected TPB clones along with a parental CHO-K1 control. This was done to further fully characterise the advantageous Library and TBP transfected clones isolated in this study and investigate as to whether the significant upregulation of TBP expression was correlated with a subsequent increase in TBP protein concentration.
Cell pellets were harvested on day 3 from actively growing suspension cultures and lysed. Total protein was then purified and quantified using a Bradford assay (see 2.7.1)

Anti-TBP antibody: Abcam (cat. ab51841) mouse monoclonal

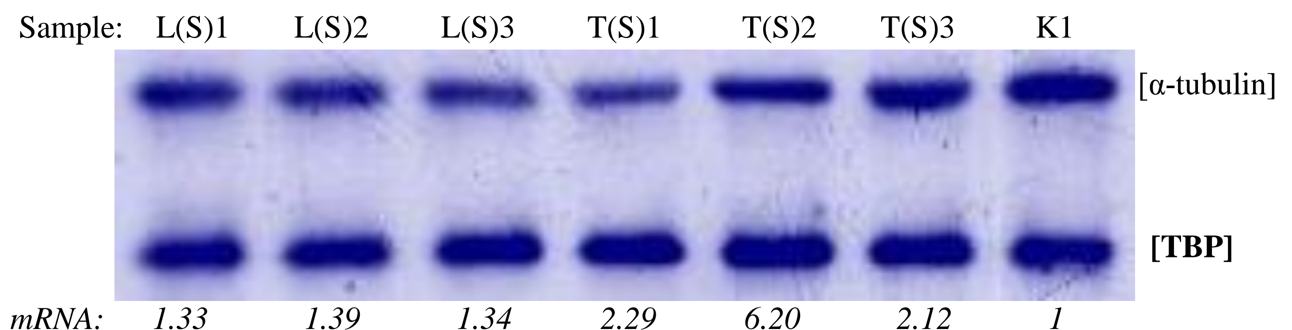

Figure 3.2.17.1: Western blot of intracellular TBP isolated from transfected TBP and Library clones alongside an untransfected K1 control. 10μg protein was added per well, as measured by a Bradford assay. The α-tubulin housekeeping gene was used as a loading control. Also noted below the figure are the corresponding mRNA expression levels (relative to the K1 control) as measured by qPCR in a previous experiment (section 3.2.10).

3.2.17.1 Discussion of intracellular TBP protein concentration

From the results of the western blot carried out above (see figure 3.2.17.1), it would appear to indicate that TBP concentration did not vary considerably in the clones isolated when compared to the CHO-K1 control at this time point. This was to be expected based on mRNA expression levels measured in a previous qPCR experiment on selected post-stress clones. Originally wild-type and mutant library TBP mRNA were found to be 5.7 and 9.6 fold upregulated in mixed populations, however the selected advantageous clones generally exhibit more moderate TBP upregulation (with the exception of T(S)2, all other selected clones exhibit mRNA upregulation between 1.3 and 2.3 fold). This may be indicative of selection for a more optimal range of expression, mediated by epigenetic, post-transcriptional and post-translational regulatory processes.

Due to the nature of TBP and its close integration with cell-wide transcriptional activity, modest changes (less than two fold) in concentration are associated with marked changes in gene expression (Johnson et al. 2003b). Because of TBP's ability to effect widespread metabolic changes, it is likely that this protein is also subject to tight post-transcriptional regulation, with expression and activity mediated by factors such as Ras-signalling and p53 binding (Johnson et al. 2003b, 2000). Increasing TBP concentration also promotes a form of autoregulation, with homodimerization preventing DNA binding and further transcriptional activation (Gokhale et al. 2010), however dimerized molecules were not detected by this assay. It is also possible that TBP molecules with sufficient mutations (in the first 100 amino acid residues to which the antibody used is reactive) may have been undetected by this assay.

Auto-regulatory factors such as these may have contributed to these cell lines' inability to stably maintain high TBP concentrations. While the TBP-linked zeocin resistance gene was used to estimate gene copy number, effective TBP expressing gene copies may have been silenced or removed during the stress selection process, in the same manner as recombinant gene silencing leading to production instability (Kim et al. 2011). However, as an evolutionary adaptation to this stress these mutations are likely to be advantageous, as suggested by the phenotypes generated.

3.2.18 Mutant TBP sequencing

The post-stress clone data gathered with respect to both TBP and Library transfected clones demonstrated both populations as having an improved ability to adapt to the stress applied when compared to the empty vector control. While this data indicated a generally consistent change in phenotype with regard to the wild type gene, a wide range of performance was noted in the Library transfected cells. This result is in line with what one might predict; TBP overexpression producing a relatively similar phenotype in transfected cells while mutant library transfection results in a mixed heterogeneous pool of clones. As with any random mutagenesis technique, we are left with a resulting mixture of both positively and negatively acting sequences, with which we used a selective stress to try and eliminate deleterious mutants. This was successful in reducing the levels variation in performance, leaving us with a selection of advantageous clones.
To examine the sequences present in these advantageous clones, and investigate if these beneficial mutants shared any common properties a series of samples were prepared from Library selected clones and sent for sequence analysis (carried out by Eurofins MWG Operon using chain-termination 'Sanger' sequencing).

Initially a PCR was carried out to amplify the mutant sequences in each clone and sent for sequencing. However, due to the mixture of different sequences within each clone the result appeared as an 'average' of these combined sequences, essentially the wild type sequence. To overcome this, each PCR mixture was cloned into a plasmid vector and transformed into bacteria. This allowed us to select bacterial clones containing single plasmids, which were grown and purified for mutant TBP sequencing.

3.2.18.1 Mutant TBP sequencing results

From each of L(S)1-3, five individual sequences were isolated (for a total of 15 sequences) and analysed using both *forward* and *reverse* primers for separate sequencing reactions. A consensus sequence was generated from each pair, and analysed at the nucleotide and protein level.

An average of 3.6 mutations per sequence was noted, a reduction from the original 9.5 average mutations noted in the original mixed plasmid library. This was to be expected, as sequences with large numbers of deleterious mutations were likely to be eliminated from the population as a result of the stress selection procedure. The larger average mutation rate ('medium' mutant library) was originally chosen over a lower rate to generate a more diverse collection of sequences and distinguish the Library cells' phenotypes from the effect of wild type TBP. This allowed us to demonstrate that, despite an initially negative effect on overall phenotype, the stress selection procedure could be successful in eliminating the majority of these deleterious mutants (or perhaps suppress their expression in selected clones) as well as providing sufficient diversity as to allow the selection and isolation of clones with the potential to outperform those isolated from a comparatively homogenous (WT-TBP) group.

The following 3 tables (figures 3.2.18.1.1-3.2.18.1.3) detail the 5 mutant sequences isolated from each clone. Details noted include the residue number within the CHO TBP sequence, mutation type (base changes, deletions and frame shifts), amino acid change as well as further details such as their location in particular structural domains (β-sheets and α-helices).

L(S)1

Sequence	Residue	Change	Mutation	Notes
A	69	Base	Stop	Truncated N-terminal protein
B	33	Base	P-L	
	96	Base	Q-H	
	182	Base	E-D	
	185	Base	T-I	B4 β-sheet
	274	Base	P-H	
	311	Deletion	F-S	
	312	Frame shift	3 residues	
	314	-	Stop	
C	33	Base	P-L	
	127	Base	P-R	
	221	Base	G-D	
D	57	Base	R-K	
	84	Base	Q-H	
	144	Base	I-M	B1 β-sheet
E	9	Base	P-L	

Figure 3.2.18.1.1: Details of the mutations found in the five (A-E) mutant sequences isolated from Library clone L(S)1. Details noted include the residue number within the CHO TBP sequence, mutation type (base changes, deletions and frame shifts), amino acid change as well as further details such as their location in particular structural domains (β-sheets and α-helices). Data above was generated from 5 mutant sequences isolated from cell line L(S)1, achieved by PCR amplification, subcloning and subsequent sequencing of individual mutant TBP sequences.

L(S)2	Sequence	Residue	Change	Mutation	Notes
	F	33	Deletion	P-L	
		34	Frame shift	37 residues	Nonsense protein
		71	-	Stop	Truncated N-terminal protein
	G	7	Base	L-P	
		8	Base	P-S	
		9	Base	P-T	
		120	Base	T-S	
		205	Base	Q-H	A'2 α-helix
		228	Base	D-N	
	H	7	Base	L-H	
		9	Base	P-L	
		120	Base	T-S	
		205	Base	Q-H	A'2 α-helix
		299	Base	E-A	A'2 α-helix
		300	Base	A-R	A'2 α-helix
	I	120	Base	T-S	
		299	Base	E-A	A'2 α-helix
		300	Base	A-P	A'2 α-helix
	J	60	Base	Q-E	
		114	Base	L-V	
		130	Base	P-Q	
		149	Base	N-T	B1 β-sheet
		164	Base	R-P	
		174	Base	A-D	S1 stirrup loop
		228	Base	D-Y	
		263	Base	L-I	S2 stirrup loop

Figure 3.2.18.1.2: Details of the mutations found in the five (F-J) mutant sequences isolated from Library clone L(S)2. Details noted include the residue number within the CHO TBP sequence, mutation type (base changes, deletions and frame shifts), amino acid change as well as further details such as their location in particular structural domains (β-sheets and α-helices). Data above was generated from 5 mutant sequences isolated from cell line L(S)2, achieved by PCR amplification, subcloning and subsequent sequencing of individual mutant TBP sequences.

L(S)3 Sequence	Residue	Change	Mutation	Notes
K	33	Deletion	P-R	
	34	Frame shift	37 residues	
	71	Frame shift	Stop	Truncated N-terminal protein
L	46	Base	T-A	
	85	Base	Stop	Truncated N-terminal protein
M	8	Base	P-L	
	9	Base	P-T	
	110	Base	G-D	
	162	Base	R-H	A1 α-helix
	174	Base	A-T	S1 stirrup loop
	179	Base	R-G	B3 β-sheet
	184	Base	R-W	
N	301	Frame shift	5 residues	A'2 α-helix
	308	-	Stop	A'2 α-helix
O	114	Base	L-V	
	220	Base	L-S	
	308	Deletion	Stop	A'2 α-helix

Figure 3.2.18.1.3: Details of the mutations found in the five (K-O) mutant sequences isolated from Library clone L(S)3. Details noted include the residue number within the CHO TBP sequence, mutation type (base changes, deletions and frame shifts), amino acid change as well as further details such as their location in particular structural domains (β-sheets and α-helices). Data above was generated from 5 mutant sequences isolated from cell line L(S)3, achieved by PCR amplification, subcloning and subsequent sequencing of individual mutant TBP sequences.

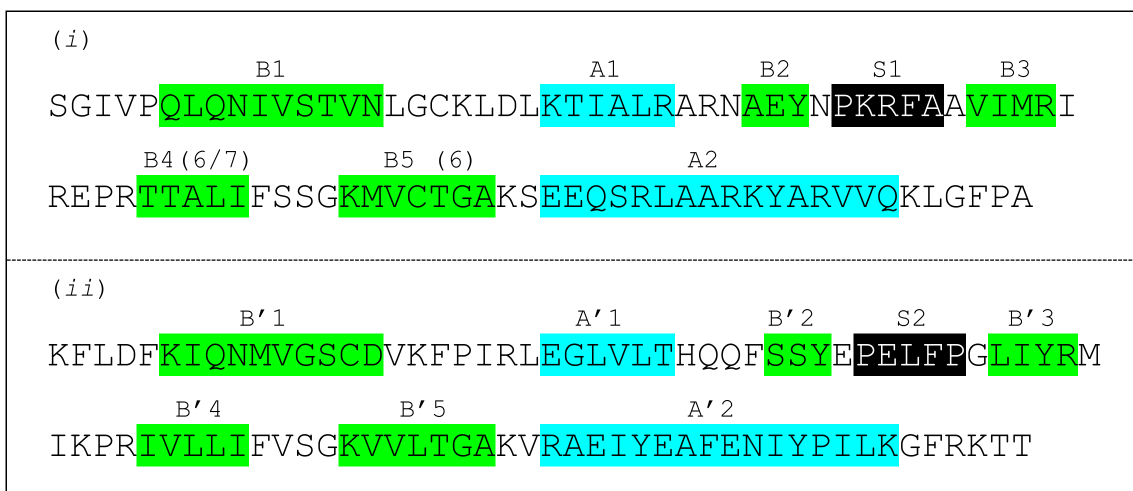

Figure 3.2.18.1.4: Protein sequence representing the conserved C-terminus of CHO TBP (amino acid residues 135-315) involved in DNA binding (adapted from Juo et al. 1996). This terminus displays internal symmetry, consisting of two identically folded domains (*i* and *ii*) of 90 amino acids each. Each consists of 5 β-sheets (B1-5, highlighted in green) and 2 α-helices (A1/2, highlighted in blue). β-sheets form the main surface by which TBP interacts with DNA, with α-helices acting to form a strengthening scaffold. The stirrup loops (S1/2, highlighted in black) assist in bending DNA and compressing the major groove.

3.2.18.2 Mutant TBP Sequencing Discussion

The first obvious characteristic of the sequences analysed is the diversity observed. TBP copy number qPCR estimates anywhere from 18 to 36 different mutant sequences in each clone, and the 5 sequences from each clone analysed above represent only a fraction of these. No sequence is represented twice in any clone, and with an original library complexity of 10^5 individual mutant sequences it is possible that each transfected cell received an entirely different complement of mutants.

To date, a number of mutagenesis studies have been carried out in human and yeast cells in order to elucidate the numerous functions and interactions that TBP is involved with. A wide range of non-functional and distinctly deleterious mutants have been discovered, usually for the purpose of further understanding of TBP's constituent domains (Bryant et al. 1996; Hampsey 1998). However, using random mutagenesis or by targeting specific regions, researchers have also generated mutants with altered function;

- Altered TATA sequence binding specificity (Arndt et al. 1992, 2004; Spencer and Arndt 2002)
- Dimerisation impaired mutants, preventing autorepression of TBP activity and inducing heightened reporter gene activity (Jackson-Fisher et al. 1999)
- Transcriptional de-repression (Kou et al. 2003)
- Enhanced ability to overcome promoter repression of certain co-repressor complexes (Geisberg and Struhl 2000)
- Bypassed transcriptional necessity for particular TBP associated factors, selectively altering gene expression (Sprouse, Wells and Auble 2009, Blair and Cullen 1997)

From these examples, we can see that TBP mutation can serve in generating a range of molecules with alternative functional capacity, with possible downstream implications for the expression of a large number of genes. While improved function is not guaranteed, this at least permits the creation of a more heterogeneous population with a mix of altered metabolic landscapes from which beneficial mutations can ultimately be selected.

Initially TBP mutants in which altered function was generated as described in the literature were catalogued (mainly in human and yeast homologs), and their mutations were mapped onto the corresponding CHO TBP residues. However, of the thirty or so mutations with demonstrable changes in activity or specificity, none were found to correspond to the mutations noted in the mutant sequences isolated above. Unfortunately this does not allow us relate the sequences in the advantageous clones isolated to any previously characterised phenotypes or activities.

As such we can only speculate as to the possible effects such mutations may have had on the structure or function of these sequences. As β-sheets are generally involved in the main DNA interacting domains, broadly speaking we can infer that mutations to these residues are more likely to affect the sequence specificity or DNA binding kinetics of TBP. These are also far less common in the clones sequenced in this experiment, with only 4 of these mutations appearing separately in isolated sequences, a possible indication that such mutations play a more active role in altering TBP function and thus are tolerated less by the cell's metabolism and selected against. This is in contrast to mutations in α-helices that play a more structural role in TBP. These mutations appear more frequently in 7 of the sequences analysed above. This may suggest that these mutations are more tolerated by the structure itself and have less impact on overall function, or are actually beneficial in themselves through means of altering the protein's interactions with other transcription factors. However, without experimental analysis of the effects of single sequences, this is impossible to verify.

A SIFT (Sorting Intolerant From Tolerant) analysis of all mutations was carried out to predict changes most likely to result in functional changes (Ng and Henikoff 2006). This method compares a multiple sequence alignment of a database of similar sequences, and makes predictions based on residue conservation and the physiochemical properties of the substituted amino acid. However, low confidence in predictions relating to N-terminal mutations resulted due to its non-conserved nature across species, making sequence comparisons difficult. Of 44 non-synonymous base changes, 32 were predicted to affect function. However, only 12 of these (generally in C-terminal positions) were identified with statistical confidence.

While most of the sequences analysed appear to hold a different set of individual point mutations, present at least once in each clone (sequences '*A*', '*F*', '*K*' and '*L*') is a truncated N-terminal sequence encompassing between the first 69 and 85 residues of TBP. Frame shift deletions in both '*F*' and '*K*' ensure that the C-terminal portion of this code is nonsense. Mutation and deletion studies have found this N-terminus to be involved in modulating the effect of TBP, indicating interactions with other transcription factors as well as the core of the TBP protein itself, and removal of this portion is not lethal in yeast. Its sequence is also highly variable across species, and may have evolved a number of varying functions in different organisms (Friedman et al. 2007; Lee and Struhl 2001). It is difficult to predict if such a polypeptide solely made up of this portion could be successfully folded and possess any useful structure or function in isolation, but its occurrence in all 3 selected library clones may mark it as potentially interesting, or perhaps merely benign as to remain unremoved by the selection process.

With each cell expressing a mix of different mutant sequences at different concentrations, it is impossible to ascribe the phenotype of each clone to a particular mutant. No sequence appears to be duplicated in each clone from the sample set analysed above, and from the insert copy number data we can see there may be between 18 to 36 individual different mutant sequences in each clone. TBP expression level data (along with TBP concentrations estimated by western blot) also demonstrates that these sequences are expressed at relatively low levels in selected clones. While we cannot be certain how many or which of these sequences are being expressed, we can hypothesise from this data that the beneficial phenotypes witnessed in these clones is a result of a number of different subtle interactions acting in tandem, rather than an obvious consequence of one single sequence.

3.2.19 Expression profiling in experimental CHO cell lines

Having carried out the process of transfecting with both TBP and mutant library and subsequent selection for advantageous clones, a transcriptomic profiling experiment was carried out on a selection of cell lines including control, unstressed and stressed populations as well as a number of successful clonal cell lines isolated from stressed mixed population. This would permit an investigation into the changes in gene expression caused by the genetic and evolutionary pressures utilised over the course of this study and potentially allow us to identify the genes responsible for the advantageous phenotype we see in advantageous clones.

For this experiment, the GeneChip 3' IVT Express Kit (Affymetrix, 901229) was used for the processing of CHO RNA samples and generation of suitable antisense RNA (cRNA). The arrays used for this experiment were 'Hamster 3a-520384' chips (Lot no. 4115243, ref no. 520384), developed by Affymetrix. Based on more recent CHO-specific transcriptome sequencing, these chips contain nucleotide probes with the ability to measure roughly 19,000 various gene transcripts and ~8,000 unique annotated genes. At this time not all genes measured are identified and annotated due in part to the historical lack of public CHO sequencing data. However identification of function based on similarities to human, mouse and rat orthologues is possible, allowing us to analyse this data and identify the factors that may contribute to a given change in phenotype.

To generate samples for this experiment, a number of CHO cell lines were seeded at 2 x 10^5 cells/ml (using 50ml flasks) in a typical batch culture situation. Triplicate samples of 2 x 10^6 viable cells were removed at day 5, and processed to isolate mRNA for transcriptional profiling. Day 5 was originally chosen as a suitable time point as this was often the point of maximum cell density or beginning of the decline phase, as well as the point when viability percentages in all cell lines appear to drop in batch culture. This would mark it as a suitable time to identify changes in gene expression related to any stress, the key phenotypic response we originally sought to investigate and characterise with this experiment.

3.2.19.1 Selection of profiling samples and comparisons

The first step in this study was deciding on a number of representative samples from throughout the study to investigate. Due to the varied number of samples and experimental steps involved, such as the effect of TBP and Library (as well as a control) combined with the changes invoked by the stress procedure, we initially decided on a range of samples to cover this variety and hopefully allow us to describe the changes brought about by each sequential step.
Ultimately being limited to 10 samples (carried out in triplicate for a total of 30 arrays), the following samples were chosen for analysis:

- pcDNA transfected mixed population (unstressed)
- pcDNA transfected mixed population (stressed)
- TBP transfected mixed population (unstressed)
- TBP transfected mixed population (stressed)
- Strong performing stress selected clones (T(S)1, T(S)2, L(S)2 and L(S)3)
- Control cell lines (CHO-K1 and CHO-SEAP)

The use of both stressed and unstressed versions of pcDNA and TBP mixed populations was initially decided upon to try and investigate the main genetic components responsible for the apparent benefits of transfection and selection. With pcDNA acting as a control the relative effects of TBP could be assessed, and pre- and post-stress mixed populations would allow for the identification of genes whose expression was affected by the stress procedure. The use of control cell lines would also provide a means of comparing successful cell lines to a relatively poor performing line (in the case of K1), or determine what the differences were that set apart the more successful selected clones from an known high density cell line, such as CHO-SEAP. Due to the limit of sample numbers Library transfected populations were not chosen for profiling. Due to the inherent heterogeneity of these populations, each cell is likely to contain transcriptomic profiles with variety exceeding that of more homogenous populations such as TBP transfected or clonal lines, thus generating a potentially noisy or otherwise uninformative signal when analysed.

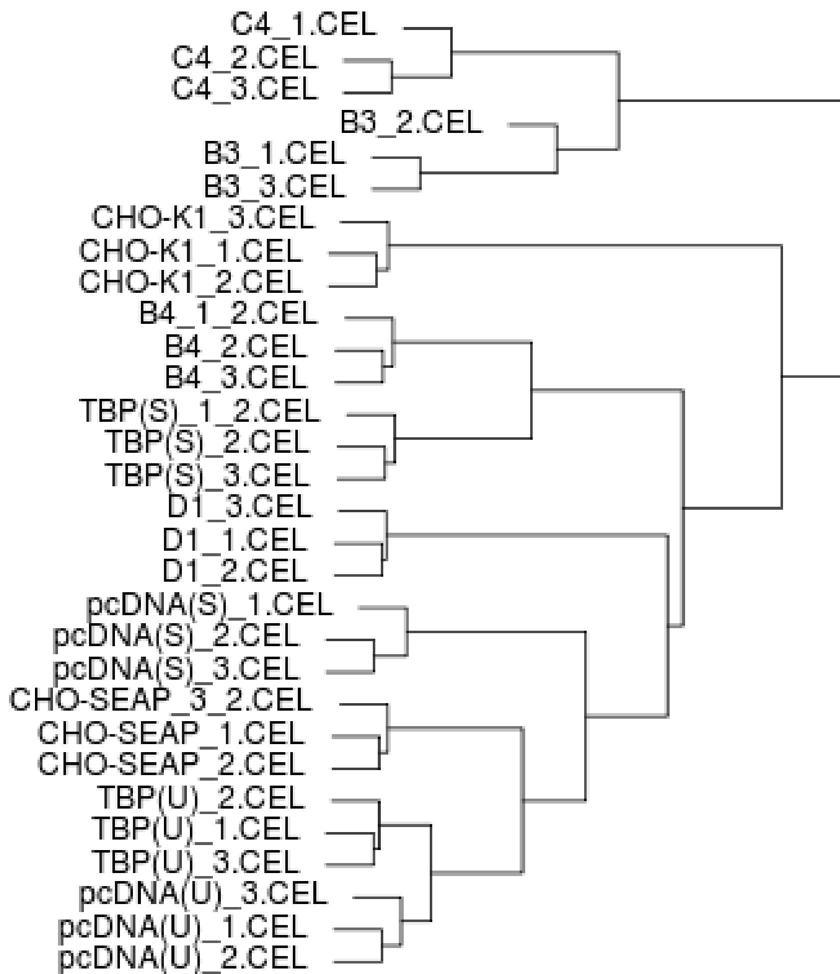

Figure 3.2.19.1.1: Dendrogram representing the final clustering of profiling samples and cell lines analysed. Samples were generated from RNA extracted from standard batch cultures on day 5 and analysed in triplicate. Differential expression analysis was carried out to determine the transcriptomic differences between cell lines, with greater separation between samples in the diagram above indicating larger degrees of dissimilarity with regards to gene expression. In general triplicate samples cluster together tightly. With the exception of control CHO-K1, unstressed cell lines appear separated by less distance. The application of stress introduces greater changes in gene expression, with cell lines incorporating combined stress and genetic intervention appearing the most distant from the unstressed pcDNA control (i.e. TBP-S and selected clones L(S)2, L(S)3, T(S)1 and T(S)2, noted in this diagram by their original designations B3, B4, C4 and D1)

3.2.19.2 Analysis of comparisons

In order to begin to understand the effects of both the two main components, a number of binary comparisons were carried out in which profile pairs were compared. This allows us to see the differences in gene expression between pairs, and isolate interesting gene targets that may have contributed to any advantageous changes in phenotype. The original analysis of raw array data and identification of statistically relevant genes was carried out by resident bioinformatician Dr. Colin Clarke. Analysis of gene lists to identify enriched cellular pathways and bioprocesses was done with DAVID, an online tool functional annotation tool (Huang, Sherman and Lempicki 2009a, 2009b)

3.2.19.3 TBP(U) v pcDNA(U) – 104 genes (23 up, 81 down)

Comparison examining the effect of TBP overexpression

The first comparison carried out was that between the TBP transfected cell line and control (pcDNA) line, both unstressed. This allowed us to investigate the effects of TBP overexpression compared to an empty vector transfected control cell line.

The most striking initial detail noticed about this comparison is the relatively short list of genes significantly dysregulated at this time point, despite the apparent difference in phenotype between these two cell lines (mainly in terms of cell density). Also of note is the range of differential expression noted in this comparison, with all genes expression changes falling within a range of 1.6 and -1.59 fold-change, suggesting that the overexpression of TBP does not appear to have a drastic effect on overall gene expression at this time point.

Firstly, examining the individual genes differentially upregulated, we see no specific pattern or any biological pathways significantly affected. The majority display a fold change just above the significance limit (between 1.2 and 1.3). The most significantly upregulated genes with notable function are GAS1, a tumor suppressor known to suppress growth and metastasis (Del Sal et al. 1992; Wang et al. 2012) as well as a role in transcriptional silencing (Koch and Pillus 2009), and the PEDF gene, demonstrated to have apoptotic/anti-tumorigenic properties (Garcia et al. 2004) with the ability to facilitate G_0 cell cycle quiescence (Tombran-Tink and Barnstable 2003).

When we examine the remaining list of downregulated genes we find that the most enriched processes are almost exclusively related to cell cycle progression and mitosis related categories such as chromosome segregation and nuclear division. Examples of genes with prominent negative fold changes that are representative of these categories include CCNB1 (promotion of mitosis), Nusap1 (mitotic spindle stabilisation), Spc25 (chromosome segregation), CDC20 (chromatid separation), Ccna2 (transition of cell cycle phases) and Prc1 (cytokinesis).
The downregulation of these genes (that, when active, are involved in cell cycle and cell division) as well as the upregulation of anti-tumorigenic proteins would suggest a slower growing culture. However, from our growth curve data we can see that higher density growth is achieved in these cells. An explanation for this may lie in temporal gene changes as the culture progresses. As stably transfected TBP cells (found to be overexpressed in actively growing cells) produce a higher density of viable cells, the gene profile measured here after a plateau in growth may reflect the cells' changes in gene expression once growth has stopped. Instead of identifying genes associated with growth, this profile may be a cellular reaction to prevent overgrowth, thus we see a profile indicative of growth restriction. For this reason an earlier time point may provide a better indication of the affected genes involved in growth, rather than the pattern we see here.

As this is the unstressed TBP culture, we do not see any significant stress responsive genes differentially regulated at this point. While phenotypically TBP has been demonstrated to perturb gene expression in early stages to benefit growth, the phenotypic differences between TBP and control cell lines in the later stage of culture would suggest that TBP does not play a significant role in the survival or viability aspect of these cells. For example, if we look at the percentage viability graph of the two cell lines compared here, we find that their profiles are mostly identical in the decline phase. From this profile we can see that, while TBP may be beneficial in early stage growth, its effects are mostly suppressed by day 5 in this culture, with an apparent shift to lower levels of mitotic proteins and a profile apparently geared towards growth suppression.

3.2.19.4 pcDNA(S) v pcDNA(U) – 351 genes (179 down, 172 up)

Comparison examining the effect of the stress procedure (no genetic manipulation)

The next comparison examined was between the control (empty vector transfected) population and its stressed counterpart. This comparison allowed us to investigate the differences in gene expression brought about by the stress procedure in isolation, without the added effects of TBP or Library overexpression.

Initially we can see a greater number of genes affected by the stress procedure in isolation than by TBP overexpression alone, with small changes in a wide range of genes accounting for the change in phenotype we see here (less than 7% of genes are found with <±2-fold dysregulation). While TBP transfection was found to affect a positive change in maximum cell density, the stress procedure generally appears to help prolong later stage survival, especially in TBP and Library stressed populations. However, without any genetic intervention we only see modest improvements here in viability in later stages (see *3.2.13.1*), compared to greater improvements to late stage viability gained in conjunction with stress and genetic manipulation combined (i.e. TBP and Library stressed cultures). This would suggest that the heterogeneous perturbations brought about by gene overexpression play a role in allowing a population to present cells more adapted to stress, with the phenotypic response from the genes dysregulated in this instance providing the most nominal improvements as a result of the stress procedure.

A broad range of metabolic processes and pathways are found to be affected by the stress procedure at this time point, with no single areas appearing to be disproportionately affected in any greater proportion by the procedure. Dysregulated genes appear in highly enriched pathways affecting growth and apoptosis, ion homeostasis and the metabolism of various compounds (such as phosphorous, amines and glutathione). This broad range makes it difficult to attribute phenotype to any specific pathways. Phenotypically we see a reduction in the rate of loss of viable cells, however this reduction may possibly be contributed to by a wide range of targets such as antiapoptotic genes like BCL10 (Chen at al. 2012). This beneficial effect may also be inhibited by the reduction in oncogenic proteins such as BMI1 (known to promote growth in certain cancers; Wu et al. 2011).

An interesting area of dysregulated expression was noted consisting of 10 genes (found to be upregulated between 1.23- and 1.51-fold), all implicated in glutathione metabolism and reaction to oxidative stress. These are generally found to be upregulated as a response to reactive oxygen species such as peroxides (Cantoni et al. 1993) and other toxic compounds (Dickinson and Forman 2002). Genes found upregulated in this instance include Catalase (prominent in the degradation of hydrogen peroxide and cellular pH maintenance), glutathione peroxidise (Gpx4) involved in the protection against oxidative damage, PSMB5 increasing proteasomal response to oxidative stress (Chondrogianni et al. 2005) and GCLM (involved in glutathione synthesis). In the context of the stressed population it seems possible that the pre-emptive upregulation of these genes was selected for by high concentrations of both intracellular and environmental waste products, with their increased activity playing a role in maintaining a healthy cell. A look at the transcriptional motifs found in these genes indicates no common factor, suggesting their upregulation together is a specific adaptation of the cell rather than a side effect of a related transcription factor.

The most statistically enriched functional group of genes (with 20 targets falling under its definition) are those implicated in the cells response to organic substances. This definition lends itself to a broad range of receptors, kinases, transcription factors and other functional enzymes, and their dysregulation is possibly an adaptive response in stress selected cells to the build up of organic waste products that are characteristic of late stage culture. An example in this category with a known positive function is RNA stabilising protein RBM3 (upregulated in this instance) is a proto-oncogene shown to induce growth and proliferation (Sureban et al. 2008), a beneficial effect in CHO.

However, while investigation into function allows us to pinpoint certain targets that may be beneficial to a viable phenotype, this functional category appeared to contain a number of genes with dysregulation that appears to be detrimental. While late stage viability in stressed CHO remains higher post day 5, until this time point viability is slightly lower (-8.2% on day 5) in stressed pcDNA culture. These apparently detrimental genes may be responsible for this reduced viability at this time point, however we can not know if their impact persists in the later stages of culture when

the stressed pcDNA culture phenotype retains a higher number of viable cells compared to the unstressed population.
A number of these genes were noted. Examples of genes whose expression suggests a detrimental function in this instance include; HERPUD1 (function in ERAD protein degradation, however elevated levels are shown to induce apoptosis; Hendriksen et al. 2006), Cyclin D1 (we see 1.7 fold downregulation in this comparison, while overexpression is known to be beneficial in CHO; Zhao et al. 2011), SGK1 (while downregulated here, promotes apoptotic resistance in various cancer types; Fagerli et al. 2011) and Growth Hormone Receptor (whose stimulation is shown to inhibit programmed cell death in CHO; Costoya et al. 1999).

As with the investigation into the effects of TBP overexpression, it is suggested from the data that some of these changes may be temporal, reflecting the changes around this time point that begin to restrict growth as cells enter the stationary phase. We can correlate some genes with a function likely to be beneficial (such as those involved in the oxidative stress responses) while the negative viable cell density at this particular time point is possibly a result of the genes with detrimental effects such as those described above.

3.2.19.5 **TBP(S) v TBP(U)** – 576 genes (289 down, 287 up)

Comparison examining the effect of the stress procedure with TBP overexpression

The next key comparison to be investigated is a comparison between the stressed and unstressed versions of the TBP transfected population. Similarly to comparing pcDNA stressed and unstressed cultures, this comparison can direct us to the pathways and genes affected by the effect of the stress procedure

Phenotypically, it is noted that of the beneficial changes from the stress procedure gained here (in conjunction with TBP) are greater than that of the stressed control (empty vector) culture. A greater number of genes are also found dysregulated than in the pcDNA(s) vs pcDNA(u) comparison, once again suggesting that the effect of TBP overexpression plays a role in facilitating a greater adaptive response to the effects of the stress environment. These two responses also appear to be divergent, with a comparison of gene expression from both stressed pcDNA and TBP populations identifying an even greater number (>700) of dysregulated genes, suggesting that the stress procedure has affected these two populations in fundamentally different ways, generating a different response in each. However, in this case the TBP stress response is certainly the most optimal as demonstrated by its phenotype.

Again, a variety of pathways are found to be dysregulated. The most significantly affected areas in this case appear to be cell cycle related (mitosis, cell cycle, chromosome segregation etc), lipid metabolism (cholesterol, steroid and lipid biosynthetic bioprocesses) as well as translation related genes. Stress related genes affected by the pcDNA stress comparison (such as those involved in oxidative stress response and glutathione metabolism) also appear to be dysregulated in this case, implicating their role once again in the stress response.

Previously it had been noted in TBP transfected cells that a range of genes involved in mitosis and the cell cycle were found to be down regulated, indicating the restriction of growth once maximum density was reached. When we look at the expression of these genes in the stressed population we find that a large proportion are again upregulated in comparison and restored to levels that would again suggest a population more involved in growth. However, day 5 is when the TBP-U population has begun to decline in cell number, while TBP-S population has reached peak

density. As with the earlier comparison between a day 5 peak density pcDNA and declining TBP culture, this may just be a temporal reflection of the cells metabolism in relation to mitotic and growth related processes. Examples of genes having apparently 'restored' levels of expression include AURKA (whose activity peaks in the G2/M phase; Bischoff and Plowman 1999), CCNA1 and CCNB2 (promotion of mitotic cell cycle transition), NCD80 and NUSAP1 (involved in spindle formation), SMC4 and Spc25 (chromosomal condensation and segregation).
A number of genes involved cell cycle progression and mitotic pathways are found to be dysregulated that were not originally identified in TBP transfected cells alone, or dysregulated to a greater extent than originally found in unstressed TBP. Due to their appearance post-stress, they may suggest at their involvement in the increase in maximum cell density we see in stress selected TBP cells. For example, GAS1 (a tumor suppressor noted previously as 1.6-fold upregulated in TBP transfected cells) is found down regulated by over 2.4-fold. Downregulation of a tumor suppressor such as this is likely to promote growth to a greater extent. DUSP1 (originally unchanged by TBP and now downregulated 1.58-fold) is suggested to be involved in cell cycle due to its interactions with p53, however it is also shown to induce apoptosis when upregulated in response to oxidative stress, and its downregulation here may promote survival despite the environmental conditions (Liu et al. 2008). Tnfrsf21 (aka 'Death Receptor 6') is found downregulated 1.7-fold, and its reduced effect is likely to have a positive impact on apoptosis (Kasof et al. 2001).
Other significantly upregulated genes identified with growth arrest and survival promoting characteristics are PLK2 and GADD45A. PLK2 is a stress responsive gene, and its upregulation in times of stress can mediate survival (for example in times of compromised respiration; Matsumoto et al. 2009). It was also found upregulated to the same degree in pcDNA stressed cells, suggesting it expression may be an authentically useful stress response. GADD45A is another stress responsive gene that is induced in conditions such as medium depletion to suppress growth (Zhan et al. 1994), and also confirmed to promote viability in tumor cells when overexpressed (Schneider et al. 2006).

An area with a consistent level of increased activity is genes involved in protein translation. Of the 23 enriched for in this comparison, 18 are found with gentle increases (1.2-1.31 fold) in expression, with the remaining 5 similarly downregulated.

Of the upregulated genes, we see many eukaryotic initiation factor 2, 3 and 6 subunits (EIF2A, 3A, 3C, 3H, 3G and 6) overexpressed, many of which are directly and indirectly implicated in cell growth and tumorigenesis (Benelli et al. 2012; Hershey 2010; Zhang, Pan and Hershey 2007). A selection of mitochondrial ribosomal proteins are also found upregulated alongside a downregulated ribosomal protein S5, an expression profile matching that of a prolific cancer line suggesting that these genes are involved in persistent growth and/or survival (Ma et al. 2009). While the remaining downregulated genes such as EIF4G2 and 3 are likely to mitigate this effect (such as the effects of EIF4G downregulation known to suppress translational efficiency; Park et al. 2011) it is possible from their implications in the growth and survival of cancerous cells that the majority of translational genes' upregulation here may also have a beneficial impact on CHO growth and survival.

A number of genes identified as playing a role in the regulation of cell death or survival were investigated, and was one of the principle categories of targets that were investigated in this study. Some were correlated for dysregulation in the same direction in both stressed pcDNA and TBP populations, implicating their change as beneficial. For example, the most downregulated gene (ERCC1, -1.95 fold regulated) is involved in DNA repair and its knockdown is associated with increased tumorigenesis in mice (although this may be a result of increased oncogenesis due to faulty DNA repair, and not necessarily related to an increase in antiapoptotic factors; Doig et al. 2006). A putative tumor suppressor protein SOD1 is also found downregulated, possibly a beneficial change as its upregulation is found to suppress growth in a number of tumors (Zhang et al. 2002; Weydert et al. 2006).
The gene Plac8 (aka Onzin) was initially found to be substantially downregulated (4.4 fold) in pcDNA stressed cells, while it is found as one of the more upregulated (2.3 fold) in TBP stressed culture. The comparative increase in this genes expression may play a role in the improved phenotype of TBP selected cells compared to the control stressed culture, as its overexpression is shown to promote survival and growth while, conversely, its knockdown is shown to increase sensitivity to apoptotic stimuli (Li et al. 2006; Rogulski et al. 2005). The gene SPRR1A is found upregulated in increasing levels in TBP transfected, pcDNA stressed and TBP stressed populations. It expression is also demonstrated to correlate with the transformed phenotype of mouse

cells (Iacovoni et al. 2004), however it is unclear whether this is an effector molecule or merely a marker of growth initiated by other oncogenes such as c-jun.

Not all genes investigated appeared beneficial, with a number of genes identified in both stressed populations suggesting a deleterious function at the levels expressed at this time point. For example, the transcription factor BMI1 is found to be upregulated in cancer, and its depletion (as seen in post-stress populations) is known to sensitize tumor cells to apoptosis (Alajez et al. 2009; Nowak et al. 2006). Similarly, receptor Fr2 (aka PAR1) is known to mediate antiapoptotic signals, yet is also found downregulated at day 5 (Yang et al. 2009; Guo et al. 2004). However, as both are found downregulated in stressed populations, it may be a result of this process or the side effect of a beneficial gene's expression.

Similarly to pcDNA stressed cells, genes responsive to oxidative stress and those involved in glutathione (a cellular anti-oxidant) metabolism. While this may be a similar reaction to this particular cellular stress, the genes involved vary to those dysregulated in pcDNA. Also, while pcDNA stressed cells produced a more definite pattern of upregulation in this area, the response here is a mixture of both up and downregulated genes. Genes upregulated include glutathione synthase (GSS), glutathione S-transferase omega 1 (GSTO1), GCLM (modulates the activity of another Glu anabolic enzyme) and heme oxygenase (HMOX1). Downregulated genes include glutathione peroxidise (Gpx1), glutathione S-transferases 1 & 7 (Gstm1/7) and IDH1 (demonstrated as having a protective effect with regards oxidative stress; Lee et al. 2002). The downregulation of genes such as these does not appear to be an optimal response, and is strange considering the more uniform response in this regard in pcDNA stressed cells. An interesting related gene involved in protein synthesis, QARS, was found to have an antiapoptotic role. Upregulated in this instance, it was determined to prevent the activity of the apoptotic signal ASK1 with increases in a glutamine depend fashion, and may have a positive impact in this instance (Ko et al. 2001).

A response unique to TBP stressed cells compared to the control stress is the downregulation of the majority (22 out of 24) of genes enriched for involvement in lipid, sterol and terpenoid (steroid precursor) metabolic and synthesis pathways. With the exception of upregulated CYB5R1 (involved in the desaturation of fatty acids) and CMAS (a transferase for a precursor of glycolipid production), the remainder are between 1.21 and 1.84 downregulated. Examples include HMGCS1, HMGCR, Insig1 and FDPS (all involved in various stages or cholesterol biosynthesis and its regulation) and others involved in the synthesis of fatty acids such as Fasn, Cyb5r3 and Cyb5r1. The sustained expression of these genes may be unnecessary for improved growth and survival, and this reduction in expression appears to be tolerated compared to the less successful unstressed population. This may also mark a shift from energy sources derived from fatty acid catabolism to leave more key substrates available for glycolysis and carbohydrate based forms of energy production, however we see no substantial changes in the regulation of respiratory genes. After an investigation into the function of these genes the phenotypic advantage in their reduction of expression for all these genes is not immediately obvious, making speculation difficult, with future metabolome analysis a possibly useful means to investigate this phenomenon.

In these patterns of gene expression brought about by the stress procedure we find small changes in a wide variety of genes in order to affect phenotype, subtly shifting the metabolic landscape to better respond to the environmental stresses inflicted. Larger changes in gene expression are likely not tolerated and are generally not found in these comparisons. When investigated, many genes with significant fold change did not appear to hold important functions or appear likely to substantially affect phenotype in isolation, and were possibly a side effect or marker of the function of more important dysregulated gene targets (or in some cases expressed at a small enough level for dysregulation to produce a significant fold change). Examples include NCAM1 (a cell adhesion molecule and the most upregulated in this instance, 6.5 fold), CD36 (a cell surface antigen, found here <9 fold downregulated), and WDFY4 (found downregulated 2-4 fold in stressed populations). These genes do not appear to hold substantial function outside of *in vivo* tissue situations.

3.2.19.6 Selected Clones v pcDNA(S)

B3/L(S)2 comparison – 1440 genes (726 down, 714 up)

B4/L(S)3 comparison – 998 genes (556 down, 442 up)

C4/T(S)1 comparison – 1303 genes (640 down, 663 up)

D1/T(S)2 comparison – 942 genes (461 down, 481 up)

Comparisons examining the unique dysregulated genes of selected stressed clones compared to the stressed control

The next comparison was carried out to investigate the changes in expression found in the four selected advantageous clones. Each was compared to the stressed pcDNA to control for the generic changes brought about by the stress procedure that have been identified, and allow us to isolate targets that are found in a number or indeed all of these more unique cell lines that may have contributed to their improvement.

The most obvious characteristic of this comparison is the gross number of genes found dysregulated; between 942 and 1440 from the lowest and highest levels of gene changes. This appears appropriate for two reasons; first because they are the phenotypically the highest performing cell lines from this study and are likely to have undergone more changes in their transcriptome to alter their phenotype. Secondly is the fact that they are clonally derived cell lines. While the profile of the mixed populations represents the response from an entire heterogeneous mix of cells, these cell lines represent a more specific and distinct profile that is by definition more likely to vary from the average.

The first step in analysing this data was to take a broad look at the bioprocesses affected in these clones compared to the stressed control. In general, the processes found affected resemble the functional groups most dysregulated in the stressed populations previously identified. Again, perturbed genes appear in a wide of metabolic processes in order to generate the improved phenotype we see in these cells. Cell cycle progress, transcription and translation, RNA metabolism and processing, lipid metabolism and glutathione/oxidative stress all appear as the most common areas of dysregulation across the four beneficial clones isolated.

Similarly to TBP transfected cells, we see a reduction across the board in genes relating to mitotic and cell cycle processes such as spindle assembly, chromosome

alignment and Cyclins. As with the peak density reached in TBP cells, this relative downregulation is possibly a reaction to the previous high activity of these genes in the preceding days of exponential growth.

A common feature noted in stressed populations is once again highlighted in the downregulation of a range of genes involved in lipid and sterol metabolism. Across the 4 cell lines a key group of 28 genes is found downregulated, most pertaining to specific enzymatic functions such as ACAT2 (aceyltransferase), SC4MOL (sterol monooxegenase), EBP (sterol isomerise) and SQLE (squalene monooxygenase). As noted in TBP selected mixed cells, determining any advantages related to the suppression of this area of metabolism is difficult. One paper describes a feedback loop in cholesterol production mediated by a number of genes in the mevalonate pathway that is known to affect growth (Brown and Goldstein 1980). A number of genes prominently downregulated are concentrated in this pathway, including the rate-limiting HMG-CoA Reductase (average 2 fold down), HMG-CoA Synthase (average 2 fold down), the LDL receptor (average 1.8 fold down) and thiolase subunits HADHA and HADHB. Ultimately, a reduction in the production of mevalonate is shown to induce G1 arrest and a reduction in DNA synthesis (Elson et al. 1999; Kawata et al. 1994). While we can infer from the data that these changes are somehow beneficial, the mechanism is unclear at this time.

One likely downstream effect of reduced cholesterol synthesis is changes to the lipid membranes composing cellular structures. Changes in cholesterol and lipid concentrations affect membrane fluidity and the organisation of lipid rafts (Sinensky 1978), and in selected cells this may be linked to changes in expression and activity in genes related to intracellular transport (Caliceti et al. 2012), protein localisation and signalling amongst others (Hao et al. 2001; Spector and Yorek 1985). In this case greater reductions in gene expression associated with cholesterol metabolism are also correlated with increased numbers of these dysregulated genes (for example the differences between samples L(S)2 and T(S)2, with the former maintaining the reduced levels of expression for genes such as ACAT2 and HMGCR etc, and more than twice the number of dysregulated genes involved in intracellular transport).

Another possible change in the cell resulting from decreased cholesterol is the increase in cellular oxygen concentration. A study was carried out demonstrating an increased oxygen gradient with the inclusion of higher levels of cholesterol in plasma membranes, with lower concentrations permitting an increase in oxygen diffusion into cells (Khan et al. 2003). This may be the case in the selected clones we have isolated in this instance, however an increase in cellular oxygen is contradicted by the hampered response in genes related to oxidative stress. In general, we would expect this response to increase. Similarly to TBP stressed cells we see a mixed response, with some increases in selected genes unique to each of the 4 lines tested (for example, DNA damage induced Ddit3 in T(S)1. However the trend is generally a reduction in these genes compared to the stressed pcDNA cell line in this comparison. This may suggest a reduced capacity to cope with oxidative damage (for example IDH1, previously demonstrated as providing a protective benefit, is consistently downregulated in all selected clones), or reduced need for such a response. However, elevated levels of reactive oxygen species (ROS) have also been shown to promote tumorigenesis and stimulate growth via activation of signalling pathways such as NF-KB (Hsu et al. 2000). Downregulation of genes such as Catalase and SOD (both found between 1.23 and 1.94 downregulated in all selected clones) have been demonstrated to induce such effects via the modulation of ROS concentrations (Reiners et al. 1991; Gupta et al. 2001), and may contribute the improved growth and survival we see here. Evidence of NF-KB over activity in promoting growth is also seen in the persistent downregulation of the NF-KB inhibitor NFKBIA, a putative tumor suppressor gene that is >2-fold downregulated in all selected clones (Cabannes et al. 1999).

Another area of significant dysregulation is the processing and transport of mRNA. Across the four cell lines investigated, 3 have significant numbers of genes dysregulated in this area (>30) with the remaining clone (T(S)2) containing ~20 affected genes. While the exact compliment of different dysregulated genes in this area are different in each cell line, on average two thirds of each group are found to be upregulated. In a subset of two clones, a group of Polymerase II related genes were also found to be upregulated, suggesting a possible increase in activity in the processes involved in mRNA generation and processing. While many genes did not provide compelling evidence to link them to a direct improvement in phenotype, some

examples were found that may impact in a positive way. For example, PRMT5 is implicated in the repression of genes involved in cell cycle progression and proliferation (Pal et al. 2004). Its downregulation in all clones tested may suggest the upregulation of these genes and related improvements in cell proliferation. The gene THOC7 is also upregulated in selected clones. Forming part of the TREX mRNA transport complex, it is found upregulated in certain aggressive breast cancers and may play a role in promoting growth (Guo et al. 2005). The expression of a number of genes known as 'heterogeneous nuclear ribonucleoproteins' (HNRNP's) are also found to be perturbed, including HNRNPs-A,D,K and P (downregulated) and HNRNPs C, H and N (upregulated). They play a number of functional roles, including splicing, mRNA transport and inhibition of apoptosis (Choi et al. 1986). Upregulation of HNRNPC is also linked to expression of XIAP (noted in clone L(S)3), which is known to inhibit apoptosis. Conversely, HNRNPK expression is known to stimulate c-*myc* expression, and both are found ~1.2 downregulated in T(S)2 (Carpenter et al. 2006). This may be detrimental, as c-*myc* is known to increase proliferation and is slightly upregulated in the other selected cell lines (Ifandi and Al-Rubeai 2003). Related to the area of mRNA processing are the genes related to Polymerase II transcription expressed in a subset (L(S)2 and C3) of the clones analysed. These are almost exclusively upregulated, and suggest at an increase in Pol II activity. These include subunits of RNA polymerase II (POLR2F, POLR2G, POLR2L), transcription factor TBP-like protein (TBPL1) and general transcription factors GTF2A (known to interact with TBP and TBPL1) and GFT2A2. These genes are correlated with the downregulation of SSU72 (found to be in 3 of the samples tested). As a phosphatase, it plays an apparent role in limiting the rate of polymerase II activity by functioning as a molecular 'brake' (Dichtl et al. 2002). Together with the pattern of gene expression we see here, its downregulation may play a role in increasing the expression of a number of Pol II transcribed genes.

A number of genes previously associated with improved growth and survival in TBP and pcDNA selected cells were also found at levels likely to be advantageous in at least one or more selected clones. For example, the oncogenic protein BMI1 (associated with high growth in certain tumors) is found upregulated between 1.4 and 2.3 fold in all selected clones. The pro-apoptotic gene HERPUD1 is downregulated in selected cells, an effect likely to impact cell survival. Plac8 (aka Onzin), a gene found

whose expression is found to vary in to a large degree, is found between 1.6 and 17.3 fold upregulated, and its overexpression may promote cell survival. CyclinD1 is found upregulated between 1.5 and 3.3 fold upregulated, and its activity in cell cycle progression may benefit or be indicative of growth.

While these are the most common across all selected clones, other potentially beneficial targets are found in one or two of the advantageous cell lines such as PEDF (downregulated in L(S)2/T(S)1), DUSP1 (downregulated in L(S)3/T(S)1), GADD45A (upregulated in L(S)2/T(S)1) and AATF (upregulated in T(S)1 apoptotic protection; Xie and Guo 2006).

3.2.19.7 Stressed v Unstressed populations – 292 genes (203 down, 89 up)

Comparison examining most prominent gene changes related to the stress procedure

Previous comparisons were originally intended to examine the overall effects of discreet steps of the experiment by comparing single cultures to one another (such as the effect of TBP overexpression, or the effects of stress on both control and transfected cell lines). While the genetic interventions carried out were shown to elicit demonstrable phenotypic changes, benefits were mainly gained as a result of their combination with the stress procedure. To investigate the most common genetic changes underlying the benefits gained from the stress procedure a comparison was carried out that combined sample sets incorporating all stressed (pcDNA-S, TBP-S, selected clones) and unstressed (pcDNA-U, TBP-U, CHO-K1, CHO-SEAP) cultures. The average number of viable cells in each group was found to be significantly different at the day 5 time point and beyond (p=0.01) when the combined average was measured so, by comparing a larger combined number of different samples under this one common characteristic, this comparison was hoped to further elucidate the common differences in expression that were brought about by the effects of stress selection.

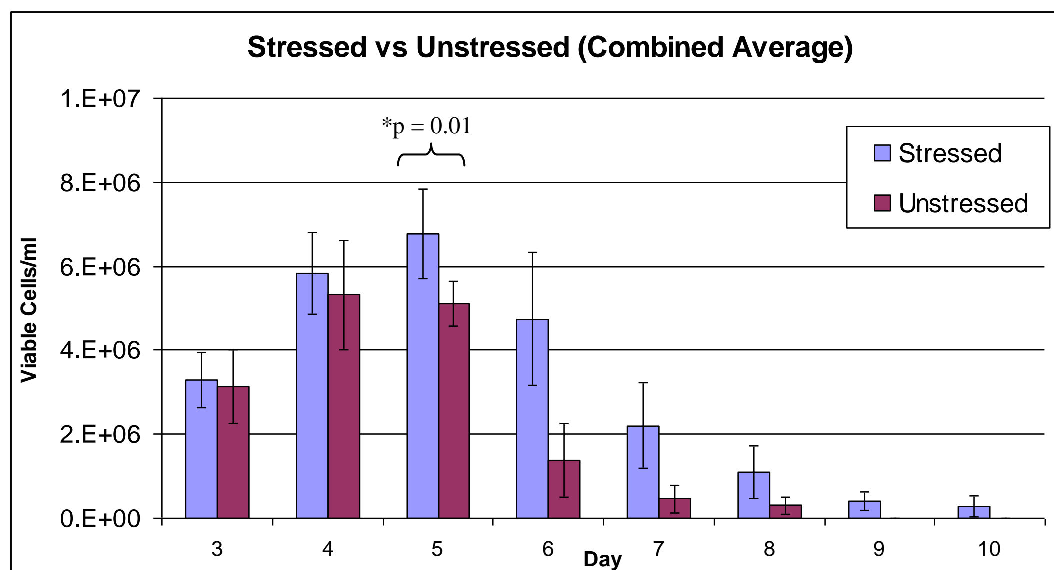

Figure 3.2.19.7.1: Graph displaying the average viable cell concentrations of the grouped stressed and unstressed populations when analysed together, with error representing the standard deviation of viable cells within each group. The most significant differences between stressed and unstressed populations appear from day 5 onwards (when RNA sampling occurred, p=0.01), suggesting group analysis of these samples may expose the genetic elements most common to their phenotypes.

Using this method of analysis we see a smaller cohort of genes significantly associated with the changes elicited by stress selection (203 down- and 89 up-regulated). This is to be expected due to the wider range of phenotypically distinct samples contributing to each sample set. This variety of input also provides us with a greater level of statistical power in determining the more significant genes and pathways associated with improvements in phenotype.

When the pathways affected were analysed, the only pathways highlighted for with significant adjusted p-values (<0.05, Benjamini Hochberg method) were those involved in lipid metabolism (sterol, steroid, lipid and cholesterol biosynthesis/metabolism). These categories encompassed a subgroup of 22 downregulated genes. Once again, this was strongly indicative that the reduction in the expression and activity of this specific category of genes is related. A selection of these genes are involved in fatty acid synthesis (SCD1,) elongation (TECR) and desaturation (SCD3, DEGS1).
At least 12 genes identified play a direct role in the synthesis and uptake of cholesterol;

- **Mevalonate pathway** - HMG-CoA Synthase (HMGCS), HMG-CoA Reductase (HMGCR), isopentenyl-diphosphate delta isomerise (IDI1), farnesyl diphosphate synthase (FDPS), farnesyl-diphosphate farnesyltransferase (FDFT1), squalene epoxidase (SQLE)
- **Post-squalene sterol modification** - Emopamil binding protein (EBP), sterol 14 alpha-demethylase (CYP51), sterol-C5-desaturase (SC5D), sterol-C4-methyl oxidase (SC4MOL), NADH-cytochrome b5 reductase 3 (CYB5R3) (Fukushima, Grinstead and Gaylor 1980; Herman 2003)
- **Cholesterol uptake -** LDL receptor (LDLR), acyl-CoA:cholesterol acyltransferase (ACAT2, deficiency is shown to reduce cholesterol uptake; Buhman et al. 2000; Repa et al. 2004)

These proteins play key roles in pathways that ultimately lead to increases in the concentration of intracellular cholesterol. As speculated previously, a reduction in cholesterol and the activity of these pathways may affect a number of characteristics, such as membrane fluidity, oxygen permeability and intracellular transport rates.

A study by Lee et al. (2011) measured the effects of cholesterol accumulation in CHO, and its effects on oxidative stress and related apoptotic pathways. This work followed from previous studies demonstrating the link between increased cholesterol concentrations and oxidative stress (Sanguigni et al. 2002). Experiments were carried out to show that mutants with naturally higher levels of free cholesterol were more susceptible to induced oxidative stress via menadione induced production of superoxide free radicals and nitric oxide. Dramatically increased levels of these reactive species then induced greater levels of apoptotic cell death, marking the downstream negative consequence of increased cholesterol accumulation. Free cholesterol is also found to aggravate this response by further inhibiting antioxidant enzyme activity, such as catalase, glutathione peroxidase and superoxide dismutase (Lu and Chiang 2001). Interestingly, these 3 genes are also marked as downregulated in the majority of stressed cells (between 1.2 and 1.36 fold down). However, with data suggesting the downregulation of cholesterol synthesis, this may be a reaction to a reduced level of intracellular oxidative stress. This idea is supported when we look at the pcDNA stressed population. As the only stressed population with no significant change in lipid metabolic enzymes, it also demonstrates a stress response in which the portion of genes related to oxidative stress are most upregulated. This is in contrast to other TBP and Library transfected cell lines which appear to make greater phenotypic gains with reductions in cholesterol metabolism, suggesting that this is a more optimal response in maintaining survival.

Of course, as we have seen previously the improvements to phenotype seen here are the result of a wide range of interacting genes generated by the transcriptomic shift (affected by both genetic modification and stress procedure). As this comparison is the result from a wider range of distinct cell lines than previous comparisons, fewer affected genes and pathways are identified with statistical significance (with the exception of those discussed above). Nevertheless, a number of single genes with more significant fold-changes that were noted previously are also present in this list, including SPRR1A and Apoptosis-antagonizing transcription factor (upregulated), as well as GAS1, DUSP1 and ERCC1 (downregulated). As well as being noted in previous binary comparisons, the presence of genes such as this in this comparison may signify a more significant relationship with the beneficial effects of stress, marking them as potential targets for future engineering work.

3.2.19.8 Profiling conclusions

Having generated a number of beneficial cell lines (both clonal and mixed populations), this experiment was carried out to give an oversight into the numbers and types of genes that were found to be affected by both the genetic intervention and stress procedure. From this data, we can make the following conclusions;

- In almost all cases, changes in phenotype after the stress procedure were brought about by large numbers of small changes in expression. This method of reaching a complex phenotype is in contrast to single gene engineering approaches, in which a smaller number of genes have a more direct effect on a discreet selection of cellular processes (also seen here in the unstressed TBP population).
- Phenotypically the effects of TBP transfection alone are minimal, as is the stress procedure. However, when combined we see a synergistic improvement in selected mixed populations and resulting clones. This synergy is reflected in the wider range of dysregulated genes in these cell lines.
- Significantly affected areas of gene expression include cell cycle, regulation of growth and apoptosis, protein translation, mRNA processing, response to oxidative stress and lipid metabolism. Cholesterol synthesis proved to be a prominent area of dysregulation associated with improved phenotype, and is worthy of further investigation.
- The selected day 5 time point provided a look at mid culture gene expression. However, further work would best incorporate samples in both exponential (day 3) and decline (day 7) samples. This would give a more detailed look at the specific genes involved in growth to high density that are likely deactivated by day 5, while the later sample would give a better indication of the genes activated when environmental stresses are more prominent.
- While previous experiments indicate a benefit to using a mutant library over the wild type expression of TBP, the data generated here from only 2 clones is not enough to determine the genetic mechanisms underlying this. Instead, all advantageous clones were analysed together to determine the common features that separate them from unstressed and control populations with poorer performance.

Section 4.0

Discussion, Conclusions & Future Work

4.1 Viral Targeting Discussion

4.1.1 Summary of results

This study was originally conceived as an investigation into the use of adeno-associated viral vectors and their potential application for gene targeting in a CHO-K1 cell line. For this study, a pair of model genes (*DHFR* and *HPRT*) was targeted using recombinant AAV vectors. These were generated to contain upwards of 1800 bp of DNA sequence homologous to a specific region in the genes targeted to promote the occurrence of homologous recombination. This, as demonstrated previously in other studies using AAV in mammalian cell lines, can be used effectively to introduce pre-determined mutations, deletions and insertions at specific chromosomal loci, facilitating the precise modification of the CHO genome. This technique also permits targeting rates higher than those acquired using similar plasmid based vectors whilst also using considerably smaller homologous sequences.

Initially, targeting vectors were constructed containing a selectable marker flanked by two homologous arm sequences. A 4.7 kb upper limit recommended for efficient packaging has been demonstrated (Dong, Fan and Frizzell 1996). In these instances, both *DHFR* (a 4186 bp constructed viral genome, including 2333 bp of homologous sequence) and *HPRT* (a 3710 bp constructed viral genome, including 1855 bp of homologous sequence) vector constructs were designed to remain under this limit and avoid the negative effects on viral propagation associated with oversized vectors. These constructs were used to successfully generate viral stocks, which were then concentrated and used to transduce CHO-K1 cells. These transduced cells were then placed under G418 selection to remove the untransduced cell population.

A control GFP virus was used to estimate the number of viral particles produced using the method followed, resulting in a final estimated MOI of 2.08×10^5 viral particles per cell applied in actual targeting experiments. The GFP virus was also used to verify the functional ability of AAV to successfully transduce CHO cells, with an MOI of less than 1000 particles per cell found to generate GFP fluorescence in 74.8% of cells.

A PCR screen was carried out on DHFR targeted clones in order to estimate the frequency of targeted homologous recombination events in comparison to the total number of recombinants. 195 clones were isolated with viral integration events, of these 5.6% had correctly integrated the 5' arm, while 3.1% of the total fraction (6 clones) were found to have integrated both 5' and 3' homologous arms, resulting a number of cell lines featuring a correctly targeted locus.

Unfortunately, a screen in selective medium for both *DHFR* and *HPRT* targeted cells did not result in the discovery of homozygous knockout clones. qPCR and PCR screening of heterozygous DHFR (+/-) clones confirmed a functional allele of the gene existing within the cells characterised. A qPCR technique was also employed to estimate the frequency of off-target insertions in a number of correctly and incorrectly targeted clones. Of the 8 clones analysed, an average number of 24 integration events per clone was reported, with the highest insert number clone containing 77 inserts. This high frequency of random integration is likely due to the high MOI used in this study, with targeted as well as random integration known to increase in frequency with increases in MOI. This result may also have been augmented by the use of the drug based selective procedure used to enrich for cells having integrated viral genomes, skewing the population toward high insert copy numbers. Such high copy numbers may be a disadvantage in gene knockout experiments such as this, but may prove a useful method for delivering a product transgene or other situations where high copy numbers are beneficial.

Alternative techniques were then explored in an attempt to generate null mutants. These included a protocol in which cells are subjected to a high concentration pulse of selective antibiotic to provoke a number of cellular mechanisms such as chromosomal duplication or gene conversion, potentially inducing cells to become homozygous at the targeted locus. A second protocol was also employed, in which a secondary targeting vector was constructed. This virus was generated and applied in the same manner as previous virus vectors in an attempt to target and disrupt the second allele. Unfortunately, neither of these further techniques was successful in isolating a true null (homozygous negative) mutant.

4.1.2 Improving targeting frequency

The targeting efficiency for single correctly targeted alleles achieved in this study was found to be over 3% of cells with viral integration events. This compares favourably to the targeting frequency of up to 1% that was originally reported in early landmark targeting papers (Hirata et al. 2002; Russell and Hirata 1998), and would suggest that the protocol employed in this study is a good starting point for further experiments investigating the use of AAV for gene targeting in CHO cells. However, this result is from an unselected population, and represents 0.7% of all cells transfected. To examine this figure critically, we can compare it to a similar study in human fibroblasts (Hirata et al. 2002) in which G418 was used to enrich for targeted clones. Two separate attempts at this experiment yielded efficiencies of 1 in 8 and 1 in 23 (12.5% and 4.3% respectively). While this initial instance of targeting in CHO did not reach these levels of efficiency, the use of a selectable marker successfully allowed us to reduce the population necessary for screening to a manageable level.
As transduction conditions were generally kept consistent in this study, it is difficult to theorise how different factors, such as the relatively high MOI employed or the length of targeting arms used, may have affected the final outcome. However, a number of optimisations to the process of viral gene targeting have been performed, demonstrably improving targeting frequency using AAV in mammalian cell lines. These modifications could potentially be applied to further uses of AAV based gene targeting in CHO cells in order to improve targeting efficiency.

Increasing MOI

In this study, a relatively high MOI of 2.08×10^5 was used to ultimately generate the *DHFR* targeted cell lines that were isolated. This compared favourably to the original study in human fibroblasts by Russell and Hirata (1998), in which a similarly high number of viral particles (3×10^5) resulted in a 0.7% targeting rate.
In this example, decreases in MOI directly resulted in reduced targeting rates. This dose dependency relating targeting rates to viral particles applied has been confirmed in other studies examining the effects of varying MOI rates (Vasileva, Linden and Jessberger 2006; Porteus et al. 2003)

These results would lead to the conclusion that an increased MOI is favourable for isolating clones with targeted genetic loci. However, generation and purification of such high levels of virus may not be feasible or even necessary for some studies, and successful targeting has been demonstrated at MOIs as low as 2000 (Hirata and Russell 2000).

Increasing arm size

The *DHRF* targeting vector used in this study on which targeting efficiency results are based contains 2333 bp worth of sequence homology to the target locus. From the results achieved, this would appear to be sufficient for correct targeting, and is comparable to previous successful studies utilising AAV targeting. Improved targeted rates have been demonstrated in studies evaluating the recombination potential of vectors containing different sized regions of homology. In one example, two to five-fold increases in targeting are gained by increasing the region of locus homology used from 1693 bp to 2988 (Hirata and Russell 2000). Another study performed to introduce a single base pair gene correction found significant increases in targeting with the downstream (3') arm size increasing from 205 to 435 bp, coupled with a 1900 bp upstream (5') region of homology (Liu et al. 2004). A similar gene repair study reported a 2-fold increase in 3′ homology resulting in a 23-fold increase in targeting at a high vector dose, with this improvement becoming more apparent (up to 73-fold) at lower doses.

Due to the packaging limit of AAV vectors, vector construction is ultimately a trade off between the size of expression cassette that is to be inserted within the vector and the remaining space available for homologous sequence. For small transgenes, such as the selection marker included in this study, this limit does not pose a problem and allows for large regions of homology to be used. For attempts at gene knockout a large inserted transgene is unnecessary. However for situations in which the locus is to be targeted for transgene expression, this can be mediated via RCME (recombinase mediated cassette exchange; Oumard et al. 2006). To achieve this, LoxP sites can be included in the vector. Once a suitably targeted clone is generated, Cre recombinase is then transfected along with a secondary plasmid vector containing the gene of interest. Cre-Lox recombination then promotes inclusion of the secondary vector at this target site, bypassing the need for an overly large transgene to be included on the original

targeting vector (Turan et al. 2011). An elegant method devised to overcome this size limit has also been successfully employed in which 2 separate vectors are co-transduced, each encoding a separate element of the final cassette. Intracellular concatemerisation then links these viruses, allowing them to integrate as a single construct (Ghosh et al. 2008; Duan et al. 2000). This property of co-transduced viruses has not been explored yet with regard to gene targeting.

Inducing double stranded DNA breaks

Due to the nature of homologous recombination used by AAV vectors to generate targeted integrations, methods have been devised in order to exploit the cellular mechanisms used to induce homologous recombination and increase the proportion of correctly targeted cells. Protocols such as this generally seek to provoke the mechanisms involved in double stranded break (DSB) repair, one of the key apparatus by which cells maintain the integrity of their genomes. Prior to this technique being used in conjunction with viral targeting, this method was studied in yeast and mouse cells using a plasmid targeting vector. In one example, a homing endonuclease I-*Sce*I is expressed on a co-transfected plasmid. By inducing a DSB at the *HPRT* locus, this was found to increase targeted recombination rate 5000 fold higher in mouse embryonic stem cells than a control line without the endonuclease cleavage site (Donoho, Jasin and Berg 1998).

Utilising this strategy in combination with homologous AAV vectors has proved successful at improving targeting rates. In a study utilising a I-*Sce*I cleavage site, AAV gene targeting was roughly 100-fold higher in human HT1080 cells (Miller, Petek and Russell 2003), with a similar substantial increase in defective GFP correction found in HEK cells (Hirsch et al. 2010) utilising a similar I-*SceI* expression system.

The main drawback to this system however, is the need for a suitable endonuclease site at the target locus. However, the advent of zinc-finger nuclease technology has allowed us to generate DNA cleavage enzymes recognising almost any custom sequence. While this AAV and nuclease combination has proven useful in a clinical setting for introducing targeted insertions (Ellis et al. 2012), it seems likely that this approach could be successfully implemented for genome editing in cultured, industrially relevant cell lines.

Inhibition of NHEJ

Induction of DSB repair mechanisms has been shown to increase targeting efficiency. Conversely it has also been found that by inhibiting the action of a competing DNA repair mechanism, non-homologous end joining (NHEJ), increases in gene targeting rates could also be achieved. In one study the protein Ku70, known to be involved in the NHEJ mechanism, was knocked down using siRNA interference in a human cell line. This resulted in a 5-10 fold increase in successful gene targeting for a variety of loci (Fattah et al. 2008). A similar study including knockdown of both Ku70 and Xrcc4 NHEJ proteins reported an 11-fold increase in targeted HR events, with a simultaneous 70% reduction in non-homologous random integration (Bertolini et al. 2009). Methods such as this prove to be an attractive means of increasing gene targeting rates with the added benefit of reducing off-target integrations.

Exploiting cell cycle sensitivity

Two unrelated studies conducted in the 1980's revealed an interesting property of viral transduction and homologous targeting rates, namely that both are found to be more efficient in cells in the S-phase portion of the cell cycle (Russell, Miller and Alexander 1995; Wong and Capecchi 1987). This is suggested to relate to the competing DNA repair mechanisms of HR and NHEJ, with the HR machinery found to be preferentially active in proliferating mid cell-cycle populations.

This property has since been exploited to demonstrate that AAV mediated gene targeting is indeed more efficient, with one study exhibiting a 1000-fold increase in dividing cells compared to their quiescent counterparts (Trobridge, Hirata and Russell 2005). This effect was also noted in studies in which 2mM thymidine was used to enrich for cells in the G_1/S cell cycle phase, resulting in between a 2 and 24 fold increase in successful gene targeting (Liu et al. 2004; Saleh-Gohari and Helleday 2004).

Enrichment methods such as this would prove useful due to their simple unintrusive nature requiring only a chemical addition to medium, and could easily be explored as a method of improving targeting rates in CHO cells. Methods such as those described above could also be implemented in tandem, hopefully in order to generate a synergistic response in which each factor contributes to increased gene targeting rates.

4.1.3 Generating a homozygous knockout cell line

Unfortunately, a homozygous knockout cell line was not isolated in this study despite a number of alternative mechanisms explored in order to target or otherwise disrupt the second allele. Prior to further experimentation in CHO using AAV vectors, we can only speculate as to the potential reasons why this failure occurred based on observations in similar experiments and the mechanisms involved in homologous recombination.

One experiment involved the use of a secondary targeting vector, identical in target locus homology to the original albeit with a different selectable marker. This was applied under identical conditions to those used for the initial targeting. However, subsequent analysis of the cell line used for this secondary targeting determined the presence of between 17 and 20 random *neo* insertions, possibly linked to the remaining homologous *DHFR* vector sequence. These 'pseudo' target insertions may have served to increase the number of possible recombination loci for the secondary vector due to their shared homology, diluting the possibility of targeting the true *DHFR* secondary allele.

The protocol examining the use of a high pulse of selective drug to induce recombination at the targeted locus also failed to isolate null mutants. The surviving clone, *1H1*, likely survived the high concentration of G418 due to the high number of *neo* insertions measured in its genome. Unfortunately data on the number of cassette insertions was unavailable when this technique was considered. With an estimated mutation rate of 1.3×10^{-5} per cell generation for this technique (Mortensen et al. 1992), it is also possible that any successful mutants were not found due to an insufficient number of clones screened. A future repeat of this experiment would be best suited to a targeted clone with no extraneous insertions. Also, it is unknown at this time whether the peculiar chromosome structure and aneuploidy of CHO derived cell lines (Wurm and Hacker 2011) would possibly affect the likely hood of recombination between chromosomes necessary for this form of mutation.

Due to high incidence of non-homologous integrants, this may be indicative of the cell cycle phase in which the majority of cells were transduced at the time of experimentation. Non-dividing cells have been demonstrated to incorporate insertions preferentially using the NHEJ pathway (as opposed to the homologous recombination that is more prevalent in S-phase cells), and modifications to any future protocols followed could involve the use of techniques demonstrated previously to enrich for S-phase cells. An examination of cell cycling in CHO to determine an optimum time post seeding to transduce the cells would also prove useful, as the 24 hours post-seeding transduction used in this experiment may not be the optimum.

From the data generated, the high incidence of off-target random insertions seems a likely factor in the inability to successfully isolate null CHO mutants in the experiments carried out. While a high MOI was initially used in this experiment as a means of increasing gene targeting rates, this variable should be carefully considered in future CHO gene targeting research. A more successful approach would likely combine a reduction in MOI with the use of techniques described above to both increase targeted recombination rates and reduce the occurrence of random NHEJ insertions. It is improbable that techniques successfully implemented in other mammalian cells could be found impossible to implement in CHO, and further work using optimised experimental protocols based on the knowledge gained in this attempt is likely to yield success.

4.1.4 Potential uses for targeted cell lines

Gene knockout

Gene targeting has two primary potential functions in industrially relevant cell lines. The first is gene knockout, in which both alleles are targeted to remove the effect of this gene on the cellular phenotype. This is applicable for any gene deemed to be detrimental to growth or productivity for example, and as expected total gene knockout provides superior results compared to techniques such as RNAi based gene knockdown (Li et al. 2010). In most cases, this would also require the use of sequential targeting methods in order to generate homozygous knockouts in order to completely ablate gene function.

Targeted transgene insertions

Another potential use for gene targeting is the insertion and expression of a target transgene (for example, a therapeutic protein product) at a predetermined genomic location. This would hold a number of benefits with regards to the creation of industrial producer cell lines, including eliminating the range of expression variability found with random insertions associated with common transfection methods. Position effects, such as the influences of nearby regulatory elements or the chromatin structure of that target site, can impact negatively on gene expression and stability over time. While targeted gene integration will not remove these effects, the discovery of a suitable genetic locus that is found to promote good levels of gene expression means that predictable, reproducible results can be achieved in successive experiments. Discovery of such a genomic locus could be mediated through methods such as random screens (Mielke et al. 1996), or via the use of *in silico* bioinformatic methods to predict positions with beneficial characteristics, such as the presence of S/MAR elements that may shield a transgene from inactivation (Frisch et al. 2002).

Correctly employing this technique could then be used to generate a correctly targeted 'master' cell line, whose expression locus could be targeted with a number of different product genes. This would combine the benefit of predictably high levels of expression with a drastic reduction in the levels of screening necessary to select clones suitable for a bioprocess, as the phenotype of the master producer cell line could be characterised and preselected for advantageous properties.

Figure 4.1.4.1 below demonstrates the process by which a correctly targeted cell line expressing a gene of interest is generated by first targeting a specific locus, generating a single targetable LoxP site and subsequent introduction of the gene of interest. This method has proven successful in CHO for generating targeted cell lines with predictable gene expression properties (Qiao et al. 2009; Fukushige and Sauer 1992), however the generation of the primary targeted site using traditional plasmid based recombination is a drawback due to the inherent difficulty of homologous targeting using plasmid vectors compared to AAV targeting. By combining this protocol with the advantages of AAV gene targeting with regards to efficiency, this could prove an interesting method for generating a useful, versatile system for protein production.

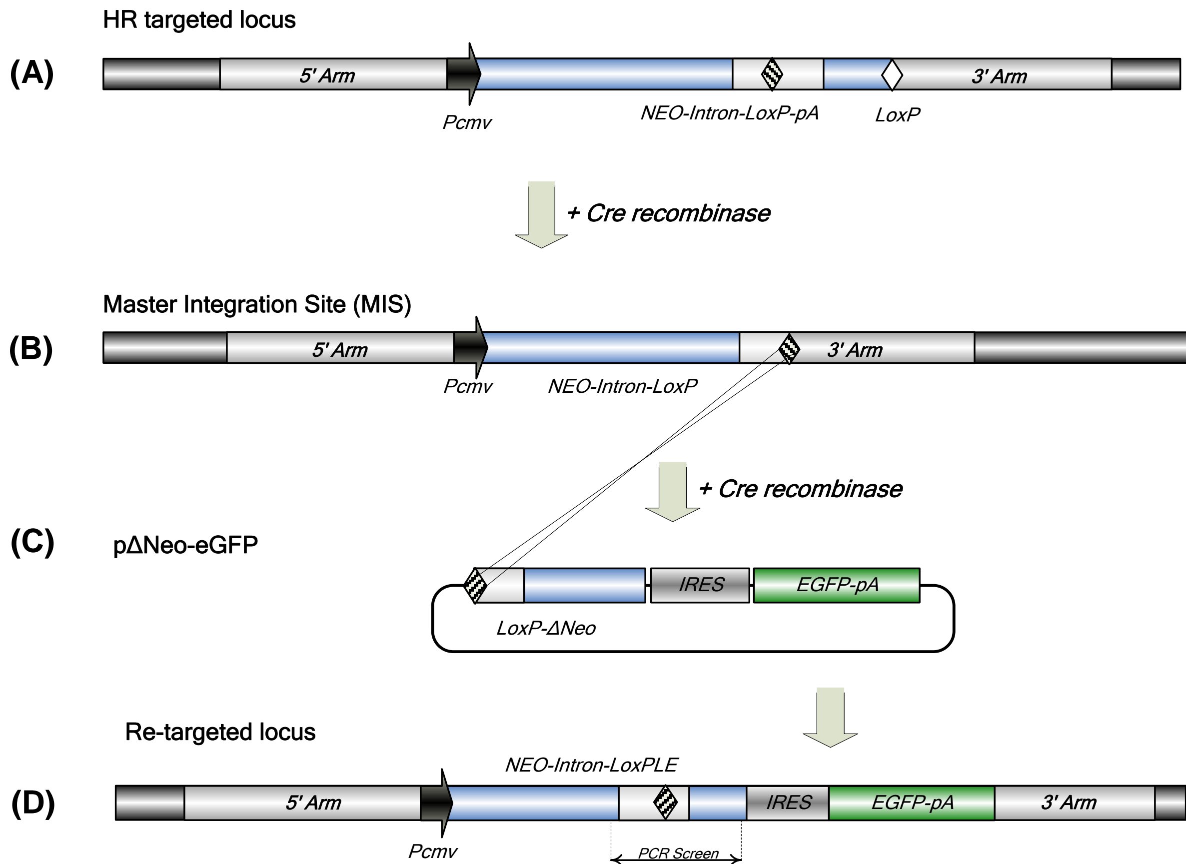

Figure 4.1.4.1: A brief description of the generation of a 'master' targeted expression system, and the introduction of a model gene of interest (GFP).

(A) Singly targeted cell line is first generated. The cassette configuration in this example is identical to that used in this experiment.

(B) Expression of Cre recombinase within the cell catalyses the excision of the sequence between the two artificial LoxP sites, leaving 1 remaining targetable 'Master' LoxP integration site and simultaneously inactivating the *neo* resistance marker.

(C) The re-addition of Cre recombinase in the presence of a vector constructed to contain a gene of interest (GFP), the Δ*neo* exon (reactivating the *neo* selection marker) and an internal ribosome entry site (IRES) permitting the expression of GFP from the CMV promoter.

(D) Cre-Lox recombination integrates this vector specifically within the target site, expressing the gene of interest in a predictable manner. G418 selection and a subsequent PCR screen at the highlighted position can also be used to determine successful recombination.

4.2 Directed Evolution Discussion

4.2.1 Summary of results

This study was originally carried out to investigate if a directed evolutionary technique known as 'Global Transcriptional Machinery Engineering' (gTME), demonstrated to generate beneficial phenotypes in *E. coli* and yeast, could be successfully applied in CHO cells. In this technique a gene is chosen for mutagenesis, ideally one involved at a high level in the cells' transcriptional activity. This is then used to generate a mutant library that, when transfected, provides a heterogeneous pool of cells. Using a suitable environmental stress, advantageous clones can then be selected for.

Mutation and Stress

Initially, a gene was selected for mutagenesis. TBP was chosen based on its almost universal involvement in the transcription of the majority of genes, providing a likely means to alter the transcriptional profile by introducing mutant copies with functional variation. After cloning the CHO TBP mRNA sequence into a plasmid expression vector, this sequence was then subjected to a low fidelity polymerase under varying conditions to induce mutations. Libraries of different mutation rates were generated, with a library of 9.5 mutations per kb (or ~9 mutations per gene sequence) chosen for transfection. This would reduce the deleterious effects of an exceedingly high mutation rate, while providing sufficient heterogeneity of mutant sequences to distinguish this library from the effects of the wild type gene. This was transformed into bacterial cells to generate a plasmid library suitable for transfection, and a library complexity of ~10^5 was measured. This library, along with an empty vector control plasmid and the wild type TBP gene were transfected separately into a CHO-K1 cell line and drug selected to generate stable cell lines.

These stable cell lines were then subjected to two separate environmental pressures for 18 passages each; one in which the culture was allowed to proceed until a low level of viability after which the remaining living cells were re-passaged, and a second in which cells were passaged at a low density to allow potentially faster growing cells to become the dominant subpopulation after exponential growth.

Clone analysis (pre- and post-stress)

After the stress procedure, single cell cloning was carried out on both pre- and post-stress mixed populations. Due to the nature of the mutant library, it was theorised that cells expressing different mutant sequences would provide a wide variety of phenotypes, with a mixed population only indicating the average performance of all its constituent cells. 12 single cell clones were isolated from each population and analysed in culture to assess the relative performance of both unstressed and stressed populations.

Pre-stress clones from parental K1, pcDNA (empty vector), wild type TBP and Library transfected clones were analysed to assess the effect of transfection without the application of a selective stress. In this instance, TBP transfection alone was found to play a beneficial role in increasing maximum cell density, ultimately leading to a greater overall performance as measured by cumulative cell hours (AIVCD). This performance was also relatively consistent across the 12 clones isolated from this population, with the smallest variance in phenotype found in TBP clones.

The Library transfected population on the other hand performed worse on average than all other comparative lines. However, this average was coupled with an extremely wide variance across the 12 clones analysed, including some potentially beneficial clones and despite the lack of any selective pressure. This is in contrast to the relative homogeneity of TBP transfected cells, confirming the hypothesis that a mutant library would produce a wider variety of phenotypes (both advantageous and deleterious).

A similar analysis was carried out on 12 clones from both post-stress TBP and Library transfected cultures to investigate if the stress procedure had indeed selected for cells with a beneficial phenotype and reduced the numbers of Library transfected clones expressing deleterious mutant TBP sequences.

In the instance of ‘viability’ stressed cultures, Library clones were found, on average, to outperform wild type TBP clones after the stress-selection procedure. A wider range of heterogeneity was still present, however a number of Library clones with AIVCD performance in the upper percentiles provided a phenotype apparently worthy of further investigation and characterisation.

In the instance of ‘low density’ stressed cultures, no significant benefit to growth rate was found to be generated in Library clones post-stress, and further work was concentrated on the more successful ‘viability’ stressed cultures.

Due to difficulties in comparing the inter-experimental performances of particular cell lines, a final experiment was performed to confirm that stress selected cells were superior when directly compared to their pre-stress counterparts. The top 3 clones from each group were compared, representing the upper potential limits of performance from each group. Compared side by side, the benefit of the stress procedure was confirmed in both TBP and Library cells, however in general the selected top performing Library clones ultimately appeared to receive the greatest benefit to phenotype. This confirmed that, while the stress procedure was beneficial to both populations, the wide heterogeneity in phenotype provided by the mutant library allowed for the selection of clones with growth characteristics superior to those provided by wild type TBP alone.

TBP concentrations (mRNA and protein)

To investigate the levels of TBP expression in both mixed populations and clonal cell lines, qPCR was carried out. Initially, in post stress mixed populations, a relatively high level of TBP overexpression was measured, with upregulation of 5.7 and 9.6 fold in TBP and Library transfected populations respectively. However, when the selection of post-stress clones was isolated from this population, the average expression level was found to be considerably lower (2.5 and 3.34 fold in TBP and Library, respectively). The most successful clones, as determined previously by their growth curves and AIVCD performances, were mostly found in the range of 1.3-2.2. This may suggest that TBP overexpression is detrimental outside of this range, with smaller changes in expression levels able to bring about changes in global gene suitable for the advantageous phenotypes measured in these clones, a sentiment also suggested by the literature. A western blot for intracellular TBP concentrations also found expressed TBP concentrations to vary little compared to an untransfected control cell line.

Mutant TBP sequencing

A selection of 15 mutant TBP sequences was isolated from 3 selected Library transfected clones. This was carried out to investigate the extent of variety in mutant sequences, and if particular mutations were more prevalent. A qPCR to determine the number of sequence inserts estimated between 18 and 36 individual sequences in the clones analysed, and of the 5 sequences isolated from each clone no mutant sequence was duplicated, suggesting a variety integrated into each different genome and expressed by each cell. A range of individual point mutations and deletions were characterised, however no definitive conclusions could be made as to their effects on structure or function. Any cumulative effect on phenotype caused by these mutant TBPs is likely the result of a range of more subtle interactions rather than the effect of one dominant mutant sequence. The limited level of overexpression measured in these cells also suggests that expression of any given single sequence is likely quite low, and a number may be epigenetically silenced by the cell or located in unexpressed regions of heterochromatin.

Functional characterisation of selected clones

Further experiments were then carried out a number of selected TBP and Library transfected clones to further characterise their phenotypic performances. Growth curves performed in 50ml disposable flasks confirmed that their advantageous phenotypes could be replicated in larger scale suspension culture vessels. A known high density control cell line (CHO-SEAP) and parental CHO-K1 cell line were also included, with the selected TBP and Library clones demonstrated to generally outperform both in terms of growth and survival.

Productivity assays using transient plasmid transfection of 3 different products then demonstrated that the genetic and directed evolution interventions did not significantly affect these cell lines' ability to generate and secrete a relevant protein product, a key characteristic of industrial bioprocess cell lines. A final study was then carried out to investigate the performance of these cell lines in a temperature shifted fed-batch situation, a common industrial bioprocess configuration. This was found to promote a longer period of viable cell time, with advantages in cell density in the early stages of culture and sustained viability in later stages promoting a greater overall performance in selected clones compared to K1 and SEAP controls.

4.2.2 gTME in a mammalian cell line

In this study, the effects of two key experimental components were examined; the effect of a genetic intervention (in this case both a TBP mutant library and the wild type gene were examined), and the effect of a stress selective procedure. The use of the mutant gene library was inspired by its successful use in studies by Alper in lower organisms such as *E. coli* (Alper and Stephanopoulos 2007) and yeast (Alper et al. 2006). The basic theory was applied in CHO, however the use of a mammalian cell line presents unique difficulties not encountered in the organisms previously examined. Generation time is one obvious factor, with sub-culturing times for *E. coli* of less than 20 hours providing a means of selecting for advantageous mutants with greater speed and efficiency.

The overall complexity of mammalian cells when compared to simpler producer organisms also makes the determination of the unique effects of target sequences more difficult. For example, in previous gTME experiments single successful gene sequences could be isolated and subjected to subsequent rounds of mutation This sequence could also be re-transfected to elicit a similar phenotype, suggesting that the stress selection procedure did not induce changes in gene expression of itself (as opposed to the result seen in this experiment, and similar directed evolution experiments in mammalian cells detailed in section 1.6.1).

In this instance, the integration of multiple mutant sequences in combination with position effects and variable expression levels makes attributing the benefits to one specific TBP mutant practically impossible. While the isolation of single copy transfectants is possible in theory, for the purposes of this study this method would have been impractical. The use of a low copy number would have also likely reduced the expression and phenotypic effect of the TBP transgenes, masking their effects and reducing the effectiveness of the treatment (Majors, Chiang and Betenbaugh 2009). Currently the use of the gTME technique in mammalian cells is relatively novel. While directed evolution experiments have proven successful in eliciting an improved phenotype, the results generated here suggest that directed evolution in conjunction with a suitable genetic intervention augments the selective process by providing a greater level of heterogeneity, and is a potentially useful tool in the generation of improved mammalian cell lines for biopharma production.

4.2.3 The effects of TBP/Library transfection

While initially used as a control to measure the relative performance of the mutant library, wild-type TBP overexpression was found to be beneficial in its own right with regards to maximum cell density. The unselected mutant library was initially found to be detrimental, although this result was to be expected. After the stress procedure, both appeared to bestow additional benefits in terms of growth and survival compared to the use of the stress procedure alone in empty vector control cells. Immediately, this appears to confirm one of the original hypotheses that an increase in heterogeneity allows for a greater variety of potential responses to an environmental stress, a percentage of which are statistically likely to be beneficial and survive.

However, the comparison between successful TBP clones and successful Library clones begs the question as to whether the added mutagenesis step is more beneficial compared to the use of the wild type gene. When we look at the selection of post stress clones isolated, the differences in final day AIVCD return a p value of 0.08, indicating the differences between groups is not strictly significant. However, a number of factors distinguish one group from the other;

- The performance of Library selected clones is higher on average, with a 16.1% increase in AIVCD compared to the TBP group of clones.
- Due to greater variability as a result of Library expression, clones at the highest percentile performance generally outperform their TBP counterparts. For example, when compared to the stressed pcDNA population, the top 3 TBP selected clones provided a benefit of 28% to final day AIVCD when scaled up to 50ml, while the top 3 Library clones provided an increase 44.5%.
- Inequalities in the stress selection procedure may have reduced its effectiveness with regards to the Library transfected population. Individual populations were measured and passaged in parallel, and the results of section 3.2.6.1 show that in later rounds of selection Library cultures were relatively healthy (>40% viability) at the time of passage. This is compared to TBP cultures whose steep drop in viability prompted the passage of all cell lines. Had Library cultures been allowed to endure further selective pressure, this may have further enhanced the phenotypic change seen in selected Library clones, however this is speculation.

- Productivity is generally found to be higher in the 3 selected Library clones in all transient transfection experiments carried out above. However, transfection efficiencies were also found to be marginally lower in TBP clones, and this difference in apparent productivity may be a direct result. How these clones would function as stable producers (or if any differences would be observed) is as of yet unknown.

While these differences are by no means definitive, in general the Library clones isolated in this experiment generally appear to outperform the TBP equivalents. It may also be inferred that, with an improved experimental process (such as an increase in library complexity or more equal application of stress to cell lines), the use of a genetic library could produce more definitive improvements in phenotype.

TBP was chosen for its similarity to high-level transcription factors used in similar gTME experiments. Future work in the area of directed evolution and mutagenesis could investigate a number of similar various targets, while adhering to the basic theory behind this method. For example, a number of other high level transcription factors involved in stress and apoptotic responses that also interact with a range of similar proteins could be investigated, including c-Jun, c-Myc, p53 and NF-κB family proteins such as NFKB1 and NFKB2. As with TBP, the potential downstream effects of the mutagenesis of these genes cannot be predicted, however their involvement in a range of cellular stress responses and their interaction with a range of pathways and other transcription factors make them attractive prospects for further development of this method.

4.2.4 The effects of selective stress

For the purposes of this experiment two selective stresses were originally investigated. Once stably transfected cell lines were generated, cells were placed at low density to promote selection of fast growing cells, while a second culture was allowed to grow until deleterious late stage conditions selected for cells with a greater resistance to the apoptotic environment.
‘Viability’ stressed cultures provided the most beneficial changes to phenotype, while ‘low density’ selected Library cultures were found to provide no benefit (or indeed a reduction in early growth rates) compared to the wild type control. This difference in results speaks of the need for the use of a well defined selective pressure that will promote the survival of cells with improved phenotypes. In the viability stress, we see the direct negative selection for cells that were more suited to survival under harsher conditions. This ensured that with each round of selection, the next seeded culture was enriched for resistant cells (as well as unexpected improvements in maximum cell densities). The failure of low density seeding to elicit a beneficial change in phenotype may be a result of its less stringent nature. While cells with potentially higher growth rates were originally intended to outgrow their competitors, no defined selective pressure can be applied to isolate these cells or fully eliminate slower growing cells from subsequent passages. This may also be a failure of the mutant library to affect growth rates in any significant way, preventing clones of this nature to become more prevalent. This could be overcome in a future experiment involving a gene more directly involved in the stimulation of growth and proliferation, however in this instance we found the combination of this stress and TBP mutagenesis to be ineffective.

The effects of directed evolution and selective stress have been shown previously to be beneficial, for example in an experiment in which a culture was selected using a similar ‘late stage survival’ method before rescue (Prentice, Ehrenfels and Sisk 2007). In this experiment, the pcDNA control culture did gain a benefit to late stage survival, however this improvement was overshadowed by the far greater levels of overall growth and survival seen in TBP and Library selected cultures. The results of this study would suggest that the combination of a genetic intervention with the use of a selective stress is preferential to the use of a selection process alone.

Further work in this area could easily be carried out to investigate the use of similarly useful selective pressures. For the purposes of improving bioprocess relevant characteristics, future stress procedures could be tailored to select for cells with resistance to specific deleterious environmental factors commonly found in culture. In this instance, the overall negative effect of regular late stage culture conditions was used. However, through the controlled introduction or removal of particular compounds into the cell medium, resistance to adverse pH as well as increasing concentrations of waste products such as lactate and ammonia could be gradually selected for, as well as the induction of alternative metabolic pathways to cope with the depletion of particular nutrients, as previously demonstrated (Bort, Stern and Borth 2010).

4.3 Conclusions

The experiments carried out to date using AAV in CHO were successful in demonstrating the use of a custom viral vector for introducing a targeted gene cassette to a defined genomic locus via homologous recombination.

- A targeted integration rate of 3.1% of all cells having integrated the targeting cassette was achieved. While this is favourable compared to the figure of ~1% often reported in previous literature, this percentage is improved through the use of a selectable marker to eliminate untransduced cells.
- This study was not successful in another of its original aims; that of generating a knockout cell line in which both alleles are targeted and disrupted. While a 'one-shot' method using a single vector to generate null mutants is not generally reported in the literature, a number of alternative techniques (such as the use of a secondary viral targeting vector) unfortunately did not achieve this goal when attempted.
- Further work with AAV using this protocol may benefit from refinements based on data gathered in this instance. For instance the use of a lower MOI as well as pre-screening of insertion numbers in targeted clones prior to the use of an alternative vector for secondary allele targeting. This may help eliminate potential complications arising from high levels of off-target insertions.

The portion of this thesis in which mutagenesis and directed evolution was applied in CHO cells was successful in isolating a number of derivative clonal cell lines with improved phenotypic properties.

- Initially, wild type TBP overexpression was found to be beneficial to growth, improving maximum cell density by 16.8%.
- When compared to the wild type gene, the unselected mutant library was found to be deleterious in its overall effect, as was predicted.
- The stress procedure was found to have a positive impact on the survival of pcDNA control cells. However, a more substantial benefit was gained in TBP selected cells, increasing maximum density by a further 9% and AIVCD by 39.9%.
- After the elimination of deleterious mutants, a selection of Library clones with exceptional phenotypes was isolated, with characteristics of the highest performers exceeding those of the stressed control cell line (maximum cell density and AIVCD increased by 53% and 44.5% respectively).
- While exceptional stressed TBP clones were also isolated, their relative increases in maximum cell density (49.8%) and AIVCD (28%) were not as great as the highest performing Library clones, suggesting that additional heterogeneity is beneficial to the selective process.
- Transcriptional profiling of a selection of mixed populations and selected clones indicated a wide range of small changes in expression resulting from the effects of stress selection. Changes in the areas of lipid metabolism and regulation of growth appeared to have the most consistent impact in beneficial cell lines.

Overall, this investigation suggests that global transcriptional machinery engineering is a useful technique for cell line engineering in CHO, with direct benefits gained from a combination of both the mutant library and stress selection procedures. This study also demonstrates the plasticity of CHO, and their innate ability to adapt to changes in their environment that can be successfully exploited to elicit an improved phenotype.

4.3.1 Future work (AAV)

Future work on this topic would likely be focussed on efforts to generate a homozygous -/- knockout cell line. This would especially be true in experiments in which gene knockout is the primary concern, as opposed to targeted gene insertion.

A number of modifications (suggested by the data generated to date as well as successful techniques demonstrated in the literature) could be applied in an effort to improve this protocol and increase the chances of generating a null mutant clone while reducing the numbers of off-target insertions;

- Reduction in MOI to investigate the effect this may have on targeting rates compared to its effect on non-targeted insertions.
- Enrichment for cells in the S-phase of the cell cycle to improve rates of homologous recombination (targeted) and reduce non-homologous end joining (non-targeted) insertion rates.
- Further inhibition of NHEJ using simultaneous knockdown of genes such as Ku70 and Xrcc4 prior to transfection.
- Investigation of secondary allele targeting techniques in clones with reduced numbers of off-target insertions, as these may possibly provide a successful result.

Using a successfully targeted cell line, utilising the loxP sites contained within this cassette to introduce a gene of interest would also be a logical follow up to this study. The cassette designed and used in this instance was created to allow the deactivation of the selectable marker and subsequent introduction of a target product gene using loxP recombination. Targeting a region of high transcriptional activity would act as a proof of concept study, and comparisons of the expression levels of similarly targeted clones would demonstrate the ability to generate cell lines with predictable gene expression patterns. This ability to generate a 'master' cell line would prove useful for future bioprocesses, reducing the levels of screening necessary by generating cell lines with a predictable phenotype and productivity characteristics.

Also, the ability to target specific genomic loci would allow for the investigation of a selection of putative target expression sites prior to generating a master cell line,

possibly identified using in-silico methods or via random insertion screening. Once a sites or sites with potentially high transcriptional activity are identified, viral targeting vectors could be produced by simply swapping the homologous arm segments, and an experiment could be then carried out in an effort to experimentally determine the true transcriptional potential of a number of given loci. This could be carried out prior to the generation of a master cell line in order to identify the site most likely to provide the highest level of productivity while also remaining stably active, key characteristics for an industrial cell line.

4.3.2 Future work (Mutagenesis & Directed Evolution)

The investigation carried out here into the use of mutagenesis and directed evolution was mostly successful in its aims of demonstrating the use of this technique in CHO cells, however future work could be carried out in a number of areas to both further characterise the clones generated in this study, and improve on this technique in further iterations to gain greater benefits if applied using other genes and environmental stresses.

Based on the results of this experiment, a number of alterations could be made to this protocol in subsequent iterations in order to improve the results of any mutation and stress procedure, and maximise the potential gains in phenotypic improvement.

- A further investigation into the mutation rates most likely to provide beneficial mutant clones. In this investigation the mutation rate measured in successful clones was substantially lower than that initially used in the original mutant library, suggesting that the optimal rate for generating non-deleterious sequences may be lower than our original target rate.
- An optimisation of the transfection procedure could be employed to reduce the incidence of multiple mutant insertions per clone, isolating the effects of single sequences and making their analysis less complex.
- An alternative method of mutagenesis may also be explored in CHO. As opposed to the low fidelity PCR method used in this study, gene shuffling or site saturation of key residues in a target gene could be employed, depending on knowledge of the gene selected and its key functional regions.
- Only one of the two environmental stresses investigated in this study was found to be suitable in eliciting an improved phenotype. Further iterations of this study (including further work with mutant TBP) would be suited to investigating any number of defined, industrially relevant environmental stresses that could be replicated in a selective process such as resistance to the build-up of waste products such as ammonia or adaptation to lower nutrient concentration.
- The use of alternative genes should also be considered for further work in this area. TBP was chosen in this experiment due to its high level involvement in the expression of a wide number of genes. However, a number of alternative genes with similar characteristics could be investigated (i.e. c-Jun, c-Myc, p53 etc.)

The clones isolated in this study were characterised based on properties such as growth, viability and productivity. However, further characterisation could be carried out to increase our understanding of these cells.

- A cell cycle analysis of late stage cells could determine if new cell growth is responsible for the persistence of viable cells, or a halt to the cell cycle and prevention of apoptosis thereby maintaining current living cells as being the cause of this phenomenon.
- Media analysis of metabolic products secreted (such as lactate and ammonia) would shed light on the environmental conditions created by these cells that may differentiate their ability to survive from that of less successful clones.
- A larger volume culture (such as a 1 litre or greater bioreactor) could demonstrate if phenotypic advantages were still apparent at scales more typical of a real bioprocess.
- Clonal stability was not addressed over the course of this study. While phenotypes did not appear to change significantly over the course of a small number of passages when clones were finally isolated and stocked, their ability to retain their unique pattern of gene expression over long periods of time (with and without the addition of zeocin selection) is currently unknown.

A gene profiling experiment was carried out on selected clones and mixed cell populations as part of this investigation. The data generated and conclusions draw from this portion of this thesis could act as a prompt for further studies.

- Genes consistently identified as deregulated in advantageous clones could be targeted for knockdown or overexpression in order to elicit a beneficial change. Counter to this, we may find that the interaction of a discreet combination of genes and/or mutants is necessary to unlock such a complex phenotype, with the process followed in this study a more attractive prospect than traditional single target engineering.
- Due to economic constraints sample numbers in this study were limited. Analysis of the data suggested that an investigation of more time points would be useful. While day 5 was chosen as a compromise, further in depth investigation of both

the exponential and decline phases could further highlight genes involved in the two distinct states of growth and survival.

- While a number of targets involving the regulation of growth and survival were noted as having a possible beneficial effect when dysregulated, downregulation of cholesterol and lipid biosynthesis appeared to have the most consistent impact on the survival of all stressed cell lines. Further work on the cell lines generated in this experiment could be useful in the study of the effects of lipid metabolism on producer cell lines, with measurements of cellular cholesterol concentrations in various cell lines and the artificial modulation of these genes providing useful means of further investigating the role of this area in CHO metabolism.

Section 5.0

Bibliography and Appendices

5.1 Bibliography

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5.2 Appendices

5.2.1 pAAV-DHFR sequence (7081 bp)

Feature	Region	Size (bp)
pAAV Backbone	1-149	149
5’ HR Arm	150-1069	920
CMV	1070-1645	576
Neo	1646-2199	554
Intron (including LoxP1)	2200-2345	146
LoxP1	2276-2309	34
Δneo	2346-2618	273
PolyA signal	2619-2888	270
LoxP2	2889-2922	34
3’ HR Arm	2923-4335	1413
pAAV Backbone	4336-7081	2746

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   1  CCTGCAGGCA GCTGCGCGCT CGCTCGCTCA CTGAGGCCGC CCGGGCAAAG CCCGGGCGTC
  61  GGGCGACCTT TGGTCGCCCG GCCTCAGTGA GCGAGCGAGC GCGCAGAGAG GGAGTGGCCA
 121  ACTCCATCAC TAGGGGTTCC TGCGGCCGCG CAGACGTGGA AGTGCGAAGT CTCCCGTGGG
 181  AATCTGGGAA CTTTGCTTCT TGGCAGAAAT TTTTGTGCTG TTCCCAGAGT TTATTAAGCA
 241  TCCTCTTTAA TATACAAATA TTTGAAATTT TGTTAGCAAG AGCAGTTAAC TGGACAGAAT
 301  TCTCAGCAAA ATTTAGCGCT TTAAATATAA AGATCAGAGC CAGTATTTAG AATTGCCGCT
 361  GGAAGACAGG AAACCTGAGT AAATAGAAAA GAATGCAGGG AGAATAGGCT AAAATACAAT
 421  TAAACACTAA TGTGCGCTGT TGGAATTCTA GAGGCAGGAA GGCATGGTTG AAGGTATGAC
 481  CTTCAGTGGG GCAGTCTGGG GGTCTGACTT GAAATAAAAG AATGGGGAGT GGTGTGCTAT
 541  TATAAGGCAG TTTCCAGTTG CTTGTAGGAC ACTCATTGTA ATTTCTCTCT AACAGACATA
 601  TTTGTTTATT CTAGTAGGAG AGGAATGGCT CTGTGGGTTT CAACCTCTTC GGGGGGTTGA
 661  ATGACCCTTT CACAGGGGTG GCTGAAGACC ATTGGAAAAC AGTTATTTAC ATTTTGATTC
 721  ATGACAGCAA AATTACATGT ATGAAGTAAC AACAAAAATA ATTTCACAGT TGGGGGGTCA
 781  CCACAACATG AGGAACTATA TTAAAGGGTC ATAGCATTAG CATGCTTGAG AACCACTGGC
 841  AAAGAGAATG GGGAAACAGG TCAGCAGAGC AGATGTTGAA CAGCATTCAT AGGACAGGTA
 901  GAGAACATAG TAGGTCACTG GTAGACAAGT TGGTAAATGC CATTAACATG GTCTACTCTC
 961  AAACTAAATA TCAGGATTGG GGAAATAGCT CAGTCAGTAA AGTTTTGAAG ACCTGAGTTA
1021  AAATCTCAGC ACCCAAATAA AAATGTGGGC CTGGTGGGAC ACAGCTAGCT AGTAATCAAT
1081  TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA
1141  TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
1201  TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
1261  AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT
1321  CAATGACGGT AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC
1381  TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA
1441  GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT
1501  TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA AATGTCGTAA
1561  CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG
1621  CAGAGCTGGT TTAGTGAACC GTCAGGGATC CGCATGATTG AACAAGATGG ATTGCACGCA
1681  GGTTCTCCGG CCGCTTGGGT GGAGAGGCTA TTCGGCTATG ACTGGGCACA ACAGACAATC
1741  GGCTGCTCTG ATGCCGCCGT GTTCCGGCTG TCAGCGCAGG GGCGCCCGGT TCTTTTTGTC
1801  AAGACCGACC TGTCCGGTGC CCTGAATGAA CTGCAAGACG AGGCAGCGCG GCTATCGTGG
1861  CTGGCCACGA CGGGCGTTCC TTGCGCAGCT GTGCTCGACG TTGTCACTGA AGCGGGAAGG
1921  GACTGGCTGC TATTGGGCGA AGTGCCGGGG CAGGATCTCC TGTCATCTCA CCTTGCTCCT
1981  GCCGAGAAAG TATCCATCAT GGCTGATGCA ATGCGGCGGC TGCATACGCT TGATCCGGCT
2041  ACCTGCCCAT TCGACCACCA AGCGAAACAT CGCATCGAGC GAGCACGTAC TCGGATGGAA
2101  GCCGGTCTTG TCGATCAGGA TGATCTGGAC GAAGAGCATC AGGGGCTCGC GCCAGCCGAA
2161  CTGTTCGCCA GGCTCAAGGC GAGCATGCCC GACGGCGAGT AAGGAATGGA GGGAAATTAT
2221  CCTTATGCAT GGCAGAAATT TCCAGGGTTT CTATAGGGTT TTGTGGCACG TCGACTACCG
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2281 TTCGTATAGC ATACATTATA CGAAGTTATA TCGATTTTTT AACTGCTAAT GCACTATGTC
2341 TTAGGGATCT CGTCGTGACA CATGGCGATG CCTGCTTGCC GAATATCATG GTGGAAAATG
2401 GCCGCTTTTC TGGATTCATC GACTGTGGCC GGCTGGGTGT GGCGGACCGC TATCAGGACA
2461 TAGCGTTGGC TACCCGTGAT ATTGCTGAAG AGCTTGGCGG CGAATGGGCT GACCGCTTCC
2521 TCGTGCTTTA CGGTATCGCC GCTCCCGATT CGCAGCGCAT CGCCTTCTAT CGCCTTCTTG
2581 ACGAGTTCTT CTGAATCAAG CTTATCGATA CCGTCGACGG GGGAGGCTAA CTGAAACACG
2641 GAAGGAGACA ATACCGGAAG GAACCCGCGC TATGACGGCA ATAAAAAGAC AGAATAAAAC
2701 GCACGGTGTT GGGTCGTTTG TTCATAAACG CGGGGTTCGG TCCCAGGGCT GGCACTCTGT
2761 CGATACCCCA CCGAGACCCC ATTGGGGCCA ATACGCCCGC GTTTCTTCCT TTTCCCCACC
2821 CCACCCCCCA AGTTCGGGTG AAGGCCCAGG GCTCGCAGCC AACGTCGGGG CGGCAGGCCC
2881 TGCCATAGAT AACTTCGTAT AGCATACATT ATACGAAGTT ATCTCGAGGG AGGCAGAGAC
2941 AGAAAGATCC CTGGGGCTTG TTCCTAGCCA AATTTGTGGG TTCCAGATTC AGTGAGAAAT
3001 CTTGTCTCAA AAAAATAAAG TGAAGACACT CATCTTCACT GTCTGGCCTG TGCAAACAGG
3061 TGCACAGTGC ATACAAAATA AAAATAATAA TTTTTTCAAG ATTATTTTTC TTTCTAATAT
3121 GTTTTGAGAA TGCCTATATA CATGTATGTA CAAAATATAT GTATGTGCCT GGTCCCCACA
3181 GAAGCAGAAG AAAATGTCAG TTCCCTAGAA CTGCCTCTAG ATGTGGGTGC TGAGAACCAA
3241 ACCCAGGTCC TCTACTATGC TTTTAACTGC TAAACCATCT CTCTAGCCCC CAAAGTCATT
3301 TTTCATTAAA GAATCCTAAG TATGACCTGG TAATTGTTAC TGTTCCTTTG ATCCAGATGT
3361 CTGACACTCA AGGTTTTTTC CCCAAGCAAT TAAATAAGAG ATGGAAGCTG CATGTCTAAA
3421 GTTTTCCTAA AAGTGCTTTT TAAATGTGTT TTCTATTCTT TGAAGTTTTT ATACATTTAT
3481 ACAGTGTTTA TAGAGCATAT CCATTTATCT TCTCACTCCT CCTTGTGCCC TCTTCTCCTT
3541 CCCTAATTCA TGTTGTAGTT GTTATCAATA ACCCTGTGTG GTGGTCAACT GGGGCATGAG
3601 CAATCTACAA CCAGCCCAAC CTAAGACTGA CTCTCCCTCT CTCAGCAGCC CCCAATAACC
3661 AATAGCTGTC TGATAGGGTG GGACCTCGGG AGCCCCTTCT GCATGCTGGA ATTTTGACTG
3721 ACTGTGAGTT GGTGCATACA GACATCAGCC ATGTCTCATC CTGTAGTCAG TTTTCACATC
3781 TCTCCCTCTT TCAGCAACTC TTATGTTCTA TCTGCCCCCT CTTCCTCAAT GGTCCCTTAA
3841 CCTTTGAATA GTGGGAGCTG GTATAGATGA CCCAGAGAAC AGTAGTTTTA GATGGTAAAT
3901 ACAAGTTATG AGTCTTTAAT CTTCTCAGAT TTCCAGGGGT AGGAGCATCT TTTCTCCATT
3961 TTAGAATTTA ATAAAGCATA TGGGGTTATC TAGTGGGACA AATCCTGTCA GCCTGAGCAC
4021 TTTGAACCAA AATTATGTGA GGTTGCAATT GACTAAAACC TAAGTAGAAG ATATAAATTG
4081 GATAAACCAA AAAGAAAAAT AAGTATTATA TCTAGTTAAT GAGTACTAAA TATCTGAAAT
4141 TTGGGTGAAG CTGTTGGTCT TAATTTTTCT AAAGGATATC CACTAGATGT CACTATGACA
4201 ATGGTATCTT GCCTGTGTAT GACACTAAAA ATGTGTGTTT TCAAAAATAT CCTTCCTGCT
4261 TAATTTTGTA GGTAAACAGA ACCTGGTGAT TATGGGCCGG AAAACCTGGT TCTCCATTCC
4321 TGAGAAGAAT CGACCGCGGC CGCAGGAACC CCTAGTGATG GAGTTGGCCA CTCCCTCTCT
4381 GCGCGCTCGC TCGCTCACTG AGGCCGGGCG ACCAAAGGTC GCCCGACGCC CGGGCTTTGC
4441 CCGGGCGGCC TCAGTGAGCG AGCGAGCGCG CAGCTGCCTG CAGGGGCGCC TGATGCGGTA
4501 TTTTCTCCTT ACGCATCTGT GCGGTATTTC ACACCGCATA CGTCAAAGCA ACCATAGTAC
4561 GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT
4621 ACACTTGCCA GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG
4681 TTCGCCGGCT TTCCCCGTCA AGCTCTAAAT CGGGGGCTCC CTTTAGGGTT CCGATTTAGT
4741 GCTTTACGGC ACCTCGACCC CAAAAAACTT GATTTGGGTG ATGGTTCACG TAGTGGGCCA
4801 TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT CCACGTTCTT TAATAGTGGA
4861 CTCTTGTTCC AAACTGGAAC AACACTCAAC CCTATCTCGG GCTATTCTTT TGATTTATAA
4921 GGGATTTTGC CGATTTCGGC CTATTGGTTA AAAAATGAGC TGATTTAACA AAAATTTAAC
4981 GCGAATTTTA ACAAAATATT AACGTTTACA ATTTTATGGT GCACTCTCAG TACAATCTGC
5041 TCTGATGCCG CATAGTTAAG CCAGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA
5101 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC
5161 ATGTGTCAGA GGTTTTCACC GTCATCACCG AAACGCGCGA GACGAAAGGG CCTCGTGATA
5221 CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT
5281 TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG
5341 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
5401 ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT
5461 GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA
5521 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC
5581 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC
5641 CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG
5701 GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA
5761 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC
5821 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT
5881 GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG

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5941  CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT
6001  TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC
6061  TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT
6121  CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC
6181  ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC
6241  TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT
6301  TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG
6361  ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC
6421  AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA
6481  CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG
6541  GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
6601  GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA
6661  CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG
6721  TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG
6781  GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG
6841  CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
6901  CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC
6961  CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA
7021  AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG
7081  T
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5.2.2 pAAV-HPRT sequence (6605 bp)

Feature	Region	Size (bp)
pAAV Backbone	1-149	149
5’ HR Arm	150-1084	935
CMV	1085-1660	576
Neo	1660-2214	554
Intron (including LoxP1)	2215-2360	146
LoxP1	2290-2324	34
Δneo	2361-2633	273
PolyA signal	2634-2903	270
LoxP2	2904-2937	34
3’ HR Arm	2938-3859	922
pAAV Backbone	3860-6605	2746

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   1  CCTGCAGGCA GCTGCGCGCT CGCTCGCTCA CTGAGGCCGC CCGGGCAAAG CCCGGGCGTC
  61  GGGCGACCTT TGGTCGCCCG GCCTCAGTGA GCGAGCGAGC GCGCAGAGAG GGAGTGGCCA
 121  ACTCCATCAC TAGGGGTTCC TGCGGCCGCT TCCTCATGGA GTGATTATGG ACAGGTAAGT
 181  AAGATCTTAA TTGAAGGGGC TGGAGAGATG GCTCAGCGGT TAAGAGCACT GACTGCTCTT
 241  CCAGAGGTTC TGAGTTCAAT TCCCAGCAAC CACATGATGG CTCACAACCA TCCCTAATGA
 301  GATCTGGTGC CCTCTTCTGG CCTGCAGGGA CACATGCAGA CAGAACAGAT CTTAATTGAA
 361  GTTTTATGGG TTTATTTTAG ATGTGTCTGT GTGTATGTTT GTGATAGGCA TGTATTTGAT
 421  ACATATGTAT AATGTATATA TTTATGTAAA AGCTAGAGAA GGACATTGGG TGCCATACTC
 481  TGTTGCTCTC TGTCTTGCTC CCTTGAGTTG GTGTCTCTCA CTGAACCTGG GGACATGCTG
 541  ATTCGGTTAG GCTCTGTCCC CTATTGCTGA AGTTTCAAGC AGATGCGGCC ACACCTGGTT
 601  TTTATATGGG TATTAAGGAT CTGAATTTAG GTCCTGATGC TTGCACAAGT GCTCCCAATG
 661  GCCTGCATGT ATGTGCAGCA TCTGCATTCC AGGAGACCAC AAAAGTGTGA TGGGTCCTCT
 721  GGAACTGGAG TTGCAGGCCA TTGGGAGCTG CTGTGTGGCT GGGAACTGGA CTTAGGGCCT
 781  CTGCCCAAAC AGCAAATGGG CTTAACTCTC TCCAGCCCCA ATATTTTCTA GTTTTAAAGT
 841  TCTTTTTCAC AAAAAGAAAA ACACCAGGAA GTCCTACTAG TTATTCAGTT TGAGATATAA
 901  ATAGTGGTAC TCCATAATGA GTCCAGCAAA TGTTGAAACG AGGTTGGAAG TCTGTAAATG
 961  AGATTATGAT TCTGTTGGTT TGCTATACAC ATTTCTTTTA AAATTTTTTT ATAAATTTTA
1021  TTTAAATTAG AAACAATCTT ATTTTACATA TCAATCCCAG TTCACTCTCA CTCCCATCGC
1081  TAGCTAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC
1141  ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC CATTGACGTC
1201  AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT
1261  GGAGTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC
1321  GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC
1381  CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT
1441  GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTCC
1501  AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT
1561  TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG GGCGGTAGGC GTGTACGGTG
1621  GGAGGTCTAT ATAAGCAGAG CTGGTTTAGT GAACCGTCAG GGATCCGCAT GATTGAACAA
1681  GATGGATTGC ACGCAGGTTC TCCGGCCGCT TGGGTGGAGA GGCTATTCGG CTATGACTGG
1741  GCACAACAGA CAATCGGCTG CTCTGATGCC GCCGTGTTCC GGCTGTCAGC GCAGGGGCGC
1801  CCGGTTCTTT TTGTCAAGAC CGACCTGTCC GGTGCCCTGA ATGAACTGCA AGACGAGGCA
1861  GCGCGGCTAT CGTGGCTGGC CACGACGGGC GTTCCTTGCG CAGCTGTGCT CGACGTTGTC
1921  ACTGAAGCGG GAAGGGACTG GCTGCTATTG GGCGAAGTGC CGGGGCAGGA TCTCCTGTCA
1981  TCTCACCTTG CTCCTGCCGA GAAAGTATCC ATCATGGCTG ATGCAATGCG GCGGCTGCAT
2041  ACGCTTGATC CGGCTACCTG CCCATTCGAC CACCAAGCGA AACATCGCAT CGAGCGAGCA
2101  CGTACTCGGA TGGAAGCCGG TCTTGTCGAT CAGGATGATC TGGACGAAGA GCATCAGGGG
2161  CTCGCGCCAG CCGAACTGTT CGCCAGGCTC AAGGCGAGCA TGCCCGACGG CGAGTAAGGA
2221  ATGGAGGGAA ATTATCCTTA TGCATGGCAG AAATTTCCAG GGTTTCTATA GGGTTTTGTG
2281  GCACGTCGAC TACCGTTCGT ATAGCATACA TTATACGAAG TTATATCGAT TTTTTAACTG
2341  CTAATGCACT ATGTCTTAGG GATCTCGTCG TGACACATGG CGATGCCTGC TTGCCGAATA
2401  TCATGGTGGA AAATGGCCGC TTTTCTGGAT TCATCGACTG TGGCCGGCTG GGTGTGGCGG
2461  ACCGCTATCA GGACATAGCG TTGGCTACCC GTGATATTGC TGAAGAGCTT GGCGGCGAAT
2521  GGGCTGACCG CTTCCTCGTG CTTTACGGTA TCGCCGCTCC CGATTCGCAG CGCATCGCCT
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2581  TCTATCGCCT TCTTGACGAG TTCTTCTGAA TCAAGCTTAT CGATACCGTC GACGGGGGAG
2641  GCTAACTGAA ACACGGAAGG AGACAATACC GGAAGGAACC CGCGCTATGA CGGCAATAAA
2701  AAGACAGAAT AAAACGCACG GTGTTGGGTC GTTTGTTCAT AAACGCGGGG TTCGGTCCCA
2761  GGGCTGGCAC TCTGTCGATA CCCCACCGAG ACCCCATTGG GGCCAATACG CCCGCGTTTC
2821  TTCCTTTTCC CCACCCCACC CCCCAAGTTC GGGTGAAGGC CCAGGGCTCG CAGCCAACGT
2881  CGGGGCGGCA GGCCCTGCCA TAGATAACTT CGTATAGCAT ACATTATACG AAGTTATCTC
2941  GAGGTAGGGA CCATGCTTTA TATTTTTTCT CAAATGGATA AGTAATTGAC CAGATGCCAT
3001  TTGTTGAATA ATTCATCTTT TAATTACTGA CTCAAGATGC CATCTTTGTC ATATACTAAA
3061  TTGCCATATG TTTTTGGGTC TGTTTCTGGA TTTTACTTTG TTCCTTTAAC TTTTTGTCAA
3121  TCACTGTACT GATTGACACA AGGGAATTAC TGTAGCTTTA TGGTACTTGT CATTTTCCTA
3181  GTAGAGAAAG TCCAGGCTTT TTATTTATCT GCTTTCAATT CAGTTTTCTG TCTTCATTTC
3241  AGCACATCCA AATAACAGTT ATTCCCACCA ACTACAGTGA GTGTTAAGTG CTCTCTTTTG
3301  AAACCCCTAT AAGGGTTTCT GCTAATTTCT ATGAGAGCAG AGTGAAAACC TAAGAAGTGT
3361  GTTCTAACAG ACTGGTACTC AGACCTGGTT TCTCAGTTCT GAAGATGACT TGGGTATGGG
3421  CTGGACTTTG AGGGGGCATA TCCTCAGGGT TGGATGTGAT ACATACCAGC TCCTTCAACT
3481  TGTGACTCAC AGGAATGTTA CAGGGCCAGA AGCAGACACC TGCCATGCTA ATAAATTGTC
3541  CTTTGTGGTT GAGAACTGCC TGATTGATGA TGAGCAGCTA TATTATGCTC ACTCAGAAAT
3601  GGATGAACTC TATTTTTAGG ACTCTTCAGA ACATTAAAAA CCAGCAATGG CCAGTTATGA
3661  TTTTTAAATT TTACTAATCT CTATTTACCT AGTCATTAAT TTCTAGAAAC CTAATACTTA
3721  TAATACATGA TTTAGAATAC CATAATGTTG TAGCCATTAG ATAGCTAACT TCTTACATCT
3781  AATAATTTTT TGTGTGTATT CAAGAATATG CATGTAAATG ATGTTTTTTT TTAACTAGAA
3841  TGATCAGTCA ACAGGGGACG CGGCCGCAGG AACCCCTAGT GATGGAGTTG GCCACTCCCT
3901  CTCTGCGCGC TCGCTCGCTC ACTGAGGCCG GGCGACCAAA GGTCGCCCGA CGCCCGGGCT
3961  TTGCCCGGGC GGCCTCAGTG AGCGAGCGAG CGCGCAGCTG CCTGCAGGGG CGCCTGATGC
4021  GGTATTTTCT CCTTACGCAT CTGTGCGGTA TTTCACACCG CATACGTCAA AGCAACCATA
4081  GTACGCGCCC TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC GCAGCGTGAC
4141  CGCTACACTT GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT CCTTTCTCGC
4201  CACGTTCGCC GGCTTTCCCC GTCAAGCTCT AAATCGGGGG CTCCCTTTAG GGTTCCGATT
4261  TAGTGCTTTA CGGCACCTCG ACCCCAAAAA ACTTGATTTG GGTGATGGTT CACGTAGTGG
4321  GCCATCGCCC TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT TCTTTAATAG
4381  TGGACTCTTG TTCCAAACTG GAACAACACT CAACCCTATC TCGGGCTATT CTTTTGATTT
4441  ATAAGGGATT TTGCCGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT AACAAAAATT
4501  TAACGCGAAT TTTAACAAAA TATTAACGTT TACAATTTTA TGGTGCACTC TCAGTACAAT
4561  CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG CCAACACCCG CTGACGCGCC
4621  CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG
4681  CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT
4741  GATACGCCTA TTTTTATAGG TTAATGTCAT GATAATAATG GTTTCTTAGA CGTCAGGTGG
4801  CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA TACATTCAAA
4861  TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA
4921  GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTTGCGG CATTTTGCCT
4981  TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG
5041  TGCACGAGTG GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG
5101  CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT
5161  ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA
5221  CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA
5281  ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC
5341  GATCGGAGGA CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACTCG
5401  CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC
5461  GATGCCTGTA GCAATGGCAA CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT
5521  AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT
5581  GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG
5641  GTCTCGCGGT ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT
5701  CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG
5761  TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA TACTTTAGAT
5821  TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT
5881  CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA
5941  GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA
6001  AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA GAGCTACCAA CTCTTTTTCC
6061  GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA
6121  GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT
6181  GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG
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6241  ATAGTTACCG GATAAGGCGC AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG
6301  CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC
6361  CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG
6421  AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTCGGGTT
6481  TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG
6541  GAAAAACGCC AGCAACGCGG CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA
6601  CATGT
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5.2.3 pAAV-DHFR/Zeo Sequence (6479 bp)

Feature	Region	Size (bp)
pAAV Backbone	1-149	149
5' HR Arm	150-1069	920
CMV	1070-1645	576
Zeo (incl. polyA)	1646-2320	675
3' HR Arm	2321-3733	1413
pAAV Backbone	3734-6479	2746

1 CCTGCAGGCA GCTGCGCGCT CGCTCGCTCA CTGAGGCCGC CCGGGCAAAG CCCGGGCGTC
61 GGGCGACCTT TGGTCGCCCG GCCTCAGTGA GCGAGCGAGC GCGCAGAGAG GGAGTGGCCA
121 ACTCCATCAC TAGGGGTTCC TGCGGCCGCG CAGACGTGGA AGTGCGAAGT CTCCCGTGGG
181 AATCTGGGAA CTTTGCTTCT TGGCAGAAAT TTTTGTGCTG TTCCCAGAGT TTATTAAGCA
241 TCCTCTTTAA TATACAAATA TTTGAAATTT TGTTAGCAAG AGCAGTTAAC TGGACAGAAT
301 TCTCAGCAAA ATTTAGCGCT TTAAATATAA AGATCAGAGC CAGTATTTAG AATTGCCGCT
361 GGAAGACAGG AAACCTGAGT AAATAGAAAA GAATGCAGGG AGAATAGGCT AAAATACAAT
421 TAAACACTAA TGTGCGCTGT TGGAATTCTA GAGGCAGGAA GGCATGGTTG AAGGTATGAC
481 CTTCAGTGGG GCAGTCTGGG GGTCTGACTT GAAATAAAAG AATGGGGAGT GGTGTGCTAT
541 TATAAGGCAG TTTCCAGTTG CTTGTAGGAC ACTCATTGTA ATTTCTCTCT AACAGACATA
601 TTTGTTTATT CTAGTAGGAG AGGAATGGCT CTGTGGGTTT CAACCTCTTC GGGGGGTTGA
661 ATGACCCTTT CACAGGGGTG GCTGAAGACC ATTGGAAAAC AGTTATTTAC ATTTTGATTC
721 ATGACAGCAA AATTACATGT ATGAAGTAAC AACAAAAATA ATTTCACAGT TGGGGGGTCA
781 CCACAACATG AGGAACTATA TTAAAGGGTC ATAGCATTAG CATGCTTGAG AACCACTGGC
841 AAAGAGAATG GGGAAACAGG TCAGCAGAGC AGATGTTGAA CAGCATTCAT AGGACAGGTA
901 GAGAACATAG TAGGTCACTG GTAGACAAGT TGGTAAATGC CATTAACATG GTCTACTCTC
961 AAACTAAATA TCAGGATTGG GGAAATAGCT CAGTCAGTAA AGTTTTGAAG ACCTGAGTTA
1021 AAATCTCAGC ACCCAAATAA AAATGTGGGC CTGGTGGGAC ACAGCTAGCT AGTAATCAAT
1081 TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA
1141 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
1201 TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
1261 AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT
1321 CAATGACGGT AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC
1381 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA
1441 GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT
1501 TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA AATGTCGTAA
1561 CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG
1621 CAGAGCTGGT TTAGTGAACC GTCAGGGATC CCAAGGTGAG GAACTAAACC ATGGCCAAGT
1681 TGACCAGTGC CGTTCCGGTG CTCACCGCGC GCGACGTCGC CGGAGCGGTC GAGTTCTGGA
1741 CCGACCGGCT CGGGTTCTCC CGGGACTTCG TGGAGGACGA CTTCGCCGGT GTGGTCCGGG
1801 ACGACGTGAC CCTGTTCATC AGCGCGGTCC AGGACCAGGT GGTGCCGGAC AACACCCTGG
1861 CCTGGGTGTG GGTGCGCGGC CTGGACGAGC TGTACGCCGA GTGGTCGGAG GTCGTGTCCA
1921 CGAACTTCCG GGACGCCTCC GGGCCGGCCA TGACCGAGAT CGGCGAGCAG CCGTGGGGGC
1981 GGGAGTTCGC CCTGCGCGAC CCGGCCGGCA ACTGCGTGCA CTTCGTGGCC GAGGAGCAGG
2041 ACTGACACGT GCTACGAGAT TTCGATTCCA CCGCCGCCTT CTATGAAAGG TTGGGCTTCG
2101 GAATCGTTTT CCGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC ATGCTGGAGT
2161 TCTTCGCCCA CCCCAACTTG TTTATTGCAG CTTATAATGG TTACAAATAA AGCAATAGCA
2221 TCACAAATTT CACAAATAAA GCATTTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC
2281 TCATCAATGT ATCTTATCAT GTCTGTATAC CGTCGACCTC CTCGAGGGAG GCAGAGACAG
2341 AAAGATCCCT GGGGCTTGTT CCTAGCCAAA TTTGTGGGTT CCAGATTCAG TGAGAAATCT
2401 TGTCTCAAAA AAATAAAGTG AAGACACTCA TCTTCACTGT CTGGCCTGTG CAAACAGGTG
2461 CACAGTGCAT ACAAAATAAA AATAATAATT TTTTCAAGAT TATTTTTCTT TCTAATATGT
2521 TTTGAGAATG CCTATATACA TGTATGTACA AAATATATGT ATGTGCCTGG TCCCCACAGA
2581 AGCAGAAGAA AATGTCAGTT CCCTAGAACT GCCTCTAGAT GTGGGTGCTG AGAACCAAAC
2641 CCAGGTCCTC TACTATGCTT TTAACTGCTA AACCATCTCT CTAGCCCCCA AAGTCATTTT
2701 TCATTAAAGA ATCCTAAGTA TGACCTGGTA ATTGTTACTG TTCCTTTGAT CCAGATGTCT
2761 GACACTCAAG GTTTTTTCCC CAAGCAATTA AATAAGAGAT GGAAGCTGCA TGTCTAAAGT
2821 TTTCCTAAAA GTGCTTTTTA AATGTGTTTT CTATTCTTTG AAGTTTTTAT ACATTTATAC

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2881  AGTGTTTATA GAGCATATCC ATTTATCTTC TCACTCCTCC TTGTGCCCTC TTCTCCTTCC
2941  CTAATTCATG TTGTAGTTGT TATCAATAAC CCTGTGTGGT GGTCAACTGG GGCATGAGCA
3001  ATCTACAACC AGCCCAACCT AAGACTGACT CTCCCTCTCT CAGCAGCCCC CAATAACCAA
3061  TAGCTGTCTG ATAGGGTGGG ACCTCGGGAG CCCCTTCTGC ATGCTGGAAT TTTGACTGAC
3121  TGTGAGTTGG TGCATACAGA CATCAGCCAT GTCTCATCCT GTAGTCAGTT TTCACATCTC
3181  TCCCTCTTTC AGCAACTCTT ATGTTCTATC TGCCCCCTCT TCCTCAATGG TCCCTTAACC
3241  TTTGAATAGT GGGAGCTGGT ATAGATGACC CAGAGAACAG TAGTTTTAGA TGGTAAATAC
3301  AAGTTATGAG TCTTTAATCT TCTCAGATTT CCAGGGGTAG GAGCATCTTT TCTCCATTTT
3361  AGAATTTAAT AAAGCATATG GGGTTATCTA GTGGGACAAA TCCTGTCAGC CTGAGCACTT
3421  TGAACCAAAA TTATGTGAGG TTGCAATTGA CTAAAACCTA AGTAGAAGAT ATAAATTGGA
3481  TAAACCAAAA AGAAAAAATAA GTATTATATC TAGTTAATGA GTACTAAATA TCTGAAATTT
3541  GGGTGAAGCT GTTGGTCTTA ATTTTTCTAA AGGATATCCA CTAGATGTCA CTATGACAAT
3601  GGTATCTTGC CTGTGTATGA CACTAAAAAT GTGTGTTTTC AAAAATATCC TTCCTGCTTA
3661  ATTTTGTAGG TAAACAGAAC CTGGTGATTA TGGGCCGGAA AACCTGGTTC TCCATTCCTG
3721  AGAAGAATCG ACCGCGGCCG CAGGAACCCC TAGTGATGGA GTTGGCCACT CCCTCTCTGC
3781  GCGCTCGCTC GCTCACTGAG GCCGGGCGAC CAAAGGTCGC CCGACGCCCG GGCTTTGCCC
3841  GGGCGGCCTC AGTGAGCGAG CGAGCGCGCA GCTGCCTGCA GGGGCGCCTG ATGCGGTATT
3901  TTCTCCTTAC GCATCTGTGC GGTATTTCAC ACCGCATACG TCAAAGCAAC CATAGTACGC
3961  GCCCTGTAGC GGCGCATTAA GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG TGACCGCTAC
4021  ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC TCGCCACGTT
4081  CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGGCTCCCT TTAGGGTTCC GATTTAGTGC
4141  TTTACGGCAC CTCGACCCCA AAAAACTTGA TTTGGGTGAT GGTTCACGTA GTGGGCCATC
4201  GCCCTGATAG ACGGTTTTTC GCCCTTTGAC GTTGGAGTCC ACGTTCTTTA ATAGTGGACT
4261  CTTGTTCCAA ACTGGAACAA CACTCAACCC TATCTCGGGC TATTCTTTTG ATTTATAAGG
4321  GATTTTGCCG ATTTCGGCCT ATTGGTTAAA AAATGAGCTG ATTTAACAAA AATTTAACGC
4381  GAATTTTAAC AAAATATTAA CGTTTACAAT TTTATGGTGC ACTCTCAGTA CAATCTGCTC
4441  TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCCGCCAACA CCCGCTGACG CGCCCTGACG
4501  GGCTTGTCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGTCTCCG GGAGCTGCAT
4561  GTGTCAGAGG TTTTCACCGT CATCACCGAA ACGCGCGAGA CGAAAGGGCC TCGTGATACG
4621  CCTATTTTTA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGACGTCAG GTGGCACTTT
4681  TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA
4741  TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT
4801  GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT
4861  TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG
4921  AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA
4981  AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG
5041  TATTGACGCC GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT
5101  TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG
5161  CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG
5221  AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA
5281  TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC
5341  TGTAGCAATG GCAACAACGT TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC
5401  CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC
5461  GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG
5521  CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC
5581  GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC
5641  ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT
5701  AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC
5761  CAAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA
5821  AGGATCTTCT TGAGATCCTT TTTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAAACC
5881  ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT
5941  AACTGGCTTC AGCAGAGCGC AGATACCAAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG
6001  CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC
6061  AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT
6121  ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA
6181  GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT
6241  TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG
6301  CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCG GGTTTCGCCA
6361  CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA
6421  CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGT
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5.2.4 pcDNA3.1/Zeo(+)-TBP sequence (5929 bp)

Feature	Region	Size (bp)
CMV promoter	236-852	617
TBP	935-1891	957
Zeocin resistance	3097-3472	375

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   1  GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG
  61  CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG
 121  CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC
 181  TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT
 241  GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
 301  TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC
 361  CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC
 421  ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT
 481  ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT
 541  ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
 601  TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG
 661  ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC
 721  AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
 781  GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
 841  CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
 901  GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCATGGAC CAGAACAACA GCCTTCCACC
 961  TTATGCTCAG GGCTTGGCCT CCCCACAGGG TGCCATGACT CCTGGAATTC CCATCTTTAG
1021  TCCAATGATG CCTTATGGCA CAGGACTTAC TCCACAGCCT ATTCAGAACA CCAATAGTCT
1081  ATCTATTTTG GAAGAGCAGC AAAGAGAGCA GCAGCAGCAA CAGCAGCAGC AGCAACAGCA
1141  GCAGCAGCAA GCAGTAGCAA CTGCAGCAGC CTCAGTACAG CAATCGACAT CTCAGCAATC
1201  CACACAGGGT GCCTCAGGCC AGACTCCACA GCTCTTCCAT TCTCAGACTC TGACCACTGC
1261  ACCATTGCCA GGCACCACCC CCTTGTACCC TTCACCTATG ACCCCTATGA CCCCTATCAC
1321  TCCTGCCACA CCAGCCTCCG AGAGCTCTGG GATCGTACCC CAGCTGCAAA ATATTGTATC
1381  CACGGTGAAT CTTGGATGTA AACTTGACCT AAAGACCATT GCACTTCGTG CCCGAAATGC
1441  TGAATATAAT CCCAAGCGGT TTGCTGCTGT CATCATGAGA ATAAGAGAGC CTCGGACAAC
1501  TGCGCTGATT TTCAGTTCTG GGAAAATGGT GTGCACAGGA GCCAAGAGTG AAGAACAATC
1561  CAGGTTAGCA GCAAGAAAAT ACGCTAGAGT TGTGCAAAAG TTGGGCTTTC CAGCAAAGTT
1621  CTTAGACTTC AAGATCCAGA ACATGGTGGG GAGCTGTGAT GTGAAGTTCC CCATAAGGCT
1681  GGAAGGCCTT GTGCTGACCC ACCAGCAGTT CAGCAGCTAT GAGCCAGAGT TATTTCCTGG
1741  CTTAATCTAC AGAATGATCA AACCCAGGAT TGTTCTCCTT ATTTTTGTTT CTGGAAAAGT
1801  TGTATTAACA GGTGCTAAAG TTAGAGCAGA GATTTATGAA GCATTTGAAA ACATCTACCC
1861  CATCTTAAAG GGATTCAGGA AGACCACATA GGCGGCCGCT CGAGTCTAGA GGGCCCGTTT
1921  AAACCCGCTG ATCAGCCTCG ACTGTGCCTT CTAGTTGCCA GCCATCTGTT GTTTGCCCCT
1981  CCCCCGTGCC TTCCTTGACC CTGGAAGGTG CCACTCCCAC TGTCCTTTCC TAATAAAATG
2041  AGGAAATTGC ATCGCATTGT CTGAGTAGGT GTCATTCTAT TCTGGGGGGT GGGGTGGGGC
2101  AGGACAGCAA GGGGGAGGAT TGGGAAGACA ATAGCAGGCA TGCTGGGGAT GCGGTGGGCT
2161  CTATGGCTTC TGAGGCGGAA AGAACCAGCT GGGGCTCTAG GGGGTATCCC CACGCGCCCT
2221  GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG
2281  CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG
2341  GCTTTCCCCG TCAAGCTCTA AATCGGGGCA TCCCTTTAGG GTTCCGATTT AGTGCTTTAC
2401  GGCACCTCGA CCCCAAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT
2461  GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT
2521  TCCAAACTGG AACAACACTC AACCCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATTT
2581  TGGGGATTTC GGCCTATTGG TTAAAAAATG AGCTGATTTA ACAAAAATTT AACGCGAATT
2641  AATTCTGTGG AATGTGTGTC AGTTAGGGTG TGGAAAGTCC CCAGGCTCCC CAGGCAGGCA
2701  GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT
2761  CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC
2821  CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG
2881  GCTGACTAAT TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCTGCCTCT GAGCTATTCC
2941  AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTC CCGGGAGCTT
3001  GTATATCCAT TTTCGGATCT GATCAGCACG TGTTGACAAT TAATCATCGG CATAGTATAT
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3061  CGGCATAGTA TAATACGACA AGGTGAGGAA CTAAACCATG GCCAAGTTGA CCAGTGCCGT
3121  TCCGGTGCTC ACCGCGCGCG ACGTCGCCGG AGCGGTCGAG TTCTGGACCG ACCGGCTCGG
3181  GTTCTCCCGG GACTTCGTGG AGGACGACTT CGCCGGTGTG GTCCGGGACG ACGTGACCCT
3241  GTTCATCAGC GCGGTCCAGG ACCAGGTGGT GCCGGACAAC ACCCTGGCCT GGGTGTGGGT
3301  GCGCGGCCTG GACGAGCTGT ACGCCGAGTG GTCGGAGGTC GTGTCCACGA ACTTCCGGGA
3361  CGCCTCCGGG CCGGCCATGA CCGAGATCGG CGAGCAGCCG TGGGGGCGGG AGTTCGCCCT
3421  GCGCGACCCG GCCGGCAACT GCGTGCACTT CGTGGCCGAG GAGCAGGACT GACACGTGCT
3481  ACGAGATTTC GATTCCACCG CCGCCTTCTA TGAAAGGTTG GGCTTCGGAA TCGTTTTCCG
3541  GGACGCCGGC TGGATGATCC TCCAGCGCGG GGATCTCATG CTGGAGTTCT TCGCCCACCC
3601  CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC
3661  AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG TCCAAACTCA TCAATGTATC
3721  TTATCATGTC TGTATACCGT CGACCTCTAG CTAGAGCTTG GCGTAATCAT GGTCATAGCT
3781  GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG CCGGAAGCAT
3841  AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC ACATTAATTG CGTTGCGCTC
3901  ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG CATTAATGAA TCGGCCAACG
3961  CGCGGGGAGA GGCGGTTTGC GTATTGGGCG CTCTTCCGCT TCCTCGCTCA CTGACTCGCT
4021  GCGCTCGGTC GTTCGGCTGC GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT
4081  ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
4141  CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA
4201  GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA
4261  CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC
4321  CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG
4381  TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
4441  CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG
4501  ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT
4561  AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT
4621  ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG
4681  ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
4741  GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA
4801  GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC
4861  CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC
4921  TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT
4981  TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
5041  ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT
5101  ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC
5161  CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
5221  TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG
5281  TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
5341  GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC
5401  AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT
5461  AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG
5521  GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC
5581  TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC
5641  GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT
5701  TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG
5761  AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG
5821  CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA
5881  ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTC
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5.2.5 TBP(U) v pcDNA(U) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
6430548M08Rik	0.360	5.381	5.130	3.05E-05	1.90E-03	2.465	1.28
Acat2	-0.378	6.811	-5.724	6.91E-06	6.36E-04	3.883	-1.30
Akap12	-0.292	7.889	-4.376	2.06E-04	6.96E-03	0.642	-1.22
ANLN	-0.502	6.950	-4.353	2.18E-04	7.12E-03	0.588	-1.42
Asf1b	-0.369	5.939	-4.405	1.91E-04	6.79E-03	0.712	-1.29
ASPM	-0.418	6.112	-6.381	1.39E-06	2.27E-04	5.414	-1.34
Aurka	-0.390	6.331	-4.964	4.63E-05	2.38E-03	2.065	-1.31
C18orf49	0.279	6.640	3.377	2.51E-03	4.42E-02	-1.722	1.21
C3	-0.331	7.330	-6.035	3.21E-06	3.72E-04	4.614	-1.26
Ccl2	-0.586	7.806	-4.403	1.92E-04	6.79E-03	0.708	-1.50
Ccna2	-0.433	6.788	-3.385	2.46E-03	4.42E-02	-1.703	-1.35
CCNB1	-0.564	5.956	-3.961	5.87E-04	1.52E-02	-0.354	-1.48
Ccnb1	-0.533	7.434	-6.346	1.51E-06	2.29E-04	5.332	-1.45
CCNB2	-0.289	6.467	-4.356	2.16E-04	7.12E-03	0.594	-1.22
CDC20	-0.467	7.231	-4.847	6.23E-05	2.90E-03	1.783	-1.38
CDK1	-0.271	8.673	-4.239	2.91E-04	8.79E-03	0.312	-1.21
CDKN3	-0.443	8.297	-5.123	3.10E-05	1.90E-03	2.449	-1.36
Cenpa	-0.497	7.615	-5.286	2.06E-05	1.43E-03	2.838	-1.41
Cenpf	-0.434	6.632	-5.278	2.10E-05	1.43E-03	2.820	-1.35
CENPL	-0.416	6.032	-5.999	3.51E-06	3.82E-04	4.529	-1.33
Cep55	-0.332	6.066	-3.931	6.34E-04	1.60E-02	-0.426	-1.26
CES1	0.420	3.649	4.192	3.28E-04	9.65E-03	0.200	1.34
Cflar	-0.271	7.205	-3.432	2.19E-03	4.03E-02	-1.595	-1.21
CHMP2A	0.276	7.416	5.551	1.06E-05	9.08E-04	3.473	1.21
CKAP2	-0.473	5.818	-7.734	5.97E-08	7.16E-05	8.390	-1.39
CKAP5	-0.273	6.097	-3.505	1.83E-03	3.56E-02	-1.428	-1.21
CKS2	-0.338	8.689	-4.324	2.35E-04	7.50E-03	0.517	-1.26
Cpn2	0.445	6.648	5.624	8.85E-06	7.76E-04	3.646	1.36
Creg1	0.326	6.806	3.465	2.02E-03	3.80E-02	-1.520	1.25
Ctla2b	-0.667	7.178	-7.798	5.18E-08	7.16E-05	8.523	-1.59
Cxcl1	-0.329	6.634	-3.841	7.94E-04	1.88E-02	-0.639	-1.26
D5Ertd579e	0.361	6.408	7.498	1.02E-07	7.50E-05	7.889	1.28
DBF4	-0.334	6.260	-4.320	2.37E-04	7.50E-03	0.508	-1.26
DCTPP1	-0.298	7.125	-4.877	5.78E-05	2.80E-03	1.853	-1.23
DLGAP5	-0.428	6.521	-4.632	1.07E-04	4.42E-03	1.262	-1.35
EBP	-0.395	5.933	-6.555	9.14E-07	2.06E-04	5.810	-1.32
ECT2	-0.477	7.232	-4.934	5.00E-05	2.53E-03	1.993	-1.39
EFEMP1	-0.390	9.515	-6.411	1.29E-06	2.24E-04	5.482	-1.31
EMP1	-0.374	7.772	-7.486	1.04E-07	7.50E-05	7.865	-1.30
EPR1	-0.392	7.818	-4.861	6.01E-05	2.84E-03	1.817	-1.31
EPS8	-0.335	8.261	-3.734	1.04E-03	2.36E-02	-0.892	-1.26
EPS8	-0.315	7.562	-3.921	6.50E-04	1.61E-02	-0.449	-1.24
ERAP1	-0.302	6.611	-6.404	1.31E-06	2.24E-04	5.467	-1.23
ERCC1	0.315	6.529	4.130	3.83E-04	1.09E-02	0.051	1.24
FDPS	-0.299	8.124	-5.114	3.18E-05	1.90E-03	2.425	-1.23
FOSL1	-0.438	7.032	-4.969	4.57E-05	2.38E-03	2.077	-1.35
GAS1	0.674	6.643	7.132	2.35E-07	1.32E-04	7.098	1.60
GPAM	0.271	7.859	6.504	1.03E-06	2.06E-04	5.694	1.21
Gstm1	0.366	9.649	5.286	2.06E-05	1.43E-03	2.839	1.29
Gstm7	0.329	8.876	4.727	8.44E-05	3.79E-03	1.493	1.26
H2afx	-0.296	6.799	-3.753	9.90E-04	2.26E-02	-0.847	-1.23
KIF23	-0.417	6.746	-6.452	1.17E-06	2.21E-04	5.575	-1.34
KIFC1	-0.452	6.589	-7.094	2.57E-07	1.32E-04	7.013	-1.37
KPNA2	-0.318	7.512	-5.097	3.32E-05	1.92E-03	2.384	-1.25
LOC685953	0.397	9.130	3.805	8.70E-04	2.03E-02	-0.725	1.32
Mad2l1	-0.309	7.100	-4.719	8.62E-05	3.82E-03	1.472	-1.24
MMD	0.336	6.497	4.140	3.74E-04	1.08E-02	0.074	1.26
MRPL39	-0.292	6.894	-5.524	1.14E-05	9.49E-04	3.408	-1.22
Mthfd2	-0.284	7.826	-6.316	1.62E-06	2.34E-04	5.263	-1.22
Mthfd2l	-0.340	6.000	-5.324	1.87E-05	1.41E-03	2.929	-1.27
Ncam1	0.587	5.358	6.529	9.73E-07	2.06E-04	5.751	1.50
Ncapd3	-0.309	5.993	-4.387	2.00E-04	6.95E-03	0.670	-1.24
Ncaph	-0.322	6.789	-4.872	5.85E-05	2.80E-03	1.843	-1.25
NDC80	-0.437	6.736	-5.323	1.88E-05	1.41E-03	2.927	-1.35
Nek2	-0.394	8.036	-5.978	3.69E-06	3.90E-04	4.481	-1.31
NFKBIA	-0.320	8.519	-4.550	1.32E-04	5.06E-03	1.064	-1.25
NFKBIA	-0.270	8.243	-4.354	2.18E-04	7.12E-03	0.589	-1.21
Nusap1	-0.506	6.996	-6.341	1.53E-06	2.29E-04	5.322	-1.42
Prc1	-0.488	7.462	-5.091	3.36E-05	1.92E-03	2.372	-1.40
PRDX4	-0.292	8.079	-6.648	7.33E-07	1.88E-04	6.020	-1.22
PTTG1	-0.339	6.979	-4.071	4.45E-04	1.20E-02	-0.091	-1.26

Pxdn	0.277	7.848	4.808	6.88E-05	3.17E-03	1.688	1.21
Reep5	0.320	7.899	6.527	9.77E-07	2.06E-04	5.748	1.25
RGD1560010	-0.274	6.651	-3.951	6.02E-04	1.53E-02	-0.377	-1.21
RNASEH2C	-0.430	6.445	-6.876	4.28E-07	1.54E-04	6.531	-1.35
RPS5	0.276	11.111	6.112	2.66E-06	3.30E-04	4.794	1.21
SBSN	-0.275	9.801	-4.635	1.07E-04	4.42E-03	1.270	-1.21
SEMA3C	-0.335	6.612	-5.805	5.65E-06	5.49E-04	4.075	-1.26
Serpinf1	0.447	8.278	5.178	2.70E-05	1.77E-03	2.580	1.36
SGK1	-0.506	7.327	-8.133	2.48E-08	7.16E-05	9.215	-1.42
SMC4	-0.450	6.591	-4.079	4.36E-04	1.20E-02	-0.072	-1.37
SOD2	-0.387	6.599	-6.154	2.41E-06	3.09E-04	4.889	-1.31
SOD2	-0.351	6.729	-5.181	2.68E-05	1.77E-03	2.587	-1.28
Spc25	-0.469	6.631	-6.053	3.07E-06	3.68E-04	4.656	-1.38
SPRR1A	0.334	8.643	3.880	7.21E-04	1.75E-02	-0.547	1.26
STMN1	-0.319	7.260	-5.314	1.92E-05	1.41E-03	2.907	-1.25
Tcf19	-0.307	5.912	-5.336	1.82E-05	1.41E-03	2.960	-1.24
Tdgf1	0.291	6.194	4.317	2.39E-04	7.50E-03	0.501	1.22
TFB1M	-0.267	6.682	-3.827	8.22E-04	1.93E-02	-0.672	-1.20
TMEM158	0.351	6.933	3.592	1.48E-03	3.07E-02	-1.226	1.28
Tnfsf9	-0.386	6.765	-3.619	1.38E-03	2.97E-02	-1.161	-1.31
Top2a	-0.527	9.103	-6.684	6.72E-07	1.86E-04	6.102	-1.44
Tpst1	-0.293	6.787	-6.932	3.75E-07	1.54E-04	6.656	-1.22
TPX2	-0.429	6.219	-4.607	1.14E-04	4.54E-03	1.202	-1.35
TUBA1C	-0.299	9.068	-6.212	2.09E-06	2.89E-04	5.025	-1.23
TUBB2C	-0.309	8.450	-6.158	2.38E-06	3.09E-04	4.900	-1.24
Tubb6	-0.270	7.128	-6.792	5.21E-07	1.56E-04	6.344	-1.21

5.2.6 pcDNA(S) v pcDNA(U) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40790	-0.539	5.984	-6.104	2.72E-06	5.42E-05	4.013	-1.45
1700052N19Rik	0.362	6.422	7.543	9.19E-08	3.24E-06	7.450	1.29
1810037I17Rik	-0.387	6.983	-7.248	1.80E-07	5.78E-06	6.765	-1.31
2310001A20Rik	0.396	7.257	7.057	2.80E-07	8.25E-06	6.316	1.32
2610002J02Rik	-0.281	6.639	-5.302	1.98E-05	2.69E-04	2.008	-1.22
4833417J20Rik	-0.287	7.312	-4.094	4.20E-04	3.41E-03	-1.040	-1.22
6430548M08Rik	-1.301	5.381	-18.558	1.13E-15	2.89E-13	25.960	-2.46
9530053H05Rik	1.406	10.002	24.358	2.36E-18	9.41E-16	32.088	2.65
A330042M18Rik	-0.793	7.205	-12.420	6.71E-12	8.61E-10	17.161	-1.73
ABHD6	0.697	6.783	8.864	5.19E-09	2.78E-07	10.379	1.62
Acp2	-0.317	6.448	-4.720	8.59E-05	9.26E-04	0.538	-1.25
ADD3	-0.527	6.120	-4.466	1.64E-04	1.59E-03	-0.106	-1.44
ADD3	-0.414	7.763	-8.646	8.21E-09	4.10E-07	9.912	-1.33
AGAP1	-0.310	7.787	-3.202	3.84E-03	1.97E-02	-3.200	-1.24
Agpat6	0.273	6.784	3.127	4.60E-03	2.27E-02	-3.374	1.21
AI646531	-0.337	7.286	-4.206	3.16E-04	2.71E-03	-0.759	-1.26
AI646531	-0.323	6.560	-5.427	1.45E-05	2.11E-04	2.325	-1.25
Akap12	-0.805	7.889	-12.084	1.18E-11	1.33E-09	16.583	-1.75
AMD1	-0.616	8.027	-7.175	2.13E-07	6.55E-06	6.594	-1.53
AMD1	-0.596	7.422	-5.943	4.03E-06	7.47E-05	3.613	-1.51
Amotl1	0.366	7.099	3.203	3.84E-03	1.97E-02	-3.199	1.29
ANP32E	-0.327	8.064	-4.700	9.05E-05	9.56E-04	0.485	-1.25
AP1B1	-0.969	6.806	-18.368	1.42E-15	3.39E-13	25.729	-1.96
APP	-0.361	8.605	-3.729	1.05E-03	7.08E-03	-1.942	-1.28
Arhgap25	0.428	6.665	5.144	2.94E-05	3.71E-04	1.610	1.35
ASAP1	0.308	5.925	5.779	6.03E-06	1.03E-04	3.207	1.24
Asf1b	0.316	5.939	3.769	9.50E-04	6.56E-03	-1.843	1.24
Atp10a	0.328	7.352	7.689	6.61E-08	2.53E-06	7.785	1.26
Atp5o	-0.313	9.052	-6.584	8.53E-07	2.13E-05	5.185	-1.24
AZIN1	-0.609	6.431	-8.401	1.38E-08	6.22E-07	9.378	-1.52
Azin1	-0.477	6.442	-5.757	6.37E-06	1.07E-04	3.151	-1.39
B230219D22Rik	-0.547	8.128	-10.382	2.53E-10	2.07E-08	13.461	-1.46
B230220B15Rik	2.634	7.506	55.056	1.22E-26	2.19E-23	49.455	6.21
BAZ1A	0.271	6.516	3.161	4.24E-03	2.13E-02	-3.296	1.21
BCL10	0.335	8.488	6.617	7.89E-07	2.01E-05	5.265	1.26
BCLAF1	-0.387	6.690	-5.419	1.48E-05	2.13E-04	2.303	-1.31
BCLAF1	-0.337	7.984	-5.407	1.52E-05	2.18E-04	2.273	-1.26
BDNF	0.300	6.480	5.754	6.42E-06	1.08E-04	3.143	1.23
BLVRB	0.280	8.858	2.921	7.51E-03	3.31E-02	-3.842	1.21
BMI1	-0.925	6.386	-11.152	6.09E-11	5.91E-09	14.914	-1.90
BRP44L	-0.567	7.208	-3.552	1.63E-03	1.02E-02	-2.371	-1.48
BRP44L	-0.508	8.068	-11.357	4.21E-11	4.24E-09	15.290	-1.42
Bsg	-0.343	8.496	-3.879	7.22E-04	5.32E-03	-1.574	-1.27
C12orf10	-0.279	6.223	-4.008	5.22E-04	4.11E-03	-1.254	-1.21
C4orf27	-1.022	6.966	-20.607	1.07E-16	3.48E-14	28.316	-2.03
CAMK2N1	-0.284	6.214	-3.786	9.12E-04	6.35E-03	-1.802	-1.22
CANX	0.282	7.461	3.237	3.53E-03	1.85E-02	-3.120	1.22
Cat	0.312	8.131	3.127	4.61E-03	2.27E-02	-3.375	1.24
Ccl2	0.515	7.806	3.873	7.33E-04	5.36E-03	-1.588	1.43
Ccnd1	-0.756	6.627	-8.905	4.77E-09	2.60E-07	10.465	-1.69
CD36	-3.803	8.162	-19.069	6.12E-16	1.73E-13	26.571	-13.96
CHMP2A	-0.700	7.416	-14.088	4.71E-13	7.05E-11	19.864	-1.62
CHPT1	0.282	8.437	5.060	3.64E-05	4.51E-04	1.398	1.22
Cisd2	-0.332	6.498	-6.993	3.25E-07	9.35E-06	6.164	-1.26
CITED2	0.532	6.419	5.636	8.60E-06	1.39E-04	2.848	1.45
CKS1B	0.302	7.858	6.066	2.98E-06	5.88E-05	3.919	1.23
Clstn1	-0.381	7.732	-5.532	1.11E-05	1.72E-04	2.589	-1.30
Clu	-0.291	10.477	-3.444	2.13E-03	1.27E-02	-2.631	-1.22
Col5a1	-0.389	6.209	-6.254	1.89E-06	4.06E-05	4.380	-1.31
COL5A2	0.455	7.606	6.256	1.88E-06	4.06E-05	4.387	1.37
Commd3	-0.392	7.861	-9.346	1.93E-09	1.24E-07	11.388	-1.31
CPD	-0.372	7.815	-6.754	5.70E-07	1.52E-05	5.596	-1.29
Cpn2	-0.287	6.648	-3.625	1.36E-03	8.87E-03	-2.194	-1.22
Cr1l	0.264	8.857	6.210	2.10E-06	4.41E-05	4.273	1.20
Cr1l	0.341	7.762	4.857	6.08E-05	6.96E-04	0.883	1.27
CRELD2	0.347	7.396	4.828	6.54E-05	7.37E-04	0.810	1.27
CROT	0.392	6.877	8.606	8.94E-09	4.32E-07	9.824	1.31
CSE1L	0.334	7.389	6.342	1.52E-06	3.45E-05	4.597	1.26
Csnk2a1	-0.486	6.763	-4.416	1.86E-04	1.76E-03	-0.230	-1.40

Cxcl1	0.569	6.634	6.649	7.31E-07	1.90E-05	5.342	1.48
DAG1	0.269	8.711	4.153	3.61E-04	3.03E-03	-0.891	1.20
DCTPP1	0.323	7.125	5.270	2.14E-05	2.84E-04	1.928	1.25
Ddhd2	-0.285	7.529	-6.330	1.57E-06	3.52E-05	4.568	-1.22
DDX3X	-0.287	9.847	-3.311	2.95E-03	1.62E-02	-2.947	-1.22
DNAJC3	0.283	6.786	5.523	1.14E-05	1.76E-04	2.564	1.22
Dock9	0.493	6.569	7.624	7.65E-08	2.83E-06	7.637	1.41
DST	0.289	6.933	4.109	4.05E-04	3.30E-03	-1.003	1.22
DUSP1	-0.412	6.221	-6.382	1.38E-06	3.19E-05	4.694	-1.33
Dusp3	-0.388	5.641	-4.219	3.06E-04	2.64E-03	-0.726	-1.31
EBNA1BP2	-0.276	8.826	-4.836	6.40E-05	7.26E-04	0.831	-1.21
EIF5	0.286	8.217	5.808	5.61E-06	9.64E-05	3.279	1.22
EIF5	0.356	6.761	3.500	1.86E-03	1.13E-02	-2.497	1.28
EPHX1	0.424	7.673	2.735	1.16E-02	4.57E-02	-4.252	1.34
EPHX1	0.453	8.644	6.107	2.70E-06	5.41E-05	4.020	1.37
ERAP1	0.297	6.611	6.284	1.75E-06	3.91E-05	4.456	1.23
ERCC1	-0.628	6.529	-8.239	1.97E-08	8.51E-07	9.021	-1.55
ERGIC2	-0.279	8.645	-5.980	3.67E-06	6.92E-05	3.707	-1.21
Erp29	0.360	7.835	6.488	1.07E-06	2.61E-05	4.952	1.28
ERP29	0.375	6.775	4.474	1.60E-04	1.56E-03	-0.084	1.30
ESD	0.341	8.915	8.268	1.84E-08	8.07E-07	9.087	1.27
ETHE1	-0.630	7.237	-9.019	3.77E-09	2.11E-07	10.706	-1.55
EWSR1	-0.448	7.376	-12.214	9.50E-12	1.18E-09	16.807	-1.36
Exosc1	-0.307	7.548	-4.126	3.87E-04	3.20E-03	-0.959	-1.24
F2r	-0.289	8.207	-3.200	3.86E-03	1.98E-02	-3.205	-1.22
Fam100b	-0.402	6.914	-5.643	8.45E-06	1.37E-04	2.866	-1.32
FAM107B	-0.470	6.765	-9.153	2.86E-09	1.66E-07	10.988	-1.38
Fam125b	-0.358	6.716	-6.934	3.73E-07	1.05E-05	6.025	-1.28
FAM40B	0.322	6.679	5.483	1.26E-05	1.88E-04	2.465	1.25
FBXO8	-1.223	6.542	-24.731	1.66E-18	7.75E-16	32.430	-2.33
FCGRT	-1.214	6.491	-17.744	3.06E-15	6.87E-13	24.955	-2.32
Fhl1	-0.623	7.505	-14.259	3.64E-13	5.68E-11	20.126	-1.54
Fkbp1a	-0.313	9.533	-5.486	1.25E-05	1.88E-04	2.471	-1.24
Fkbp9	0.310	7.631	6.013	3.39E-06	6.51E-05	3.788	1.24
FMNL2	-0.399	7.362	-6.043	3.15E-06	6.15E-05	3.863	-1.32
FOSL1	0.317	7.032	3.592	1.48E-03	9.49E-03	-2.276	1.25
FOXJ3	-0.389	7.777	-7.035	2.95E-07	8.62E-06	6.264	-1.31
Foxp2	-0.306	7.336	-4.848	6.22E-05	7.07E-04	0.860	-1.24
G3BP2	-0.269	8.617	-5.032	3.91E-05	4.77E-04	1.326	-1.21
GADD45B	0.460	6.746	6.776	5.41E-07	1.45E-05	5.648	1.38
GALNT7	-0.997	7.466	-9.833	7.30E-10	5.38E-08	12.381	-2.00
GAS1	-0.725	6.643	-7.667	6.94E-08	2.62E-06	7.736	-1.65
GCLM	0.591	9.225	11.352	4.25E-11	4.24E-09	15.281	1.51
GHR	-0.421	6.655	-6.690	6.63E-07	1.75E-05	5.441	-1.34
GJA1	-0.383	10.715	-5.221	2.43E-05	3.17E-04	1.804	-1.30
Gm15104	0.278	6.022	4.934	5.01E-05	5.91E-04	1.077	1.21
Gm6377	-0.332	8.134	-4.423	1.83E-04	1.74E-03	-0.215	-1.26
Gmfb	-0.375	8.592	-6.563	8.98E-07	2.21E-05	5.134	-1.30
GNAI1	-0.478	7.278	-7.228	1.88E-07	5.94E-06	6.719	-1.39
GOLGA7	-0.277	8.199	-5.500	1.21E-05	1.83E-04	2.508	-1.21
GPC6	1.405	3.584	16.760	1.08E-14	2.29E-12	23.683	2.65
Gpd2	0.348	6.879	4.567	1.27E-04	1.28E-03	0.150	1.27
GPHN	-0.270	6.426	-4.138	3.75E-04	3.14E-03	-0.928	-1.21
Gpnmb	-1.557	5.966	-24.691	1.73E-18	7.75E-16	32.393	-2.94
Gpr56	-1.125	6.194	-14.319	3.32E-13	5.65E-11	20.217	-2.18
Gpx4	0.445	10.579	8.509	1.10E-08	5.00E-07	9.614	1.36
GSS	0.292	6.247	3.173	4.12E-03	2.08E-02	-3.268	1.22
GSS	0.333	6.709	5.041	3.82E-05	4.68E-04	1.349	1.26
Gss	0.402	8.655	8.590	9.25E-09	4.32E-07	9.789	1.32
GSS	0.413	7.023	7.582	8.42E-08	2.99E-06	7.540	1.33
GSTA5	0.381	9.606	7.147	2.28E-07	6.93E-06	6.528	1.30
Gstm1	0.293	9.649	4.232	2.96E-04	2.58E-03	-0.694	1.23
Gstm6	0.311	7.960	4.209	3.14E-04	2.70E-03	-0.752	1.24
Gtf2h5	-0.264	6.911	-4.399	1.94E-04	1.82E-03	-0.273	-1.20
GUSB	0.282	6.935	5.614	9.09E-06	1.44E-04	2.793	1.22
HADHA	0.353	7.683	4.763	7.70E-05	8.44E-04	0.646	1.28
HADHB	0.425	8.394	8.638	8.36E-09	4.11E-07	9.893	1.34
Herpud1	0.395	6.811	8.909	4.73E-09	2.60E-07	10.474	1.32
HEXIM1	-0.337	7.032	-4.871	5.86E-05	6.80E-04	0.919	-1.26
HMGB1	-0.301	7.626	-5.234	2.35E-05	3.09E-04	1.837	-1.23
HMGN2	-0.318	8.395	-7.443	1.15E-07	3.98E-06	7.221	-1.25
HMOX1	0.383	7.105	4.435	1.77E-04	1.70E-03	-0.184	1.30
Hnrnpa1	0.372	6.520	5.583	9.81E-06	1.54E-04	2.715	1.29

HSP90B1	0.421	10.245	6.599	8.24E-07	2.07E-05	5.221	1.34
Hsp90b1	0.547	8.375	2.861	8.65E-03	3.69E-02	-3.976	1.46
HSPA5	0.427	11.437	9.107	3.14E-09	1.79E-07	10.893	1.34
HSPB1	0.340	9.142	5.224	2.40E-05	3.15E-04	1.813	1.27
Hyou1	0.541	6.015	6.626	7.72E-07	1.98E-05	5.288	1.45
IMMP1L	-0.309	6.798	-4.651	1.02E-04	1.06E-03	0.362	-1.24
Insig1	0.292	8.024	5.980	3.68E-06	6.92E-05	3.706	1.22
Irak1	-0.287	6.508	-4.535	1.37E-04	1.37E-03	0.069	-1.22
Irak2	0.379	6.582	5.322	1.88E-05	2.60E-04	2.059	1.30
ITIH5	0.359	6.180	3.236	3.54E-03	1.85E-02	-3.121	1.28
Jub	0.563	7.280	14.884	1.44E-13	2.59E-11	21.062	1.48
LAMC1	0.274	7.195	5.602	9.35E-06	1.47E-04	2.763	1.21
LAYN	-0.449	6.973	-6.210	2.10E-06	4.41E-05	4.274	-1.37
LOC100294264	0.405	6.932	4.858	6.05E-05	6.95E-04	0.887	1.32
LOC685953	-3.528	9.130	-33.801	1.18E-21	1.06E-18	39.385	-11.53
LOC732360	0.270	6.927	5.430	1.44E-05	2.11E-04	2.332	1.21
LPP	-0.270	7.853	-4.850	6.18E-05	7.05E-04	0.867	-1.21
Lrp10	0.853	7.803	11.928	1.55E-11	1.68E-09	16.311	1.81
LRRC8D	0.396	6.697	4.717	8.66E-05	9.29E-04	0.529	1.32
MANF	0.439	8.744	7.953	3.67E-08	1.52E-06	8.385	1.36
MAPK6	0.420	7.276	5.812	5.56E-06	9.64E-05	3.288	1.34
MARK1	0.378	6.616	8.092	2.71E-08	1.16E-06	8.694	1.30
MBNL2	-0.292	8.281	-6.841	4.64E-07	1.26E-05	5.804	-1.22
MBNL2	-0.273	7.563	-4.764	7.69E-05	8.44E-04	0.648	-1.21
ME1	0.342	7.376	7.333	1.48E-07	4.98E-06	6.964	1.27
MGC112830	-0.264	6.352	-4.495	1.52E-04	1.49E-03	-0.031	-1.20
MIR21	-0.291	6.244	-3.649	1.28E-03	8.45E-03	-2.136	-1.22
Mknk2	-0.342	6.338	-4.872	5.84E-05	6.79E-04	0.923	-1.27
Mknk2	-0.271	6.736	-3.039	5.68E-03	2.66E-02	-3.576	-1.21
Mmp14	0.408	6.694	6.055	3.06E-06	6.01E-05	3.892	1.33
MMP14	0.481	6.141	4.711	8.80E-05	9.42E-04	0.513	1.40
MOBKL3	-0.298	6.352	-6.390	1.36E-06	3.17E-05	4.713	-1.23
MRPS22	-0.278	6.359	-5.940	4.06E-06	7.47E-05	3.606	-1.21
MTBP	0.302	6.107	5.722	6.95E-06	1.15E-04	3.064	1.23
Mthfd2l	0.413	6.000	6.481	1.09E-06	2.63E-05	4.936	1.33
Ncam1	0.678	5.358	7.535	9.35E-08	3.26E-06	7.433	1.60
Ncapd3	0.351	5.993	4.980	4.45E-05	5.33E-04	1.195	1.28
NCEH1	0.272	6.694	5.376	1.64E-05	2.33E-04	2.196	1.21
NEIL3	0.384	7.798	5.431	1.43E-05	2.11E-04	2.335	1.31
NFKBIA	0.499	8.519	7.083	2.64E-07	7.83E-06	6.379	1.41
NFKBIA	0.540	8.243	8.716	7.09E-09	3.64E-07	10.061	1.45
Nid1	0.304	8.249	4.215	3.10E-04	2.67E-03	-0.738	1.23
NUCB2	0.519	7.514	9.817	7.54E-10	5.42E-08	12.347	1.43
Nudt3	-0.276	6.423	-3.269	3.27E-03	1.75E-02	-3.045	-1.21
OAZ1	0.439	7.602	8.015	3.20E-08	1.34E-06	8.523	1.36
OAZ1	0.553	7.862	7.301	1.59E-07	5.16E-06	6.891	1.47
OAZ2	0.446	6.880	7.707	6.34E-08	2.45E-06	7.827	1.36
PALLD	0.583	7.134	11.895	1.64E-11	1.73E-09	16.252	1.50
PCGF5	0.308	6.328	6.266	1.83E-06	4.04E-05	4.411	1.24
PCK2	0.350	6.594	5.313	1.93E-05	2.63E-04	2.036	1.27
PDIA3	0.334	9.730	5.375	1.65E-05	2.33E-04	2.194	1.26
PDIA4	0.409	7.671	8.696	7.39E-09	3.74E-07	10.018	1.33
PDIA5	0.349	7.082	5.420	1.47E-05	2.13E-04	2.307	1.27
PDIA6	0.431	10.091	10.636	1.57E-10	1.38E-08	13.948	1.35
PDLIM1	0.434	7.184	9.228	2.45E-09	1.47E-07	11.145	1.35
Pdyn	-0.321	7.034	-3.722	1.07E-03	7.19E-03	-1.960	-1.25
Pex19	-0.311	6.163	-6.399	1.33E-06	3.14E-05	4.736	-1.24
Pgs1	0.401	6.290	5.937	4.08E-06	7.47E-05	3.600	1.32
Pir	0.538	6.603	7.899	4.14E-08	1.66E-06	8.262	1.45
PJA1	-0.510	7.598	-12.142	1.07E-11	1.24E-09	16.683	-1.42
PLAC8	-2.139	5.438	-21.416	4.45E-17	1.60E-14	29.185	-4.40
PLK2	0.711	7.605	13.952	5.79E-13	8.33E-11	19.653	1.64
PLTP	-0.372	7.226	-2.834	9.22E-03	3.86E-02	-4.036	-1.29
POLR2G	-0.320	8.219	-7.897	4.15E-08	1.66E-06	8.258	-1.25
Ppcs	-0.297	7.062	-5.275	2.12E-05	2.82E-04	1.940	-1.23
PPP1CB	-0.283	9.069	-4.325	2.34E-04	2.13E-03	-0.460	-1.22
PPP2R1A	-0.771	6.065	-6.839	4.66E-07	1.26E-05	5.799	-1.71
PRCP	0.274	7.535	6.572	8.78E-07	2.18E-05	5.156	1.21
PRKCDBP	0.303	6.389	2.813	9.66E-03	4.00E-02	-4.080	1.23
Prmt5	0.690	7.197	9.281	2.20E-09	1.37E-07	11.254	1.61
Procr	0.571	6.220	8.555	9.96E-09	4.59E-07	9.714	1.49
PSMB5	0.459	9.145	4.934	5.00E-05	5.91E-04	1.079	1.37
PSMC4	-0.278	7.500	-3.967	5.78E-04	4.46E-03	-1.355	-1.21

PSMC5	-0.278	7.802	-6.967	3.46E-07	9.85E-06	6.103	-1.21
PSMC5	-0.276	7.329	-3.127	4.61E-03	2.27E-02	-3.376	-1.21
PSME4	0.552	7.404	9.278	2.21E-09	1.37E-07	11.248	1.47
PTPRG	2.300	5.545	45.692	9.97E-25	1.19E-21	45.776	4.93
PURB	-0.330	7.313	-5.774	6.10E-06	1.04E-04	3.194	-1.26
RAB11A	0.307	5.948	2.938	7.21E-03	3.21E-02	-3.804	1.24
Rab31	-0.463	6.713	-5.725	6.90E-06	1.15E-04	3.071	-1.38
Rapgef1	-0.264	9.228	-5.438	1.41E-05	2.09E-04	2.352	-1.20
Rbck1	-0.504	6.528	-6.022	3.32E-06	6.43E-05	3.809	-1.42
RBM3	0.315	7.415	3.133	4.54E-03	2.25E-02	-3.361	1.24
Rere	-0.400	6.955	-4.719	8.61E-05	9.26E-04	0.536	-1.32
RFX7	-0.297	7.777	-4.113	4.01E-04	3.27E-03	-0.993	-1.23
RGS17	-1.022	7.346	-10.533	1.90E-10	1.63E-08	13.752	-2.03
RIOK3	-0.293	7.168	-4.106	4.07E-04	3.31E-03	-1.008	-1.23
RNF10	-0.295	7.472	-5.339	1.80E-05	2.53E-04	2.103	-1.23
RPP14	0.291	6.393	4.312	2.42E-04	2.18E-03	-0.492	1.22
Rps28	0.311	8.402	3.260	3.34E-03	1.78E-02	-3.066	1.24
RPS5	-0.414	11.111	-9.162	2.81E-09	1.65E-07	11.006	-1.33
SAT1	0.427	6.704	8.816	5.75E-09	3.04E-07	10.275	1.34
SBSN	0.350	9.801	5.918	4.28E-06	7.70E-05	3.552	1.27
SBSN	0.452	6.788	5.059	3.65E-05	4.51E-04	1.394	1.37
SCD	-0.429	7.564	-4.585	1.21E-04	1.23E-03	0.195	-1.35
Scd3	-0.417	8.588	-10.020	5.07E-10	3.88E-08	12.751	-1.34
SEC24D	0.341	7.004	4.720	8.59E-05	9.26E-04	0.538	1.27
SEL1L	0.580	7.016	6.135	2.52E-06	5.14E-05	4.089	1.49
SELS	0.277	6.598	5.942	4.04E-06	7.47E-05	3.611	1.21
SEMA3C	-0.617	6.612	-10.704	1.38E-10	1.24E-08	14.078	-1.53
Sepp1	-0.492	9.267	-7.592	8.23E-08	2.99E-06	7.562	-1.41
Serpinh1	0.363	8.949	6.003	3.48E-06	6.61E-05	3.763	1.29
SERPINH1	0.379	8.798	4.130	3.83E-04	3.18E-03	-0.949	1.30
SERPINH1	0.395	9.252	12.158	1.04E-11	1.24E-09	16.712	1.32
SF3B3	0.269	7.166	3.518	1.77E-03	1.09E-02	-2.452	1.20
SGCE	-0.729	6.005	-5.656	8.18E-06	1.33E-04	2.898	-1.66
SGK1	-0.593	7.327	-9.533	1.32E-09	8.96E-08	11.774	-1.51
SHMT2	0.310	6.208	3.041	5.65E-03	2.65E-02	-3.572	1.24
SLC7A1	0.266	7.369	2.709	1.23E-02	4.78E-02	-4.308	1.20
Slc7a11	0.362	7.310	4.303	2.47E-04	2.22E-03	-0.515	1.28
SMPD1	0.311	6.885	5.172	2.75E-05	3.52E-04	1.680	1.24
Smpd1	0.326	6.110	3.465	2.02E-03	1.22E-02	-2.580	1.25
SNAP23	-0.350	7.105	-7.884	4.28E-08	1.69E-06	8.229	-1.27
Snn	0.451	6.495	5.833	5.27E-06	9.24E-05	3.341	1.37
SNRPA1	0.318	7.859	5.622	8.89E-06	1.42E-04	2.814	1.25
SNRPD2	-0.423	8.788	-6.352	1.49E-06	3.39E-05	4.621	-1.34
SPIN1	-0.263	7.093	-3.760	9.73E-04	6.69E-03	-1.866	-1.20
SPRR1A	0.741	8.643	8.596	9.12E-09	4.32E-07	9.804	1.67
Srpr	-0.516	10.118	-3.101	4.90E-03	2.37E-02	-3.434	-1.43
Srpr	-0.460	10.831	-3.831	8.15E-04	5.78E-03	-1.692	-1.38
Srpr	0.335	8.041	7.608	7.93E-08	2.91E-06	7.600	1.26
SSU72	0.596	7.371	9.375	1.82E-09	1.19E-07	11.449	1.51
STIM2	-0.293	6.458	-3.584	1.51E-03	9.61E-03	-2.293	-1.23
STMN1	-0.590	7.260	-9.831	7.33E-10	5.38E-08	12.376	-1.51
SUPT16H	0.274	10.402	3.780	9.24E-04	6.41E-03	-1.816	1.21
SYNCRIP	-1.013	6.547	-15.239	8.67E-14	1.64E-11	21.579	-2.02
SYNCRIP	-0.875	6.514	-4.587	1.21E-04	1.23E-03	0.200	-1.83
Tbc1d1	-0.291	7.646	-5.143	2.95E-05	3.71E-04	1.608	-1.22
Tcf19	-0.354	5.912	-6.157	2.39E-06	4.93E-05	4.142	-1.28
TDP2	0.265	6.488	6.383	1.38E-06	3.19E-05	4.696	1.20
Tes	0.417	6.550	8.595	9.15E-09	4.32E-07	9.801	1.34
Tes	0.500	7.629	8.383	1.44E-08	6.39E-07	9.338	1.41
TFPI	0.552	7.291	6.892	4.12E-07	1.14E-05	5.925	1.47
Thbd	-0.557	7.746	-6.436	1.22E-06	2.89E-05	4.826	-1.47
TIMP2	-0.403	8.474	-5.828	5.35E-06	9.33E-05	3.327	-1.32
Timp2	-0.381	7.474	-4.925	5.11E-05	5.98E-04	1.057	-1.30
Tinagl1	0.442	6.534	3.906	6.75E-04	5.03E-03	-1.506	1.36
TKT	0.281	6.994	6.210	2.10E-06	4.41E-05	4.272	1.21
TM2D3	0.274	6.682	6.647	7.35E-07	1.90E-05	5.336	1.21
Tm9sf3	-0.370	6.640	-7.198	2.02E-07	6.32E-06	6.648	-1.29
Tmem138	-0.322	6.341	-3.197	3.89E-03	1.99E-02	-3.212	-1.25
Tmem167b	-0.309	6.863	-6.917	3.88E-07	1.08E-05	5.984	-1.24
TMEM38B	-0.359	6.934	-6.181	2.25E-06	4.68E-05	4.201	-1.28
TMSB4X	-0.386	10.469	-7.319	1.53E-07	4.99E-06	6.932	-1.31
Tnfaip3	0.498	5.235	5.447	1.37E-05	2.05E-04	2.376	1.41
Tnfsf9	1.035	6.765	9.703	9.43E-10	6.52E-08	12.119	2.05

TRIB1	-0.287	6.683	-4.125	3.88E-04	3.21E-03	-0.962	-1.22
TSC22D3	-0.379	6.584	-7.319	1.53E-07	4.99E-06	6.933	-1.30
TSPO	-0.292	8.531	-3.891	6.99E-04	5.19E-03	-1.542	-1.22
TUBA1A	-0.579	8.193	-6.614	7.95E-07	2.01E-05	5.257	-1.49
UAP1	0.320	6.881	5.192	2.61E-05	3.39E-04	1.732	1.25
UAP1L1	-0.292	6.485	-3.264	3.31E-03	1.77E-02	-3.057	-1.22
Ube2s	-0.424	7.516	-5.147	2.92E-05	3.69E-04	1.618	-1.34
USP46	-0.421	6.354	-6.256	1.88E-06	4.06E-05	4.387	-1.34
UTP18	0.300	7.550	7.324	1.51E-07	4.99E-06	6.944	1.23
VAMP7	-0.394	6.650	-4.882	5.70E-05	6.65E-04	0.948	-1.31
VCAM1	-0.611	6.411	-7.178	2.12E-07	6.55E-06	6.601	-1.53
VIM	-0.321	8.032	-5.580	9.87E-06	1.54E-04	2.710	-1.25
VMA21	-0.356	7.724	-5.511	1.17E-05	1.79E-04	2.535	-1.28
VNN1	0.850	6.056	15.542	5.65E-14	1.13E-11	22.012	1.80
Vps36	-0.304	6.947	-6.097	2.76E-06	5.49E-05	3.994	-1.23
Wdfy4	-1.071	6.002	-10.082	4.49E-10	3.51E-08	12.875	-2.10
WWC3	0.268	6.647	5.336	1.82E-05	2.53E-04	2.094	1.20
Ybx1	-0.475	11.304	-10.367	2.60E-10	2.08E-08	13.431	-1.39
YPEL3	0.264	7.458	5.100	3.29E-05	4.11E-04	1.498	1.20
Ywhab	-0.284	7.203	-4.457	1.68E-04	1.62E-03	-0.128	-1.22
YWHAQ	-0.306	7.581	-6.560	9.03E-07	2.21E-05	5.128	-1.24
ZC3HAV1	-0.385	7.214	-7.139	2.32E-07	6.97E-06	6.510	-1.31
Zfp110	-0.507	6.538	-12.599	4.98E-12	6.63E-10	17.464	-1.42
ZFR	-0.349	8.429	-4.435	1.77E-04	1.70E-03	-0.184	-1.27
ZMYND11	-0.270	7.803	-7.583	8.40E-08	2.99E-06	7.542	-1.21

5.2.7 TBP(S) v TBP(U) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40790	-0.920	5.984	-10.426	2.33E-10	1.09E-08	13.635	-1.89
1810037I17Rik	-0.284	6.983	-5.324	1.87E-05	1.74E-04	2.182	-1.22
2310014D11Rik	-0.417	6.536	-5.710	7.16E-06	7.91E-05	3.149	-1.33
2610002J02Rik	-0.369	6.639	-6.956	3.54E-07	6.43E-06	6.188	-1.29
2810025M15Rik	0.388	6.900	7.035	2.95E-07	5.41E-06	6.375	1.31
2810474O19Rik	-0.317	7.272	-6.092	2.80E-06	3.71E-05	4.098	-1.25
3110062M04Rik	-0.317	7.493	-6.576	8.69E-07	1.36E-05	5.279	-1.25
4833417J20Rik	-0.643	7.312	-9.178	2.71E-09	9.41E-08	11.139	-1.56
6430548M08Rik	-1.508	5.381	-21.499	4.08E-17	1.63E-14	29.246	-2.84
9530053H05Rik	0.650	10.002	11.267	4.95E-11	3.08E-09	15.209	1.57
9530053H05Rik	0.675	9.988	13.204	2.85E-13	2.12E-11	20.257	1.60
ABHD6	0.971	6.783	12.352	7.53E-12	6.06E-10	17.119	1.96
ABHD6	0.985	6.770	14.034	6.74E-14	5.87E-12	21.715	1.98
Acat2	-0.541	6.811	-8.186	2.20E-08	5.70E-07	9.009	-1.46
ACLY	-0.355	8.199	-6.349	1.50E-06	2.18E-05	4.727	-1.28
ACO1	-0.466	6.759	-14.486	2.59E-13	3.88E-11	20.526	-1.38
Acp2	-0.319	6.448	-4.751	7.95E-05	5.85E-04	0.734	-1.25
ACTB	-0.270	7.958	-3.245	3.46E-03	1.33E-02	-2.977	-1.21
Adam10	-0.301	8.078	-4.640	1.05E-04	7.28E-04	0.454	-1.23
ADD3	-0.595	7.763	-12.417	6.75E-12	5.77E-10	17.230	-1.51
ADD3	-0.586	6.120	-4.971	4.55E-05	3.68E-04	1.291	-1.50
ADH5	-0.406	8.532	-2.993	6.34E-03	2.14E-02	-3.556	-1.33
ADH5	-0.348	9.619	-4.755	7.87E-05	5.80E-04	0.745	-1.27
ADNP	-0.277	7.851	-2.881	8.26E-03	2.64E-02	-3.808	-1.21
AF085738	-0.334	10.908	-7.604	8.00E-08	1.71E-06	7.699	-1.26
AFTPH	0.426	7.139	5.937	4.09E-06	4.98E-05	3.714	1.34
AIMP1	0.274	8.513	6.637	7.52E-07	1.22E-05	5.425	1.21
Akap12	-0.393	7.889	-5.893	4.55E-06	5.43E-05	3.607	-1.31
AKIRIN2	0.448	6.069	5.886	4.63E-06	5.47E-05	3.588	1.36
AKR1B10	0.349	9.869	10.843	1.07E-10	5.59E-09	14.426	1.27
Akr7a5	-0.280	6.496	-3.228	3.61E-03	1.37E-02	-3.016	-1.21
AMD1	0.502	7.422	5.004	4.19E-05	3.42E-04	1.376	1.42
AMD1	0.538	8.027	6.257	1.87E-06	2.67E-05	4.501	1.45
AMD1	0.570	7.999	7.163	1.08E-07	1.47E-06	7.222	1.48
Amotl1	0.385	7.099	3.371	2.55E-03	1.04E-02	-2.680	1.31
AMOTL2	-0.528	8.057	-5.126	3.08E-05	2.60E-04	1.683	-1.44
Anxa4	-0.367	7.155	-6.401	1.32E-06	1.96E-05	4.854	-1.29
ARHGAP24	-0.558	7.747	-7.440	1.16E-07	2.42E-06	7.321	-1.47
ARHGEF6	-0.316	7.955	-4.923	5.14E-05	4.06E-04	1.170	-1.24
Arl15	-0.458	6.329	-7.884	4.28E-08	9.92E-07	8.334	-1.37
ARL6IP5	-0.277	7.904	-6.589	8.42E-07	1.33E-05	5.311	-1.21
ARPC3	-0.275	7.652	-2.984	6.47E-03	2.17E-02	-3.576	-1.21
Asf1b	0.326	5.939	3.888	7.06E-04	3.57E-03	-1.429	1.25
ASNSD1	0.359	8.235	5.539	1.09E-05	1.13E-04	2.721	1.28
ASRGL1	0.315	6.420	6.029	3.26E-06	4.18E-05	3.942	1.24
Atf5	-0.343	9.270	-2.954	6.95E-03	2.29E-02	-3.644	-1.27
Atf6	0.288	8.404	6.089	2.82E-06	3.72E-05	4.090	1.22
Atp10a	-0.264	7.352	-6.185	2.23E-06	3.06E-05	4.326	-1.20
ATP5G2	0.301	8.724	2.880	8.26E-03	2.64E-02	-3.808	1.23
Aurka	0.460	6.331	5.854	5.02E-06	5.85E-05	3.508	1.38
B230220B15Rik	2.133	7.506	44.582	1.78E-24	6.40E-21	44.832	4.39
B230220B15Rik	2.134	7.476	47.135	2.11E-27	7.54E-24	51.591	4.39
B630006N21Rik	0.347	7.660	5.137	2.99E-05	2.54E-04	1.711	1.27
BCL2L2	0.288	6.311	6.361	1.46E-06	2.15E-05	4.756	1.22
BDNF	0.627	6.480	12.041	1.28E-11	9.60E-10	16.585	1.54
BDNF	0.634	6.492	12.627	8.06E-13	5.53E-11	19.203	1.55
BHLHE41	-0.423	6.897	-7.335	1.47E-07	2.96E-06	7.078	-1.34
BMI1	-0.569	6.386	-6.861	4.43E-07	7.79E-06	5.963	-1.48
Bnip2	0.337	6.652	4.333	2.29E-04	1.40E-03	-0.318	1.26
BTG3	0.353	7.374	3.905	6.76E-04	3.45E-03	-1.386	1.28
Bub3	0.268	7.159	5.989	3.60E-06	4.47E-05	3.844	1.20
C11orf31	0.397	6.301	7.771	5.51E-08	1.23E-06	8.078	1.32
C12orf10	0.432	6.223	6.205	2.12E-06	2.94E-05	4.375	1.35
C18orf49	-0.889	6.640	-10.766	1.23E-10	6.24E-09	14.281	-1.85
C1GALT1C1	-0.374	7.060	-7.542	9.21E-08	1.95E-06	7.555	-1.30
C1R	-0.434	9.482	-5.506	1.19E-05	1.20E-04	2.639	-1.35
C3	-1.251	7.330	-22.795	1.08E-17	5.52E-15	30.548	-2.38
C78376	-0.460	7.959	-7.350	1.42E-07	2.87E-06	7.113	-1.38
C80142	0.673	6.839	6.075	2.91E-06	3.81E-05	4.056	1.59
C80142	0.676	6.833	7.034	1.49E-07	1.96E-06	6.896	1.60
CACHD1	0.301	6.534	5.869	4.83E-06	5.69E-05	3.546	1.23
CALD1	-0.723	7.673	-5.660	8.10E-06	8.79E-05	3.025	-1.65
CALD1	-0.643	7.225	-7.252	1.78E-07	3.45E-06	6.884	-1.56
CAMK2N1	-0.392	6.214	-5.228	2.38E-05	2.13E-04	1.941	-1.31
CAMLG	0.276	7.728	6.244	1.93E-06	2.71E-05	4.472	1.21

CAPZB	-0.271	7.784	-4.947	4.84E-05	3.86E-04	1.231	-1.21
CBX5	0.319	7.118	3.077	5.19E-03	1.81E-02	-3.365	1.25
CCDC80	-0.468	8.173	-5.440	1.40E-05	1.37E-04	2.475	-1.38
CCDC80	-0.282	7.753	-2.751	1.12E-02	3.40E-02	-4.092	-1.22
Ccl2	0.664	7.826	5.410	1.03E-05	7.33E-05	2.631	1.58
Ccl2	0.744	7.806	5.591	9.62E-06	1.00E-04	2.852	1.67
Ccna2	0.393	6.788	3.073	5.23E-03	1.82E-02	-3.373	1.31
CCNB1	0.506	5.956	3.556	1.61E-03	7.21E-03	-2.238	1.42
Ccnb1	0.534	7.434	6.349	1.50E-06	2.18E-05	4.727	1.45
CCNB1	0.585	5.963	4.418	1.46E-04	7.20E-04	-0.017	1.50
Ccnb1	0.590	7.409	7.337	6.97E-08	1.04E-06	7.662	1.50
Ccnd1	0.437	6.627	5.144	2.94E-05	2.51E-04	1.728	1.35
CCNDBP1	0.331	7.359	5.210	2.49E-05	2.19E-04	1.895	1.26
CD36	-3.316	8.162	-16.626	1.29E-14	2.73E-12	23.539	-9.96
Cd81	-0.378	8.075	-7.328	1.50E-07	2.99E-06	7.061	-1.30
CDC20	0.415	7.231	4.306	2.46E-04	1.48E-03	-0.388	1.33
CDK1	0.323	8.673	5.037	3.86E-05	3.18E-04	1.458	1.25
CDKN3	0.294	8.297	3.399	2.38E-03	9.88E-03	-2.614	1.23
Cenpa	0.629	7.615	6.686	6.70E-07	1.10E-05	5.543	1.55
Cenpa	0.699	7.609	7.657	3.17E-08	5.25E-07	8.463	1.62
CES1	-0.737	3.649	-7.357	1.40E-07	2.85E-06	7.128	-1.67
Chchd3	0.284	6.879	6.427	1.24E-06	1.87E-05	4.918	1.22
CHMP2A	-0.837	7.416	-16.841	9.72E-15	2.18E-12	23.822	-1.79
Cidec	0.288	6.285	5.718	7.02E-06	7.80E-05	3.170	1.22
CKS1B	0.415	7.858	8.337	1.59E-08	4.39E-07	9.342	1.33
Clec2e	-0.634	5.756	-6.004	3.47E-06	4.36E-05	3.880	-1.55
Cmas	0.322	8.324	9.176	2.72E-09	9.41E-08	11.135	1.25
COBLL1	-0.296	6.463	-5.541	1.09E-05	1.13E-04	2.728	-1.23
Col12a1	-0.657	6.218	-13.697	8.58E-13	1.14E-10	19.317	-1.58
COL16A1	-0.632	6.726	-8.919	4.63E-09	1.48E-07	10.596	-1.55
Col5a1	-1.209	6.209	-19.447	3.94E-16	1.29E-13	27.012	-2.31
COPG2	-0.341	6.694	-2.802	9.91E-03	3.08E-02	-3.980	-1.27
CPD	-0.420	7.815	-7.618	7.76E-08	1.68E-06	7.730	-1.34
Cpn2	-2.253	6.648	-28.478	6.40E-20	4.60E-17	35.464	-4.77
CRELD1	0.358	6.519	3.598	1.45E-03	6.60E-03	-2.136	1.28
CRELD2	0.271	7.396	3.781	9.24E-04	4.47E-03	-1.693	1.21
CSDA	0.269	7.938	3.813	8.51E-04	4.17E-03	-1.612	1.20
Csf1	-0.702	7.153	-12.334	7.76E-12	6.06E-10	17.089	-1.63
CSNK2A2	0.296	7.248	4.272	2.68E-04	1.58E-03	-0.472	1.23
Ctla2b	0.293	7.178	3.424	2.24E-03	9.39E-03	-2.554	1.23
CTNNB1	-0.275	7.234	-3.022	5.91E-03	2.02E-02	-3.489	-1.21
CTSH	-0.384	6.886	-6.392	1.35E-06	2.00E-05	4.831	-1.31
Cxx1a	-0.354	6.694	-5.511	1.17E-05	1.20E-04	2.651	-1.28
CYB5R1	0.400	6.816	6.240	1.95E-06	2.72E-05	4.461	1.32
Cyb5r3	-0.293	7.652	-4.679	9.53E-05	6.72E-04	0.553	-1.22
Cyp51	-0.427	9.644	-7.939	3.78E-08	9.01E-07	8.459	-1.34
D5Ertd579e	-0.474	6.408	-9.845	7.12E-10	2.94E-08	12.499	-1.39
D5Ertd579e	-0.377	7.211	-9.375	1.82E-09	6.74E-08	11.546	-1.30
DAG1	-0.408	7.183	-6.151	2.42E-06	3.28E-05	4.243	-1.33
DAG1	-0.359	8.711	-5.541	1.09E-05	1.13E-04	2.726	-1.28
DBF4	0.327	6.260	4.237	2.93E-04	1.70E-03	-0.561	1.25
Dbi	-0.424	8.412	-6.835	4.71E-07	8.22E-06	5.900	-1.34
DCTPP1	0.294	7.125	4.810	6.84E-05	5.19E-04	0.884	1.23
DDIT3	0.415	6.496	4.349	2.20E-04	1.35E-03	-0.278	1.33
DDX1	0.265	6.903	5.858	4.97E-06	5.83E-05	3.518	1.20
DDX39	0.303	7.395	4.745	8.07E-05	5.88E-04	0.719	1.23
Dkc1	0.292	7.108	4.479	1.59E-04	1.03E-03	0.047	1.22
DLGAP5	0.441	6.521	4.778	7.42E-05	5.52E-04	0.803	1.36
Dmtf1	0.464	6.932	5.593	9.56E-06	1.00E-04	2.858	1.38
DNAJA3	0.308	7.151	7.939	3.79E-08	9.01E-07	8.458	1.24
DNAJA3	0.338	6.546	2.999	6.25E-03	2.11E-02	-3.543	1.26
DNTTIP1	0.291	6.557	5.229	2.38E-05	2.13E-04	1.943	1.22
Dpp3	-0.310	6.723	-4.331	2.31E-04	1.40E-03	-0.325	-1.24
Dram1	-0.581	6.300	-9.574	1.22E-09	4.81E-08	11.953	-1.50
DUSP1	-0.661	6.221	-10.244	3.29E-10	1.48E-08	13.284	-1.58
E130203B14Rik	-0.415	8.135	-5.084	3.43E-05	2.87E-04	1.576	-1.33
ECT2	0.529	7.232	5.472	1.29E-05	1.29E-04	2.555	1.44
ECT2	0.586	7.200	6.441	6.79E-07	7.23E-06	5.362	1.50
EEF1E1	0.313	7.790	5.258	2.21E-05	2.01E-04	2.016	1.24
EFEMP1	0.619	9.518	11.287	1.04E-11	4.52E-10	16.608	1.54
EFEMP1	0.632	9.515	10.398	2.46E-10	1.13E-08	13.581	1.55
EIF2A	0.349	7.926	7.036	2.94E-07	5.41E-06	6.376	1.27
EIF2AK1	0.296	7.446	5.893	4.56E-06	5.43E-05	3.605	1.23
EIF3A	0.272	6.909	4.815	6.75E-05	5.13E-04	0.898	1.21
EIF3C	0.264	9.095	5.705	7.24E-06	7.98E-05	3.138	1.20
EIF3G	0.313	9.161	6.125	2.58E-06	3.46E-05	4.178	1.24
EIF3H	0.271	8.828	5.923	4.23E-06	5.08E-05	3.679	1.21
EIF3H	0.297	8.385	2.569	1.69E-02	4.77E-02	-4.480	1.23
Eif4a1	-0.554	8.429	-3.208	3.79E-03	1.42E-02	-3.064	-1.47

Eif4a1	-0.490	9.235	-11.418	3.78E-11	2.51E-09	15.483	-1.40
Eif4ebp1	0.323	7.889	2.771	1.07E-02	3.27E-02	-4.049	1.25
Eif4g2	-0.297	8.282	-5.531	1.12E-05	1.15E-04	2.703	-1.23
EIF4G3	-0.317	7.477	-4.128	3.86E-04	2.15E-03	-0.833	-1.25
EIF5	-0.491	8.217	-9.970	5.59E-10	2.39E-08	12.746	-1.41
EIF5	-0.418	6.761	-4.113	4.01E-04	2.22E-03	-0.871	-1.34
EIF6	0.268	8.152	3.205	3.81E-03	1.43E-02	-3.070	1.20
EMP1	0.340	7.772	6.812	4.97E-07	8.59E-06	5.844	1.27
EPR1	0.284	7.818	3.522	1.76E-03	7.73E-03	-2.320	1.22
EPS8	0.396	8.261	4.412	1.88E-04	1.19E-03	-0.121	1.32
ERCC1	-0.963	6.529	-12.634	4.71E-12	4.45E-10	17.595	-1.95
Erlin1	0.367	6.942	7.325	1.51E-07	2.99E-06	7.055	1.29
Ern1	0.338	6.120	5.424	1.46E-05	1.42E-04	2.434	1.26
ETHE1	-0.926	7.237	-13.266	1.69E-12	1.96E-10	18.632	-1.90
Exosc1	0.290	7.548	3.891	7.01E-04	3.56E-03	-1.422	1.22
F2r	-0.756	8.207	-8.373	1.47E-08	4.10E-07	9.420	-1.69
Fam102b	-0.430	7.655	-5.605	9.29E-06	9.88E-05	2.887	-1.35
FAM107B	-0.694	6.765	-13.525	1.12E-12	1.44E-10	19.046	-1.62
Fasn	-0.349	6.330	-3.496	1.87E-03	8.16E-03	-2.382	-1.27
FCGRT	-0.789	6.491	-11.527	3.11E-11	2.19E-09	15.680	-1.73
FDPS	-0.307	8.124	-5.243	2.30E-05	2.08E-04	1.977	-1.24
Fez2	0.281	7.112	6.295	1.71E-06	2.45E-05	4.596	1.22
Fhl1	-0.569	7.505	-13.027	2.48E-12	2.70E-10	18.245	-1.48
Fhl2	0.775	6.253	18.283	1.06E-16	2.22E-14	28.220	1.71
Fhl2	0.808	6.258	18.389	1.38E-15	3.82E-13	25.769	1.75
FN1	-0.696	8.505	-13.910	6.18E-13	8.53E-11	19.649	-1.62
FNIP2	-0.342	7.370	-5.206	2.52E-05	2.20E-04	1.885	-1.27
FOSL1	0.704	7.029	8.555	3.71E-09	7.88E-08	10.637	1.63
FOSL1	0.730	7.032	8.282	1.79E-08	4.83E-07	9.220	1.66
FOXJ2	0.276	5.870	2.653	1.40E-02	4.06E-02	-4.302	1.21
FSTL1	-0.409	8.751	-9.808	7.67E-10	3.13E-08	12.423	-1.33
FUCA1	0.482	7.522	9.586	1.19E-09	4.75E-08	11.976	1.40
GADD45A	0.703	8.855	10.536	1.89E-10	9.21E-09	13.846	1.63
GADD45A	0.743	8.839	12.190	1.82E-12	1.01E-10	18.378	1.67
GADD45B	-0.389	6.746	-5.725	6.89E-06	7.68E-05	3.188	-1.31
GALNT7	-0.395	7.466	-3.891	7.00E-04	3.56E-03	-1.421	-1.31
GAMT	-0.455	6.280	-5.665	7.99E-06	8.70E-05	3.038	-1.37
GAS1	-1.275	6.643	-13.490	1.19E-12	1.47E-10	18.991	-2.42
GCLM	0.579	9.225	11.125	6.39E-11	3.89E-09	14.949	1.49
GCLM	0.614	9.205	12.496	1.03E-12	6.20E-11	18.958	1.53
GGA2	-0.504	8.255	-13.270	1.68E-12	1.96E-10	18.639	-1.42
GGA2	-0.391	6.153	-4.943	4.88E-05	3.88E-04	1.222	-1.31
GHR	-0.658	6.655	-10.451	2.22E-10	1.06E-08	13.683	-1.58
Ghr	0.344	6.285	6.063	3.00E-06	3.88E-05	4.026	1.27
GJA1	-0.319	10.715	-4.357	2.16E-04	1.33E-03	-0.258	-1.25
Gm10397	-0.283	7.405	-5.431	1.43E-05	1.40E-04	2.452	-1.22
Gm6377	-0.347	8.134	-4.621	1.10E-04	7.54E-04	0.407	-1.27
GNAI1	-0.508	7.278	-7.690	6.60E-08	1.45E-06	7.894	-1.42
GPAM	-0.317	7.859	-7.605	7.99E-08	1.71E-06	7.699	-1.25
GPC6	0.755	3.584	9.011	3.82E-09	1.27E-07	10.790	1.69
GPC6	0.765	3.568	9.727	2.65E-10	7.57E-09	13.316	1.70
Gpd2	0.341	6.879	4.485	1.56E-04	1.02E-03	0.062	1.27
Gpnmb	-0.960	5.966	-15.221	8.90E-14	1.68E-11	21.602	-1.94
GPR176	-0.903	7.295	-20.666	1.00E-16	3.59E-14	28.366	-1.87
Gpr56	0.904	6.210	12.539	9.48E-13	6.16E-11	19.038	1.87
Gpr56	0.946	6.194	12.037	1.28E-11	9.60E-10	16.579	1.93
Gpx1	-0.389	7.884	-5.595	9.52E-06	1.00E-04	2.863	-1.31
GSS	0.319	6.709	4.834	6.44E-05	4.93E-04	0.945	1.25
GSS	0.358	7.023	6.579	8.63E-07	1.35E-05	5.286	1.28
GSS	0.411	6.247	4.459	1.67E-04	1.08E-03	-0.003	1.33
Gss	0.419	8.655	8.964	4.21E-09	1.39E-07	10.691	1.34
Gstm1	-0.404	9.649	-5.834	5.27E-06	6.11E-05	3.458	-1.32
Gstm7	-0.378	8.876	-5.442	1.39E-05	1.37E-04	2.478	-1.30
GSTO1	0.422	6.462	8.208	2.10E-08	5.58E-07	9.057	1.34
Gt(ROSA)26Sor	-0.326	6.526	-5.652	8.26E-06	8.91E-05	3.005	-1.25
Gyg	-0.464	8.497	-5.676	7.79E-06	8.51E-05	3.064	-1.38
Gypc	-0.439	7.466	-7.544	9.17E-08	1.95E-06	7.560	-1.36
H6pd	-0.628	6.439	-9.339	1.95E-09	7.09E-08	11.472	-1.55
HBXIP	0.286	8.226	5.446	1.38E-05	1.36E-04	2.489	1.22
HEG1	-0.548	8.653	-8.871	5.12E-09	1.63E-07	10.492	-1.46
HEXA	-0.347	6.609	-4.708	8.86E-05	6.35E-04	0.626	-1.27
HEXB	-0.361	7.769	-7.281	1.67E-07	3.25E-06	6.951	-1.28
HEXIM1	-0.369	7.032	-5.339	1.80E-05	1.70E-04	2.220	-1.29
HMGCR	-0.442	6.572	-6.589	8.43E-07	1.33E-05	5.311	-1.36
Hmgcs1	-0.877	6.887	-15.899	3.44E-14	6.87E-12	22.555	-1.84
HMOX1	0.516	7.105	5.968	3.79E-06	4.66E-05	3.791	1.43
Hnrnpa1	0.358	6.520	5.365	1.69E-05	1.61E-04	2.285	1.28
Hsd17b12	-0.315	7.796	-6.601	8.20E-07	1.30E-05	5.338	-1.24
HSP90B1	0.266	10.245	4.176	3.41E-04	1.95E-03	-0.713	1.20

HTRA1	-0.469	10.001	-7.911	4.03E-08	9.47E-07	8.394	-1.38
Hyou1	0.502	6.015	6.155	2.40E-06	3.26E-05	4.253	1.42
IDH1	-0.816	8.313	-10.840	1.07E-10	5.59E-09	14.422	-1.76
IDH1	-0.813	6.178	-3.610	1.41E-03	6.43E-03	-2.107	-1.76
Idi1	-0.610	6.124	-10.226	3.41E-10	1.51E-08	13.248	-1.53
IFRD2	0.324	6.584	4.898	5.48E-05	4.29E-04	1.105	1.25
Igf2bp2	-0.394	7.260	-5.508	1.18E-05	1.20E-04	2.644	-1.31
ING1	0.283	6.587	6.000	3.50E-06	4.38E-05	3.870	1.22
Ing1	0.367	7.161	10.250	3.25E-10	1.48E-08	13.296	1.29
Insig1	-0.401	8.024	-8.191	2.18E-08	5.68E-07	9.020	-1.32
IPO5	0.307	7.087	4.885	5.67E-05	4.42E-04	1.072	1.24
IPO7	0.376	8.117	6.251	1.90E-06	2.69E-05	4.488	1.30
IPO7	0.420	8.188	8.695	7.41E-09	2.24E-07	10.118	1.34
Irak1	-0.345	6.508	-5.440	1.40E-05	1.37E-04	2.474	-1.27
Irak2	-0.308	6.582	-4.317	2.39E-04	1.44E-03	-0.359	-1.24
Itfg3	-0.305	6.192	-5.810	5.59E-06	6.44E-05	3.399	-1.24
Jak2	-0.294	6.679	-4.109	4.05E-04	2.24E-03	-0.881	-1.23
KARS	0.289	8.766	3.879	7.22E-04	3.63E-03	-1.451	1.22
Kdelc2	0.291	7.107	3.235	3.55E-03	1.35E-02	-3.001	1.22
Kdelr3	-0.345	6.846	-5.169	2.77E-05	2.39E-04	1.790	-1.27
KIF23	0.332	6.746	5.131	3.04E-05	2.58E-04	1.696	1.26
KLF6	0.403	6.300	5.208	2.51E-05	2.20E-04	1.889	1.32
KLF6	0.407	8.141	8.193	2.17E-08	5.68E-07	9.025	1.33
KLF6	0.455	7.705	7.793	5.24E-08	1.19E-06	8.128	1.37
Kpna4	0.353	7.353	6.416	1.28E-06	1.91E-05	4.891	1.28
KRR1	0.445	6.347	8.130	2.49E-08	6.30E-07	8.885	1.36
LAMA5	0.281	6.977	4.126	3.87E-04	2.16E-03	-0.837	1.21
LAMP2	-0.479	6.487	-7.410	1.24E-07	2.56E-06	7.252	-1.39
LASS5	-0.320	6.341	-4.629	1.08E-04	7.42E-04	0.427	-1.25
LAYN	-0.517	6.973	-7.142	2.30E-07	4.39E-06	6.628	-1.43
LOC100131463	0.296	10.883	8.681	7.63E-09	2.27E-07	10.087	1.23
LOC100131826	-0.384	7.320	-4.515	1.45E-04	9.55E-04	0.139	-1.30
LOC100288436	0.293	6.402	5.840	5.19E-06	6.04E-05	3.473	1.23
LOC401387	-0.438	9.012	-7.715	6.23E-08	1.37E-06	7.952	-1.35
LOC685953	-3.022	9.130	-28.957	4.35E-20	3.91E-17	35.828	-8.12
LOC728758	0.267	6.767	5.335	1.82E-05	1.71E-04	2.210	1.20
LPCAT3	-0.287	6.684	-3.111	4.78E-03	1.69E-02	-3.287	-1.22
Lrig1	-0.423	8.856	-4.340	2.25E-04	1.38E-03	-0.301	-1.34
Lrrc58	-0.331	8.100	-8.206	2.11E-08	5.58E-07	9.053	-1.26
LRRC8D	0.369	6.697	4.404	1.92E-04	1.21E-03	-0.142	1.29
LRRFIP1	0.437	6.403	5.140	2.97E-05	2.53E-04	1.718	1.35
Ly6e	-0.431	7.105	-5.790	5.87E-06	6.68E-05	3.349	-1.35
LYSMD4	-0.381	7.687	-8.080	2.78E-08	6.93E-07	8.773	-1.30
Mad2l1	0.341	7.100	5.200	2.55E-05	2.23E-04	1.870	1.27
Mad2l1	0.463	6.292	3.767	9.56E-04	4.60E-03	-1.726	1.38
MAGED1	-0.353	7.518	-5.783	5.97E-06	6.74E-05	3.333	-1.28
MAMDC2	-0.763	7.429	-6.602	8.17E-07	1.30E-05	5.341	-1.70
MAP4K4	0.305	6.730	4.950	4.80E-05	3.85E-04	1.238	1.24
MAPK6	0.316	7.276	4.368	2.10E-04	1.30E-03	-0.231	1.24
MBNL2	-0.337	7.563	-5.888	4.62E-06	5.47E-05	3.592	-1.26
MCM7	0.276	7.318	3.894	6.95E-04	3.54E-03	-1.414	1.21
MDP1	0.277	6.783	5.285	2.07E-05	1.90E-04	2.083	1.21
MEF2A	-0.424	7.229	-7.043	2.89E-07	5.38E-06	6.394	-1.34
Met	-0.643	9.659	-10.890	9.80E-11	5.42E-09	14.515	-1.56
METTL9	-0.472	6.724	-7.789	5.28E-08	1.19E-06	8.121	-1.39
MGST1	-0.361	11.519	-6.107	2.70E-06	3.59E-05	4.133	-1.28
Mgst1	-0.333	11.656	-8.114	2.58E-08	6.48E-07	8.849	-1.26
MIR21	-0.266	6.244	-3.340	2.75E-03	1.10E-02	-2.753	-1.20
MITF	-0.311	7.306	-4.347	2.21E-04	1.36E-03	-0.284	-1.24
MMD	-1.055	6.497	-12.988	2.64E-12	2.79E-10	18.180	-2.08
MMP14	-0.611	6.141	-5.981	3.67E-06	4.53E-05	3.823	-1.53
Mmp14	-0.539	6.694	-7.998	3.33E-08	8.19E-07	8.590	-1.45
MMP9	-0.429	6.731	-6.794	5.18E-07	8.87E-06	5.802	-1.35
MRPL24	0.355	7.227	6.890	4.14E-07	7.33E-06	6.030	1.28
MRPL37	0.381	7.536	5.222	2.42E-05	2.15E-04	1.924	1.30
MRPL37	0.438	6.243	3.147	4.39E-03	1.58E-02	-3.205	1.35
MRPS18B	0.265	7.064	3.349	2.69E-03	1.08E-02	-2.732	1.20
MRPS22	0.281	6.359	5.998	3.52E-06	4.39E-05	3.865	1.22
MRPS30	0.350	6.293	5.927	4.19E-06	5.05E-05	3.690	1.27
MRPS35	0.400	8.616	8.166	2.30E-08	5.87E-07	8.963	1.32
MRPS9	0.364	6.453	7.785	5.34E-08	1.20E-06	8.110	1.29
MTHFD1L	0.615	7.277	16.273	1.89E-15	3.06E-13	25.325	1.53
MTHFD1L	0.623	7.267	14.601	2.19E-13	3.56E-11	20.697	1.54
Mthfd2	0.459	7.826	10.195	3.61E-10	1.58E-08	13.189	1.37
Mthfd2l	0.951	6.000	14.914	1.38E-13	2.37E-11	21.158	1.93
Mthfd2l	0.970	5.991	17.297	4.20E-16	8.31E-14	26.837	1.96
MYO1B	0.630	8.889	12.510	1.00E-12	6.16E-11	18.984	1.55
MYO1B	0.631	8.879	11.265	4.97E-11	3.08E-09	15.205	1.55
Nars	0.344	7.775	6.906	3.99E-07	7.09E-06	6.069	1.27

NARS	0.377	8.331	6.295	1.71E-06	2.45E-05	4.596	1.30
NARS	0.381	6.984	3.330	2.81E-03	1.12E-02	-2.777	1.30
Nbl1	-0.392	6.440	-4.544	1.34E-04	8.90E-04	0.212	-1.31
Ncam1	2.710	5.415	35.704	3.32E-24	5.92E-21	44.999	6.54
Ncam1	2.712	5.358	30.155	1.69E-20	3.04E-17	36.710	6.55
Ncapd3	-0.441	5.993	-6.255	1.88E-06	2.67E-05	4.497	-1.36
NCK2	-0.477	6.349	-6.019	3.35E-06	4.26E-05	3.916	-1.39
NDC80	0.417	6.736	5.086	3.41E-05	2.86E-04	1.582	1.34
Neat1	-0.614	8.207	-9.367	1.85E-09	6.77E-08	11.530	-1.53
NEIL3	0.621	7.798	8.780	6.19E-09	1.92E-07	10.299	1.54
NEIL3	0.629	7.792	10.051	1.32E-10	4.09E-09	14.026	1.55
Nek2	0.554	8.036	8.422	1.32E-08	3.75E-07	9.526	1.47
Nek2	0.610	8.014	9.624	3.32E-10	9.32E-09	13.089	1.53
Nfib	-0.700	6.638	-8.395	1.40E-08	3.94E-07	9.467	-1.62
NGDN	0.430	7.269	8.687	7.53E-09	2.25E-07	10.100	1.35
NINJ1	-0.383	6.177	-6.036	3.21E-06	4.13E-05	3.959	-1.30
NOL7	0.286	8.228	4.734	8.30E-05	6.02E-04	0.691	1.22
NR1D1	-0.330	6.614	-5.577	9.94E-06	1.03E-04	2.818	-1.26
NR1H3	-0.461	6.661	-5.446	1.38E-05	1.36E-04	2.489	-1.38
NUP54	0.265	6.875	3.961	5.88E-04	3.07E-03	-1.249	1.20
Nusap1	0.392	6.996	4.913	5.27E-05	4.14E-04	1.145	1.31
OAZ2	-0.303	6.880	-5.245	2.28E-05	2.07E-04	1.984	-1.23
OBFC1	0.289	6.436	4.553	1.31E-04	8.73E-04	0.235	1.22
Optn	-0.480	7.088	-5.593	9.57E-06	1.00E-04	2.857	-1.39
PALLD	-0.487	7.134	-9.932	6.02E-10	2.51E-08	12.671	-1.40
Parp3	-0.264	6.322	-4.370	2.09E-04	1.30E-03	-0.226	-1.20
Pbk	0.272	6.261	3.101	4.90E-03	1.72E-02	-3.310	1.21
PCGF5	0.283	6.328	5.762	6.29E-06	7.06E-05	3.279	1.22
Pcolce	-0.479	8.526	-6.358	1.47E-06	2.15E-05	4.749	-1.39
Pcyox1	-0.305	6.602	-3.923	6.46E-04	3.33E-03	-1.342	-1.24
PDIA6	0.353	10.091	8.710	7.17E-09	2.18E-07	10.150	1.28
Pdyn	-0.370	7.034	-4.294	2.53E-04	1.51E-03	-0.418	-1.29
PECI	-0.567	6.867	-10.881	9.96E-11	5.42E-09	14.498	-1.48
PHGDH	-0.305	7.601	-4.253	2.81E-04	1.64E-03	-0.519	-1.24
Pir	0.313	6.603	4.598	1.17E-04	7.89E-04	0.349	1.24
PJA1	-0.418	7.598	-9.951	5.80E-10	2.45E-08	12.708	-1.34
PLAC8	1.171	5.487	12.097	2.16E-12	1.17E-10	18.201	2.25
PLAC8	1.266	5.438	12.678	4.37E-12	4.25E-10	17.669	2.41
Plekha5	0.440	7.039	10.850	1.06E-10	5.59E-09	14.439	1.36
PLIN2	0.338	9.911	3.057	5.45E-03	1.89E-02	-3.412	1.26
PLIN2	0.457	9.393	11.020	7.73E-11	4.55E-09	14.756	1.37
PLK2	0.742	7.605	14.572	2.28E-13	3.56E-11	20.654	1.67
PLK2	0.784	7.596	15.885	3.41E-15	4.86E-13	24.730	1.72
PLS3	-0.331	8.295	-5.345	1.78E-05	1.68E-04	2.234	-1.26
PLTP	-0.566	7.226	-4.315	2.40E-04	1.45E-03	-0.364	-1.48
PPAP2A	-0.828	6.987	-10.968	8.50E-11	4.93E-09	14.660	-1.77
Ppcs	-0.303	7.062	-5.377	1.64E-05	1.57E-04	2.316	-1.23
PPM1G	0.281	7.133	5.151	2.89E-05	2.48E-04	1.745	1.21
PPP2R1A	-0.620	6.065	-5.502	1.20E-05	1.21E-04	2.630	-1.54
PPP2R5C	0.279	7.119	4.259	2.76E-04	1.62E-03	-0.504	1.21
Prc1	0.502	7.462	5.233	2.35E-05	2.12E-04	1.953	1.42
Prc1	0.557	7.457	6.231	1.17E-06	1.13E-05	4.812	1.47
Procr	0.545	6.220	8.173	2.27E-08	5.82E-07	8.980	1.46
Pros1	-0.760	7.149	-13.220	1.82E-12	2.04E-10	18.558	-1.69
PRUNE2	-0.329	7.588	-5.934	4.12E-06	4.98E-05	3.706	-1.26
PTPRM	-0.269	7.071	-6.447	1.18E-06	1.80E-05	4.966	-1.20
PTTG1	0.306	6.979	3.674	1.21E-03	5.62E-03	-1.954	1.24
Pttg1ip	-0.266	8.427	-6.530	9.71E-07	1.50E-05	5.167	-1.20
PUM2	0.306	7.024	7.317	1.54E-07	3.03E-06	7.035	1.24
PWP1	0.408	7.363	7.409	1.25E-07	2.56E-06	7.249	1.33
Pxdn	-0.515	7.848	-8.949	4.35E-09	1.42E-07	10.658	-1.43
QARS	0.287	9.208	7.036	2.94E-07	5.41E-06	6.378	1.22
RAB6A	0.270	6.620	4.089	4.25E-04	2.32E-03	-0.929	1.21
RARRES2	-0.599	9.322	-8.434	1.29E-08	3.68E-07	9.552	-1.51
Rbpj	0.786	7.011	10.931	9.10E-11	5.11E-09	14.591	1.72
Rbpj	0.790	7.631	12.694	7.14E-13	4.99E-11	19.326	1.73
Rbpj	0.795	7.019	12.515	9.90E-13	6.16E-11	18.993	1.73
Rbpj	0.799	7.628	11.422	3.75E-11	2.51E-09	15.491	1.74
RBPJ	0.836	7.585	14.977	1.26E-13	2.27E-11	21.249	1.79
RBPJ	0.843	7.583	16.903	7.42E-16	1.26E-13	26.265	1.79
RCBTB2	-0.738	7.042	-12.542	5.48E-12	5.05E-10	17.441	-1.67
RCN2	-0.268	7.783	-6.292	1.72E-06	2.46E-05	4.588	-1.20
Reep5	-0.578	7.899	-11.797	1.94E-11	1.42E-09	16.159	-1.49
REEP5	-0.458	6.759	-4.690	9.29E-05	6.61E-04	0.580	-1.37
Rere	-0.456	6.955	-5.382	1.62E-05	1.56E-04	2.328	-1.37
RETSAT	-0.507	5.458	-5.402	1.54E-05	1.49E-04	2.377	-1.42
REV3L	-0.304	7.281	-4.631	1.08E-04	7.42E-04	0.433	-1.23
RFX7	-0.594	7.777	-8.238	1.97E-08	5.28E-07	9.123	-1.51
RGD1560010	0.316	6.651	4.560	1.29E-04	8.61E-04	0.253	1.25

RGS17	-0.469	7.346	-4.835	6.42E-05	4.93E-04	0.948	-1.38
RNASET2	0.348	10.189	8.074	2.82E-08	6.98E-07	8.759	1.27
RPF2	0.281	7.016	6.615	7.93E-07	1.28E-05	5.372	1.21
RPS5	-0.426	11.111	-9.423	1.65E-09	6.31E-08	11.645	-1.34
RYBP	-0.482	8.342	-9.432	1.62E-09	6.26E-08	11.663	-1.40
SC4MOL	-0.444	8.194	-8.799	5.95E-09	1.86E-07	10.340	-1.36
Sc5d	-0.517	6.364	-5.482	1.26E-05	1.26E-04	2.579	-1.43
Scarb1	-0.473	6.897	-6.834	4.71E-07	8.22E-06	5.899	-1.39
SCD	-0.674	7.564	-7.214	1.95E-07	3.74E-06	6.795	-1.60
Scd3	-0.582	8.588	-13.985	5.50E-13	7.91E-11	19.765	-1.50
Sdc4	0.300	8.779	5.944	4.01E-06	4.91E-05	3.733	1.23
Sdc4	0.362	7.039	3.886	7.08E-04	3.57E-03	-1.432	1.28
Sec22a	0.297	6.119	7.135	2.34E-07	4.44E-06	6.611	1.23
SEC24D	-0.266	7.004	-3.689	1.16E-03	5.44E-03	-1.917	-1.20
SEMA3C	-0.278	6.612	-4.830	6.51E-05	4.96E-04	0.935	-1.21
Sepp1	-0.557	9.267	-8.600	9.06E-09	2.65E-07	9.913	-1.47
SERPINH1	-0.265	8.798	-2.886	8.15E-03	2.61E-02	-3.795	-1.20
SGCE	-1.172	6.005	-9.091	3.24E-09	1.10E-07	10.957	-2.25
Sh2b3	0.263	6.519	4.797	7.07E-05	5.32E-04	0.852	1.20
Shc1	-0.312	8.134	-7.919	3.96E-08	9.35E-07	8.414	-1.24
SLC25A20	-0.370	8.555	-5.199	2.56E-05	2.23E-04	1.867	-1.29
Slc30a4	0.276	6.781	5.596	9.50E-06	1.00E-04	2.865	1.21
Slc6a6	-0.268	8.040	-5.602	9.36E-06	9.92E-05	2.880	-1.20
Slc7a11	0.300	7.310	3.570	1.56E-03	7.00E-03	-2.204	1.23
SMC4	0.283	6.591	2.562	1.72E-02	4.83E-02	-4.494	1.22
SMC6	0.301	6.823	4.898	5.48E-05	4.29E-04	1.107	1.23
SNAI2	-0.434	6.399	-6.608	8.07E-07	1.29E-05	5.355	-1.35
Snn	-0.576	6.495	-7.453	1.13E-07	2.37E-06	7.350	-1.49
Snora28	-0.481	7.421	-9.297	2.13E-09	7.58E-08	11.385	-1.40
SNRPA1	0.298	7.859	5.257	2.22E-05	2.01E-04	2.013	1.23
SNRPB2	0.280	7.682	4.572	1.25E-04	8.38E-04	0.283	1.21
SNRPD2	-0.531	8.788	-7.977	3.49E-08	8.46E-07	8.543	-1.44
SOD1	-0.500	8.506	-8.942	4.42E-09	1.43E-07	10.643	-1.41
Spc25	0.444	6.631	5.726	6.88E-06	7.68E-05	3.189	1.36
SPIN1	-0.633	7.093	-9.039	3.61E-09	1.21E-07	10.848	-1.55
SPRR1A	2.022	8.643	23.454	5.61E-18	3.36E-15	31.182	4.06
SPRR1A	2.087	8.682	26.345	9.48E-21	5.64E-18	37.430	4.25
SPTBN1	-0.372	9.556	-6.630	7.64E-07	1.24E-05	5.410	-1.29
Sqle	-0.591	9.031	-9.379	1.80E-09	6.74E-08	11.554	-1.51
SQLE	-0.520	7.073	-7.818	4.96E-08	1.13E-06	8.185	-1.43
SQLE	-0.491	6.333	-3.192	3.94E-03	1.46E-02	-3.101	-1.41
SSR2	0.324	9.195	6.016	3.36E-06	4.27E-05	3.911	1.25
STMN1	-0.458	7.260	-7.638	7.42E-08	1.62E-06	7.775	-1.37
STT3B	-0.289	8.430	-4.842	6.30E-05	4.85E-04	0.966	-1.22
Tapbpl	0.273	5.845	2.898	7.94E-03	2.57E-02	-3.770	1.21
Tbc1d1	-0.734	7.646	-12.955	2.78E-12	2.86E-10	18.127	-1.66
TBPL1	0.448	7.435	6.806	5.05E-07	8.68E-06	5.830	1.36
Tcf19	-0.540	5.912	-9.408	1.70E-09	6.43E-08	11.614	-1.45
TDP2	0.321	6.488	7.733	5.99E-08	1.33E-06	7.992	1.25
Tes	0.513	6.550	10.572	1.77E-10	8.83E-09	13.914	1.43
Tes	0.676	7.629	11.331	4.41E-11	2.83E-09	15.326	1.60
Tes	0.693	7.604	12.981	4.24E-13	3.03E-11	19.853	1.62
TFB1M	0.331	6.682	4.746	8.04E-05	5.88E-04	0.723	1.26
Tgfbr2	-0.829	6.364	-10.535	1.90E-10	9.21E-09	13.844	-1.78
Thbd	-0.630	7.746	-7.279	1.68E-07	3.25E-06	6.948	-1.55
THBS3	-0.277	6.147	-5.047	3.76E-05	3.11E-04	1.484	-1.21
TIMM10	0.292	7.656	5.287	2.05E-05	1.90E-04	2.090	1.22
TIMM16	0.324	8.011	6.186	2.22E-06	3.06E-05	4.329	1.25
TIMM50	0.391	7.608	7.940	3.78E-08	9.01E-07	8.461	1.31
TIMM9	0.316	7.636	6.460	1.15E-06	1.75E-05	4.998	1.24
TIMP2	-0.465	8.474	-6.724	6.12E-07	1.02E-05	5.634	-1.38
Timp2	-0.414	7.474	-5.362	1.70E-05	1.61E-04	2.278	-1.33
TJP2	-0.446	6.785	-8.668	7.83E-09	2.31E-07	10.060	-1.36
TMED3	-0.269	6.990	-5.617	9.01E-06	9.67E-05	2.917	-1.20
TMEM101	0.593	6.466	7.113	2.46E-07	4.65E-06	6.558	1.51
TMEM101	0.603	6.460	7.892	1.79E-08	3.19E-07	9.043	1.52
Tmem109	-0.271	6.566	-5.208	2.50E-05	2.20E-04	1.890	-1.21
TMEM156	0.476	7.139	8.308	1.69E-08	4.60E-07	9.279	1.39
TMEM158	-1.079	6.933	-11.033	7.55E-11	4.52E-09	14.780	-2.11
TMEM66	0.289	8.790	7.994	3.35E-08	8.20E-07	8.582	1.22
TMSB10	-0.333	10.371	-5.367	1.68E-05	1.61E-04	2.290	-1.26
Tnfaip3	-0.326	5.235	-3.559	1.60E-03	7.18E-03	-2.231	-1.25
Tnfrsf21	-0.767	7.387	-10.824	1.11E-10	5.69E-09	14.390	-1.70
Tnfsf9	0.992	6.765	9.300	2.12E-09	7.58E-08	11.391	1.99
Tnfsf9	0.998	6.721	10.915	2.19E-11	8.77E-10	15.852	2.00
TNIP1	-0.294	6.401	-3.976	5.65E-04	2.97E-03	-1.210	-1.23
Top2a	0.480	9.103	6.083	2.86E-06	3.76E-05	4.076	1.39
TPM1	-0.327	7.136	-7.309	1.57E-07	3.07E-06	7.017	-1.25
Tpst1	-0.351	6.787	-8.318	1.66E-08	4.54E-07	9.299	-1.28

TPX2	0.486	6.219	5.223	2.41E-05	2.15E-04	1.927	1.40
TPX2	0.565	6.178	6.197	1.28E-06	1.21E-05	4.724	1.48
TRAK2	-0.493	6.593	-6.162	2.36E-06	3.22E-05	4.269	-1.41
Tram1	-0.290	6.401	-3.655	1.26E-03	5.85E-03	-1.998	-1.22
TRAP1	0.283	6.783	3.462	2.04E-03	8.70E-03	-2.465	1.22
TRAPPC4	0.304	7.791	5.483	1.26E-05	1.26E-04	2.582	1.23
Trib3	0.441	8.754	9.455	1.55E-09	6.04E-08	11.710	1.36
TRIB3	0.477	5.753	3.509	1.81E-03	7.93E-03	-2.351	1.39
Trmt6	0.388	6.854	7.089	2.60E-07	4.89E-06	6.502	1.31
TSC22D1	-0.534	10.051	-7.415	1.23E-07	2.55E-06	7.263	-1.45
TSC22D3	-0.359	6.584	-6.940	3.68E-07	6.61E-06	6.150	-1.28
TUBA1A	-0.591	8.193	-6.741	5.88E-07	9.87E-06	5.675	-1.51
UAP1	0.417	6.881	6.760	5.62E-07	9.52E-06	5.721	1.34
UAP1L1	-0.434	6.485	-4.849	6.20E-05	4.78E-04	0.983	-1.35
UBASH3B	0.361	8.046	8.819	5.70E-09	1.80E-07	10.383	1.28
UBE2E3	-0.345	7.271	-5.258	2.21E-05	2.01E-04	2.015	-1.27
Ube2s	-0.277	7.516	-3.364	2.59E-03	1.05E-02	-2.698	-1.21
UCK2	0.474	6.008	7.388	1.31E-07	2.67E-06	7.200	1.39
VAMP7	-0.269	6.650	-3.329	2.82E-03	1.12E-02	-2.780	-1.20
VCAM1	-1.064	6.411	-12.507	5.81E-12	5.22E-10	17.382	-2.09
Vps36	-0.265	6.947	-5.324	1.87E-05	1.74E-04	2.182	-1.20
VWA5A	0.314	7.095	6.132	2.54E-06	3.41E-05	4.196	1.24
WBP5	-0.447	8.222	-8.516	1.08E-08	3.11E-07	9.732	-1.36
Wdfy4	-1.984	6.002	-18.671	9.83E-16	2.94E-13	26.106	-3.96
Wdr1	-0.282	8.251	-2.888	8.12E-03	2.61E-02	-3.792	-1.22
WDR67	0.302	6.118	3.374	2.53E-03	1.04E-02	-2.674	1.23
Wdyhv1	-0.303	7.392	-6.012	3.40E-06	4.30E-05	3.900	-1.23
WHSC1	0.276	6.747	3.302	3.02E-03	1.18E-02	-2.844	1.21
WWC2	-0.538	6.927	-9.196	2.61E-09	9.21E-08	11.177	-1.45
XBP1	-0.372	7.896	-6.729	6.05E-07	1.01E-05	5.646	-1.29
ZC3HAV1	0.277	7.214	5.126	3.08E-05	2.60E-04	1.684	1.21
Zcchc24	-0.418	6.916	-6.827	4.80E-07	8.33E-06	5.881	-1.34
ZEB1	-0.416	7.606	-11.366	4.14E-11	2.71E-09	15.389	-1.33
Zfp110	-0.721	6.538	-17.923	2.45E-15	6.28E-13	25.199	-1.65
ZFP36L1	-0.575	8.280	-9.671	1.01E-09	4.06E-08	12.148	-1.49
ZFP36L2	-0.737	7.753	-12.386	7.10E-12	5.94E-10	17.178	-1.67

5.2.8 L(S)2 v pcDNA(S) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40607	0.282	7.921	6.877	4.26E-07	1.95E-06	5.369	1.22
40790	-0.340	5.984	-3.859	7.60E-04	1.70E-03	-2.170	-1.27
0610039K10Rik	0.554	8.116	12.262	8.76E-12	1.39E-10	16.451	1.47
1110003E01Rik	-0.310	7.572	-2.988	6.42E-03	1.15E-02	-4.244	-1.24
1110004F10Rik	0.327	6.588	5.956	3.90E-06	1.41E-05	3.117	1.25
1700020I14Rik	0.427	8.162	10.034	4.93E-10	4.79E-09	12.302	1.34
1700025O18Rik	0.507	7.116	8.284	1.78E-08	1.17E-07	8.617	1.42
1700052N19Rik	-0.837	6.422	-17.427	4.56E-15	2.06E-13	24.235	-1.79
1810013D10Rik	0.356	7.076	6.673	6.90E-07	2.98E-06	4.878	1.28
2310001A20Rik	-0.500	7.257	-8.914	4.68E-09	3.56E-08	9.989	-1.41
2310044H10Rik	0.361	6.864	4.908	5.34E-05	1.49E-04	0.472	1.28
2810474O19Rik	-0.385	7.272	-7.409	1.25E-07	6.55E-07	6.626	-1.31
2810482I07Rik	-0.412	6.348	-6.926	3.80E-07	1.76E-06	5.485	-1.33
2900097C17Rik	0.602	9.676	12.386	7.11E-12	1.16E-10	16.666	1.52
3010026O09Rik	0.381	6.003	2.582	1.64E-02	2.70E-02	-5.130	1.30
3010026O09Rik	0.632	8.162	12.023	1.31E-11	1.99E-10	16.033	1.55
4930535I16Rik	0.304	8.352	6.953	3.57E-07	1.66E-06	5.549	1.23
4930539H15Rik	0.497	6.597	11.249	5.11E-11	6.45E-10	14.634	1.41
5830485P09Rik	-0.398	7.542	-7.659	7.08E-08	4.02E-07	7.204	-1.32
5930416I19Rik	0.450	6.436	8.169	2.29E-08	1.46E-07	8.361	1.37
6330406I15Rik	-0.492	8.622	-7.733	5.98E-08	3.48E-07	7.376	-1.41
6330578E17Rik	0.317	6.733	6.163	2.35E-06	8.96E-06	3.629	1.25
6430548M08Rik	0.884	5.381	12.604	4.94E-12	8.54E-11	17.041	1.85
9430072B17Rik	-0.329	9.474	-7.458	1.11E-07	5.95E-07	6.740	-1.26
9430083M05Rik	0.296	12.230	9.810	7.64E-10	7.09E-09	11.852	1.23
9530053H05Rik	-0.937	10.002	-16.234	2.18E-14	8.34E-13	22.625	-1.91
A330042M18Rik	0.855	7.205	13.390	1.39E-12	3.04E-11	18.349	1.81
A730071L15Rik	0.374	6.649	5.551	1.06E-05	3.43E-05	2.102	1.30
Aars	-0.316	7.664	-5.464	1.32E-05	4.19E-05	1.881	-1.24
ABCC5	-0.507	7.507	-5.774	6.11E-06	2.09E-05	2.661	-1.42
ABHD5	-0.744	6.637	-7.393	1.29E-07	6.78E-07	6.587	-1.68
ABHD6	0.297	6.783	3.783	9.18E-04	2.01E-03	-2.357	1.23
ACAT1	-0.799	8.170	-6.421	1.26E-06	5.07E-06	4.264	-1.74
ACAT1	-0.562	8.742	-17.424	4.58E-15	2.06E-13	24.230	-1.48
Acat2	-1.799	6.811	-27.215	1.83E-19	3.76E-17	34.593	-3.48
ACLY	-0.479	8.199	-8.560	9.85E-09	6.85E-08	9.225	-1.39
ACO1	-0.576	6.759	-17.899	2.52E-15	1.22E-13	24.845	-1.49
ACOT7	0.549	7.565	10.207	3.53E-10	3.64E-09	12.644	1.46
ACTL6A	-0.276	7.119	-4.484	1.56E-04	3.94E-04	-0.602	-1.21
ACTR1A	0.290	7.024	3.677	1.20E-03	2.55E-03	-2.617	1.22
ACTR3	-0.595	8.121	-3.563	1.59E-03	3.30E-03	-2.894	-1.51
ACTR3	-0.385	8.697	-6.489	1.07E-06	4.40E-06	4.429	-1.31
Actr3	-0.267	10.274	-4.645	1.04E-04	2.72E-04	-0.195	-1.20
ADAM9	-0.527	8.339	-6.990	3.28E-07	1.54E-06	5.637	-1.44
ADD3	0.400	6.120	3.395	2.40E-03	4.83E-03	-3.297	1.32
ADH5	-0.699	8.532	-5.149	2.91E-05	8.57E-05	1.085	-1.62
ADH5	-0.587	9.619	-8.035	3.07E-08	1.90E-07	8.060	-1.50
ADNP	-0.582	7.851	-6.047	3.12E-06	1.15E-05	3.343	-1.50
Ado	0.331	7.572	8.225	2.03E-08	1.31E-07	8.486	1.26
ADO	0.342	6.943	5.919	4.27E-06	1.52E-05	3.024	1.27
ADSS	-0.357	7.645	-7.041	2.91E-07	1.39E-06	5.759	-1.28
AFF4	0.494	7.576	11.762	2.06E-11	2.94E-10	15.570	1.41
AFTPH	0.306	7.139	4.265	2.73E-04	6.55E-04	-1.156	1.24
Agrn	0.754	7.203	15.854	3.66E-14	1.28E-12	22.091	1.69
AHNAK	-0.835	8.479	-11.129	6.34E-11	7.76E-10	14.413	-1.78
AIDA	-0.519	6.309	-3.210	3.77E-03	7.17E-03	-3.733	-1.43
AIFM1	-0.452	8.277	-9.786	8.01E-10	7.36E-09	11.803	-1.37
AIMP1	0.452	8.513	10.963	8.59E-11	1.01E-09	14.100	1.37
Ak2	0.523	9.209	9.695	9.59E-10	8.66E-09	11.618	1.44
Akap12	-1.519	7.889	-22.796	1.08E-17	1.17E-15	30.440	-2.87
AKIRIN2	0.661	6.069	8.689	7.49E-09	5.39E-08	9.506	1.58
AKTIP	-0.307	6.876	-4.969	4.57E-05	1.29E-04	0.629	-1.24
ALDH2	-0.397	8.281	-6.384	1.38E-06	5.50E-06	4.172	-1.32
Amacr	-0.572	6.539	-8.486	1.15E-08	7.83E-08	9.062	-1.49
AMD1	1.302	7.422	12.977	2.69E-12	5.06E-11	17.669	2.47
AMD1	1.363	8.027	15.862	3.62E-14	1.28E-12	22.103	2.57
ANKRD10	-0.316	6.403	-4.466	1.64E-04	4.12E-04	-0.649	-1.25
ANLN	-1.230	6.950	-10.665	1.49E-10	1.65E-09	13.535	-2.35
ANP32E	0.310	8.064	4.456	1.68E-04	4.21E-04	-0.673	1.24
ANXA2	-0.898	9.836	-5.882	4.67E-06	1.65E-05	2.933	-1.86
ANXA2P3	-0.574	10.669	-8.209	2.10E-08	1.35E-07	8.450	-1.49
AP1B1	0.704	6.806	13.356	1.46E-12	3.12E-11	18.293	1.63
Ap3d1	0.485	7.148	4.942	4.90E-05	1.38E-04	0.559	1.40
APEH	-0.321	6.410	-5.104	3.26E-05	9.48E-05	0.970	-1.25
APEX1	-0.342	7.431	-6.322	1.60E-06	6.28E-06	4.022	-1.27

API5	-0.338	6.606	-3.231	3.58E-03	6.87E-03	-3.683	-1.26
APP	0.288	8.605	2.974	6.62E-03	1.18E-02	-4.275	1.22
APP	0.345	10.319	8.056	2.93E-08	1.83E-07	8.107	1.27
ARFGEF2	-0.298	6.640	-7.011	3.12E-07	1.48E-06	5.688	-1.23
ARHGAP18	-0.821	6.699	-3.622	1.37E-03	2.89E-03	-2.751	-1.77
ARHGAP18	-0.583	6.623	-5.822	5.43E-06	1.88E-05	2.781	-1.50
ARHGAP18	-0.434	7.541	-4.988	4.36E-05	1.24E-04	0.677	-1.35
ARHGAP18	-0.397	7.448	-5.117	3.15E-05	9.20E-05	1.004	-1.32
ARHGAP24	-1.023	7.747	-13.630	9.52E-13	2.24E-11	18.737	-2.03
Arhgap25	-0.298	6.665	-3.575	1.54E-03	3.21E-03	-2.864	-1.23
Arhgap29	-0.342	7.970	-7.295	1.62E-07	8.24E-07	6.358	-1.27
ARHGEF6	-0.606	7.955	-9.458	1.54E-09	1.32E-08	11.132	-1.52
ARIH1	0.507	6.918	9.607	1.14E-09	1.00E-08	11.440	1.42
Arl10	0.622	6.690	14.412	2.89E-13	7.99E-12	19.964	1.54
Arl2bp	0.478	7.421	8.002	3.30E-08	2.03E-07	7.986	1.39
ARL3	-0.280	8.358	-5.785	5.94E-06	2.04E-05	2.690	-1.21
ARL6IP1	-0.423	8.692	-7.359	1.40E-07	7.24E-07	6.509	-1.34
ARL6IP4	-0.297	6.141	-4.444	1.73E-04	4.33E-04	-0.703	-1.23
ARL8B	0.364	7.987	7.885	4.27E-08	2.57E-07	7.721	1.29
ARPC1B	-0.384	9.617	-9.621	1.11E-09	9.83E-09	11.466	-1.30
ARPC4	0.442	6.197	4.655	1.01E-04	2.66E-04	-0.169	1.36
Arpp19	-0.263	6.929	-4.680	9.52E-05	2.51E-04	-0.106	-1.20
Asf1b	-1.183	5.939	-14.107	4.57E-13	1.22E-11	19.492	-2.27
ASNS	-0.580	9.701	-14.073	4.82E-13	1.25E-11	19.438	-1.49
ASNS	-0.444	9.296	-9.061	3.45E-09	2.73E-08	10.301	-1.36
ASPM	-0.803	6.112	-12.259	8.79E-12	1.39E-10	16.447	-1.75
ATAD2	-0.363	6.552	-4.088	4.26E-04	9.88E-04	-1.599	-1.29
Atf5	0.476	9.270	4.097	4.17E-04	9.69E-04	-1.578	1.39
Atp10a	-0.957	7.352	-22.398	1.61E-17	1.61E-15	30.029	-1.94
ATP2A2	0.329	6.749	5.505	1.19E-05	3.82E-05	1.985	1.26
ATP5A1	-0.620	7.427	-5.806	5.65E-06	1.95E-05	2.741	-1.54
ATP5A1	-0.392	8.837	-9.370	1.84E-09	1.56E-08	10.950	-1.31
ATP5B	-0.680	9.171	-4.534	1.38E-04	3.52E-04	-0.476	-1.60
Atp5b	-0.327	10.066	-9.087	3.27E-09	2.60E-08	10.358	-1.25
ATP5B	-0.274	10.189	-5.526	1.13E-05	3.64E-05	2.040	-1.21
ATP5G2	-0.288	8.724	-2.750	1.12E-02	1.90E-02	-4.772	-1.22
ATP5H	0.320	9.664	7.123	2.40E-07	1.16E-06	5.954	1.25
Atp5o	0.298	9.052	6.269	1.82E-06	7.07E-06	3.892	1.23
ATP6V1B2	0.515	7.365	8.629	8.52E-09	6.01E-08	9.374	1.43
ATP6V1D	0.428	9.487	12.699	4.23E-12	7.56E-11	17.201	1.35
Atp6v1e1	0.312	8.150	6.454	1.17E-06	4.73E-06	4.344	1.24
ATXN10	-0.477	6.608	-2.429	2.30E-02	3.68E-02	-5.446	-1.39
ATXN7L3B	0.661	7.040	9.208	2.55E-09	2.09E-08	10.612	1.58
ATXN7L3B	0.666	6.370	13.355	1.47E-12	3.12E-11	18.291	1.59
AU015740	0.580	6.605	8.725	6.95E-09	5.05E-08	9.582	1.49
Aurka	-0.994	6.331	-12.649	4.59E-12	8.09E-11	17.116	-1.99
AXL	-0.526	7.254	-9.144	2.91E-09	2.35E-08	10.476	-1.44
Azin1	0.928	6.442	11.188	5.71E-11	7.07E-10	14.521	1.90
AZIN1	1.228	6.431	16.946	8.48E-15	3.42E-13	23.597	2.34
B230219D22Rik	0.540	8.128	10.255	3.23E-10	3.37E-09	12.738	1.45
B230220B15Rik	-2.677	7.506	-55.955	8.31E-27	4.98E-24	51.314	-6.40
B2m	0.354	7.614	7.366	1.37E-07	7.14E-07	6.527	1.28
BAZ1A	-0.367	6.516	-4.289	2.56E-04	6.18E-04	-1.095	-1.29
BC013529	-0.304	7.343	-7.136	2.33E-07	1.13E-06	5.984	-1.23
BC017643	0.287	6.869	6.798	5.14E-07	2.30E-06	5.178	1.22
BC046331	0.366	6.418	5.406	1.53E-05	4.78E-05	1.735	1.29
BCL2L2	0.451	6.311	9.953	5.77E-10	5.57E-09	12.141	1.37
BCLAF1	0.294	7.984	4.718	8.63E-05	2.29E-04	-0.008	1.23
BCLAF1	0.365	6.690	5.106	3.24E-05	9.43E-05	0.976	1.29
Bloc1s2	0.746	6.230	9.802	7.76E-10	7.19E-09	11.836	1.68
BMI1	0.848	6.386	10.225	3.41E-10	3.53E-09	12.681	1.80
BNIP3L	-0.430	7.109	-2.342	2.79E-02	4.37E-02	-5.621	-1.35
BRE	-0.274	6.906	-4.871	5.87E-05	1.62E-04	0.378	-1.21
BRIX1	0.366	7.665	7.553	8.97E-08	4.94E-07	6.961	1.29
BTBD1	0.371	8.357	5.901	4.46E-06	1.58E-05	2.979	1.29
BTG3	0.274	7.374	3.029	5.82E-03	1.05E-02	-4.151	1.21
BUD31	0.493	7.762	8.388	1.42E-08	9.46E-08	8.847	1.41
BZW1	0.370	6.951	6.653	7.25E-07	3.12E-06	4.827	1.29
C11orf73	-0.302	8.188	-4.589	1.20E-04	3.09E-04	-0.337	-1.23
C12orf10	0.932	6.223	13.377	1.42E-12	3.08E-11	18.328	1.91
C12orf11	-0.269	6.952	-3.176	4.10E-03	7.72E-03	-3.813	-1.20
C12orf24	0.363	6.765	6.591	8.39E-07	3.55E-06	4.678	1.29
C14orf166	-0.482	9.852	-12.634	4.70E-12	8.20E-11	17.092	-1.40
C19orf62	0.374	6.759	5.868	4.85E-06	1.70E-05	2.895	1.30
C1orf131	0.305	6.302	4.507	1.47E-04	3.74E-04	-0.544	1.24
C1orf25	0.462	6.127	10.093	4.40E-10	4.41E-09	12.420	1.38
C1orf77	0.274	7.460	8.006	3.27E-08	2.02E-07	7.996	1.21
C1R	-1.176	9.482	-14.913	1.38E-13	4.36E-12	20.723	-2.26
C20orf111	0.652	6.588	13.069	2.32E-12	4.45E-11	17.821	1.57

C20orf24	0.394	9.165	11.159	6.01E-11	7.40E-10	14.468	1.31
C20orf4	0.703	6.497	13.355	1.47E-12	3.12E-11	18.293	1.63
C22orf32	-0.316	8.845	-5.503	1.20E-05	3.84E-05	1.980	-1.24
C2orf24	0.789	6.408	6.929	3.78E-07	1.75E-06	5.493	1.73
C2orf56	0.357	6.861	8.073	2.82E-08	1.78E-07	8.146	1.28
C3	-3.164	7.330	-57.665	4.07E-27	2.93E-24	51.988	-8.97
C3orf48	1.042	7.672	18.149	1.85E-15	9.36E-14	25.163	2.06
C4orf27	1.252	6.966	25.249	1.03E-18	1.77E-16	32.832	2.38
C4orf34	-0.459	6.275	-3.193	3.92E-03	7.43E-03	-3.772	-1.37
C5orf51	0.461	6.308	5.973	3.74E-06	1.36E-05	3.159	1.38
C78376	-0.953	7.959	-15.224	8.86E-14	2.95E-12	21.183	-1.94
CAB39	0.305	7.933	5.890	4.59E-06	1.62E-05	2.951	1.24
CAB39	0.368	7.377	6.140	2.49E-06	9.38E-06	3.571	1.29
CACHD1	0.359	6.534	7.001	3.19E-07	1.50E-06	5.665	1.28
CAD	-0.337	6.534	-7.521	9.65E-08	5.23E-07	6.887	-1.26
CALD1	-1.108	7.225	-12.508	5.80E-12	9.83E-11	16.876	-2.16
CALD1	-0.925	7.673	-7.244	1.82E-07	9.16E-07	6.239	-1.90
CAMK2D	-0.317	9.232	-5.638	8.56E-06	2.82E-05	2.319	-1.25
CAMK2N1	0.600	6.214	8.005	3.27E-08	2.02E-07	7.994	1.52
CAMSAP1L1	0.312	6.300	7.355	1.41E-07	7.29E-07	6.500	1.24
CAPG	-0.905	7.250	-8.529	1.05E-08	7.23E-08	9.157	-1.87
CAPG	-0.593	7.270	-7.478	1.06E-07	5.71E-07	6.787	-1.51
CAPN2	-0.827	6.868	-10.743	1.29E-10	1.44E-09	13.684	-1.77
Capn7	0.273	6.641	4.593	1.19E-04	3.07E-04	-0.326	1.21
Capns1	0.345	7.079	5.363	1.70E-05	5.30E-05	1.627	1.27
CAPZA2	0.309	9.291	4.646	1.04E-04	2.72E-04	-0.192	1.24
CAPZA2	0.357	10.363	8.059	2.91E-08	1.82E-07	8.115	1.28
Cat	-0.301	8.131	-3.020	5.95E-03	1.07E-02	-4.172	-1.23
CAV2	-0.711	6.234	-9.834	7.29E-10	6.84E-09	11.900	-1.64
CBX5	-0.571	7.118	-5.517	1.16E-05	3.72E-05	2.016	-1.49
Cbx6-Nptxr	0.376	6.780	5.003	4.20E-05	1.20E-04	0.715	1.30
CCDC132	0.419	7.485	7.574	8.55E-08	4.73E-07	7.010	1.34
CCDC43	0.512	6.315	10.209	3.52E-10	3.63E-09	12.649	1.43
CCDC50	0.316	7.337	5.568	1.02E-05	3.30E-05	2.143	1.25
CCDC75	0.616	6.959	8.783	6.15E-09	4.49E-08	9.709	1.53
CCDC85B	-0.463	6.897	-6.305	1.67E-06	6.53E-06	3.979	-1.38
Ccl2	-2.737	7.806	-20.572	1.11E-16	8.85E-15	28.051	-6.67
Ccna2	-1.017	6.788	-7.959	3.63E-08	2.21E-07	7.888	-2.02
CCNB1	-1.347	5.956	-9.460	1.53E-09	1.32E-08	11.137	-2.54
Ccnb1	-1.155	7.434	-13.744	7.98E-13	1.91E-11	18.918	-2.23
CCNB2	-0.409	6.467	-6.156	2.39E-06	9.07E-06	3.613	-1.33
Ccnd1	0.612	6.627	7.202	2.00E-07	9.92E-07	6.142	1.53
CCNL2	-0.348	7.749	-7.506	1.00E-07	5.40E-07	6.851	-1.27
CCT2	-0.499	7.481	-2.491	2.01E-02	3.26E-02	-5.320	-1.41
CCT8	-0.388	7.783	-3.560	1.60E-03	3.32E-03	-2.901	-1.31
CD36	4.087	8.162	20.494	1.21E-16	9.23E-15	27.964	17.00
CDC20	-1.127	7.231	-11.694	2.32E-11	3.23E-10	15.448	-2.18
Cdca7	-0.647	7.256	-12.120	1.11E-11	1.70E-10	16.203	-1.57
CDIPT	0.694	7.020	10.690	1.42E-10	1.58E-09	13.584	1.62
CDK1	-0.441	8.673	-6.890	4.14E-07	1.90E-06	5.399	-1.36
CDK11A	0.360	6.593	5.693	7.47E-06	2.49E-05	2.458	1.28
Cdk5rap3	-0.326	7.163	-5.324	1.87E-05	5.76E-05	1.528	-1.25
CDK6	0.408	6.642	3.668	1.22E-03	2.61E-03	-2.638	1.33
Cdk6	0.559	8.613	8.823	5.65E-09	4.16E-08	9.795	1.47
Cdk6	0.720	7.547	6.672	6.92E-07	2.99E-06	4.874	1.65
CDKN3	-1.202	8.297	-13.900	6.27E-13	1.55E-11	19.167	-2.30
Cdv3	0.500	8.233	8.619	8.70E-09	6.13E-08	9.352	1.41
Cebpb	-0.523	9.362	-13.001	2.58E-12	4.94E-11	17.709	-1.44
CEBPZ	0.289	7.942	3.413	2.30E-03	4.63E-03	-3.254	1.22
Cenpa	-0.781	7.615	-8.303	1.71E-08	1.12E-07	8.659	-1.72
Cenpf	-0.915	6.632	-11.128	6.35E-11	7.76E-10	14.411	-1.89
CENPL	-0.417	6.032	-6.008	3.44E-06	1.26E-05	3.245	-1.34
Cep55	-0.357	6.066	-4.226	3.01E-04	7.19E-04	-1.254	-1.28
CETN3	-0.495	8.092	-6.606	8.09E-07	3.44E-06	4.716	-1.41
Cfl1	0.376	5.881	2.632	1.47E-02	2.43E-02	-5.025	1.30
Cflar	-0.575	7.205	-7.298	1.61E-07	8.21E-07	6.366	-1.49
CHD2	0.634	6.581	10.967	8.51E-11	1.00E-09	14.109	1.55
CHD4	0.290	7.060	2.790	1.02E-02	1.75E-02	-4.685	1.22
CHMP2A	0.481	7.416	9.677	9.92E-10	8.92E-09	11.583	1.40
Chordc1	0.889	6.453	12.397	6.97E-12	1.15E-10	16.686	1.85
Chpf	0.608	6.081	5.243	2.29E-05	6.92E-05	1.323	1.52
CHPT1	-0.679	7.085	-6.232	1.99E-06	7.67E-06	3.799	-1.60
CHPT1	-0.546	8.437	-9.786	8.00E-10	7.36E-09	11.804	-1.46
CHUK	0.334	7.119	6.616	7.90E-07	3.37E-06	4.740	1.26
Cidec	-0.355	6.285	-7.057	2.80E-07	1.34E-06	5.798	-1.28
CIRBP	-0.835	7.470	-12.295	8.28E-12	1.33E-10	16.510	-1.78
CISD3	-0.308	6.884	-4.948	4.83E-05	1.36E-04	0.575	-1.24
CITED2	0.625	6.419	6.622	7.80E-07	3.33E-06	4.753	1.54
CKAP2	-1.177	5.818	-19.230	5.07E-16	3.04E-14	26.492	-2.26

CKAP5	-0.583	6.097	-7.482	1.06E-07	5.67E-07	6.795	-1.50
CKS1B	-0.723	7.858	-14.503	2.53E-13	7.15E-12	20.103	-1.65
CKS2	-0.405	8.689	-5.178	2.70E-05	8.03E-05	1.159	-1.32
Clec2e	-2.580	5.756	-24.439	2.19E-18	3.03E-16	32.068	-5.98
Clic4	-0.534	8.809	-8.882	5.00E-09	3.77E-08	9.922	-1.45
CLIP1	0.276	6.403	4.549	1.33E-04	3.40E-04	-0.439	1.21
Clstn1	0.745	7.732	10.805	1.15E-10	1.31E-09	13.802	1.68
CLTA	0.471	8.281	12.643	4.64E-12	8.13E-11	17.106	1.39
CLTC	0.288	9.014	6.325	1.59E-06	6.25E-06	4.028	1.22
Cmtm3	-0.348	6.630	-6.583	8.56E-07	3.62E-06	4.658	-1.27
CNBP	-0.342	9.436	-3.109	4.81E-03	8.91E-03	-3.967	-1.27
CNOT7	0.280	7.521	6.151	2.42E-06	9.16E-06	3.601	1.21
CNTD1	0.349	6.556	8.703	7.28E-09	5.26E-08	9.536	1.27
COBLL1	-0.670	6.463	-12.531	5.58E-12	9.50E-11	16.915	-1.59
COL5A2	-0.346	7.606	-4.756	7.84E-05	2.09E-04	0.088	-1.27
Commd3	0.492	7.861	11.727	2.19E-11	3.08E-10	15.506	1.41
COMMD7	0.748	6.618	15.867	3.60E-14	1.28E-12	22.110	1.68
COMMD8	0.469	7.001	6.841	4.64E-07	2.10E-06	5.281	1.38
COMTD1	-0.506	6.280	-7.520	9.68E-08	5.24E-07	6.883	-1.42
COPE	0.442	8.277	8.158	2.34E-08	1.49E-07	8.337	1.36
COPG2	-0.499	6.694	-4.104	4.09E-04	9.54E-04	-1.559	-1.41
Cops8	0.568	7.245	11.485	3.35E-11	4.43E-10	15.069	1.48
CORO1B	-0.436	7.585	-6.044	3.14E-06	1.16E-05	3.335	-1.35
Coro1b	-0.408	8.179	-6.459	1.15E-06	4.68E-06	4.357	-1.33
Cox4nb	0.738	7.112	13.419	1.33E-12	2.94E-11	18.396	1.67
Cpn2	0.428	6.648	5.406	1.52E-05	4.78E-05	1.736	1.35
Cpsf2	0.566	7.072	7.211	1.96E-07	9.82E-07	6.161	1.48
CPT2	-0.412	6.454	-7.281	1.67E-07	8.48E-07	6.326	-1.33
Creg1	-0.274	6.806	-2.909	7.74E-03	1.35E-02	-4.422	-1.21
CRELD1	0.608	6.519	6.109	2.68E-06	1.01E-05	3.497	1.52
CRELD2	0.415	7.396	5.782	5.99E-06	2.05E-05	2.681	1.33
CRIPT	0.395	8.324	10.071	4.59E-10	4.54E-09	12.375	1.31
Crkl	0.526	6.176	4.401	1.93E-04	4.77E-04	-0.812	1.44
Crkl	0.556	6.976	13.208	1.85E-12	3.74E-11	18.051	1.47
CROT	-0.339	6.877	-7.450	1.14E-07	6.04E-07	6.720	-1.27
CSDAP1	0.311	10.028	6.104	2.71E-06	1.02E-05	3.484	1.24
CSDE1	-1.097	7.004	-2.842	9.03E-03	1.56E-02	-4.570	-2.14
CSDE1	-0.307	7.867	-2.388	2.52E-02	3.99E-02	-5.529	-1.24
CSE1L	-1.052	7.389	-19.943	2.24E-16	1.46E-14	27.332	-2.07
Csnk1d	0.586	7.166	10.087	4.45E-10	4.45E-09	12.408	1.50
CSNK1E	0.368	6.379	7.207	1.98E-07	9.87E-07	6.153	1.29
CSNK1E	0.500	6.600	7.736	5.95E-08	3.47E-07	7.382	1.41
CSNK2A1	0.483	6.970	9.311	2.07E-09	1.73E-08	10.827	1.40
Csnk2a1	0.700	6.763	6.363	1.45E-06	5.74E-06	4.122	1.62
CSNK2A2	0.331	7.248	4.779	7.40E-05	1.99E-04	0.145	1.26
CTAGE1	-0.368	8.036	-6.343	1.52E-06	6.00E-06	4.073	-1.29
CTBP2	0.299	7.102	5.568	1.02E-05	3.30E-05	2.144	1.23
Ctla2b	-3.920	7.178	-45.815	9.36E-25	4.20E-22	46.768	-15.14
Ctnnbl1	0.951	7.066	14.758	1.74E-13	5.38E-12	20.489	1.93
Ctsb	-0.390	9.036	-6.724	6.12E-07	2.68E-06	5.000	-1.31
CTSF	-0.364	6.525	-4.893	5.54E-05	1.54E-04	0.436	-1.29
Ctsz	-0.930	9.672	-20.294	1.51E-16	1.06E-14	27.736	-1.91
CUL3	0.299	7.260	4.055	4.63E-04	1.07E-03	-1.681	1.23
CUL3	0.306	8.127	6.576	8.70E-07	3.66E-06	4.642	1.24
CUL4B	-0.321	6.379	-6.047	3.12E-06	1.15E-05	3.343	-1.25
Cul5	0.406	7.214	8.145	2.41E-08	1.53E-07	8.307	1.33
Cxcl1	-1.357	6.634	-15.852	3.67E-14	1.28E-12	22.089	-2.56
CYB5R1	0.430	6.816	6.707	6.37E-07	2.77E-06	4.960	1.35
Cyb5r3	-0.422	7.652	-6.747	5.80E-07	2.55E-06	5.055	-1.34
CYFIP1	-0.456	7.533	-8.325	1.63E-08	1.07E-07	8.708	-1.37
CYP20A1	-0.464	7.126	-8.180	2.23E-08	1.43E-07	8.385	-1.38
Cyp51	-0.943	9.644	-17.540	3.95E-15	1.86E-13	24.381	-1.92
D10Jhu81e	-0.722	6.385	-11.300	4.66E-11	5.98E-10	14.730	-1.65
D15Ertd621e	0.569	7.356	13.496	1.17E-12	2.64E-11	18.522	1.48
D17Wsu104e	-0.445	6.741	-6.615	7.93E-07	3.38E-06	4.736	-1.36
D5Ertd579e	-0.359	7.211	-8.942	4.42E-09	3.38E-08	10.049	-1.28
D5Ertd579e	0.573	6.408	11.903	1.62E-11	2.41E-10	15.820	1.49
D830024F11Rik	-0.422	7.236	-6.670	6.95E-07	3.00E-06	4.870	-1.34
DAG1	-0.698	8.711	-10.782	1.20E-10	1.35E-09	13.758	-1.62
DAG1	-0.509	7.183	-7.682	6.71E-08	3.85E-07	7.258	-1.42
DARS	-0.516	6.678	-2.291	3.11E-02	4.82E-02	-5.721	-1.43
DARS	-0.446	8.202	-2.554	1.75E-02	2.86E-02	-5.189	-1.36
DBF4	-0.663	6.260	-8.582	9.41E-09	6.56E-08	9.272	-1.58
Dbi	-0.996	8.412	-16.056	2.78E-14	1.03E-12	22.376	-1.99
DCAF13	0.401	7.411	9.518	1.36E-09	1.19E-08	11.257	1.32
DCAF7	0.344	7.819	6.667	7.00E-07	3.01E-06	4.863	1.27
DCN	-0.505	10.903	-9.089	3.25E-09	2.60E-08	10.362	-1.42
DCTN3	0.301	7.288	5.128	3.06E-05	8.97E-05	1.033	1.23
DCTPP1	-0.504	7.125	-8.239	1.96E-08	1.27E-07	8.517	-1.42

Ddhd2	0.469	7.529	10.434	2.29E-10	2.46E-09	13.090	1.38
DDHD2	0.595	9.554	2.535	1.82E-02	2.98E-02	-5.229	1.51
DDIT3	0.667	6.496	6.993	3.25E-07	1.53E-06	5.645	1.59
Ddx17	0.533	8.800	11.678	2.39E-11	3.29E-10	15.418	1.45
DDX27	-0.292	6.735	-3.719	1.08E-03	2.33E-03	-2.515	-1.22
Ddx54	-0.450	7.085	-6.392	1.35E-06	5.41E-06	4.193	-1.37
DDX6	0.505	6.386	7.149	2.26E-07	1.11E-06	6.015	1.42
Def8	0.365	6.413	7.211	1.96E-07	9.82E-07	6.161	1.29
Dek	-0.302	7.745	-4.889	5.60E-05	1.55E-04	0.426	-1.23
Dennd4a	0.452	6.236	9.070	3.39E-09	2.69E-08	10.321	1.37
DENND5A	0.286	6.500	3.986	5.51E-04	1.26E-03	-1.854	1.22
DERL1	0.304	7.056	5.181	2.68E-05	7.99E-05	1.166	1.23
DERL1	0.316	8.644	6.411	1.29E-06	5.18E-06	4.238	1.24
Derl1	0.324	8.334	6.569	8.85E-07	3.71E-06	4.624	1.25
Derl1	0.523	7.959	7.322	1.52E-07	7.81E-07	6.422	1.44
DHRS7	-0.524	7.060	-12.694	4.26E-12	7.57E-11	17.193	-1.44
DLAT	0.329	6.860	6.065	2.99E-06	1.11E-05	3.386	1.26
DLGAP5	-0.959	6.521	-10.388	2.50E-10	2.67E-09	13.000	-1.94
Dlst	0.443	6.415	6.582	8.58E-07	3.62E-06	4.655	1.36
Dmtf1	0.307	6.932	3.693	1.15E-03	2.46E-03	-2.577	1.24
DNAJA2	-0.335	6.750	-4.889	5.60E-05	1.55E-04	0.426	-1.26
DNAJB2	0.500	6.280	4.712	8.76E-05	2.32E-04	-0.023	1.41
Dnajb6	0.609	6.727	6.471	1.12E-06	4.57E-06	4.385	1.53
DNAJC15	0.359	7.484	6.577	8.68E-07	3.66E-06	4.644	1.28
DNAJC8	0.314	7.300	6.051	3.09E-06	1.14E-05	3.351	1.24
DNTTIP1	0.707	6.557	12.692	4.28E-12	7.57E-11	17.189	1.63
DNTTIP2	0.373	7.857	6.489	1.07E-06	4.40E-06	4.430	1.30
DOCK7	-0.322	7.938	-6.473	1.11E-06	4.55E-06	4.390	-1.25
Dock9	-0.789	6.569	-12.189	9.91E-12	1.54E-10	16.324	-1.73
DPF2	0.353	6.623	5.707	7.20E-06	2.41E-05	2.494	1.28
DPM1	-0.463	8.866	-10.727	1.33E-10	1.48E-09	13.653	-1.38
Dpp3	-0.434	6.723	-6.069	2.96E-06	1.10E-05	3.398	-1.35
DR1	0.362	8.097	4.172	3.45E-04	8.17E-04	-1.389	1.29
Dram1	-1.132	6.300	-18.661	9.94E-16	5.33E-14	25.801	-2.19
DUS1L	-0.299	7.044	-5.809	5.59E-06	1.94E-05	2.750	-1.23
Dusp3	1.266	5.641	13.772	7.64E-13	1.85E-11	18.964	2.41
DYNC1H1	0.429	6.945	4.853	6.14E-05	1.69E-04	0.333	1.35
DYNLRB1	0.608	9.137	11.766	2.05E-11	2.94E-10	15.576	1.52
DYNLT3	0.299	6.979	6.802	5.09E-07	2.28E-06	5.187	1.23
DYX1C1	-0.384	7.256	-7.449	1.14E-07	6.04E-07	6.720	-1.30
E130203B14Rik	-0.773	8.135	-9.475	1.49E-09	1.29E-08	11.167	-1.71
EBNA1BP2	0.275	8.826	4.811	6.82E-05	1.85E-04	0.228	1.21
EBP	-1.345	5.933	-22.289	1.80E-17	1.74E-15	29.915	-2.54
ECT2	-0.742	7.232	-7.677	6.78E-08	3.88E-07	7.247	-1.67
EFEMP1	-1.230	9.515	-20.244	1.59E-16	1.08E-14	27.679	-2.35
Ehd4	-0.692	6.254	-19.135	5.67E-16	3.23E-14	26.377	-1.62
Eif1	0.278	9.538	5.987	3.61E-06	1.32E-05	3.194	1.21
EIF1AX	0.387	7.613	7.140	2.31E-07	1.12E-06	5.995	1.31
EIF2B4	0.291	6.151	3.291	3.09E-03	6.04E-03	-3.543	1.22
EIF3B	0.377	7.197	2.726	1.18E-02	2.00E-02	-4.824	1.30
EIF3D	0.396	7.558	3.389	2.44E-03	4.89E-03	-3.311	1.32
EIF3D	0.563	9.026	8.985	4.03E-09	3.15E-08	10.141	1.48
EIF3IP1	-0.297	10.139	-5.124	3.10E-05	9.06E-05	1.021	-1.23
EIF3J	0.457	7.214	8.835	5.52E-09	4.12E-08	9.820	1.37
EIF3J	0.492	6.961	4.780	7.39E-05	1.99E-04	0.148	1.41
Eif4a1	-0.502	8.429	-2.905	7.81E-03	1.36E-02	-4.431	-1.42
EIF4G1	0.328	7.266	2.875	8.36E-03	1.45E-02	-4.497	1.26
EIF6	0.903	8.152	10.788	1.18E-10	1.34E-09	13.770	1.87
ELAVL1	0.266	6.699	4.535	1.38E-04	3.52E-04	-0.474	1.20
ELK3	-0.619	7.108	-7.715	6.24E-08	3.61E-07	7.333	-1.54
ELOVL1	-0.472	6.439	-9.125	3.03E-09	2.43E-08	10.436	-1.39
Elp2	0.474	5.950	4.481	1.58E-04	3.97E-04	-0.611	1.39
EMP1	-1.234	7.772	-24.720	1.68E-18	2.63E-16	32.336	-2.35
Enah	0.290	8.200	5.339	1.80E-05	5.57E-05	1.567	1.22
Endod1	-0.403	6.475	-6.575	8.71E-07	3.67E-06	4.640	-1.32
ENG	0.549	7.236	4.698	9.09E-05	2.40E-04	-0.061	1.46
Ensa	0.292	6.811	7.419	1.22E-07	6.42E-07	6.650	1.22
EPDR1	-0.344	7.424	-8.272	1.83E-08	1.19E-07	8.590	-1.27
EPR1	-0.488	7.818	-6.045	3.14E-06	1.16E-05	3.336	-1.40
EPRS	-0.419	6.781	-3.375	2.53E-03	5.05E-03	-3.346	-1.34
EPRS	-0.290	8.814	-7.808	5.06E-08	3.00E-07	7.547	-1.22
EPS8	-1.063	8.261	-11.829	1.84E-11	2.68E-10	15.690	-2.09
EPS8	-0.913	7.562	-11.367	4.14E-11	5.38E-10	14.853	-1.88
ERAP1	-0.498	6.611	-10.537	1.89E-10	2.07E-09	13.288	-1.41
ERCC1	0.279	6.529	3.666	1.23E-03	2.62E-03	-2.644	1.21
ERGIC3	0.297	7.529	3.153	4.32E-03	8.11E-03	-3.866	1.23
ERGIC3	0.386	7.853	7.705	6.38E-08	3.68E-07	7.310	1.31
Ermp1	-0.429	7.114	-6.899	4.05E-07	1.87E-06	5.421	-1.35
Ern1	0.660	6.120	10.594	1.70E-10	1.87E-09	13.400	1.58

ERP29	-0.719	6.775	-8.580	9.45E-09	6.58E-08	9.268	-1.65
Erp29	-0.536	7.835	-9.667	1.01E-09	9.08E-09	11.561	-1.45
ERP44	-0.507	6.554	-3.447	2.11E-03	4.30E-03	-3.172	-1.42
ESD	-0.266	8.915	-6.453	1.17E-06	4.73E-06	4.343	-1.20
ETFDH	-0.347	7.133	-5.744	6.58E-06	2.23E-05	2.586	-1.27
ETHE1	0.535	7.237	7.665	6.97E-08	3.97E-07	7.219	1.45
ETNK1	0.472	8.465	8.190	2.18E-08	1.40E-07	8.408	1.39
ETS1	-0.295	7.088	-5.850	5.06E-06	1.77E-05	2.852	-1.23
EWSR1	0.277	7.376	7.547	9.10E-08	4.98E-07	6.947	1.21
Exosc1	0.686	7.548	9.214	2.52E-09	2.08E-08	10.624	1.61
EXOSC9	0.458	6.576	7.282	1.66E-07	8.47E-07	6.330	1.37
EZH2	-0.379	6.593	-5.511	1.17E-05	3.77E-05	2.000	-1.30
F2r	-0.909	8.207	-10.070	4.60E-10	4.54E-09	12.373	-1.88
FAF1	0.613	6.458	4.403	1.92E-04	4.75E-04	-0.808	1.53
Fam102b	-0.567	7.655	-7.388	1.30E-07	6.82E-07	6.578	-1.48
FAM107B	-1.009	6.765	-19.660	3.08E-16	1.94E-14	27.002	-2.01
FAM113A	-0.546	6.737	-8.499	1.12E-08	7.64E-08	9.091	-1.46
FAM120A	-0.377	9.029	-9.512	1.38E-09	1.20E-08	11.243	-1.30
Fam125b	0.740	6.716	14.336	3.24E-13	8.89E-12	19.847	1.67
FAM32A	0.346	6.710	6.329	1.57E-06	6.19E-06	4.038	1.27
Fam32a	0.608	7.017	12.443	6.46E-12	1.08E-10	16.764	1.52
FAM40B	-0.494	6.679	-8.413	1.35E-08	9.02E-08	8.903	-1.41
FAM49B	-0.284	7.847	-5.838	5.22E-06	1.82E-05	2.821	-1.22
FAM54B	0.347	6.657	5.020	4.03E-05	1.15E-04	0.757	1.27
Fam82a2	0.410	6.323	9.832	7.31E-10	6.84E-09	11.896	1.33
Fam92a	0.268	6.361	4.813	6.79E-05	1.85E-04	0.232	1.20
FAM98A	-0.399	6.939	-9.660	1.03E-09	9.15E-09	11.546	-1.32
Farp2	0.276	6.968	5.070	3.55E-05	1.03E-04	0.884	1.21
FARSB	-0.311	7.344	-6.336	1.55E-06	6.10E-06	4.055	-1.24
Farsb	-0.295	7.210	-6.344	1.52E-06	6.00E-06	4.075	-1.23
Fasn	-0.794	6.330	-7.941	3.77E-08	2.29E-07	7.850	-1.73
FAT1	-0.305	8.629	-6.031	3.25E-06	1.19E-05	3.302	-1.24
FBXL5	-0.291	6.539	-4.382	2.03E-04	4.99E-04	-0.861	-1.22
FBXO18	-0.271	6.618	-3.292	3.09E-03	6.03E-03	-3.541	-1.21
FBXO28	0.458	7.050	8.916	4.66E-09	3.56E-08	9.993	1.37
FBXO8	1.172	6.542	23.688	4.47E-18	5.18E-16	31.337	2.25
FBXW11	0.345	7.396	6.494	1.06E-06	4.35E-06	4.443	1.27
FCGRT	0.509	6.491	7.445	1.15E-07	6.08E-07	6.709	1.42
FCHO2	-0.361	7.134	-6.238	1.96E-06	7.58E-06	3.814	-1.28
FDFT1	-0.724	6.917	-16.287	2.03E-14	7.84E-13	22.700	-1.65
FDPS	-1.079	8.124	-18.441	1.30E-15	6.71E-14	25.528	-2.11
Fem1b	0.617	7.723	12.247	8.98E-12	1.41E-10	16.426	1.53
FERMT2	-0.281	8.924	-6.054	3.07E-06	1.13E-05	3.360	-1.21
FH	-0.269	8.282	-5.284	2.07E-05	6.31E-05	1.427	-1.21
Fhl1	0.358	7.505	8.205	2.11E-08	1.36E-07	8.441	1.28
Fhl2	-0.429	6.258	-9.755	8.51E-10	7.76E-09	11.740	-1.35
Fkbp1a	-0.293	9.533	-5.131	3.04E-05	8.91E-05	1.040	-1.23
Fkbp9	-0.338	7.631	-6.561	9.02E-07	3.78E-06	4.605	-1.26
FLJ44342	0.379	7.768	7.410	1.24E-07	6.54E-07	6.629	1.30
FN1	-0.523	8.505	-10.464	2.17E-10	2.34E-09	13.148	-1.44
FNTA	0.374	8.174	11.735	2.16E-11	3.05E-10	15.520	1.30
FOXJ2	0.535	5.870	5.140	2.97E-05	8.75E-05	1.062	1.45
FOXJ3	0.513	7.777	9.274	2.23E-09	1.86E-08	10.750	1.43
FOXO3B	0.296	6.763	4.747	8.02E-05	2.14E-04	0.065	1.23
Frmd6	-0.590	7.433	-10.826	1.10E-10	1.27E-09	13.843	-1.50
FRMD6	-0.414	8.522	-7.816	4.97E-08	2.96E-07	7.566	-1.33
FRY	-1.023	7.685	-14.671	1.97E-13	5.83E-12	20.358	-2.03
FTSJ3	0.314	7.362	4.779	7.40E-05	1.99E-04	0.146	1.24
G3BP2	0.527	8.617	9.854	7.01E-10	6.62E-09	11.941	1.44
Gaa	0.552	9.198	7.966	3.57E-08	2.17E-07	7.905	1.47
GABARAP	0.537	9.365	11.600	2.74E-11	3.74E-10	15.278	1.45
Gabarapl1	0.291	7.833	5.326	1.86E-05	5.74E-05	1.533	1.22
GADD45A	0.499	8.855	7.474	1.08E-07	5.76E-07	6.776	1.41
GADD45B	-0.689	6.746	-10.136	4.05E-10	4.11E-09	12.505	-1.61
GALNT7	0.522	7.466	5.151	2.89E-05	8.54E-05	1.090	1.44
GAPDH	-0.713	10.322	-4.693	9.19E-05	2.43E-04	-0.072	-1.64
GAPDH	-0.675	10.582	-4.948	4.83E-05	1.36E-04	0.575	-1.60
GAPDH	-0.664	8.290	-5.353	1.74E-05	5.40E-05	1.602	-1.58
GAPDH	-0.663	7.912	-5.278	2.10E-05	6.39E-05	1.411	-1.58
GAPDH	-0.588	7.234	-5.030	3.93E-05	1.13E-04	0.782	-1.50
GAPDH	-0.529	7.620	-5.957	3.89E-06	1.40E-05	3.119	-1.44
Gapdh	-0.437	9.031	-7.978	3.48E-08	2.13E-07	7.931	-1.35
Gapdh	-0.419	9.326	-8.507	1.10E-08	7.53E-08	9.109	-1.34
GAPVD1	0.391	6.971	5.764	6.26E-06	2.13E-05	2.636	1.31
GAS1	0.458	6.643	4.844	6.28E-05	1.72E-04	0.311	1.37
GHITM	0.356	9.871	7.587	8.32E-08	4.64E-07	7.038	1.28
GJA1	-0.267	10.715	-3.639	1.32E-03	2.78E-03	-2.710	-1.20
GLG1	0.514	6.270	2.773	1.06E-02	1.81E-02	-4.722	1.43
GLO1	-0.405	6.844	-2.534	1.83E-02	2.99E-02	-5.231	-1.32

GLOD4	-0.310	6.950	-3.536	1.70E-03	3.51E-03	-2.958	-1.24
Glrx	-0.272	7.177	-5.416	1.49E-05	4.68E-05	1.760	-1.21
GLRX5	0.277	8.281	6.489	1.07E-06	4.40E-06	4.431	1.21
GLUD1	-0.375	7.039	-4.437	1.76E-04	4.40E-04	-0.722	-1.30
GLUL	-0.448	6.564	-6.764	5.57E-07	2.47E-06	5.096	-1.36
Gm10291	-0.411	10.903	-8.422	1.32E-08	8.87E-08	8.922	-1.33
Gm10291	-0.360	10.446	-6.089	2.82E-06	1.05E-05	3.446	-1.28
Gm10359	-0.545	10.886	-6.707	6.37E-07	2.77E-06	4.959	-1.46
Gm12033	-0.573	8.805	-6.153	2.41E-06	9.14E-06	3.604	-1.49
Gm12033	-0.559	9.387	-5.901	4.46E-06	1.58E-05	2.979	-1.47
Gm14148	-0.461	9.820	-9.240	2.39E-09	1.98E-08	10.678	-1.38
Gm15104	-0.374	6.022	-6.639	7.49E-07	3.21E-06	4.794	-1.30
Gm2223	-0.280	7.713	-3.103	4.87E-03	9.00E-03	-3.980	-1.21
Gm2260	-0.472	8.341	-7.552	9.00E-08	4.95E-07	6.958	-1.39
Gm5069	-0.574	9.657	-10.339	2.75E-10	2.90E-09	12.904	-1.49
Gm5578	0.353	6.726	6.603	8.16E-07	3.46E-06	4.707	1.28
Gm70	-0.273	7.287	-2.476	2.08E-02	3.36E-02	-5.351	-1.21
Gm9174	0.320	7.020	5.753	6.43E-06	2.18E-05	2.609	1.25
GMFB	0.411	7.391	2.854	8.79E-03	1.52E-02	-4.544	1.33
Gmfb	0.668	8.592	11.683	2.37E-11	3.27E-10	15.426	1.59
GNAI1	0.324	7.278	4.908	5.34E-05	1.49E-04	0.473	1.25
GNAI2	-0.373	6.846	-4.840	6.34E-05	1.74E-04	0.301	-1.29
GNL2	0.281	6.688	4.623	1.10E-04	2.86E-04	-0.251	1.22
GNPAT	-0.723	6.582	-13.209	1.85E-12	3.74E-11	18.053	-1.65
GNPTAB	-0.738	7.124	-13.804	7.27E-13	1.78E-11	19.015	-1.67
GPBP1L1	0.352	6.653	5.111	3.20E-05	9.34E-05	0.987	1.28
GPC6	-1.701	3.584	-20.301	1.50E-16	1.06E-14	27.744	-3.25
Gpd2	-0.465	6.879	-6.108	2.69E-06	1.01E-05	3.494	-1.38
Gpnmb	1.397	5.966	22.165	2.04E-17	1.88E-15	29.786	2.63
GPR176	-0.519	7.295	-11.879	1.68E-11	2.48E-10	15.779	-1.43
Gpr56	1.920	6.194	24.439	2.18E-18	3.03E-16	32.069	3.78
Gpsm2	-0.584	6.902	-8.827	5.61E-09	4.14E-08	9.802	-1.50
Gpx1	-1.024	7.884	-14.715	1.85E-13	5.58E-12	20.425	-2.03
Gpx4	-0.571	10.579	-10.933	9.07E-11	1.05E-09	14.045	-1.49
GSK3B	0.449	6.314	4.927	5.09E-05	1.42E-04	0.521	1.36
Gstm5	-0.436	10.418	-7.611	7.88E-08	4.43E-07	7.093	-1.35
Gstm6	-0.318	7.960	-4.305	2.46E-04	5.95E-04	-1.054	-1.25
Gstm7	-0.347	8.876	-4.996	4.27E-05	1.22E-04	0.697	-1.27
GTF2A1	0.422	6.434	6.250	1.91E-06	7.39E-06	3.843	1.34
Gtf2i	0.432	6.845	9.154	2.85E-09	2.30E-08	10.499	1.35
GTPBP4	0.266	7.814	4.403	1.92E-04	4.75E-04	-0.808	1.20
GUSB	-0.954	6.935	-18.969	6.89E-16	3.83E-14	26.177	-1.94
Gusb	-0.775	7.029	-13.142	2.06E-12	4.00E-11	17.943	-1.71
Gyg	-0.485	8.497	-5.923	4.23E-06	1.51E-05	3.034	-1.40
Gypc	-0.567	7.466	-9.743	8.72E-10	7.93E-09	11.715	-1.48
H1f0	0.948	8.525	15.035	1.16E-13	3.76E-12	20.905	1.93
H2afx	-0.329	6.799	-4.172	3.45E-04	8.17E-04	-1.390	-1.26
H3F3B	0.425	9.428	10.045	4.83E-10	4.71E-09	12.324	1.34
H3F3C	0.396	10.004	7.988	3.40E-08	2.09E-07	7.956	1.32
H6pd	-0.382	6.439	-5.680	7.70E-06	2.57E-05	2.426	-1.30
HADHB	-0.358	8.394	-7.277	1.69E-07	8.55E-07	6.317	-1.28
HARS	-0.322	6.414	-4.690	9.28E-05	2.45E-04	-0.081	-1.25
HAT1	-0.305	6.594	-4.355	2.17E-04	5.31E-04	-0.928	-1.24
HAX1	0.445	7.314	8.827	5.60E-09	4.14E-08	9.804	1.36
HBP1	-0.273	6.407	-2.626	1.49E-02	2.46E-02	-5.038	-1.21
HBS1L	0.533	6.390	11.362	4.18E-11	5.42E-10	14.842	1.45
HBXIP	0.673	8.226	12.832	3.40E-12	6.20E-11	17.427	1.59
HDDC3	-0.375	7.397	-10.352	2.68E-10	2.84E-09	12.929	-1.30
HEG1	-0.299	8.653	-4.840	6.35E-05	1.74E-04	0.300	-1.23
Hibadh	-0.304	7.744	-5.601	9.38E-06	3.06E-05	2.226	-1.23
HIP1	-0.618	6.987	-12.241	9.07E-12	1.42E-10	16.415	-1.53
HIST1H2BN	0.315	7.314	3.695	1.14E-03	2.45E-03	-2.572	1.24
HMBS	-0.665	7.118	-14.228	3.81E-13	1.04E-11	19.680	-1.59
HMGCR	-1.175	6.572	-17.534	3.98E-15	1.86E-13	24.374	-2.26
Hmgcs1	-1.236	6.887	-22.397	1.61E-17	1.61E-15	30.029	-2.35
HMGN2	-0.313	8.395	-7.325	1.51E-07	7.76E-07	6.430	-1.24
HMGN5	-0.519	8.999	-9.506	1.40E-09	1.21E-08	11.231	-1.43
HMOX1	-0.519	7.105	-6.005	3.46E-06	1.26E-05	3.238	-1.43
Hnrnpa3	-0.299	8.503	-3.234	3.56E-03	6.84E-03	-3.678	-1.23
HNRNPC	0.310	9.033	6.523	9.88E-07	4.10E-06	4.512	1.24
Hnrnpd	-0.397	9.164	-7.158	2.22E-07	1.09E-06	6.036	-1.32
HNRNPH1	0.312	8.470	5.630	8.73E-06	2.87E-05	2.299	1.24
HNRNPR	-0.344	8.226	-6.782	5.33E-07	2.38E-06	5.141	-1.27
Hnrpdl	-0.532	6.334	-8.336	1.59E-08	1.05E-07	8.733	-1.45
HNRPDL	-0.471	6.768	-7.209	1.97E-07	9.84E-07	6.158	-1.39
Hoxd9	0.582	5.751	5.433	1.42E-05	4.50E-05	1.804	1.50
HP1BP3	-0.312	8.095	-7.634	7.48E-08	4.21E-07	7.147	-1.24
HPRT1	-0.671	9.127	-13.540	1.10E-12	2.51E-11	18.591	-1.59
HSD17B10	-0.323	8.843	-6.268	1.82E-06	7.08E-06	3.889	-1.25

Hsd17b12	-0.309	7.796	-6.466	1.13E-06	4.61E-06	4.375	-1.24
HSPH1	0.732	7.650	12.918	2.96E-12	5.50E-11	17.570	1.66
HTRA1	-0.552	10.001	-9.318	2.04E-09	1.71E-08	10.841	-1.47
HTRA2	-0.317	7.125	-6.119	2.62E-06	9.84E-06	3.519	-1.25
IDH1	-1.534	6.178	-6.816	4.93E-07	2.22E-06	5.221	-2.90
IDH1	-1.158	8.313	-15.379	7.11E-14	2.41E-12	21.410	-2.23
IDH3B	-0.272	7.689	-3.107	4.83E-03	8.95E-03	-3.973	-1.21
Idi1	-1.320	6.124	-22.110	2.16E-17	1.94E-15	29.727	-2.50
Ifitm2	-0.438	10.142	-5.710	7.15E-06	2.40E-05	2.502	-1.35
Ifitm3	-0.761	9.676	-11.241	5.18E-11	6.49E-10	14.620	-1.69
Ifngr2	-0.516	6.377	-11.301	4.66E-11	5.98E-10	14.730	-1.43
IFRD1	0.638	6.993	3.314	2.93E-03	5.74E-03	-3.489	1.56
IFRD1	0.752	9.381	11.029	7.60E-11	9.02E-10	14.226	1.68
IFRD2	0.311	6.584	4.700	9.04E-05	2.39E-04	-0.055	1.24
Ift52	0.620	7.441	17.286	5.47E-15	2.39E-13	24.049	1.54
IGFBP4	-0.571	6.718	-5.450	1.37E-05	4.33E-05	1.847	-1.49
IK	0.268	6.681	4.620	1.11E-04	2.88E-04	-0.258	1.20
Ildr2	0.406	6.552	7.912	4.02E-08	2.43E-07	7.782	1.32
Impad1	0.327	6.929	3.941	6.18E-04	1.40E-03	-1.966	1.25
IMPDH2	-0.387	9.639	-9.677	9.93E-10	8.92E-09	11.582	-1.31
Insig1	-0.878	8.024	-17.964	2.32E-15	1.14E-13	24.928	-1.84
IP6K1	0.406	6.552	2.527	1.86E-02	3.03E-02	-5.246	1.33
IPO7	0.404	8.117	6.724	6.12E-07	2.68E-06	5.000	1.32
IPO7	0.487	8.188	10.073	4.57E-10	4.54E-09	12.380	1.40
Irak2	-0.925	6.582	-12.975	2.69E-12	5.06E-11	17.666	-1.90
IRS1	0.583	6.303	8.828	5.60E-09	4.14E-08	9.804	1.50
Isca1	-0.336	8.849	-7.967	3.56E-08	2.17E-07	7.907	-1.26
ITCH	0.474	7.087	9.780	8.11E-10	7.43E-09	11.791	1.39
ITIH5	-1.171	6.180	-10.550	1.84E-10	2.02E-09	13.313	-2.25
Jak2	-0.555	6.679	-7.741	5.89E-08	3.45E-07	7.392	-1.47
Jub	-0.305	7.280	-8.057	2.92E-08	1.83E-07	8.111	-1.24
JUN	0.745	7.869	10.484	2.09E-10	2.26E-09	13.186	1.68
KCMF1	0.279	8.700	6.456	1.16E-06	4.70E-06	4.350	1.21
Kctd10	0.333	6.615	6.733	5.99E-07	2.63E-06	5.022	1.26
KIAA0174	0.504	7.533	11.722	2.21E-11	3.09E-10	15.497	1.42
KIF23	-0.542	6.746	-8.379	1.45E-08	9.63E-08	8.827	-1.46
KIF5B	0.372	8.303	5.790	5.87E-06	2.02E-05	2.701	1.29
KIF5B	0.432	6.853	3.549	1.65E-03	3.41E-03	-2.928	1.35
KIFC1	-0.647	6.589	-10.149	3.95E-10	4.02E-09	12.529	-1.57
KLF4	-0.274	6.396	-3.147	4.39E-03	8.20E-03	-3.879	-1.21
KLF6	0.399	6.300	5.157	2.85E-05	8.44E-05	1.105	1.32
KLF6	0.414	7.705	7.095	2.56E-07	1.23E-06	5.889	1.33
KLF6	0.417	8.141	8.404	1.38E-08	9.18E-08	8.882	1.34
KPNA2	-0.684	7.512	-10.954	8.73E-11	1.02E-09	14.084	-1.61
Kpna4	0.637	7.353	11.554	2.97E-11	4.01E-10	15.193	1.55
KPNA6	0.453	6.350	6.547	9.32E-07	3.89E-06	4.572	1.37
Kpnb1	0.288	8.159	5.213	2.48E-05	7.42E-05	1.247	1.22
KPNB1	0.312	7.476	3.086	5.08E-03	9.33E-03	-4.020	1.24
KRIT1	0.288	7.004	5.185	2.66E-05	7.92E-05	1.176	1.22
KRR1	0.540	6.347	9.870	6.80E-10	6.44E-09	11.972	1.45
LAMA5	-0.351	6.977	-5.154	2.87E-05	8.50E-05	1.097	-1.28
LAMC1	-0.400	7.195	-8.183	2.22E-08	1.42E-07	8.393	-1.32
LAMP2	0.282	6.487	4.369	2.09E-04	5.14E-04	-0.894	1.22
LAP3	-0.472	6.693	-3.581	1.52E-03	3.17E-03	-2.850	-1.39
LAP3	-0.400	7.918	-10.099	4.35E-10	4.38E-09	12.431	-1.32
LAPTM4A	-0.653	7.312	-3.534	1.70E-03	3.52E-03	-2.963	-1.57
LAPTM4A	-0.531	7.522	-7.071	2.71E-07	1.30E-06	5.830	-1.45
LARP1	0.311	7.726	5.120	3.12E-05	9.13E-05	1.013	1.24
LAYN	-0.619	6.973	-8.554	9.99E-09	6.91E-08	9.211	-1.54
LDHA	-0.530	9.533	-5.232	2.36E-05	7.12E-05	1.295	-1.44
Ldlr	-1.380	6.729	-22.030	2.34E-17	2.05E-15	29.643	-2.60
Lemd2	0.280	6.690	4.651	1.02E-04	2.69E-04	-0.179	1.21
LEMD3	0.296	7.077	3.121	4.68E-03	8.69E-03	-3.941	1.23
Leprotl1	0.464	7.192	11.440	3.63E-11	4.76E-10	14.986	1.38
LGALS1	-1.120	10.053	-13.340	1.50E-12	3.16E-11	18.267	-2.17
LGALS3	-0.736	10.366	-11.687	2.35E-11	3.26E-10	15.435	-1.67
Lman2	-0.351	7.930	-4.259	2.77E-04	6.64E-04	-1.172	-1.28
LOC100043810	-0.557	7.540	-6.713	6.27E-07	2.74E-06	4.975	-1.47
LOC100130746	0.501	7.013	10.380	2.54E-10	2.71E-09	12.983	1.42
LOC100131826	-0.706	7.320	-8.307	1.70E-08	1.12E-07	8.667	-1.63
LOC100133593	0.363	6.201	3.306	2.98E-03	5.84E-03	-3.508	1.29
LOC100287146	-0.440	7.745	-6.867	4.37E-07	1.99E-06	5.344	-1.36
LOC100287239	-0.310	9.812	-2.496	1.99E-02	3.23E-02	-5.310	-1.24
LOC100287552	0.806	5.617	5.987	3.61E-06	1.32E-05	3.193	1.75
LOC100287653	0.294	7.806	5.036	3.86E-05	1.11E-04	0.799	1.23
LOC100287764	1.098	6.206	5.813	5.54E-06	1.92E-05	2.760	2.14
LOC100288436	0.363	6.402	7.229	1.88E-07	9.44E-07	6.205	1.29
LOC100294264	-0.625	6.932	-7.497	1.02E-07	5.50E-07	6.831	-1.54
LOC100294264	0.461	7.669	5.870	4.82E-06	1.70E-05	2.902	1.38

LOC294154	0.542	5.851	4.355	2.17E-04	5.31E-04	-0.928	1.46
LOC401387	-0.851	9.012	-15.001	1.22E-13	3.91E-12	20.854	-1.80
LOC547349	0.445	9.208	6.476	1.10E-06	4.52E-06	4.398	1.36
LOC553150	0.435	8.437	7.205	1.99E-07	9.90E-07	6.148	1.35
LOC642969	-0.896	6.690	-3.819	8.39E-04	1.86E-03	-2.268	-1.86
LOC685953	3.997	9.130	38.295	6.36E-23	1.90E-20	42.609	15.96
LOC728758	0.662	6.767	13.233	1.78E-12	3.66E-11	18.092	1.58
LOC728776	0.359	9.135	5.335	1.82E-05	5.63E-05	1.555	1.28
LOC732229	0.455	7.725	8.457	1.23E-08	8.28E-08	9.000	1.37
LOC732229	0.502	7.558	10.039	4.89E-10	4.76E-09	12.311	1.42
LPCAT3	-0.869	6.684	-9.414	1.68E-09	1.43E-08	11.041	-1.83
LPCAT3	-0.638	6.785	-11.039	7.47E-11	8.91E-10	14.245	-1.56
LPL	0.316	10.528	5.807	5.62E-06	1.94E-05	2.745	1.24
LPP	0.500	6.385	5.646	8.38E-06	2.77E-05	2.341	1.41
LPP	0.644	7.853	11.570	2.89E-11	3.93E-10	15.222	1.56
Lrig1	-1.924	8.856	-19.737	2.82E-16	1.81E-14	27.092	-3.79
Lrp10	-0.655	7.803	-9.166	2.78E-09	2.25E-08	10.523	-1.57
LRPPRC	0.436	7.197	5.414	1.50E-05	4.70E-05	1.755	1.35
LRRC33	0.464	8.703	12.770	3.76E-12	6.83E-11	17.322	1.38
LRRC59	0.474	8.298	10.046	4.82E-10	4.71E-09	12.325	1.39
LRRC8D	-0.484	6.697	-5.766	6.22E-06	2.12E-05	2.642	-1.40
Lsg1	0.391	7.593	5.567	1.02E-05	3.31E-05	2.141	1.31
LSG1	0.474	7.417	9.070	3.39E-09	2.69E-08	10.321	1.39
LSM14A	0.282	6.661	5.873	4.79E-06	1.69E-05	2.908	1.22
LSM3	-0.545	7.307	-9.828	7.38E-10	6.89E-09	11.888	-1.46
LUZP6	0.311	7.299	6.868	4.36E-07	1.99E-06	5.347	1.24
LUZP6	0.381	6.513	6.654	7.23E-07	3.11E-06	4.831	1.30
Ly6e	-0.987	7.105	-13.246	1.74E-12	3.60E-11	18.114	-1.98
LYAR	-0.296	7.006	-5.254	2.23E-05	6.75E-05	1.352	-1.23
MACROD1	0.354	6.433	5.420	1.47E-05	4.64E-05	1.771	1.28
Mad2l1	-0.731	6.292	-5.938	4.07E-06	1.46E-05	3.072	-1.66
Mad2l1	-0.575	7.100	-8.764	6.40E-09	4.67E-08	9.667	-1.49
MAFG	0.598	6.127	6.278	1.78E-06	6.94E-06	3.913	1.51
MAFG	0.660	6.400	6.703	6.43E-07	2.80E-06	4.949	1.58
MAFG	0.755	5.986	5.850	5.07E-06	1.77E-05	2.851	1.69
MAGOH	0.366	7.716	7.365	1.38E-07	7.15E-07	6.523	1.29
MAMDC2	-1.020	7.429	-8.828	5.59E-09	4.14E-08	9.806	-2.03
MAN2A1	-0.509	6.683	-6.916	3.89E-07	1.80E-06	5.461	-1.42
Man2a1	-0.473	6.504	-6.540	9.47E-07	3.95E-06	4.555	-1.39
MAN2C1	-0.564	6.482	-7.606	7.97E-08	4.47E-07	7.083	-1.48
Manbal	0.625	6.723	10.763	1.24E-10	1.40E-09	13.722	1.54
Map1lc3b	0.301	8.856	4.166	3.50E-04	8.26E-04	-1.403	1.23
Map1lc3b	0.433	8.566	13.201	1.87E-12	3.74E-11	18.040	1.35
MAP2K1	0.313	8.294	6.088	2.82E-06	1.05E-05	3.444	1.24
MAPK1	-0.387	6.595	-7.378	1.34E-07	6.98E-07	6.553	-1.31
MAPKAPK5	0.475	6.646	7.716	6.22E-08	3.60E-07	7.337	1.39
MAPRE1	0.554	7.902	9.271	2.25E-09	1.87E-08	10.743	1.47
MARK1	-0.960	6.616	-20.525	1.17E-16	9.12E-15	27.998	-1.95
MAX	0.296	5.929	2.578	1.66E-02	2.72E-02	-5.139	1.23
MBNL1	0.360	9.159	8.792	6.03E-09	4.42E-08	9.728	1.28
Mbp	-0.968	6.692	-14.604	2.17E-13	6.30E-12	20.258	-1.96
MCL1	0.284	7.354	6.762	5.59E-07	2.48E-06	5.092	1.22
Mcm2	-0.345	6.936	-5.935	4.11E-06	1.47E-05	3.062	-1.27
MDH1	-0.440	8.427	-3.478	1.96E-03	4.01E-03	-3.099	-1.36
MDH1	-0.275	9.418	-6.427	1.24E-06	5.01E-06	4.279	-1.21
Med29	0.467	7.001	7.846	4.66E-08	2.79E-07	7.633	1.38
Mesdc2	-0.306	9.074	-9.463	1.52E-09	1.31E-08	11.143	-1.24
METTL6	0.629	6.319	13.267	1.69E-12	3.50E-11	18.148	1.55
METTL9	-0.697	6.724	-11.510	3.21E-11	4.27E-10	15.114	-1.62
MFN2	0.383	7.176	8.613	8.81E-09	6.19E-08	9.339	1.30
Mia3	-0.311	8.330	-5.703	7.27E-06	2.43E-05	2.484	-1.24
MIB1	0.454	7.443	7.115	2.45E-07	1.18E-06	5.935	1.37
MIF	-0.462	9.040	-6.535	9.59E-07	3.99E-06	4.542	-1.38
MIF	-0.342	9.635	-5.475	1.28E-05	4.08E-05	1.910	-1.27
MIR21	-0.456	6.244	-5.717	7.04E-06	2.37E-05	2.518	-1.37
MITF	-1.221	7.306	-17.062	7.29E-15	3.08E-13	23.753	-2.33
Mknk2	-0.356	6.736	-3.989	5.48E-04	1.25E-03	-1.847	-1.28
MLF2	0.275	6.161	2.365	2.65E-02	4.17E-02	-5.575	1.21
MLL	0.347	6.439	4.541	1.35E-04	3.46E-04	-0.457	1.27
MMD	-0.333	6.497	-4.101	4.13E-04	9.60E-04	-1.568	-1.26
MMP24	0.582	6.162	8.942	4.42E-09	3.38E-08	10.049	1.50
MMP9	0.618	6.731	9.796	7.86E-10	7.26E-09	11.823	1.54
Mobkl1b	0.271	8.120	5.323	1.88E-05	5.77E-05	1.526	1.21
MOBKL3	0.553	6.352	11.846	1.78E-11	2.62E-10	15.718	1.47
MPHOSPH10	0.375	6.627	7.189	2.06E-07	1.02E-06	6.110	1.30
MPP6	-0.473	7.328	-8.534	1.04E-08	7.16E-08	9.169	-1.39
MPST	-0.402	6.645	-6.976	3.38E-07	1.58E-06	5.605	-1.32
MRPL1	0.363	7.634	7.261	1.75E-07	8.83E-07	6.280	1.29
Mrpl18	-0.302	7.610	-6.800	5.11E-07	2.29E-06	5.184	-1.23

MRPL24	0.511	7.227	9.914	6.22E-10	5.96E-09	12.062	1.42
MRPL30	0.318	8.776	5.090	3.37E-05	9.78E-05	0.936	1.25
MRPL37	0.645	7.536	8.831	5.56E-09	4.14E-08	9.811	1.56
MRPL43	0.332	7.341	5.229	2.38E-05	7.16E-05	1.287	1.26
Mrpl50	-0.394	7.408	-5.441	1.40E-05	4.42E-05	1.824	-1.31
Mrpl53	0.313	6.945	4.637	1.06E-04	2.77E-04	-0.214	1.24
Mrpl9	0.343	6.152	3.467	2.01E-03	4.11E-03	-3.124	1.27
MRPS10	0.315	6.973	3.844	7.88E-04	1.75E-03	-2.206	1.24
MRPS15	0.268	7.120	4.423	1.83E-04	4.55E-04	-0.758	1.20
MRPS18A	0.265	9.934	4.216	3.08E-04	7.36E-04	-1.279	1.20
MRPS18B	0.391	7.064	4.937	4.96E-05	1.39E-04	0.547	1.31
MRPS18C	0.321	8.020	8.045	3.00E-08	1.87E-07	8.084	1.25
MRPS22	0.638	6.359	13.625	9.60E-13	2.24E-11	18.728	1.56
Mrps23	0.434	6.974	9.252	2.33E-09	1.94E-08	10.704	1.35
MRPS35	0.382	8.616	7.799	5.17E-08	3.06E-07	7.525	1.30
MSL1	0.354	6.484	5.034	3.88E-05	1.12E-04	0.794	1.28
MSN	0.456	7.535	3.103	4.88E-03	9.00E-03	-3.981	1.37
Msrb2	-0.372	6.378	-5.605	9.28E-06	3.03E-05	2.237	-1.29
MT1A	-0.328	7.750	-3.132	4.55E-03	8.47E-03	-3.915	-1.26
Mtap4	0.377	6.079	4.386	2.00E-04	4.94E-04	-0.849	1.30
MTBP	-0.362	6.107	-6.854	4.50E-07	2.05E-06	5.314	-1.29
MTDH	0.299	7.693	5.834	5.26E-06	1.83E-05	2.813	1.23
MTHFD1	-0.481	7.372	-12.115	1.12E-11	1.71E-10	16.195	-1.40
MTHFD1L	-0.508	7.267	-11.895	1.64E-11	2.43E-10	15.806	-1.42
Mthfd2	-0.480	7.826	-10.660	1.50E-10	1.66E-09	13.526	-1.39
Mthfd2l	-0.672	6.000	-10.534	1.90E-10	2.07E-09	13.284	-1.59
MTX1	0.635	7.183	10.082	4.49E-10	4.48E-09	12.398	1.55
MTX2	-0.339	7.973	-5.795	5.79E-06	2.00E-05	2.715	-1.27
Myc	0.302	9.167	6.977	3.38E-07	1.58E-06	5.606	1.23
Myd88	-0.310	6.590	-7.484	1.05E-07	5.64E-07	6.801	-1.24
MYL12B	-0.337	10.684	-4.203	3.19E-04	7.60E-04	-1.312	-1.26
MYO1B	-0.378	8.879	-6.758	5.65E-07	2.50E-06	5.082	-1.30
N6AMT2	-0.517	6.228	-8.654	8.07E-09	5.73E-08	9.430	-1.43
NAP1L1	0.269	6.670	5.942	4.04E-06	1.45E-05	3.080	1.21
Nap1l4	0.488	6.668	7.831	4.81E-08	2.87E-07	7.599	1.40
NARS	0.292	8.331	4.873	5.83E-05	1.61E-04	0.385	1.22
Nars	0.345	7.775	6.931	3.76E-07	1.75E-06	5.497	1.27
Nbl1	-0.655	6.440	-7.584	8.37E-08	4.65E-07	7.032	-1.57
Ncam1	0.453	5.358	5.041	3.82E-05	1.10E-04	0.811	1.37
Ncapd3	-0.845	5.993	-11.988	1.40E-11	2.11E-10	15.971	-1.80
Ncaph	-0.870	6.789	-13.151	2.03E-12	3.98E-11	17.958	-1.83
NCEH1	-0.654	6.694	-12.909	3.00E-12	5.55E-11	17.556	-1.57
NCK2	-0.434	6.349	-5.478	1.27E-05	4.06E-05	1.918	-1.35
NCL	-0.347	8.619	-6.451	1.17E-06	4.75E-06	4.337	-1.27
NCOA6	0.670	5.976	12.555	5.36E-12	9.22E-11	16.957	1.59
NDC80	-0.344	6.736	-4.186	3.33E-04	7.90E-04	-1.354	-1.27
Ndufaf2	0.496	7.178	9.118	3.07E-09	2.46E-08	10.422	1.41
Ndufb5	-0.546	9.033	-8.599	9.07E-09	6.35E-08	9.310	-1.46
Ndufb9	0.285	10.546	8.134	2.47E-08	1.57E-07	8.282	1.22
NDUFS7	0.328	9.159	4.532	1.38E-04	3.54E-04	-0.481	1.25
Neat1	-0.771	8.207	-11.765	2.05E-11	2.94E-10	15.574	-1.71
NEDD8	0.331	8.511	6.852	4.53E-07	2.06E-06	5.307	1.26
Nek2	-0.727	8.036	-11.039	7.47E-11	8.91E-10	14.245	-1.65
Neu1	0.303	6.374	5.530	1.12E-05	3.61E-05	2.048	1.23
NEURL3	0.420	6.003	3.080	5.16E-03	9.46E-03	-4.035	1.34
Neurl3	0.650	6.664	9.350	1.91E-09	1.62E-08	10.908	1.57
NFKBIA	-1.215	8.519	-17.262	5.63E-15	2.44E-13	24.018	-2.32
NFKBIA	-1.161	8.243	-18.751	8.92E-16	4.86E-14	25.912	-2.24
NFU1	0.397	6.920	11.169	5.90E-11	7.29E-10	14.486	1.32
NGDN	0.651	7.269	13.144	2.05E-12	4.00E-11	17.946	1.57
NGRN	0.355	7.748	9.449	1.57E-09	1.34E-08	11.113	1.28
NHP2L1	-0.439	8.019	-7.454	1.12E-07	5.99E-07	6.731	-1.36
Nid1	-0.645	8.249	-8.943	4.41E-09	3.38E-08	10.050	-1.56
Nid1	-0.290	8.359	-2.987	6.43E-03	1.15E-02	-4.246	-1.22
NINJ1	-0.362	6.177	-5.710	7.15E-06	2.40E-05	2.502	-1.29
NIT1	-0.493	6.656	-6.231	1.99E-06	7.67E-06	3.797	-1.41
NOL7	0.556	8.228	9.190	2.65E-09	2.16E-08	10.575	1.47
NOP56	-0.335	7.327	-3.981	5.58E-04	1.27E-03	-1.866	-1.26
NOP58	0.459	8.700	5.660	8.10E-06	2.69E-05	2.375	1.37
Nox1	-0.293	7.107	-3.706	1.11E-03	2.40E-03	-2.547	-1.23
NPC2	-0.350	9.515	-7.689	6.62E-08	3.80E-07	7.273	-1.27
Nploc4	0.520	6.036	6.028	3.27E-06	1.20E-05	3.294	1.43
NR1H3	-0.724	6.661	-8.545	1.02E-08	7.01E-08	9.193	-1.65
Nr2c1	0.285	6.434	5.598	9.44E-06	3.07E-05	2.220	1.22
NRAS	0.521	7.871	10.334	2.77E-10	2.92E-09	12.895	1.43
NRD1	0.323	6.675	2.628	1.48E-02	2.45E-02	-5.034	1.25
NRP1	-0.545	7.277	-9.181	2.70E-09	2.19E-08	10.556	-1.46
NSMCE2	0.332	8.368	5.006	4.17E-05	1.19E-04	0.723	1.26
NSMCE4A	-0.306	6.768	-6.734	5.97E-07	2.62E-06	5.024	-1.24

NTAN1	-0.380	7.177	-5.094	3.34E-05	9.71E-05	0.945	-1.30
Nucb1	0.288	6.716	5.214	2.47E-05	7.41E-05	1.249	1.22
Nucks1	-0.365	6.595	-4.935	4.99E-05	1.40E-04	0.541	-1.29
NUDC	0.630	8.891	9.938	5.94E-10	5.71E-09	12.110	1.55
Nudt3	0.277	6.423	3.282	3.16E-03	6.14E-03	-3.564	1.21
NUMB	0.380	6.379	5.783	5.98E-06	2.05E-05	2.683	1.30
NUP205	-0.278	6.845	-6.292	1.72E-06	6.71E-06	3.948	-1.21
NUP93	-0.350	6.264	-5.830	5.32E-06	1.85E-05	2.801	-1.27
Nusap1	-0.968	6.996	-12.131	1.09E-11	1.68E-10	16.223	-1.96
NVL	0.862	6.210	16.059	2.77E-14	1.03E-12	22.380	1.82
Oaf	-0.433	7.439	-9.327	2.00E-09	1.69E-08	10.860	-1.35
OAZ1	-0.641	7.862	-8.460	1.22E-08	8.25E-08	9.006	-1.56
OAZ1	-0.475	7.602	-8.675	7.72E-09	5.51E-08	9.475	-1.39
OAZ2	-0.279	6.880	-4.823	6.62E-05	1.80E-04	0.258	-1.21
OBFC1	-0.504	6.436	-7.934	3.83E-08	2.32E-07	7.832	-1.42
ORC4L	0.507	6.941	10.793	1.17E-10	1.33E-09	13.780	1.42
Ormdl1	0.609	6.318	13.758	7.80E-13	1.88E-11	18.942	1.53
Ormdl3	0.321	7.280	6.613	7.96E-07	3.39E-06	4.732	1.25
OSBPL9	-0.571	6.512	-5.605	9.27E-06	3.03E-05	2.238	-1.49
Osmr	-0.865	7.659	-13.521	1.13E-12	2.55E-11	18.562	-1.82
OXR1	-0.420	6.713	-6.010	3.41E-06	1.25E-05	3.251	-1.34
OXSR1	-0.381	8.386	-6.697	6.52E-07	2.83E-06	4.935	-1.30
Pabpc4	0.405	7.915	5.478	1.28E-05	4.06E-05	1.916	1.32
PABPN1	-0.532	8.440	-10.806	1.15E-10	1.31E-09	13.804	-1.45
Paics	-0.325	9.258	-5.356	1.73E-05	5.38E-05	1.608	-1.25
PALLD	-0.953	7.134	-19.459	3.89E-16	2.37E-14	26.764	-1.94
PAM	-0.443	7.027	-6.465	1.13E-06	4.62E-06	4.371	-1.36
PAPSS1	-0.669	6.702	-10.256	3.21E-10	3.37E-09	12.742	-1.59
Parp3	-0.786	6.322	-12.995	2.61E-12	4.96E-11	17.699	-1.72
Pbk	-0.634	6.261	-7.233	1.86E-07	9.38E-07	6.214	-1.55
PCBP1	-0.345	9.084	-3.336	2.77E-03	5.47E-03	-3.436	-1.27
PCK2	-1.148	6.594	-17.427	4.56E-15	2.06E-13	24.235	-2.22
Pcolce	-0.953	8.526	-12.631	4.73E-12	8.21E-11	17.086	-1.94
PDE6D	-0.425	6.781	-3.424	2.24E-03	4.52E-03	-3.228	-1.34
PDIA3	-0.299	9.730	-4.807	6.89E-05	1.87E-04	0.218	-1.23
PDIA4	-0.798	6.027	-4.004	5.27E-04	1.21E-03	-1.810	-1.74
PDIA4	-0.266	7.671	-5.664	8.03E-06	2.67E-05	2.385	-1.20
PDIA5	-0.857	7.082	-13.298	1.60E-12	3.35E-11	18.199	-1.81
PDLIM1	-1.045	7.184	-22.198	1.97E-17	1.86E-15	29.820	-2.06
Pdrg1	2.158	7.434	38.856	4.52E-23	1.48E-20	42.949	4.46
Pdyn	-0.429	7.034	-4.977	4.48E-05	1.27E-04	0.650	-1.35
Pebp1	-0.277	9.686	-7.354	1.41E-07	7.30E-07	6.498	-1.21
PECI	-0.395	6.867	-7.572	8.60E-08	4.74E-07	7.005	-1.31
Pes1	0.602	7.542	8.114	2.58E-08	1.63E-07	8.237	1.52
Pex19	0.422	6.163	8.692	7.45E-09	5.37E-08	9.511	1.34
PGAM4	-0.443	6.574	-8.303	1.71E-08	1.12E-07	8.660	-1.36
PGK1	-0.725	9.403	-20.434	1.29E-16	9.66E-15	27.895	-1.65
Pgs1	-0.428	6.290	-6.334	1.56E-06	6.13E-06	4.050	-1.34
PHF20	0.385	6.211	5.733	6.76E-06	2.28E-05	2.559	1.31
PHF20	0.479	6.196	10.252	3.24E-10	3.37E-09	12.734	1.39
PHF5A	0.838	7.050	15.905	3.42E-14	1.24E-12	22.164	1.79
PHGDH	-0.829	7.601	-11.567	2.90E-11	3.94E-10	15.217	-1.78
PI4KA	-0.269	7.162	-4.542	1.35E-04	3.46E-04	-0.456	-1.20
PIGT	0.584	6.318	5.824	5.40E-06	1.87E-05	2.786	1.50
Pir	-0.398	6.603	-5.837	5.22E-06	1.82E-05	2.820	-1.32
PJA2	0.285	7.115	5.371	1.67E-05	5.21E-05	1.646	1.22
PJA2	0.298	7.451	6.366	1.44E-06	5.72E-06	4.128	1.23
PKN2	0.362	7.422	8.692	7.44E-09	5.37E-08	9.513	1.29
PLAA	-0.356	6.833	-7.273	1.70E-07	8.61E-07	6.309	-1.28
PLAC8	2.290	5.438	22.929	9.42E-18	1.06E-15	30.576	4.89
Plbd2	0.459	7.151	6.723	6.13E-07	2.68E-06	4.997	1.37
Plekha5	-0.694	7.039	-17.130	6.68E-15	2.86E-13	23.843	-1.62
PLK2	0.585	7.605	11.481	3.38E-11	4.45E-10	15.060	1.50
Plp2	-0.289	7.148	-3.985	5.53E-04	1.26E-03	-1.856	-1.22
PLS3	-0.277	8.295	-4.475	1.60E-04	4.03E-04	-0.626	-1.21
PLTP	1.268	7.226	9.659	1.03E-09	9.15E-09	11.546	2.41
Plxna1	-0.499	6.084	-9.873	6.76E-10	6.42E-09	11.978	-1.41
PMPCA	0.378	7.183	6.163	2.35E-06	8.96E-06	3.630	1.30
Pnn	0.348	7.266	6.545	9.37E-07	3.91E-06	4.566	1.27
Pnp2	-0.277	6.523	-4.142	3.72E-04	8.75E-04	-1.465	-1.21
POLR1C	0.297	7.898	6.091	2.80E-06	1.04E-05	3.452	1.23
POLR1D	-0.567	8.762	-7.352	1.42E-07	7.32E-07	6.493	-1.48
POLR2F	0.525	8.788	9.661	1.03E-09	9.15E-09	11.549	1.44
POLR2G	0.573	8.219	14.121	4.48E-13	1.20E-11	19.514	1.49
POLR2L	0.287	7.536	5.771	6.15E-06	2.10E-05	2.654	1.22
POM121C	0.377	6.545	4.338	2.26E-04	5.52E-04	-0.970	1.30
PPAP2A	-1.221	6.987	-16.179	2.35E-14	8.88E-13	22.549	-2.33
Ppia	-0.432	10.987	-7.445	1.15E-07	6.08E-07	6.709	-1.35
PPP1CB	0.398	9.069	6.077	2.90E-06	1.08E-05	3.417	1.32

PPP1R11	0.466	6.328	3.532	1.71E-03	3.54E-03	-2.968	1.38
PPP1R12A	0.402	6.479	4.795	7.10E-05	1.92E-04	0.187	1.32
PPP2CB	0.269	9.586	6.137	2.51E-06	9.43E-06	3.565	1.21
PPP2R1A	0.428	6.065	3.792	8.97E-04	1.97E-03	-2.334	1.34
PPP2R2A	0.291	7.150	6.440	1.21E-06	4.87E-06	4.310	1.22
Ppp2r2a	0.407	7.954	6.267	1.83E-06	7.10E-06	3.886	1.33
PPP2R5A	0.520	6.010	8.547	1.01E-08	7.00E-08	9.197	1.43
PPP3CB	0.316	6.496	5.267	2.16E-05	6.55E-05	1.385	1.24
PPPDE1	0.313	6.277	3.259	3.34E-03	6.48E-03	-3.618	1.24
Pqlc3	-0.621	6.550	-5.351	1.75E-05	5.43E-05	1.596	-1.54
Prc1	-1.257	7.462	-13.108	2.18E-12	4.20E-11	17.886	-2.39
PRCP	-0.439	7.535	-10.518	1.96E-10	2.13E-09	13.251	-1.36
Prdx3	-0.437	8.036	-6.502	1.04E-06	4.28E-06	4.462	-1.35
PRKACB	0.370	8.518	7.007	3.15E-07	1.49E-06	5.678	1.29
PRKCA	-0.419	5.864	-5.590	9.64E-06	3.13E-05	2.200	-1.34
PRKCDBP	-0.584	6.389	-5.425	1.45E-05	4.59E-05	1.784	-1.50
PRKCSH	-0.336	6.754	-3.018	5.97E-03	1.08E-02	-4.175	-1.26
Prkrir	0.442	7.275	10.154	3.91E-10	3.99E-09	12.541	1.36
Prmt5	-0.464	7.197	-6.238	1.96E-06	7.58E-06	3.815	-1.38
Procr	0.911	6.220	13.654	9.18E-13	2.18E-11	18.775	1.88
Pros1	-0.976	7.149	-16.984	8.07E-15	3.33E-13	23.648	-1.97
Prps2	-0.476	7.860	-6.222	2.04E-06	7.83E-06	3.775	-1.39
PRPS2	-0.362	9.468	-6.522	9.89E-07	4.10E-06	4.510	-1.29
PRUNE2	-0.511	7.588	-9.210	2.54E-09	2.09E-08	10.617	-1.42
Psap	0.483	8.656	8.946	4.37E-09	3.37E-08	10.058	1.40
Psat1	-0.811	8.652	-12.376	7.23E-12	1.18E-10	16.648	-1.75
Psat1	-0.683	9.538	-14.953	1.31E-13	4.16E-12	20.782	-1.61
PSIMCT-1	0.731	7.013	8.874	5.08E-09	3.81E-08	9.904	1.66
PSMA7	-0.617	10.089	-18.433	1.31E-15	6.71E-14	25.519	-1.53
PSMB3	0.442	8.872	5.626	8.81E-06	2.89E-05	2.290	1.36
PSMB3	0.617	6.534	12.261	8.77E-12	1.39E-10	16.450	1.53
PSMB5	-0.952	9.145	-10.230	3.38E-10	3.51E-09	12.690	-1.93
PSMB6	0.484	8.403	8.331	1.61E-08	1.06E-07	8.721	1.40
PSMB8	-0.366	7.695	-7.373	1.35E-07	7.05E-07	6.541	-1.29
Psmc1	0.341	8.262	6.780	5.36E-07	2.39E-06	5.135	1.27
PSMC4	0.411	7.500	5.855	5.01E-06	1.75E-05	2.863	1.33
PSMC5	0.426	7.329	4.824	6.61E-05	1.80E-04	0.259	1.34
PSMC5	0.629	7.802	15.757	4.19E-14	1.45E-12	21.954	1.55
Psmd12	0.524	7.739	5.918	4.28E-06	1.53E-05	3.021	1.44
PSMD12	0.584	8.257	12.390	7.06E-12	1.16E-10	16.673	1.50
PSMD4	0.479	8.426	8.683	7.60E-09	5.45E-08	9.492	1.39
PSMD4	0.573	8.639	10.585	1.73E-10	1.90E-09	13.381	1.49
Psmd8	-0.327	7.549	-5.794	5.81E-06	2.00E-05	2.712	-1.25
Ptdss1	-0.349	6.929	-6.245	1.93E-06	7.46E-06	3.833	-1.27
PTGR1	-0.676	7.564	-5.908	4.38E-06	1.56E-05	2.997	-1.60
PTGR1	-0.550	8.581	-11.284	4.80E-11	6.09E-10	14.700	-1.46
Ptgr2	0.343	6.491	8.446	1.26E-08	8.45E-08	8.976	1.27
PTMA	-0.267	11.781	-2.771	1.07E-02	1.82E-02	-4.726	-1.20
PTP4A2	0.420	9.890	5.616	9.04E-06	2.96E-05	2.264	1.34
Ptp4a2	0.490	9.223	3.647	1.29E-03	2.73E-03	-2.690	1.40
PTPRG	-2.656	5.545	-52.750	3.35E-26	1.72E-23	49.986	-6.30
PTS	-0.288	7.243	-3.479	1.95E-03	4.00E-03	-3.095	-1.22
PTTG1	-0.585	6.979	-7.026	3.01E-07	1.43E-06	5.724	-1.50
PUM1	0.296	6.424	5.178	2.70E-05	8.03E-05	1.159	1.23
PUM1	0.426	6.710	4.928	5.08E-05	1.42E-04	0.524	1.34
PURB	0.575	7.313	10.057	4.72E-10	4.63E-09	12.347	1.49
PWP1	0.350	7.363	6.364	1.45E-06	5.74E-06	4.124	1.27
Pxdn	-0.292	7.848	-5.077	3.49E-05	1.01E-04	0.901	-1.22
PXK	-0.626	6.721	-13.561	1.06E-12	2.44E-11	18.625	-1.54
QARS	-0.544	9.208	-13.339	1.50E-12	3.16E-11	18.266	-1.46
Qsox1	-0.371	7.440	-4.792	7.16E-05	1.93E-04	0.179	-1.29
RAB10	-0.406	6.225	-2.407	2.42E-02	3.84E-02	-5.491	-1.33
RAB11A	-0.792	5.948	-7.577	8.51E-08	4.72E-07	7.016	-1.73
RAB24	-0.282	6.607	-5.362	1.70E-05	5.30E-05	1.625	-1.22
Rab32	-0.696	6.424	-8.955	4.30E-09	3.33E-08	10.076	-1.62
RAB34	0.378	8.642	7.915	4.00E-08	2.42E-07	7.789	1.30
RAB5C	0.409	6.092	4.823	6.62E-05	1.80E-04	0.257	1.33
RAB6A	0.686	6.620	10.368	2.60E-10	2.76E-09	12.960	1.61
RAB7A	0.299	8.931	5.779	6.03E-06	2.06E-05	2.675	1.23
RAB7A	0.305	7.737	4.978	4.47E-05	1.27E-04	0.652	1.24
Rad17	0.324	6.445	6.190	2.20E-06	8.43E-06	3.697	1.25
RALGAPB	0.624	6.409	14.570	2.29E-13	6.57E-12	20.206	1.54
RALY	0.707	6.393	7.549	9.06E-08	4.97E-07	6.951	1.63
RANGRF	-0.526	7.413	-8.264	1.86E-08	1.21E-07	8.572	-1.44
Rapgef1	0.266	9.228	5.492	1.23E-05	3.93E-05	1.953	1.20
RARRES2	-1.140	9.322	-16.048	2.81E-14	1.03E-12	22.365	-2.20
Rbck1	0.625	6.528	7.464	1.10E-07	5.87E-07	6.755	1.54
RBM3	-1.411	7.415	-14.020	5.22E-13	1.33E-11	19.355	-2.66
RBM39	0.688	8.443	13.648	9.26E-13	2.19E-11	18.766	1.61

RBM5	0.289	7.044	6.447	1.18E-06	4.78E-06	4.328	1.22
RBM7	-0.278	7.727	-4.976	4.50E-05	1.28E-04	0.645	-1.21
RBMXL1	-0.879	5.851	-9.816	7.56E-10	7.03E-09	11.863	-1.84
Rbpms	-0.637	6.521	-11.046	7.38E-11	8.86E-10	14.257	-1.56
Rcan1	0.388	6.276	6.548	9.29E-07	3.89E-06	4.575	1.31
RCBTB2	-0.488	7.042	-8.283	1.79E-08	1.17E-07	8.615	-1.40
RCL1	-0.290	7.522	-7.647	7.26E-08	4.11E-07	7.178	-1.22
REEP5	1.022	6.759	10.460	2.18E-10	2.35E-09	13.140	2.03
Reep5	1.184	7.899	24.155	2.86E-18	3.66E-16	31.795	2.27
RELA	0.375	6.829	3.364	2.59E-03	5.16E-03	-3.370	1.30
Rere	0.304	6.955	3.582	1.52E-03	3.17E-03	-2.848	1.23
RETSAT	-0.933	5.458	-9.951	5.80E-10	5.59E-09	12.135	-1.91
REV3L	-0.404	7.281	-6.141	2.48E-06	9.35E-06	3.576	-1.32
Rfx5	-0.505	6.821	-8.993	3.97E-09	3.11E-08	10.158	-1.42
RFX7	-0.405	7.777	-5.609	9.18E-06	3.00E-05	2.248	-1.32
RGD1561797	-0.336	7.190	-4.318	2.38E-04	5.79E-04	-1.022	-1.26
RGS17	0.697	7.346	7.188	2.07E-07	1.02E-06	6.107	1.62
Riok1	0.384	6.689	7.573	8.58E-08	4.74E-07	7.007	1.30
RIOK3	0.521	7.168	7.311	1.56E-07	7.99E-07	6.396	1.44
RNASEH2C	-1.522	6.445	-24.323	2.44E-18	3.24E-16	31.957	-2.87
RNASET2	0.346	10.189	8.038	3.05E-08	1.89E-07	8.068	1.27
RNF10	0.319	7.472	5.783	5.97E-06	2.05E-05	2.685	1.25
RNF11	0.448	7.884	9.652	1.04E-09	9.26E-09	11.531	1.36
RNF111	0.840	6.483	12.504	5.83E-12	9.84E-11	16.870	1.79
RNF139	-0.375	7.550	-8.405	1.37E-08	9.17E-08	8.884	-1.30
RNFT1	-0.452	6.370	-7.967	3.56E-08	2.17E-07	7.907	-1.37
RNH1	-0.382	6.472	-3.050	5.54E-03	1.01E-02	-4.104	-1.30
RNH1	-0.280	7.269	-4.425	1.81E-04	4.52E-04	-0.751	-1.21
ROCK2	0.559	7.129	6.002	3.49E-06	1.27E-05	3.230	1.47
ROMO1	0.610	9.948	12.186	9.96E-12	1.54E-10	16.319	1.53
Rpia	-0.606	7.906	-10.253	3.23E-10	3.37E-09	12.736	-1.52
RPL10	-0.835	6.263	-10.699	1.40E-10	1.56E-09	13.601	-1.78
RPL11	-0.415	11.456	-14.006	5.33E-13	1.34E-11	19.334	-1.33
Rpl14	0.299	11.184	6.180	2.26E-06	8.63E-06	3.672	1.23
RPL7	-0.387	11.044	-7.447	1.14E-07	6.06E-07	6.715	-1.31
RPP14	-0.463	6.393	-6.868	4.36E-07	1.99E-06	5.345	-1.38
RPP21	0.533	8.060	8.640	8.31E-09	5.88E-08	9.399	1.45
RPS25	-0.268	11.745	-6.092	2.80E-06	1.04E-05	3.453	-1.20
Rps28	-0.321	8.402	-3.364	2.59E-03	5.16E-03	-3.372	-1.25
RPS5	0.459	11.111	10.154	3.91E-10	3.99E-09	12.541	1.37
RRAS	-0.421	6.545	-5.978	3.70E-06	1.34E-05	3.170	-1.34
Rrp1	0.712	6.040	7.221	1.91E-07	9.61E-07	6.186	1.64
RSL1D1	0.396	7.841	3.257	3.36E-03	6.50E-03	-3.623	1.32
Rsl24d1	0.397	7.715	6.928	3.78E-07	1.75E-06	5.490	1.32
RSRC1	0.335	6.889	7.706	6.37E-08	3.68E-07	7.312	1.26
RSU1	-0.449	8.196	-5.350	1.76E-05	5.44E-05	1.594	-1.37
RTN3	-0.446	7.053	-3.392	2.42E-03	4.87E-03	-3.305	-1.36
RTN3	-0.268	8.012	-5.205	2.52E-05	7.54E-05	1.228	-1.20
RTN4	0.310	7.824	7.740	5.90E-08	3.45E-07	7.390	1.24
RUVBL1	-0.311	6.682	-5.495	1.22E-05	3.91E-05	1.960	-1.24
RYBP	0.315	8.342	6.159	2.38E-06	9.03E-06	3.618	1.24
RYK	0.297	6.736	7.203	2.00E-07	9.92E-07	6.143	1.23
S100A11	-0.477	7.784	-7.096	2.56E-07	1.23E-06	5.889	-1.39
SAMD4B	0.437	8.703	9.052	3.51E-09	2.76E-08	10.284	1.35
SAMM50	-0.343	7.616	-7.698	6.48E-08	3.73E-07	7.294	-1.27
SAP18	0.272	8.656	4.782	7.34E-05	1.98E-04	0.154	1.21
SASH1	-0.468	7.769	-8.133	2.47E-08	1.57E-07	8.280	-1.38
SAT1	-0.559	6.704	-11.539	3.05E-11	4.10E-10	15.167	-1.47
SBSN	-4.003	9.801	-67.587	9.46E-29	1.17E-25	55.474	-16.03
SBSN	-3.754	6.788	-42.023	7.16E-24	2.86E-21	44.771	-13.49
SC4MOL	-1.746	6.368	-8.209	2.10E-08	1.35E-07	8.450	-3.35
SC4MOL	-1.352	8.194	-26.799	2.61E-19	4.94E-17	34.231	-2.55
Sc5d	-0.649	6.364	-6.879	4.24E-07	1.95E-06	5.373	-1.57
Scamp2	0.264	6.915	4.956	4.73E-05	1.34E-04	0.595	1.20
Scamp2	0.281	8.543	6.978	3.36E-07	1.58E-06	5.610	1.21
SCAP	-0.424	7.413	-8.253	1.91E-08	1.23E-07	8.548	-1.34
Scarb1	-0.503	6.897	-7.271	1.71E-07	8.65E-07	6.302	-1.42
SCD	-0.853	7.257	-8.023	3.14E-08	1.94E-07	8.035	-1.81
SCD	-0.703	7.564	-7.526	9.55E-08	5.18E-07	6.898	-1.63
SCD	-0.694	9.542	-16.365	1.83E-14	7.14E-13	22.807	-1.62
Scd1	-0.581	9.240	-11.025	7.66E-11	9.05E-10	14.218	-1.50
Scd3	-0.466	8.588	-11.201	5.57E-11	6.93E-10	14.546	-1.38
Scyl3	1.525	5.993	24.436	2.19E-18	3.03E-16	32.066	2.88
Sdc1	-0.419	5.513	-6.841	4.65E-07	2.10E-06	5.281	-1.34
SDHA	-0.273	7.108	-5.292	2.03E-05	6.20E-05	1.448	-1.21
Sdhd	-0.436	9.423	-6.835	4.71E-07	2.13E-06	5.266	-1.35
SEC22B	0.605	6.590	11.069	7.07E-11	8.53E-10	14.300	1.52
SEC24D	-0.311	7.004	-4.311	2.42E-04	5.88E-04	-1.039	-1.24
SELK	0.416	7.749	8.682	7.62E-09	5.45E-08	9.489	1.33

SEMA3C	0.981	6.612	17.014	7.76E-15	3.24E-13	23.688	1.97
SENP5	0.363	6.817	5.174	2.73E-05	8.10E-05	1.149	1.29
SENP6	0.451	7.501	5.153	2.87E-05	8.50E-05	1.096	1.37
SEPHS2	-0.318	8.261	-6.423	1.25E-06	5.05E-06	4.269	-1.25
SERBP1	-0.274	8.550	-3.431	2.20E-03	4.46E-03	-3.211	-1.21
SERINC3	0.309	9.592	7.089	2.60E-07	1.25E-06	5.873	1.24
Serp1	0.481	7.644	11.295	4.71E-11	6.02E-10	14.720	1.40
SERPINB1	-1.299	8.041	-18.081	2.01E-15	1.00E-13	25.076	-2.46
Serpinf1	-0.762	8.278	-8.832	5.55E-09	4.14E-08	9.814	-1.70
SERPINH1	-0.660	8.798	-7.182	2.10E-07	1.03E-06	6.093	-1.58
SERPINH1	-0.490	9.252	-15.081	1.09E-13	3.58E-12	20.971	-1.40
Serpinh1	-0.472	8.949	-7.808	5.06E-08	3.00E-07	7.547	-1.39
SERTAD2	-0.275	7.311	-4.071	4.45E-04	1.03E-03	-1.642	-1.21
SET	0.498	8.500	13.163	1.99E-12	3.93E-11	17.977	1.41
SF3A3	0.398	8.084	8.559	9.87E-09	6.85E-08	9.223	1.32
SFRS2	-0.495	6.996	-6.852	4.53E-07	2.06E-06	5.307	-1.41
SFRS2	-0.465	8.647	-13.525	1.12E-12	2.55E-11	18.567	-1.38
SFRS7	-0.508	8.831	-5.182	2.67E-05	7.96E-05	1.170	-1.42
SGCE	-0.310	6.005	-2.407	2.42E-02	3.85E-02	-5.492	-1.24
Sh2b3	-0.508	6.519	-9.252	2.33E-09	1.94E-08	10.704	-1.42
SH3BGRL	-0.692	7.329	-2.930	7.35E-03	1.29E-02	-4.374	-1.62
Shc1	0.282	8.134	7.138	2.32E-07	1.13E-06	5.990	1.22
SHMT2	-0.780	6.208	-7.642	7.35E-08	4.14E-07	7.166	-1.72
Sik3	-0.334	7.041	-7.025	3.02E-07	1.43E-06	5.721	-1.26
Sin3b	0.430	6.811	5.022	4.01E-05	1.15E-04	0.762	1.35
Slc1a5	-0.703	6.945	-14.021	5.22E-13	1.33E-11	19.357	-1.63
SLC25A1	-0.658	6.910	-7.196	2.03E-07	1.00E-06	6.126	-1.58
SLC25A13	-0.285	6.821	-5.964	3.83E-06	1.38E-05	3.135	-1.22
SLC25A17	0.265	6.764	5.937	4.08E-06	1.47E-05	3.070	1.20
SLC25A20	-0.626	6.724	-3.636	1.32E-03	2.80E-03	-2.717	-1.54
SLC25A20	-0.481	8.555	-6.758	5.64E-07	2.50E-06	5.082	-1.40
SLC25A5	-0.598	10.477	-14.014	5.27E-13	1.33E-11	19.346	-1.51
Slc35a1	-0.299	6.412	-6.099	2.75E-06	1.03E-05	3.470	-1.23
Slc35c2	0.599	6.171	8.915	4.67E-09	3.56E-08	9.992	1.51
SLC39A1	-0.287	8.219	-5.732	6.77E-06	2.28E-05	2.557	-1.22
SLC44A1	0.395	7.158	5.979	3.68E-06	1.34E-05	3.174	1.31
SLC4A7	-0.391	6.315	-3.974	5.68E-04	1.29E-03	-1.884	-1.31
Slc4a7	-0.374	6.815	-5.356	1.73E-05	5.38E-05	1.609	-1.30
Slc6a6	-0.917	8.040	-19.167	5.46E-16	3.21E-14	26.417	-1.89
Slc7a11	0.413	7.310	4.911	5.30E-05	1.48E-04	0.482	1.33
Slc7a5	-0.436	7.632	-7.809	5.05E-08	3.00E-07	7.549	-1.35
Slmo2	-0.362	7.294	-8.876	5.06E-09	3.81E-08	9.908	-1.29
SLTM	0.265	6.758	5.505	1.19E-05	3.82E-05	1.986	1.20
SMARCA5	0.317	9.386	5.623	8.88E-06	2.91E-05	2.282	1.25
SMARCC1	0.431	6.216	2.641	1.44E-02	2.38E-02	-5.006	1.35
SMC4	-0.491	6.591	-4.448	1.71E-04	4.29E-04	-0.693	-1.41
Smg1	0.458	6.069	7.371	1.36E-07	7.07E-07	6.538	1.37
SNAI2	-0.961	6.399	-14.622	2.12E-13	6.19E-12	20.284	-1.95
Snn	-1.034	6.495	-13.371	1.43E-12	3.09E-11	18.318	-2.05
SNRNP27	0.303	7.046	2.782	1.04E-02	1.77E-02	-4.701	1.23
SNRPB2	0.360	7.682	5.867	4.85E-06	1.70E-05	2.895	1.28
SNRPC	0.504	7.891	10.075	4.56E-10	4.54E-09	12.383	1.42
SNRPD2	0.478	8.788	7.176	2.13E-07	1.05E-06	6.078	1.39
SNRPF	-0.451	7.914	-9.420	1.66E-09	1.41E-08	11.054	-1.37
SNX12	0.283	6.594	5.280	2.09E-05	6.36E-05	1.418	1.22
SNX14	-0.544	6.583	-9.239	2.40E-09	1.98E-08	10.677	-1.46
Snx18	0.514	6.521	9.765	8.35E-10	7.63E-09	11.760	1.43
SNX2	-0.352	6.773	-8.667	7.85E-09	5.60E-08	9.458	-1.28
Snx5	-0.450	7.211	-6.918	3.88E-07	1.79E-06	5.465	-1.37
SOD1	-0.348	8.506	-6.221	2.04E-06	7.83E-06	3.773	-1.27
SOD2	-1.163	6.599	-18.495	1.21E-15	6.41E-14	25.596	-2.24
SOD2	-0.954	6.729	-14.086	4.72E-13	1.23E-11	19.459	-1.94
SOX12	1.495	5.451	11.085	6.86E-11	8.30E-10	14.331	2.82
SPATA2	-0.429	6.823	-8.497	1.13E-08	7.65E-08	9.087	-1.35
Spc25	-0.526	6.631	-6.787	5.28E-07	2.36E-06	5.151	-1.44
SPIN1	0.414	7.093	5.921	4.25E-06	1.52E-05	3.029	1.33
SPPL2A	-0.526	7.487	-2.356	2.70E-02	4.25E-02	-5.593	-1.44
SPRR1A	1.635	8.643	18.963	6.94E-16	3.83E-14	26.170	3.10
SQLE	-1.414	6.333	-9.197	2.61E-09	2.13E-08	10.588	-2.67
SQLE	-1.297	7.073	-19.491	3.75E-16	2.32E-14	26.802	-2.46
Sqle	-1.107	9.031	-17.577	3.77E-15	1.81E-13	24.430	-2.15
SQSTM1	0.691	7.962	14.725	1.82E-13	5.55E-12	20.440	1.61
SRP68	0.655	6.373	6.816	4.93E-07	2.22E-06	5.221	1.57
SRP72	-0.597	6.670	-2.823	9.46E-03	1.63E-02	-4.613	-1.51
Srpr	0.585	10.118	3.518	1.77E-03	3.65E-03	-3.001	1.50
Srpr	0.633	10.831	5.274	2.12E-05	6.44E-05	1.402	1.55
SSR4	-0.542	9.057	-6.298	1.70E-06	6.64E-06	3.961	-1.46
SSU72	-0.809	7.371	-12.722	4.07E-12	7.32E-11	17.240	-1.75
STIM2	0.743	6.458	9.088	3.26E-09	2.60E-08	10.359	1.67

STK39	0.421	6.315	4.627	1.09E-04	2.84E-04	-0.241	1.34
STK4	0.608	6.572	14.098	4.64E-13	1.23E-11	19.478	1.52
STMN1	-0.338	7.260	-5.631	8.71E-06	2.86E-05	2.302	-1.26
STT3A	-0.972	6.388	-4.370	2.09E-04	5.13E-04	-0.891	-1.96
STT3A	-0.566	7.885	-10.102	4.33E-10	4.37E-09	12.436	-1.48
Stx12	0.367	7.514	7.002	3.19E-07	1.50E-06	5.666	1.29
SUCLG2	-0.286	8.679	-7.143	2.29E-07	1.12E-06	6.002	-1.22
Sumf2	0.328	7.010	5.999	3.51E-06	1.28E-05	3.223	1.26
SUPT16H	-0.895	10.402	-12.364	7.38E-12	1.19E-10	16.628	-1.86
SUPT4H1	0.652	7.223	12.205	9.65E-12	1.51E-10	16.352	1.57
SUPT6H	0.707	6.509	6.829	4.78E-07	2.16E-06	5.253	1.63
Syf2	0.622	7.090	9.892	6.51E-10	6.22E-09	12.017	1.54
SYNCRIP	0.540	6.547	8.123	2.53E-08	1.60E-07	8.257	1.45
SYPL1	-0.340	7.000	-3.710	1.10E-03	2.37E-03	-2.536	-1.27
SYS1	0.911	6.956	16.605	1.33E-14	5.30E-13	23.136	1.88
Taf2	0.361	8.441	7.672	6.86E-08	3.91E-07	7.236	1.28
TAGLN2	-0.476	8.340	-6.571	8.80E-07	3.70E-06	4.630	-1.39
TAGLN2	-0.368	7.238	-6.146	2.45E-06	9.25E-06	3.588	-1.29
TALDO1	-0.294	8.276	-5.485	1.25E-05	3.99E-05	1.935	-1.23
Tapbpl	0.557	5.845	5.904	4.43E-06	1.57E-05	2.987	1.47
Tardbp	-0.443	6.769	-3.286	3.14E-03	6.11E-03	-3.555	-1.36
TARDBP	-0.301	6.505	-3.832	8.13E-04	1.81E-03	-2.237	-1.23
TAX1BP1	0.353	8.337	6.414	1.28E-06	5.14E-06	4.247	1.28
Tbc1d13	0.542	6.269	8.806	5.86E-09	4.30E-08	9.759	1.46
Tbc1d20	0.664	7.320	11.787	1.97E-11	2.85E-10	15.614	1.58
TBCE	0.550	6.484	11.747	2.12E-11	3.00E-10	15.541	1.46
TBPL1	1.089	7.435	16.524	1.48E-14	5.83E-13	23.026	2.13
TBRG1	0.486	6.867	7.547	9.10E-08	4.98E-07	6.947	1.40
TCEA1	0.394	8.260	8.969	4.17E-09	3.25E-08	10.107	1.31
Tcf19	-0.655	5.912	-11.408	3.84E-11	5.02E-10	14.928	-1.58
TECR	-0.560	8.225	-7.981	3.46E-08	2.12E-07	7.938	-1.47
TFB1M	-0.524	6.682	-7.527	9.52E-08	5.18E-07	6.900	-1.44
Tfb2m	0.366	7.005	7.199	2.02E-07	9.99E-07	6.133	1.29
TFPI	-1.079	7.291	-13.471	1.22E-12	2.73E-11	18.481	-2.11
Tgfbr2	-0.613	6.364	-7.789	5.29E-08	3.10E-07	7.503	-1.53
Tgm2	0.537	7.080	8.876	5.07E-09	3.81E-08	9.907	1.45
Tgoln2	-0.284	6.450	-3.398	2.38E-03	4.80E-03	-3.290	-1.22
Thbd	-2.274	7.746	-26.259	4.18E-19	7.51E-17	33.754	-4.84
THBS3	0.476	6.147	8.663	7.92E-09	5.64E-08	9.449	1.39
THOC7	0.454	7.681	8.582	9.40E-09	6.56E-08	9.273	1.37
TIMM10	0.508	7.656	9.182	2.69E-09	2.19E-08	10.557	1.42
TIMM17B	-0.568	6.995	-6.373	1.42E-06	5.64E-06	4.145	-1.48
TIMM23	-0.402	7.920	-2.879	8.30E-03	1.44E-02	-4.489	-1.32
TIMM50	0.359	7.608	7.299	1.60E-07	8.20E-07	6.368	1.28
TIMM9	0.319	7.636	6.531	9.69E-07	4.03E-06	4.531	1.25
TIMP1	-0.911	6.932	-13.406	1.35E-12	2.98E-11	18.376	-1.88
Timp2	0.280	7.474	3.625	1.36E-03	2.88E-03	-2.744	1.21
TIMP2	0.288	8.474	4.169	3.47E-04	8.20E-04	-1.396	1.22
Tinagl1	-1.639	6.534	-14.492	2.57E-13	7.20E-12	20.087	-3.11
TIPARP	0.342	7.024	2.990	6.38E-03	1.14E-02	-4.238	1.27
TIPARP	0.363	8.475	8.854	5.30E-09	3.97E-08	9.861	1.29
TJP2	-0.699	6.785	-13.597	1.00E-12	2.33E-11	18.683	-1.62
TKT	-0.745	6.373	-5.333	1.83E-05	5.65E-05	1.550	-1.68
TKT	-0.510	6.994	-11.292	4.73E-11	6.03E-10	14.714	-1.42
TM9SF4	1.389	5.793	24.004	3.30E-18	4.09E-16	31.648	2.62
TMBIM6	0.276	8.964	5.368	1.68E-05	5.24E-05	1.639	1.21
TMED3	-0.595	6.990	-12.434	6.56E-12	1.09E-10	16.749	-1.51
TMEM101	0.382	6.466	4.589	1.20E-04	3.09E-04	-0.337	1.30
Tmem109	-0.669	6.566	-12.839	3.36E-12	6.16E-11	17.437	-1.59
Tmem111	0.502	7.013	9.062	3.45E-09	2.73E-08	10.303	1.42
Tmem14c	-0.269	9.909	-6.160	2.37E-06	9.00E-06	3.623	-1.20
Tmem150a	-0.436	6.896	-6.306	1.66E-06	6.52E-06	3.982	-1.35
TMEM156	-3.624	7.139	-63.314	4.45E-28	4.00E-25	54.053	-12.33
Tmem161b	0.280	6.598	4.127	3.87E-04	9.06E-04	-1.503	1.21
Tmem167	0.493	7.216	7.585	8.36E-08	4.65E-07	7.034	1.41
Tmem167b	0.659	6.863	14.771	1.70E-13	5.32E-12	20.510	1.58
TMEM183A	0.374	6.182	7.144	2.29E-07	1.12E-06	6.003	1.30
Tmem189	-0.572	6.827	-7.870	4.41E-08	2.66E-07	7.687	-1.49
TMEM223	-0.499	7.260	-7.327	1.50E-07	7.74E-07	6.435	-1.41
TMEM30A	-0.470	6.995	-4.510	1.46E-04	3.71E-04	-0.536	-1.39
TMEM5	-0.360	6.602	-8.956	4.29E-09	3.33E-08	10.080	-1.28
TMEM66	0.327	8.790	9.055	3.49E-09	2.75E-08	10.290	1.25
TMSB10	-0.610	10.371	-9.841	7.19E-10	6.77E-09	11.914	-1.53
Tmx1	0.417	8.098	7.575	8.54E-08	4.73E-07	7.012	1.34
Tnfaip3	-0.864	5.235	-9.439	1.60E-09	1.36E-08	11.094	-1.82
Tnfrsf21	-1.358	7.387	-19.155	5.54E-16	3.21E-14	26.402	-2.56
Tnfsf9	-1.268	6.765	-11.884	1.67E-11	2.47E-10	15.786	-2.41
TNIP1	-0.392	6.401	-5.316	1.91E-05	5.87E-05	1.508	-1.31
Tnks2	0.324	6.639	6.409	1.30E-06	5.20E-06	4.234	1.25

Tollip	0.413	6.477	6.227	2.01E-06	7.74E-06	3.787	1.33
TOMM34	0.869	7.388	14.669	1.98E-13	5.83E-12	20.355	1.83
TOP1	0.380	7.654	5.547	1.07E-05	3.47E-05	2.091	1.30
Top2a	-0.930	9.103	-11.795	1.95E-11	2.82E-10	15.627	-1.91
TOP2B	0.282	7.259	4.440	1.75E-04	4.37E-04	-0.714	1.22
TPD52L2	-0.358	6.534	-3.762	9.68E-04	2.11E-03	-2.409	-1.28
TPI1	-0.313	9.171	-6.171	2.31E-06	8.80E-06	3.650	-1.24
Tprgl	-0.477	6.781	-7.869	4.43E-08	2.66E-07	7.685	-1.39
Tpst1	-0.528	6.787	-12.496	5.92E-12	9.93E-11	16.856	-1.44
TPX2	0.828	6.219	8.891	4.91E-09	3.71E-08	9.940	1.78
TRAK2	-0.471	6.593	-5.887	4.62E-06	1.63E-05	2.945	-1.39
Tram1	-0.303	6.401	-3.812	8.53E-04	1.89E-03	-2.285	-1.23
TRAP1	0.461	6.783	5.639	8.52E-06	2.81E-05	2.324	1.38
TRAPPC4	0.657	7.791	11.827	1.84E-11	2.68E-10	15.685	1.58
TRIAP1	0.377	6.780	8.905	4.77E-09	3.62E-08	9.969	1.30
TRIB3	1.107	5.604	7.793	5.24E-08	3.08E-07	7.512	2.15
TRIB3	1.523	5.753	11.208	5.50E-11	6.86E-10	14.559	2.87
Trib3	1.752	8.754	37.549	1.01E-22	2.79E-20	42.150	3.37
Trim44	0.365	6.505	8.029	3.11E-08	1.92E-07	8.047	1.29
Trmt6	0.477	6.854	8.722	7.00E-09	5.07E-08	9.576	1.39
TSC22D3	0.723	6.584	13.954	5.78E-13	1.44E-11	19.252	1.65
TSEN15	0.372	7.360	10.166	3.83E-10	3.93E-09	12.563	1.29
Tspan4	-0.431	6.391	-7.596	8.15E-08	4.56E-07	7.059	-1.35
TSPO	0.406	8.531	5.405	1.53E-05	4.79E-05	1.733	1.32
Ttc13	-0.275	7.694	-4.974	4.52E-05	1.28E-04	0.640	-1.21
Ttc3	0.387	7.465	6.173	2.30E-06	8.77E-06	3.654	1.31
TTC33	0.365	6.573	5.218	2.44E-05	7.34E-05	1.260	1.29
TUBA1C	-0.981	9.068	-20.362	1.40E-16	1.03E-14	27.813	-1.97
TUBB2C	-0.755	8.450	-15.057	1.13E-13	3.68E-12	20.936	-1.69
Tubb6	-0.805	7.128	-20.264	1.56E-16	1.08E-14	27.702	-1.75
Twistnb	0.363	7.652	4.488	1.55E-04	3.91E-04	-0.591	1.29
TXNDC15	0.450	6.914	11.636	2.57E-11	3.53E-10	15.342	1.37
TXNIP	1.000	8.976	14.208	3.93E-13	1.06E-11	19.649	2.00
TXNRD1	0.469	7.365	3.218	3.70E-03	7.06E-03	-3.715	1.38
TXNRD1	0.581	9.549	14.670	1.98E-13	5.83E-12	20.357	1.50
Tyms	-1.008	8.010	-12.872	3.18E-12	5.87E-11	17.494	-2.01
UAP1	-0.525	6.881	-8.508	1.10E-08	7.53E-08	9.110	-1.44
UAP1L1	-0.426	6.485	-4.759	7.78E-05	2.08E-04	0.096	-1.34
UBASH3B	-1.112	8.046	-27.180	1.89E-19	3.76E-17	34.563	-2.16
UBE2D4	0.415	6.665	6.821	4.86E-07	2.19E-06	5.234	1.33
Ube2h	0.330	6.554	2.977	6.58E-03	1.18E-02	-4.268	1.26
UBE2J2	0.272	7.348	6.062	3.01E-06	1.12E-05	3.379	1.21
UBE2Q1	-0.331	7.359	-4.258	2.77E-04	6.64E-04	-1.172	-1.26
UBE2R2	0.280	6.750	3.931	6.33E-04	1.43E-03	-1.991	1.21
Ube2s	0.681	7.516	8.271	1.83E-08	1.19E-07	8.589	1.60
Ubiad1	0.417	6.641	7.646	7.28E-08	4.12E-07	7.176	1.33
UBL3	0.398	6.206	7.527	9.51E-08	5.18E-07	6.901	1.32
UBL3	0.417	7.629	5.793	5.82E-06	2.00E-05	2.710	1.34
UBQLN1	0.520	7.255	13.229	1.79E-12	3.66E-11	18.086	1.43
UBQLN2	0.325	8.467	4.596	1.18E-04	3.05E-04	-0.318	1.25
UBQLN4	0.275	6.593	2.920	7.52E-03	1.32E-02	-4.396	1.21
UBR4	0.500	6.320	5.247	2.27E-05	6.85E-05	1.334	1.41
UCHL5	-0.370	6.364	-3.954	5.97E-04	1.36E-03	-1.933	-1.29
UCK2	-0.266	6.008	-4.139	3.75E-04	8.82E-04	-1.473	-1.20
Uck2	0.515	6.716	4.814	6.77E-05	1.84E-04	0.235	1.43
Ufd1l	0.562	6.294	11.936	1.53E-11	2.29E-10	15.879	1.48
UFD1L	0.782	8.108	17.353	5.02E-15	2.22E-13	24.137	1.72
UGCG	-0.610	6.257	-7.002	3.19E-07	1.50E-06	5.666	-1.53
UGCG	-0.429	6.614	-4.288	2.57E-04	6.20E-04	-1.099	-1.35
UGP2	-0.297	8.371	-7.098	2.55E-07	1.23E-06	5.894	-1.23
Ugt1a1	-0.356	10.340	-8.042	3.02E-08	1.88E-07	8.076	-1.28
UQCC	0.629	7.071	13.826	7.03E-13	1.73E-11	19.050	1.55
UQCC	0.639	6.686	12.945	2.83E-12	5.29E-11	17.616	1.56
USP1	-0.471	7.729	-7.591	8.24E-08	4.60E-07	7.048	-1.39
USP19	0.389	6.555	7.644	7.32E-08	4.13E-07	7.170	1.31
USP46	0.514	6.354	7.640	7.38E-08	4.15E-07	7.162	1.43
UTP18	-0.293	7.550	-7.164	2.19E-07	1.07E-06	6.050	-1.23
UTP3	0.515	6.703	11.097	6.73E-11	8.19E-10	14.352	1.43
VAMP8	-0.443	7.162	-7.780	5.40E-08	3.16E-07	7.481	-1.36
VAPA	0.268	8.901	7.597	8.13E-08	4.55E-07	7.062	1.20
Vapb	-0.472	8.100	-5.600	9.40E-06	3.06E-05	2.225	-1.39
VAT1	0.494	7.192	2.990	6.38E-03	1.14E-02	-4.239	1.41
VCAM1	-0.805	6.411	-9.470	1.50E-09	1.30E-08	11.157	-1.75
VDAC2	-0.322	8.821	-7.485	1.05E-07	5.64E-07	6.803	-1.25
VEGFB	0.374	6.878	6.951	3.59E-07	1.67E-06	5.545	1.30
VMA21	0.744	7.724	11.534	3.07E-11	4.12E-10	15.158	1.68
VNN1	-1.373	6.056	-25.098	1.19E-18	1.94E-16	32.692	-2.59
VPS33B	-0.286	6.639	-6.748	5.78E-07	2.55E-06	5.058	-1.22
VWA5A	-0.460	7.095	-8.966	4.20E-09	3.27E-08	10.100	-1.38

WAC	0.406	8.157	7.842	4.70E-08	2.81E-07	7.623	1.33
WAC	0.426	7.633	5.901	4.46E-06	1.58E-05	2.979	1.34
WAPAL	0.302	6.875	6.506	1.03E-06	4.24E-06	4.472	1.23
WBP5	0.323	8.222	6.151	2.42E-06	9.17E-06	3.599	1.25
Wdfy4	0.394	6.002	3.708	1.11E-03	2.38E-03	-2.541	1.31
Wdr1	-0.290	8.251	-2.962	6.82E-03	1.21E-02	-4.302	-1.22
WDR26	0.425	6.370	6.932	3.75E-07	1.74E-06	5.500	1.34
Wdr5	0.361	6.168	5.292	2.03E-05	6.20E-05	1.448	1.28
WDR67	-0.345	6.118	-3.859	7.59E-04	1.70E-03	-2.170	-1.27
WDR74	0.487	6.571	8.911	4.71E-09	3.58E-08	9.983	1.40
Wdr77	0.396	6.647	7.534	9.38E-08	5.12E-07	6.916	1.32
WDYHV1	-0.588	6.805	-5.943	4.03E-06	1.45E-05	3.083	-1.50
Wdyhv1	-0.504	7.392	-10.018	5.09E-10	4.93E-09	12.269	-1.42
WSB1	0.819	6.023	12.411	6.82E-12	1.13E-10	16.710	1.76
WWC3	-0.433	6.647	-8.613	8.81E-09	6.19E-08	9.340	-1.35
XBP1	-0.681	7.896	-12.340	7.68E-12	1.24E-10	16.586	-1.60
XPOT	-0.355	7.326	-3.319	2.89E-03	5.68E-03	-3.478	-1.28
Xrcc5	-0.369	6.430	-7.390	1.30E-07	6.81E-07	6.582	-1.29
YIPF1	0.498	6.174	8.954	4.30E-09	3.33E-08	10.075	1.41
Ykt6	0.337	6.652	7.395	1.29E-07	6.76E-07	6.593	1.26
YPEL5	0.623	6.204	9.035	3.64E-09	2.85E-08	10.248	1.54
YWHAB	0.377	6.224	2.735	1.16E-02	1.96E-02	-4.805	1.30
Ywhab	0.497	7.203	7.797	5.19E-08	3.07E-07	7.521	1.41
YWHAG	0.370	7.313	4.418	1.85E-04	4.60E-04	-0.770	1.29
YWHAQ	0.294	7.581	6.305	1.67E-06	6.53E-06	3.978	1.23
Ywhaz	0.463	7.652	6.361	1.46E-06	5.76E-06	4.117	1.38
ZC3HAV1	0.533	7.214	9.878	6.68E-10	6.37E-09	11.990	1.45
ZEB1	-0.275	7.606	-7.527	9.52E-08	5.18E-07	6.900	-1.21
ZFAND5	0.652	7.866	11.510	3.21E-11	4.27E-10	15.114	1.57
Zfp110	0.355	6.538	8.813	5.77E-09	4.24E-08	9.774	1.28
ZFP36L1	-0.491	8.280	-8.253	1.91E-08	1.23E-07	8.549	-1.41
ZFR	0.867	8.429	11.033	7.55E-11	8.99E-10	14.233	1.82
ZMYND11	0.403	7.803	11.320	4.50E-11	5.82E-10	14.765	1.32
ZNF664	0.330	6.480	6.613	7.96E-07	3.39E-06	4.732	1.26
ZSWIM3	0.708	6.905	14.091	4.68E-13	1.23E-11	19.468	1.63
Zyx	-0.304	7.962	-4.746	8.05E-05	2.14E-04	0.062	-1.23

5.2.9 L(S)3 v pcDNA(S) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40788	-0.278	8.571	-2.637	1.45E-02	2.93E-02	-4.623	-1.21
40790	0.566	5.984	6.418	1.27E-06	7.84E-06	4.632	1.48
1700020I14Rik	0.478	8.162	11.243	5.16E-11	1.08E-09	14.961	1.39
1700052N19Rik	-0.750	6.422	-15.626	5.02E-14	3.11E-12	22.048	-1.68
2310001A20Rik	-0.717	7.257	-12.785	3.67E-12	1.16E-10	17.666	-1.64
2310014D11Rik	-0.908	6.536	-12.442	6.47E-12	1.91E-10	17.086	-1.88
2310044H10Rik	0.291	6.864	3.953	5.99E-04	1.78E-03	-1.547	1.22
2610002M06Rik	-0.309	6.504	-5.273	2.13E-05	9.49E-05	1.781	-1.24
2810025M15Rik	-0.360	6.900	-6.529	9.74E-07	6.21E-06	4.902	-1.28
2810474O19Rik	-1.132	7.272	-21.770	3.07E-17	6.13E-15	29.544	-2.19
2810482I07Rik	-0.454	6.348	-7.636	7.45E-08	6.66E-07	7.520	-1.37
3010026O09Rik	-0.298	8.162	-5.674	7.83E-06	3.91E-05	2.790	-1.23
3110062M04Rik	-0.537	7.493	-11.137	6.26E-11	1.27E-09	14.764	-1.45
4833417J20Rik	0.384	7.312	5.482	1.26E-05	5.94E-05	2.309	1.31
6330578E17Rik	0.321	6.733	6.232	1.99E-06	1.17E-05	4.178	1.25
6430548M08Rik	0.649	5.381	9.253	2.33E-09	3.13E-08	11.062	1.57
9430072B17Rik	-0.271	9.474	-6.133	2.53E-06	1.43E-05	3.934	-1.21
9530053H05Rik	-1.151	10.002	-19.943	2.24E-16	2.77E-14	27.543	-2.22
A330042M18Rik	0.733	7.205	11.486	3.35E-11	7.52E-10	15.404	1.66
A430103D13Rik	0.299	6.913	4.182	3.36E-04	1.08E-03	-0.978	1.23
A730071L15Rik	0.376	6.649	5.584	9.77E-06	4.78E-05	2.567	1.30
Abcf1	0.326	7.651	3.728	1.05E-03	2.97E-03	-2.103	1.25
ABHD6	-0.324	6.783	-4.120	3.94E-04	1.23E-03	-1.133	-1.25
Acat2	-1.292	6.811	-19.544	3.52E-16	3.96E-14	27.083	-2.45
ACLY	-0.431	8.199	-7.704	6.40E-08	5.75E-07	7.675	-1.35
ACO1	-0.659	6.759	-20.467	1.25E-16	1.79E-14	28.134	-1.58
ACOT7	0.298	7.565	5.548	1.07E-05	5.18E-05	2.474	1.23
Acp2	0.290	6.448	4.325	2.34E-04	7.82E-04	-0.617	1.22
ACTB	0.299	7.958	3.593	1.47E-03	3.94E-03	-2.431	1.23
ACTB	0.321	7.918	3.732	1.04E-03	2.94E-03	-2.092	1.25
Adam23	-0.373	7.575	-5.864	4.89E-06	2.57E-05	3.266	-1.29
Adam23	-0.372	7.260	-5.984	3.64E-06	1.96E-05	3.563	-1.29
ADAM9	-0.328	8.339	-4.355	2.17E-04	7.33E-04	-0.542	-1.26
ADD3	-0.307	7.763	-6.400	1.32E-06	8.14E-06	4.590	-1.24
ADO	0.522	6.943	9.028	3.69E-09	4.69E-08	10.589	1.44
Ado	0.616	7.572	15.325	7.67E-14	4.31E-12	21.617	1.53
ADSS	0.343	7.645	6.764	5.56E-07	3.79E-06	5.472	1.27
ADSS	0.393	8.642	6.071	2.94E-06	1.64E-05	3.780	1.31
AFAP1	-0.421	6.434	-6.362	1.45E-06	8.82E-06	4.496	-1.34
AGAP1	0.320	7.787	3.298	3.05E-03	7.39E-03	-3.137	1.25
Agap1	0.330	7.472	6.582	8.58E-07	5.48E-06	5.031	1.26
Agrn	0.334	7.203	7.032	2.97E-07	2.22E-06	6.110	1.26
AHCYL1	-0.590	7.942	-14.231	3.79E-13	1.66E-11	19.986	-1.51
AI646531	0.637	6.560	10.695	1.41E-10	2.53E-09	13.935	1.55
AI646531	0.686	7.286	8.553	1.00E-08	1.11E-07	9.570	1.61
AIG1	-0.356	6.487	-4.691	9.25E-05	3.42E-04	0.308	-1.28
Akap12	-0.808	7.889	-12.120	1.11E-11	2.88E-10	16.531	-1.75
Amacr	-0.479	6.539	-7.109	2.48E-07	1.91E-06	6.293	-1.39
AMD1	0.804	7.422	8.010	3.24E-08	3.17E-07	8.369	1.75
AMD1	0.812	8.027	9.455	1.55E-09	2.20E-08	11.480	1.76
AMOTL2	-0.370	8.057	-3.594	1.47E-03	3.94E-03	-2.429	-1.29
ANLN	-0.772	6.950	-6.691	6.61E-07	4.36E-06	5.296	-1.71
ANO6	-0.414	7.837	-10.263	3.17E-10	5.25E-09	13.102	-1.33
ANXA1	-0.291	10.378	-4.032	4.91E-04	1.49E-03	-1.350	-1.22
Anxa4	-0.316	7.155	-5.520	1.15E-05	5.49E-05	2.406	-1.25
AP1B1	0.736	6.806	13.963	5.69E-13	2.30E-11	19.571	1.67
APP	0.572	10.319	13.337	1.51E-12	5.42E-11	18.574	1.49
APP	0.777	8.605	8.036	3.06E-08	3.03E-07	8.428	1.71
APP	0.812	6.325	3.551	1.63E-03	4.31E-03	-2.532	1.76
Arf2	-0.484	6.235	-6.791	5.22E-07	3.60E-06	5.536	-1.40
ARHGAP24	-0.427	7.747	-5.685	7.61E-06	3.82E-05	2.819	-1.34
Arhgap25	-0.478	6.665	-5.743	6.59E-06	3.35E-05	2.965	-1.39
Arhgap29	-0.284	7.970	-6.066	2.98E-06	1.65E-05	3.768	-1.22
Arl2bp	0.359	7.421	6.017	3.36E-06	1.83E-05	3.646	1.28
ARL6IP1	-0.288	8.692	-5.014	4.09E-05	1.68E-04	1.126	-1.22
ARL6IP4	-0.315	6.141	-4.716	8.68E-05	3.24E-04	0.371	-1.24
ARPC1A	0.412	8.147	7.031	2.98E-07	2.22E-06	6.107	1.33
ARPC5	0.325	8.452	4.807	6.89E-05	2.65E-04	0.602	1.25
ASAP1	0.610	5.925	11.437	3.65E-11	8.01E-10	15.314	1.53
Asf1b	-0.821	5.939	-9.795	7.87E-10	1.18E-08	12.172	-1.77
ASPM	-0.852	6.112	-12.999	2.59E-12	8.54E-11	18.022	-1.80
ATAD2	0.302	6.552	3.403	2.36E-03	5.94E-03	-2.888	1.23
Atf5	0.417	9.270	3.590	1.49E-03	3.97E-03	-2.439	1.34
ATG3	-0.297	6.477	-4.782	7.35E-05	2.79E-04	0.538	-1.23
Atp10a	-0.629	7.352	-14.727	1.82E-13	9.06E-12	20.738	-1.55

ATP2A2	-0.282	6.749	-4.726	8.47E-05	3.17E-04	0.396	-1.22
ATXN7L3B	0.362	7.040	5.038	3.84E-05	1.59E-04	1.188	1.29
ATXN7L3B	0.458	6.370	9.177	2.72E-09	3.59E-08	10.904	1.37
Aurka	-0.530	6.331	-6.739	5.91E-07	3.98E-06	5.411	-1.44
AW742525	-0.293	7.372	-7.545	9.14E-08	7.98E-07	7.311	-1.23
Azin1	0.556	6.442	6.705	6.40E-07	4.27E-06	5.329	1.47
AZIN1	0.785	6.431	10.839	1.08E-10	2.03E-09	14.207	1.72
B230219D22Rik	0.804	8.128	15.270	8.30E-14	4.53E-12	21.536	1.75
B230220B15Rik	-1.771	7.506	-37.012	1.42E-22	1.02E-19	41.602	-3.41
B2M	-0.356	10.904	-8.359	1.52E-08	1.63E-07	9.145	-1.28
B4GALT3	0.389	6.562	6.507	1.03E-06	6.47E-06	4.850	1.31
BAZ1A	-0.513	6.516	-5.989	3.59E-06	1.94E-05	3.578	-1.43
BAZ1A	-0.366	6.586	-7.433	1.18E-07	9.88E-07	7.051	-1.29
BC013529	-0.320	7.343	-7.499	1.02E-07	8.72E-07	7.204	-1.25
BCL10	-0.575	8.488	-11.344	4.31E-11	9.27E-10	15.146	-1.49
BCLAF1	0.320	6.690	4.480	1.58E-04	5.53E-04	-0.226	1.25
BCLAF1	0.331	7.984	5.308	1.95E-05	8.81E-05	1.870	1.26
BEST1	-0.373	11.907	-7.466	1.09E-07	9.34E-07	7.128	-1.29
BLVRB	-0.694	8.858	-7.234	1.86E-07	1.48E-06	6.586	-1.62
BMI1	0.563	6.386	6.781	5.35E-07	3.66E-06	5.511	1.48
Bod1	-0.293	7.775	-6.077	2.90E-06	1.62E-05	3.796	-1.23
BRD7	-0.339	7.096	-6.061	3.01E-06	1.67E-05	3.756	-1.27
BRP44L	0.326	8.068	7.287	1.65E-07	1.33E-06	6.710	1.25
BRP44L	0.380	7.208	2.382	2.55E-02	4.78E-02	-5.148	1.30
BTBD1	0.286	7.455	2.509	1.93E-02	3.76E-02	-4.890	1.22
BTBD1	0.322	8.357	5.117	3.15E-05	1.34E-04	1.386	1.25
BTBD1	0.397	8.023	3.669	1.22E-03	3.37E-03	-2.248	1.32
BUD31	0.303	7.762	5.154	2.87E-05	1.23E-04	1.480	1.23
C11orf17	-0.272	6.469	-4.826	6.57E-05	2.54E-04	0.650	-1.21
C12orf10	0.308	6.223	4.418	1.85E-04	6.38E-04	-0.384	1.24
C14orf50	-0.264	7.750	-5.716	7.04E-06	3.56E-05	2.897	-1.20
C19orf62	0.280	6.759	4.385	2.01E-04	6.87E-04	-0.465	1.21
C1R	-0.971	9.482	-12.310	8.07E-12	2.26E-10	16.860	-1.96
C22orf28	-0.267	8.171	-7.184	2.09E-07	1.65E-06	6.470	-1.20
C2orf24	0.298	6.408	2.616	1.52E-02	3.05E-02	-4.666	1.23
C3	-1.960	7.330	-35.720	3.25E-22	1.95E-19	40.810	-3.89
C4orf27	1.279	6.966	25.781	6.38E-19	2.09E-16	33.414	2.43
C5orf51	0.278	6.308	3.604	1.43E-03	3.84E-03	-2.404	1.21
C78376	-0.371	7.959	-5.924	4.22E-06	2.25E-05	3.415	-1.29
C7orf55	0.293	9.383	3.983	5.56E-04	1.67E-03	-1.474	1.23
CACHD1	0.355	6.534	6.913	3.92E-07	2.80E-06	5.827	1.28
CALD1	-0.477	7.225	-5.384	1.61E-05	7.37E-05	2.062	-1.39
CALD1	-0.426	7.673	-3.335	2.78E-03	6.86E-03	-3.049	-1.34
CAMK2N1	0.348	6.214	4.646	1.04E-04	3.78E-04	0.193	1.27
CANX	-0.548	7.461	-6.284	1.75E-06	1.05E-05	4.306	-1.46
CANX	-0.392	8.370	-9.495	1.43E-09	2.05E-08	11.562	-1.31
CAPG	-0.385	7.270	-4.862	5.99E-05	2.34E-04	0.742	-1.31
CAPG	-0.352	7.250	-3.320	2.88E-03	7.06E-03	-3.083	-1.28
Capns1	0.449	7.079	6.964	3.48E-07	2.56E-06	5.950	1.36
Cat	-0.589	8.131	-5.906	4.40E-06	2.33E-05	3.372	-1.50
CAT	-0.527	6.892	-2.649	1.41E-02	2.86E-02	-4.596	-1.44
CAV2	-0.377	6.234	-5.216	2.46E-05	1.07E-04	1.637	-1.30
CBFB	0.299	7.994	5.209	2.50E-05	1.08E-04	1.620	1.23
Cbx1	0.305	6.452	4.766	7.65E-05	2.89E-04	0.498	1.24
Cbx6-Nptxr	0.365	6.780	4.862	6.00E-05	2.34E-04	0.742	1.29
CCDC132	0.300	7.485	5.432	1.43E-05	6.61E-05	2.183	1.23
CCDC50	0.534	7.337	9.404	1.71E-09	2.40E-08	11.375	1.45
CCDC53	-0.436	7.195	-8.903	4.79E-09	5.88E-08	10.323	-1.35
CCDC80	-0.264	7.753	-2.577	1.66E-02	3.30E-02	-4.749	-1.20
Ccna2	-0.610	6.788	-4.773	7.51E-05	2.84E-04	0.516	-1.53
CCNB1	-1.171	5.956	-8.223	2.03E-08	2.13E-07	8.846	-2.25
Ccnb1	-0.927	7.434	-11.028	7.62E-11	1.50E-09	14.563	-1.90
CCNB2	-1.093	6.467	-16.455	1.62E-14	1.19E-12	23.201	-2.13
Ccnd1	1.744	6.627	20.529	1.16E-16	1.74E-14	28.204	3.35
Cd151	0.268	6.994	3.154	4.31E-03	1.01E-02	-3.472	1.20
CD36	3.337	8.162	16.730	1.12E-14	8.98E-13	23.572	10.10
CDC20	-0.938	7.231	-9.739	8.78E-10	1.31E-08	12.059	-1.92
CDC37	0.406	7.553	6.451	1.17E-06	7.33E-06	4.713	1.32
Cdc42se1	-0.353	6.268	-6.227	2.02E-06	1.19E-05	4.164	-1.28
CDIPT	0.326	7.020	5.018	4.04E-05	1.67E-04	1.138	1.25
CDK1	-0.435	8.673	-6.787	5.27E-07	3.63E-06	5.527	-1.35
Cdk5rap3	-0.330	7.163	-5.397	1.56E-05	7.16E-05	2.095	-1.26
CDKN3	-1.362	8.297	-15.752	4.22E-14	2.70E-12	22.227	-2.57
Cenpa	-0.866	7.615	-9.199	2.60E-09	3.46E-08	10.949	-1.82
Cenpf	-0.955	6.632	-11.607	2.70E-11	6.31E-10	15.623	-1.94
CENPL	-0.717	6.032	-10.335	2.77E-10	4.66E-09	13.242	-1.64
Cep55	-0.470	6.066	-5.569	1.01E-05	4.95E-05	2.528	-1.39
Cflar	-0.587	7.205	-7.451	1.13E-07	9.57E-07	7.093	-1.50
CHD2	0.412	6.581	7.126	2.38E-07	1.85E-06	6.334	1.33

Chordc1	0.343	6.453	4.789	7.22E-05	2.75E-04	0.555	1.27
Chpf	0.639	6.081	5.504	1.19E-05	5.69E-05	2.364	1.56
CHPT1	-0.277	8.437	-4.971	4.55E-05	1.84E-04	1.018	-1.21
Cisd2	0.491	6.498	10.337	2.76E-10	4.66E-09	13.245	1.41
CISD3	0.269	6.884	4.323	2.35E-04	7.86E-04	-0.623	1.21
CKAP2	-1.046	5.818	-17.098	6.96E-15	5.82E-13	24.059	-2.07
CKAP5	-0.569	6.097	-7.307	1.57E-07	1.29E-06	6.757	-1.48
CKS1B	-0.384	7.858	-7.703	6.40E-08	5.75E-07	7.674	-1.30
CKS2	-0.622	8.689	-7.960	3.62E-08	3.48E-07	8.257	-1.54
Clec2e	-0.468	5.756	-4.433	1.78E-04	6.16E-04	-0.346	-1.38
CLIC1	-0.356	8.811	-7.549	9.06E-08	7.94E-07	7.320	-1.28
Clstn1	0.580	7.732	8.412	1.35E-08	1.46E-07	9.263	1.49
Clu	0.295	10.477	3.488	1.91E-03	4.96E-03	-2.684	1.23
Cmas	-0.300	8.324	-8.549	1.01E-08	1.12E-07	9.562	-1.23
CMTM1	-0.494	8.581	-12.635	4.69E-12	1.45E-10	17.415	-1.41
Cnn2	0.421	7.200	4.150	3.64E-04	1.15E-03	-1.056	1.34
COBLL1	-0.757	6.463	-14.148	4.30E-13	1.84E-11	19.858	-1.69
Col12a1	-0.745	6.218	-15.548	5.60E-14	3.25E-12	21.937	-1.68
COL4A3BP	-0.330	6.224	-8.314	1.67E-08	1.78E-07	9.047	-1.26
COL5A2	-0.857	7.606	-11.767	2.05E-11	4.90E-10	15.908	-1.81
COMTD1	-0.276	6.280	-4.110	4.03E-04	1.26E-03	-1.157	-1.21
COPB2	-0.289	7.048	-5.086	3.40E-05	1.43E-04	1.309	-1.22
CORO1B	-0.376	7.585	-5.204	2.53E-05	1.10E-04	1.606	-1.30
Coro1b	-0.335	8.179	-5.305	1.97E-05	8.86E-05	1.862	-1.26
Cox4nb	0.388	7.112	7.045	2.88E-07	2.17E-06	6.142	1.31
COX7A2L	0.268	7.153	2.815	9.63E-03	2.05E-02	-4.238	1.20
CPD	0.425	7.815	7.714	6.25E-08	5.64E-07	7.699	1.34
Cpn2	-0.579	6.648	-7.312	1.55E-07	1.28E-06	6.769	-1.49
Cpsf2	0.298	7.072	3.793	8.96E-04	2.56E-03	-1.944	1.23
CPT2	-0.559	6.454	-9.877	6.69E-10	1.02E-08	12.338	-1.47
Cr1l	-0.708	8.857	-16.675	1.21E-14	9.25E-13	23.497	-1.63
Cr1l	-0.530	7.762	-7.561	8.81E-08	7.76E-07	7.349	-1.44
CRELD1	-0.300	6.519	-3.011	6.08E-03	1.37E-02	-3.801	-1.23
CRELD2	-0.659	7.396	-9.176	2.73E-09	3.59E-08	10.900	-1.58
Crk	-0.567	7.733	-6.192	2.19E-06	1.27E-05	4.078	-1.48
CROT	-0.402	6.877	-8.820	5.69E-09	6.73E-08	10.147	-1.32
CSDAP1	0.297	10.028	5.819	5.46E-06	2.84E-05	3.154	1.23
CSE1L	-0.385	7.389	-7.293	1.62E-07	1.32E-06	6.726	-1.31
CSNK2A1	0.323	6.970	6.231	1.99E-06	1.17E-05	4.175	1.25
Csnk2a1	0.518	6.763	4.707	8.89E-05	3.30E-04	0.348	1.43
Ctla2b	-3.209	7.178	-37.496	1.04E-22	9.37E-20	41.891	-9.24
Ctsb	0.325	9.036	5.599	9.41E-06	4.62E-05	2.604	1.25
CTSH	-0.713	6.886	-11.846	1.78E-11	4.30E-10	16.049	-1.64
Ctsz	-0.284	9.672	-6.204	2.13E-06	1.24E-05	4.108	-1.22
Cxcl1	-1.338	6.634	-15.631	4.99E-14	3.11E-12	22.055	-2.53
Cxx1a	0.303	6.694	4.714	8.73E-05	3.25E-04	0.366	1.23
CYFIP1	-0.348	7.533	-6.346	1.51E-06	9.14E-06	4.457	-1.27
Cyp51	-0.668	9.644	-12.426	6.65E-12	1.94E-10	17.058	-1.59
D15Ertd621e	0.569	7.356	13.500	1.17E-12	4.42E-11	18.837	1.48
D17Wsu104e	-0.417	6.741	-6.211	2.09E-06	1.22E-05	4.126	-1.34
D5Ertd579e	-0.351	7.211	-8.750	6.60E-09	7.70E-08	9.996	-1.28
D830024F11Rik	-0.390	7.236	-6.168	2.33E-06	1.33E-05	4.018	-1.31
DAG1	-0.302	8.711	-4.666	9.86E-05	3.61E-04	0.244	-1.23
DBF4	-0.705	6.260	-9.125	3.03E-09	3.94E-08	10.793	-1.63
Dcaf12l1	0.450	6.572	5.279	2.10E-05	9.35E-05	1.796	1.37
DCAF13	0.269	7.411	6.380	1.39E-06	8.51E-06	4.541	1.20
DCN	-0.328	10.903	-5.906	4.41E-06	2.33E-05	3.372	-1.26
Ddhd2	0.483	7.529	10.749	1.27E-10	2.32E-09	14.037	1.40
DDHD2	1.681	9.554	7.159	2.21E-07	1.74E-06	6.410	3.21
DDIT3	-0.802	6.496	-8.398	1.39E-08	1.50E-07	9.232	-1.74
DDOST	-0.302	8.414	-6.694	6.57E-07	4.35E-06	5.302	-1.23
DDOST	-0.266	6.514	-2.713	1.22E-02	2.51E-02	-4.460	-1.20
DDX21	0.280	7.918	5.366	1.69E-05	7.69E-05	2.016	1.21
DDX41	0.344	6.481	3.928	6.37E-04	1.88E-03	-1.609	1.27
Degs1	-0.345	6.778	-7.800	5.15E-08	4.77E-07	7.896	-1.27
DERL1	0.333	7.056	5.674	7.81E-06	3.91E-05	2.792	1.26
DERL1	0.366	8.644	7.430	1.19E-07	9.91E-07	7.045	1.29
Derl1	0.442	8.334	8.960	4.25E-09	5.30E-08	10.446	1.36
Derl1	0.607	7.959	8.491	1.14E-08	1.26E-07	9.435	1.52
DHRS7	-0.277	7.060	-6.713	6.29E-07	4.21E-06	5.347	-1.21
DLAT	-0.309	7.289	-5.767	6.21E-06	3.19E-05	3.025	-1.24
DLGAP5	-0.751	6.521	-8.131	2.48E-08	2.53E-07	8.641	-1.68
Dmtf1	0.276	6.932	3.325	2.85E-03	7.00E-03	-3.072	1.21
DNAJA3	0.282	7.151	7.270	1.71E-07	1.38E-06	6.672	1.22
DNAJB2	-0.300	6.280	-2.828	9.35E-03	1.99E-02	-4.210	-1.23
Dnajb6	0.299	6.727	3.179	4.06E-03	9.56E-03	-3.414	1.23
DNAJC3	-0.827	6.788	-10.809	1.14E-10	2.11E-09	14.151	-1.77
DNAJC3	-0.720	6.786	-14.033	5.12E-13	2.11E-11	19.680	-1.65
DNPEP	-0.273	7.288	-3.473	1.98E-03	5.12E-03	-2.721	-1.21

Dock9	-0.269	6.569	-4.153	3.62E-04	1.15E-03	-1.051	-1.20
Dram1	-0.871	6.300	-14.358	3.13E-13	1.43E-11	20.181	-1.83
DTYMK	0.328	7.027	6.022	3.32E-06	1.81E-05	3.658	1.26
DUSP1	-0.740	6.221	-11.474	3.42E-11	7.64E-10	15.381	-1.67
DYNC2LI1	0.299	8.006	5.770	6.17E-06	3.17E-05	3.031	1.23
DYNC2LI1	0.334	7.258	2.510	1.93E-02	3.75E-02	-4.888	1.26
E130203B14Rik	-0.290	8.135	-3.561	1.59E-03	4.21E-03	-2.508	-1.22
EBNA1BP2	0.285	7.513	5.539	1.09E-05	5.28E-05	2.453	1.22
EBNA1BP2	0.294	8.826	5.145	2.93E-05	1.26E-04	1.459	1.23
EBP	-1.246	5.933	-20.648	1.02E-16	1.67E-14	28.335	-2.37
ECT2	-0.646	7.232	-6.681	6.78E-07	4.46E-06	5.270	-1.56
EEF1B2	0.268	10.413	8.186	2.21E-08	2.28E-07	8.762	1.20
Eef2	0.401	6.433	7.537	9.32E-08	8.07E-07	7.291	1.32
EFEMP1	-0.335	9.515	-5.509	1.18E-05	5.63E-05	2.378	-1.26
EFEMP2	-0.323	6.616	-3.868	7.41E-04	2.16E-03	-1.758	-1.25
Efr3a	0.340	8.691	6.935	3.72E-07	2.69E-06	5.880	1.27
EIF2A	0.337	7.926	6.780	5.36E-07	3.66E-06	5.510	1.26
EIF2S3	0.350	6.968	5.650	8.31E-06	4.11E-05	2.731	1.27
EIF3C	0.322	9.095	6.974	3.40E-07	2.51E-06	5.973	1.25
EIF3C	0.333	9.693	6.725	6.10E-07	4.11E-06	5.377	1.26
EIF3H	0.353	8.828	7.731	6.02E-08	5.45E-07	7.737	1.28
EIF3H	0.381	8.385	3.294	3.08E-03	7.46E-03	-3.147	1.30
EIF3K	0.459	9.323	8.209	2.10E-08	2.18E-07	8.814	1.37
EIF3M	0.394	9.726	7.087	2.61E-07	2.00E-06	6.241	1.31
Eif4a1	-0.626	8.429	-3.625	1.36E-03	3.70E-03	-2.355	-1.54
Eif4a1	-0.529	9.235	-12.338	7.71E-12	2.21E-10	16.907	-1.44
EIF5	-0.915	6.761	-9.002	3.89E-09	4.93E-08	10.535	-1.89
EIF5	-0.898	8.217	-18.239	1.66E-15	1.53E-13	25.516	-1.86
EIF5A	0.304	6.847	6.288	1.74E-06	1.04E-05	4.315	1.23
EIF5A	0.399	9.394	3.232	3.57E-03	8.52E-03	-3.291	1.32
Elp2	0.342	5.950	3.231	3.58E-03	8.54E-03	-3.294	1.27
EMP1	-0.657	7.772	-13.153	2.02E-12	6.92E-11	18.275	-1.58
ENAH	-0.271	7.328	-7.113	2.46E-07	1.90E-06	6.301	-1.21
Ensa	0.353	6.811	8.970	4.16E-09	5.21E-08	10.467	1.28
EPHX1	-0.301	8.644	-4.051	4.68E-04	1.43E-03	-1.303	-1.23
EPR1	-0.684	7.818	-8.473	1.19E-08	1.30E-07	9.396	-1.61
EPS8	-0.462	7.562	-5.754	6.42E-06	3.28E-05	2.990	-1.38
EPS8	-0.428	8.261	-4.759	7.79E-05	2.94E-04	0.480	-1.34
ERAP1	-0.703	6.611	-14.888	1.44E-13	7.37E-12	20.977	-1.63
ERCC1	0.267	6.529	3.503	1.84E-03	4.80E-03	-2.649	1.20
Ern1	0.514	6.120	8.249	1.92E-08	2.03E-07	8.902	1.43
Erp29	-0.784	7.835	-14.131	4.41E-13	1.87E-11	19.831	-1.72
ERP29	-0.779	6.775	-9.293	2.15E-09	2.91E-08	11.145	-1.72
ERP44	-0.465	7.100	-9.719	9.14E-10	1.35E-08	12.018	-1.38
ERP44	-0.459	6.554	-3.122	4.66E-03	1.08E-02	-3.545	-1.37
ESD	-0.902	8.915	-21.901	2.68E-17	6.02E-15	29.680	-1.87
Esyt2	0.378	7.084	8.030	3.10E-08	3.06E-07	8.414	1.30
EWSR1	0.460	7.376	12.542	5.48E-12	1.64E-10	17.257	1.38
EXOSC9	0.280	6.576	4.453	1.69E-04	5.88E-04	-0.294	1.21
F2r	-0.547	8.207	-6.052	3.08E-06	1.71E-05	3.734	-1.46
Fam100b	0.375	6.914	5.269	2.15E-05	9.55E-05	1.772	1.30
Fam102b	-0.351	7.655	-4.571	1.26E-04	4.48E-04	0.003	-1.28
FAM107B	-0.329	6.765	-6.416	1.28E-06	7.86E-06	4.628	-1.26
Fam108b	-0.383	6.803	-5.224	2.41E-05	1.05E-04	1.658	-1.30
FAM108B1	-0.344	7.357	-6.367	1.44E-06	8.74E-06	4.508	-1.27
Fam125b	0.274	6.716	5.302	1.98E-05	8.90E-05	1.855	1.21
FAM40A	-0.287	6.463	-6.009	3.43E-06	1.86E-05	3.627	-1.22
FAM40B	-0.367	6.679	-6.259	1.86E-06	1.11E-05	4.243	-1.29
FAM49B	0.468	7.847	9.638	1.07E-09	1.57E-08	11.855	1.38
Fam92a	0.268	6.361	4.808	6.88E-05	2.64E-04	0.604	1.20
Fasn	-0.662	6.330	-6.620	7.84E-07	5.05E-06	5.123	-1.58
FAT1	-0.517	8.629	-10.219	3.45E-10	5.64E-09	13.015	-1.43
FBL	-0.688	9.015	-12.775	3.73E-12	1.17E-10	17.650	-1.61
FBXO6	0.302	7.635	3.623	1.37E-03	3.71E-03	-2.358	1.23
FBXO8	1.053	6.542	21.279	5.16E-17	9.75E-15	29.022	2.07
FCGRT	0.516	6.491	7.545	9.15E-08	7.98E-07	7.310	1.43
FDPS	-1.070	8.124	-18.294	1.55E-15	1.50E-13	25.584	-2.10
FERMT2	-0.368	9.646	-5.466	1.31E-05	6.15E-05	2.269	-1.29
FERMT2	-0.331	8.924	-7.129	2.37E-07	1.85E-06	6.340	-1.26
Fez2	-0.311	7.112	-6.959	3.52E-07	2.57E-06	5.938	-1.24
Fhl1	0.293	7.505	6.699	6.49E-07	4.31E-06	5.314	1.22
Fkbp9	-0.346	7.631	-6.708	6.36E-07	4.25E-06	5.336	-1.27
FMNL2	0.663	7.362	10.041	4.87E-10	7.70E-09	12.663	1.58
FOSL1	-0.266	7.032	-3.016	6.00E-03	1.35E-02	-3.789	-1.20
Foxp2	0.325	7.336	5.145	2.93E-05	1.26E-04	1.459	1.25
Frmd6	-0.311	7.433	-5.712	7.11E-06	3.59E-05	2.887	-1.24
Fth1	-0.350	11.320	-7.242	1.83E-07	1.46E-06	6.606	-1.27
FUBP3	0.348	6.446	6.441	1.20E-06	7.47E-06	4.689	1.27
FUCA1	0.496	7.522	9.854	7.01E-10	1.06E-08	12.290	1.41

G3BP1	0.314	7.821	6.202	2.14E-06	1.24E-05	4.104	1.24
G3BP2	0.480	8.617	8.978	4.10E-09	5.15E-08	10.483	1.40
GABARAP	0.555	9.365	11.980	1.42E-11	3.53E-10	16.285	1.47
Gabarapl1	-0.269	7.833	-4.914	5.26E-05	2.09E-04	0.873	-1.20
GABARAPL2	0.263	8.308	4.789	7.22E-05	2.75E-04	0.556	1.20
GADD45A	-0.304	8.855	-4.551	1.32E-04	4.67E-04	-0.046	-1.23
GADD45B	-0.951	6.746	-13.992	5.45E-13	2.23E-11	19.616	-1.93
GALNT7	0.971	7.466	9.580	1.20E-09	1.75E-08	11.737	1.96
Gapdh	-0.271	9.031	-4.947	4.84E-05	1.94E-04	0.956	-1.21
Gatad2a	-0.369	6.422	-5.261	2.19E-05	9.69E-05	1.751	-1.29
Gatad2a	-0.363	6.502	-6.932	3.75E-07	2.70E-06	5.874	-1.29
GCLM	-0.843	9.225	-16.193	2.31E-14	1.62E-12	22.841	-1.79
GFM1	-0.433	7.517	-11.055	7.25E-11	1.44E-09	14.613	-1.35
GHR	0.292	6.655	4.646	1.04E-04	3.78E-04	0.193	1.22
Gm10159	0.412	10.114	11.707	2.27E-11	5.40E-10	15.801	1.33
Gm10291	-0.289	10.903	-5.920	4.26E-06	2.26E-05	3.405	-1.22
Gm10397	0.310	7.405	5.955	3.91E-06	2.09E-05	3.493	1.24
Gm14148	-0.276	9.820	-5.530	1.12E-05	5.38E-05	2.429	-1.21
Gm2260	0.276	8.341	4.416	1.86E-04	6.40E-04	-0.388	1.21
Gm3811	0.343	7.340	6.362	1.45E-06	8.82E-06	4.496	1.27
Gm5069	-0.320	9.657	-5.777	6.06E-06	3.13E-05	3.049	-1.25
Gm5512	-0.371	6.531	-6.183	2.24E-06	1.30E-05	4.056	-1.29
GMCL1	0.452	6.620	6.858	4.46E-07	3.16E-06	5.696	1.37
Gmfb	0.431	8.592	7.541	9.22E-08	8.02E-07	7.302	1.35
GNAI2	-0.336	6.846	-4.366	2.11E-04	7.16E-04	-0.514	-1.26
GNB2L1	0.289	10.808	8.535	1.04E-08	1.15E-07	9.531	1.22
GNPAT	-0.484	6.582	-8.844	5.41E-09	6.48E-08	10.199	-1.40
GPC6	-1.031	3.584	-12.301	8.19E-12	2.28E-10	16.845	-2.04
Gpd2	-0.315	6.879	-4.135	3.79E-04	1.19E-03	-1.095	-1.24
Gpnmb	1.322	5.966	20.966	7.21E-17	1.23E-14	28.684	2.50
Gpr124	0.628	7.400	13.216	1.83E-12	6.32E-11	18.379	1.55
GPR176	-1.233	7.295	-28.225	7.88E-20	3.14E-17	35.483	-2.35
Gpr56	1.447	6.194	18.419	1.33E-15	1.37E-13	25.739	2.73
Gpsm2	-0.363	6.902	-5.483	1.26E-05	5.93E-05	2.312	-1.29
Gpx1	-0.847	7.884	-12.171	1.02E-11	2.72E-10	16.620	-1.80
Gpx4	-0.366	10.579	-6.994	3.24E-07	2.40E-06	6.021	-1.29
GRINA	-0.300	7.072	-3.369	2.56E-03	6.40E-03	-2.969	-1.23
GSS	-1.023	7.023	-18.801	8.41E-16	8.89E-14	26.203	-2.03
Gss	-0.942	8.655	-20.147	1.78E-16	2.39E-14	27.776	-1.92
GSS	-0.832	6.247	-9.038	3.62E-09	4.63E-08	10.609	-1.78
GSS	-0.678	6.709	-10.268	3.15E-10	5.23E-09	13.110	-1.60
GSTA5	-0.711	9.606	-13.338	1.51E-12	5.42E-11	18.576	-1.64
Gstm6	-0.357	7.960	-4.836	6.40E-05	2.48E-04	0.676	-1.28
Gt(ROSA)26Sor	-0.512	6.526	-8.855	5.29E-09	6.40E-08	10.222	-1.43
Gtf2h5	0.368	6.911	6.142	2.48E-06	1.41E-05	3.955	1.29
GULP1	0.304	6.746	3.512	1.80E-03	4.71E-03	-2.628	1.23
GUSB	-0.748	6.935	-14.878	1.46E-13	7.37E-12	20.963	-1.68
Gusb	-0.604	7.029	-10.253	3.23E-10	5.33E-09	13.082	-1.52
H1f0	0.444	8.525	7.038	2.93E-07	2.19E-06	6.125	1.36
H3F3B	0.417	9.428	9.869	6.80E-10	1.03E-08	12.322	1.34
H3F3C	0.400	10.004	8.080	2.78E-08	2.79E-07	8.527	1.32
HADHB	-0.285	8.394	-5.797	5.76E-06	2.99E-05	3.100	-1.22
HBXIP	0.354	8.226	6.740	5.89E-07	3.98E-06	5.413	1.28
HEATR3	-0.292	6.574	-6.023	3.31E-06	1.81E-05	3.660	-1.22
Herpud1	-0.477	6.811	-10.765	1.23E-10	2.26E-09	14.068	-1.39
HEXA	-0.470	6.609	-6.372	1.42E-06	8.66E-06	4.521	-1.38
HEXIM1	0.602	7.032	8.717	7.07E-09	8.20E-08	9.925	1.52
Hibadh	-0.310	7.744	-5.727	6.85E-06	3.48E-05	2.925	-1.24
Hint1	0.325	9.534	7.525	9.56E-08	8.26E-07	7.265	1.25
HMGCR	-1.060	6.572	-15.810	3.89E-14	2.59E-12	22.308	-2.08
Hmgcs1	-1.111	6.887	-20.138	1.80E-16	2.39E-14	27.764	-2.16
HMOX1	-1.041	7.105	-12.040	1.28E-11	3.25E-10	16.390	-2.06
Hnrnpa3	-0.381	8.503	-4.117	3.96E-04	1.24E-03	-1.140	-1.30
Hnrnpa3	-0.287	9.119	-5.898	4.50E-06	2.37E-05	3.350	-1.22
HNRNPC	0.286	9.033	6.004	3.47E-06	1.88E-05	3.615	1.22
HNRNPH1	0.449	8.470	8.095	2.69E-08	2.72E-07	8.559	1.36
HNRNPM	0.300	8.319	5.291	2.03E-05	9.11E-05	1.828	1.23
HNRPDL	-0.401	6.768	-6.128	2.56E-06	1.45E-05	3.921	-1.32
Hnrpdl	-0.323	6.334	-5.060	3.63E-05	1.51E-04	1.244	-1.25
HPRT1	-0.499	9.127	-10.077	4.54E-10	7.25E-09	12.735	-1.41
Hsd17b12	-0.512	7.796	-10.719	1.34E-10	2.43E-09	13.981	-1.43
Hsp90b1	-1.188	8.375	-6.209	2.10E-06	1.23E-05	4.122	-2.28
Hsp90b1	-1.118	7.905	-5.055	3.69E-05	1.53E-04	1.229	-2.17
HSP90B1	-1.037	10.245	-16.248	2.14E-14	1.54E-12	22.917	-2.05
HSPA5	-1.162	11.437	-24.785	1.58E-18	4.73E-16	32.512	-2.24
HSPA8	0.271	10.561	5.648	8.34E-06	4.12E-05	2.726	1.21
HSPB1	-0.334	9.142	-5.128	3.06E-05	1.30E-04	1.416	-1.26
Hspb8	-0.340	7.970	-6.913	3.92E-07	2.80E-06	5.827	-1.27
Hyou1	-0.825	6.015	-10.106	4.29E-10	6.94E-09	12.792	-1.77

IBTK	-0.478	6.329	-6.822	4.86E-07	3.38E-06	5.609	-1.39
IDH1	-0.321	8.313	-4.266	2.71E-04	8.90E-04	-0.765	-1.25
Idi1	-1.266	6.124	-21.204	5.58E-17	1.00E-14	28.942	-2.40
Ifngr2	-0.333	6.377	-7.291	1.63E-07	1.32E-06	6.721	-1.26
IL1R1	0.700	6.416	10.063	4.66E-10	7.41E-09	12.708	1.63
Ildr2	0.559	6.552	10.907	9.50E-11	1.83E-09	14.336	1.47
Impad1	0.395	6.929	4.756	7.84E-05	2.95E-04	0.472	1.31
Insig1	-0.582	8.024	-11.905	1.61E-11	3.91E-10	16.154	-1.50
INSIG2	0.357	6.483	7.270	1.71E-07	1.38E-06	6.671	1.28
IPO7	0.301	8.117	5.006	4.17E-05	1.71E-04	1.105	1.23
IPO7	0.523	8.188	10.827	1.10E-10	2.06E-09	14.184	1.44
Irak2	-0.337	6.582	-4.727	8.44E-05	3.16E-04	0.400	-1.26
Isca1	-0.281	8.849	-6.674	6.89E-07	4.53E-06	5.253	-1.22
ITGB5	0.365	7.557	4.090	4.24E-04	1.31E-03	-1.207	1.29
ITIH5	-0.533	6.180	-4.799	7.03E-05	2.69E-04	0.582	-1.45
Jak2	-0.415	6.679	-5.795	5.80E-06	3.01E-05	3.094	-1.33
Jub	-0.633	7.280	-16.710	1.16E-14	9.02E-13	23.545	-1.55
JUN	-0.282	7.869	-3.970	5.74E-04	1.72E-03	-1.506	-1.22
KARS	0.277	8.766	3.715	1.09E-03	3.05E-03	-2.135	1.21
Kdelr3	-0.448	6.846	-6.714	6.26E-07	4.20E-06	5.352	-1.36
Kdm1a	-0.275	7.988	-6.670	6.95E-07	4.56E-06	5.244	-1.21
KIAA0196	0.510	7.763	8.326	1.63E-08	1.74E-07	9.074	1.42
KIAA0494	0.367	6.477	9.361	1.87E-09	2.58E-08	11.285	1.29
KIF23	-0.529	6.746	-8.180	2.23E-08	2.30E-07	8.751	-1.44
KIF5B	-0.264	8.303	-4.109	4.04E-04	1.26E-03	-1.159	-1.20
KIFAP3	0.287	6.899	4.456	1.68E-04	5.85E-04	-0.287	1.22
KIFC1	-0.347	6.589	-5.444	1.39E-05	6.46E-05	2.214	-1.27
KLC1	-0.356	7.277	-6.457	1.16E-06	7.28E-06	4.729	-1.28
KLF6	0.328	7.705	5.622	8.89E-06	4.38E-05	2.661	1.26
KLF6	0.340	8.141	6.849	4.55E-07	3.21E-06	5.675	1.27
KLF6	0.380	6.300	4.905	5.38E-05	2.13E-04	0.849	1.30
KPNA1	-0.304	6.808	-4.581	1.22E-04	4.40E-04	0.029	-1.23
KPNA2	-0.663	7.512	-10.619	1.62E-10	2.87E-09	13.789	-1.58
Krcc1	-0.895	6.207	-14.647	2.04E-13	9.65E-12	20.618	-1.86
LAMC1	-0.481	7.195	-9.842	7.18E-10	1.08E-08	12.266	-1.40
LAMP2	0.431	6.487	6.669	6.97E-07	4.56E-06	5.242	1.35
LAP3	-0.277	7.918	-6.998	3.21E-07	2.38E-06	6.031	-1.21
LAPTM4A	-0.380	7.522	-5.058	3.65E-05	1.52E-04	1.238	-1.30
LAYN	0.327	6.973	4.516	1.44E-04	5.07E-04	-0.137	1.25
Ldlr	-0.767	6.729	-12.250	8.93E-12	2.45E-10	16.756	-1.70
LEMD3	0.422	7.077	4.445	1.73E-04	6.00E-04	-0.314	1.34
Leprotl1	0.326	7.192	8.037	3.05E-08	3.03E-07	8.430	1.25
LETM1	-0.476	6.495	-9.555	1.27E-09	1.84E-08	11.684	-1.39
Lgals8	-0.384	7.619	-5.921	4.25E-06	2.26E-05	3.408	-1.30
LOC100043810	0.283	7.540	3.410	2.32E-03	5.86E-03	-2.871	1.22
LOC100129096	0.281	9.294	4.022	5.04E-04	1.53E-03	-1.377	1.21
LOC100130003	0.288	8.698	5.291	2.04E-05	9.11E-05	1.827	1.22
LOC100130746	0.359	7.013	7.449	1.14E-07	9.58E-07	7.088	1.28
LOC100131826	-0.459	7.320	-5.400	1.55E-05	7.12E-05	2.102	-1.37
LOC100287803	0.325	10.185	5.448	1.37E-05	6.41E-05	2.224	1.25
LOC100288550	0.325	11.286	3.739	1.02E-03	2.90E-03	-2.074	1.25
LOC144017	-0.291	9.492	-3.375	2.52E-03	6.32E-03	-2.955	-1.22
LOC401387	-0.629	9.012	-11.082	6.91E-11	1.38E-09	14.663	-1.55
LOC624853	0.365	9.491	2.748	1.12E-02	2.35E-02	-4.383	1.29
LOC685953	3.176	9.130	30.430	1.37E-20	6.17E-18	37.195	9.04
LOC728066	-0.321	7.550	-7.481	1.06E-07	9.05E-07	7.162	-1.25
LOC732360	-0.324	6.927	-6.521	9.92E-07	6.27E-06	4.884	-1.25
LPL	0.296	10.528	5.434	1.42E-05	6.59E-05	2.187	1.23
LPP	0.319	6.385	3.605	1.43E-03	3.84E-03	-2.403	1.25
LPP	0.394	7.853	7.080	2.65E-07	2.02E-06	6.225	1.31
Lrig1	-0.449	8.856	-4.608	1.14E-04	4.14E-04	0.096	-1.37
Lrp10	-0.767	7.803	-10.737	1.30E-10	2.36E-09	14.014	-1.70
LRRFIP1	0.758	6.403	8.923	4.59E-09	5.67E-08	10.367	1.69
Ly6e	-0.410	7.105	-5.498	1.21E-05	5.75E-05	2.350	-1.33
MAFG	-0.339	6.127	-3.559	1.60E-03	4.23E-03	-2.512	-1.26
MAFG	-0.330	6.400	-3.345	2.72E-03	6.72E-03	-3.026	-1.26
MAFG	-0.326	5.986	-2.530	1.84E-02	3.62E-02	-4.847	-1.25
MAMDC2	-0.960	7.429	-8.312	1.68E-08	1.78E-07	9.043	-1.95
Man2a1	-0.355	6.504	-4.908	5.34E-05	2.12E-04	0.857	-1.28
MAN2A1	-0.307	6.683	-4.171	3.45E-04	1.10E-03	-1.004	-1.24
MAN2C1	-0.497	6.482	-6.700	6.47E-07	4.30E-06	5.318	-1.41
MANF	-1.203	8.744	-21.808	2.95E-17	6.13E-15	29.583	-2.30
Map1lc3b	0.430	8.856	5.960	3.86E-06	2.07E-05	3.504	1.35
Map1lc3b	0.585	8.566	17.852	2.67E-15	2.34E-13	25.031	1.50
MAPK14	0.290	7.353	8.161	2.33E-08	2.39E-07	8.708	1.22
MAPK6	-0.333	7.276	-4.604	1.15E-04	4.17E-04	0.087	-1.26
MARK1	-0.444	6.616	-9.491	1.44E-09	2.06E-08	11.554	-1.36
Mcm2	0.530	6.936	9.132	2.98E-09	3.90E-08	10.808	1.44
MCM6	0.487	7.043	7.332	1.48E-07	1.22E-06	6.817	1.40

MCM7	0.427	7.318	6.025	3.29E-06	1.81E-05	3.667	1.34
ME1	-0.438	7.376	-9.384	1.79E-09	2.48E-08	11.333	-1.35
Me1	-0.374	7.716	-5.692	7.48E-06	3.77E-05	2.836	-1.30
Med29	-0.617	7.001	-10.354	2.67E-10	4.57E-09	13.278	-1.53
Mesdc2	-0.288	9.074	-8.906	4.76E-09	5.86E-08	10.329	-1.22
Met	-0.381	9.659	-6.453	1.17E-06	7.32E-06	4.718	-1.30
METTL9	-0.447	6.724	-7.386	1.31E-07	1.09E-06	6.943	-1.36
MGC112830	0.585	6.352	9.977	5.51E-10	8.57E-09	12.537	1.50
Mgst1	-0.464	11.656	-11.314	4.54E-11	9.72E-10	15.091	-1.38
MGST1	-0.422	11.519	-7.124	2.40E-07	1.86E-06	6.329	-1.34
Mia3	-0.361	8.330	-6.614	7.95E-07	5.12E-06	5.108	-1.28
MMD	-0.329	6.497	-4.050	4.70E-04	1.43E-03	-1.308	-1.26
MMP14	-0.391	6.141	-3.826	8.23E-04	2.38E-03	-1.861	-1.31
MMP9	-0.708	6.731	-11.218	5.40E-11	1.13E-09	14.914	-1.63
MOBKL3	0.331	6.352	7.076	2.68E-07	2.03E-06	6.215	1.26
MPHOSPH8	-0.271	7.252	-3.905	6.75E-04	1.99E-03	-1.666	-1.21
MRPL13	0.339	9.065	9.038	3.62E-09	4.63E-08	10.610	1.26
Mrpl18	-0.317	7.610	-7.143	2.30E-07	1.80E-06	6.372	-1.25
MRPL20	0.391	8.473	8.034	3.07E-08	3.03E-07	8.424	1.31
MRPL30	0.376	8.776	6.019	3.34E-06	1.82E-05	3.651	1.30
MRPL37	0.266	7.536	3.642	1.31E-03	3.57E-03	-2.313	1.20
MRPL37	0.333	6.243	2.390	2.51E-02	4.72E-02	-5.132	1.26
MRPL39	-0.369	6.894	-6.961	3.50E-07	2.57E-06	5.943	-1.29
Mrpl50	-0.269	7.408	-3.715	1.09E-03	3.05E-03	-2.134	-1.21
MRPS18A	0.415	9.934	6.599	8.23E-07	5.29E-06	5.073	1.33
MRPS18B	0.498	7.064	6.291	1.72E-06	1.04E-05	4.322	1.41
Msrb2	0.283	6.378	4.263	2.74E-04	8.97E-04	-0.774	1.22
MTCH2	-0.268	8.629	-4.788	7.24E-05	2.76E-04	0.553	-1.20
MTERFD1	-0.293	6.708	-4.869	5.89E-05	2.31E-04	0.759	-1.23
Mthfd2l	-0.856	6.000	-13.416	1.33E-12	4.93E-11	18.703	-1.81
MYO1C	-0.528	7.651	-7.802	5.14E-08	4.77E-07	7.899	-1.44
MYO1C	-0.320	7.577	-6.743	5.85E-07	3.96E-06	5.420	-1.25
NAA20	0.479	8.064	9.055	3.49E-09	4.50E-08	10.647	1.39
NACA	0.280	10.388	5.663	8.05E-06	4.00E-05	2.763	1.21
NAGA	-0.264	6.853	-4.574	1.24E-04	4.46E-04	0.011	-1.20
NANS	-0.492	6.959	-10.104	4.31E-10	6.94E-09	12.789	-1.41
Nap1l1	0.380	6.621	6.843	4.62E-07	3.25E-06	5.660	1.30
NAP1L1	0.402	6.670	8.867	5.16E-09	6.26E-08	10.247	1.32
Nap1l4	0.284	6.668	4.565	1.27E-04	4.53E-04	-0.011	1.22
Nars	0.266	7.775	5.332	1.83E-05	8.31E-05	1.932	1.20
NARS	0.291	6.984	2.547	1.77E-02	3.49E-02	-4.811	1.22
Ncam1	1.993	5.358	22.157	2.06E-17	4.93E-15	29.946	3.98
Ncapd3	-1.286	5.993	-18.243	1.65E-15	1.53E-13	25.521	-2.44
Ncaph	-0.350	6.789	-5.292	2.03E-05	9.10E-05	1.830	-1.27
NCEH1	-0.555	6.694	-10.963	8.59E-11	1.67E-09	14.440	-1.47
NDC80	-0.371	6.736	-4.521	1.43E-04	5.02E-04	-0.124	-1.29
Ndufb9	0.516	10.546	14.715	1.85E-13	9.10E-12	20.720	1.43
NDUFV1	-0.279	7.817	-5.306	1.96E-05	8.85E-05	1.864	-1.21
Neat1	-0.607	8.207	-9.260	2.30E-09	3.10E-08	11.076	-1.52
Nek2	-0.711	8.036	-10.807	1.14E-10	2.11E-09	14.147	-1.64
Nek6	0.338	7.298	5.594	9.54E-06	4.68E-05	2.591	1.26
Neu1	-0.661	6.374	-12.086	1.18E-11	3.03E-10	16.471	-1.58
Neurl3	-0.545	6.664	-7.838	4.74E-08	4.43E-07	7.980	-1.46
NEURL3	-0.364	6.003	-2.670	1.34E-02	2.75E-02	-4.552	-1.29
NFE2L2	-0.273	9.200	-5.004	4.19E-05	1.72E-04	1.100	-1.21
Nfib	0.297	6.638	3.556	1.62E-03	4.27E-03	-2.521	1.23
Nfic	-0.359	6.666	-5.063	3.61E-05	1.51E-04	1.251	-1.28
NFKB2	-0.330	6.057	-3.033	5.77E-03	1.31E-02	-3.751	-1.26
NFKBIA	-1.096	8.519	-15.579	5.36E-14	3.24E-12	21.981	-2.14
NFKBIA	-1.054	8.243	-17.030	7.60E-15	6.20E-13	23.970	-2.08
NGDN	0.576	7.269	11.624	2.63E-11	6.17E-10	15.652	1.49
Nhlrc3	-0.264	6.385	-5.951	3.95E-06	2.11E-05	3.483	-1.20
NHP2L1	-0.351	8.019	-5.962	3.85E-06	2.07E-05	3.509	-1.28
NIT1	-0.386	6.656	-4.876	5.80E-05	2.28E-04	0.776	-1.31
NME1-NME2	0.269	9.016	6.038	3.19E-06	1.76E-05	3.699	1.20
NOL7	0.289	8.228	4.776	7.46E-05	2.83E-04	0.522	1.22
NONO	0.396	8.710	7.134	2.34E-07	1.83E-06	6.351	1.32
NOP58	0.331	8.700	4.081	4.34E-04	1.34E-03	-1.230	1.26
Nox1	0.338	7.107	4.281	2.62E-04	8.62E-04	-0.728	1.26
NPC2	-0.454	9.515	-9.990	5.38E-10	8.45E-09	12.562	-1.37
NPM1	0.319	7.805	4.360	2.14E-04	7.25E-04	-0.529	1.25
Nr2c1	0.579	6.434	11.389	3.98E-11	8.66E-10	15.228	1.49
NSA2	0.318	9.381	8.068	2.86E-08	2.86E-07	8.499	1.25
NSMCE2	0.819	8.368	12.334	7.76E-12	2.21E-10	16.900	1.76
NUCB2	-0.527	7.514	-9.972	5.56E-10	8.61E-09	12.527	-1.44
Nudt3	0.456	6.423	5.395	1.57E-05	7.18E-05	2.090	1.37
NUP98	-1.079	7.281	-16.655	1.24E-14	9.30E-13	23.470	-2.11
Nusap1	-0.692	6.996	-8.664	7.90E-09	8.98E-08	9.812	-1.62
NVL	0.292	6.210	5.442	1.39E-05	6.49E-05	2.208	1.22

OAZ1	-0.267	7.862	-3.520	1.77E-03	4.63E-03	-2.608	-1.20
OGT	0.411	7.673	7.103	2.52E-07	1.93E-06	6.279	1.33
OSBPL3	-0.471	6.826	-10.590	1.71E-10	3.00E-09	13.734	-1.39
Osmr	-0.391	7.659	-6.110	2.68E-06	1.51E-05	3.875	-1.31
OSTC	-0.461	8.595	-6.836	4.70E-07	3.29E-06	5.643	-1.38
OSTC	-0.460	9.604	-9.988	5.39E-10	8.45E-09	12.559	-1.38
OXR1	-0.506	6.713	-7.246	1.81E-07	1.45E-06	6.616	-1.42
OXSR1	-0.284	8.386	-4.990	4.34E-05	1.77E-04	1.065	-1.22
P4hb	-0.291	7.388	-4.787	7.26E-05	2.76E-04	0.550	-1.22
Pabpc4	0.475	7.915	6.428	1.24E-06	7.67E-06	4.657	1.39
PALLD	-1.502	7.134	-30.647	1.16E-20	5.97E-18	37.357	-2.83
PAPSS1	-0.470	6.702	-7.206	1.98E-07	1.57E-06	6.522	-1.39
Parp3	-0.535	6.322	-8.836	5.51E-09	6.55E-08	10.181	-1.45
PCGF5	-0.335	6.328	-6.821	4.86E-07	3.38E-06	5.608	-1.26
PCNA	0.512	8.681	11.356	4.22E-11	9.14E-10	15.167	1.43
Pcolce	-0.586	8.526	-7.768	5.54E-08	5.05E-07	7.822	-1.50
PDIA3	-0.892	9.730	-14.344	3.20E-13	1.44E-11	20.158	-1.86
PDIA4	-1.411	6.027	-7.080	2.65E-07	2.02E-06	6.225	-2.66
PDIA4	-1.236	7.671	-26.312	3.99E-19	1.43E-16	33.879	-2.36
PDIA5	-0.530	7.082	-8.215	2.07E-08	2.16E-07	8.827	-1.44
PDIA6	-0.723	10.091	-17.853	2.67E-15	2.34E-13	25.033	-1.65
PDLIM1	-0.524	7.184	-11.138	6.24E-11	1.27E-09	14.767	-1.44
Pdrg1	0.429	7.434	7.731	6.01E-08	5.45E-07	7.738	1.35
Pebp1	0.388	9.686	10.311	2.90E-10	4.87E-09	13.194	1.31
PECI	-0.359	6.867	-6.894	4.10E-07	2.91E-06	5.782	-1.28
Pex19	0.425	6.163	8.759	6.47E-09	7.57E-08	10.016	1.34
Pgs1	-0.495	6.290	-7.326	1.51E-07	1.24E-06	6.801	-1.41
PHF5A	0.303	7.050	5.745	6.57E-06	3.35E-05	2.968	1.23
PHOSPHO2	-0.428	7.478	-11.244	5.16E-11	1.08E-09	14.962	-1.35
Pir	-0.906	6.603	-13.298	1.60E-12	5.65E-11	18.512	-1.87
PITPNA	-0.308	7.555	-6.641	7.44E-07	4.83E-06	5.176	-1.24
PLAA	-0.587	6.833	-11.992	1.39E-11	3.51E-10	16.307	-1.50
PLAC8	4.114	5.438	41.191	1.15E-23	1.37E-20	43.963	17.32
Plbd2	0.303	7.151	4.442	1.74E-04	6.03E-04	-0.321	1.23
PLIN2	0.436	9.911	3.950	6.04E-04	1.79E-03	-1.556	1.35
PLIN2	0.660	9.393	15.896	3.46E-14	2.34E-12	22.429	1.58
PLK2	-0.665	7.605	-13.062	2.34E-12	7.86E-11	18.126	-1.59
PLTP	1.178	7.226	8.980	4.08E-09	5.15E-08	10.487	2.26
POLR2G	0.306	8.219	7.536	9.33E-08	8.07E-07	7.291	1.24
PPAP2A	-1.141	6.987	-15.121	1.03E-13	5.50E-12	21.320	-2.21
Ppap2c	-0.265	6.713	-4.331	2.30E-04	7.72E-04	-0.602	-1.20
Ppcs	-0.278	7.062	-4.937	4.96E-05	1.98E-04	0.932	-1.21
PPP3R1	0.284	7.691	3.827	8.22E-04	2.37E-03	-1.859	1.22
Prc1	-0.668	7.462	-6.962	3.50E-07	2.57E-06	5.944	-1.59
Prdx1	-0.264	11.449	-5.309	1.94E-05	8.80E-05	1.872	-1.20
PRKACB	0.412	8.518	7.793	5.23E-08	4.82E-07	7.880	1.33
PRKCDBP	-0.794	6.389	-7.379	1.33E-07	1.10E-06	6.927	-1.73
PRKCSH	-0.369	6.754	-3.318	2.90E-03	7.10E-03	-3.089	-1.29
Prkrir	0.399	7.275	9.177	2.72E-09	3.59E-08	10.902	1.32
Prmt5	-0.702	7.197	-9.447	1.57E-09	2.22E-08	11.464	-1.63
Procr	-0.411	6.220	-6.162	2.36E-06	1.35E-05	4.006	-1.33
Pros1	-0.632	7.149	-10.997	8.07E-11	1.57E-09	14.504	-1.55
PRPF38B	-0.357	7.449	-5.906	4.41E-06	2.33E-05	3.371	-1.28
PRPF8	-0.612	8.561	-10.241	3.31E-10	5.43E-09	13.058	-1.53
PRUNE2	-0.299	7.588	-5.398	1.55E-05	7.14E-05	2.099	-1.23
Psap	0.434	8.656	8.051	2.96E-08	2.95E-07	8.462	1.35
PSMB5	-0.506	9.145	-5.439	1.40E-05	6.52E-05	2.202	-1.42
PSMC4	-0.317	7.500	-4.517	1.44E-04	5.05E-04	-0.132	-1.25
PSMC5	0.315	7.329	3.567	1.57E-03	4.17E-03	-2.495	1.24
PSMC5	0.327	7.802	8.201	2.13E-08	2.22E-07	8.796	1.25
PSME4	-0.326	7.404	-5.472	1.29E-05	6.07E-05	2.284	-1.25
Ptdss1	-0.349	6.929	-6.258	1.87E-06	1.11E-05	4.242	-1.27
PTGR1	-0.386	7.564	-3.375	2.52E-03	6.32E-03	-2.954	-1.31
PTGR1	-0.380	8.581	-7.780	5.39E-08	4.95E-07	7.851	-1.30
Ptplad1	-0.464	7.781	-11.923	1.56E-11	3.86E-10	16.186	-1.38
Ptpmt1	-0.377	6.973	-7.772	5.49E-08	5.02E-07	7.831	-1.30
PTPRG	-2.282	5.545	-45.330	1.20E-24	2.16E-21	46.037	-4.86
PTPRM	-0.599	7.071	-14.383	3.02E-13	1.39E-11	20.219	-1.52
PTTG1	-1.023	6.979	-12.278	8.52E-12	2.35E-10	16.805	-2.03
PURB	0.873	7.313	15.267	8.33E-14	4.53E-12	21.533	1.83
QKI	-0.680	5.943	-4.981	4.44E-05	1.80E-04	1.042	-1.60
QKI	-0.571	7.862	-7.517	9.74E-08	8.39E-07	7.247	-1.49
RAB11A	-0.491	5.948	-4.699	9.07E-05	3.36E-04	0.328	-1.41
RAB22A	0.371	7.574	3.324	2.85E-03	7.01E-03	-3.074	1.29
RAB24	-0.465	6.607	-8.850	5.35E-09	6.43E-08	10.210	-1.38
Rab31	0.357	6.713	4.416	1.86E-04	6.40E-04	-0.388	1.28
Rab32	-0.632	6.424	-8.126	2.51E-08	2.55E-07	8.629	-1.55
RANBP9	-0.348	7.016	-7.648	7.25E-08	6.50E-07	7.548	-1.27
RANGRF	-0.322	7.413	-5.063	3.61E-05	1.51E-04	1.251	-1.25

RARRES2	-1.376	9.322	-19.375	4.29E-16	4.67E-14	26.886	-2.60
Rbck1	0.664	6.528	7.935	3.82E-08	3.63E-07	8.202	1.58
RBM3	-0.348	7.415	-3.459	2.05E-03	5.28E-03	-2.755	-1.27
RBPJ	0.381	7.585	6.832	4.75E-07	3.32E-06	5.633	1.30
Rbpj	0.463	7.011	6.434	1.22E-06	7.56E-06	4.673	1.38
Rbpj	0.560	7.628	8.004	3.28E-08	3.21E-07	8.356	1.47
Rcan1	-0.466	6.276	-7.863	4.48E-08	4.24E-07	8.039	-1.38
RCBTB2	-0.702	7.042	-11.920	1.57E-11	3.86E-10	16.180	-1.63
RCL1	-0.462	7.522	-12.171	1.02E-11	2.72E-10	16.619	-1.38
Rere	0.469	6.955	5.529	1.12E-05	5.39E-05	2.428	1.38
RETSAT	0.501	5.458	5.343	1.78E-05	8.12E-05	1.959	1.42
REV3L	-0.418	7.281	-6.369	1.43E-06	8.71E-06	4.513	-1.34
Rfx5	-0.297	6.821	-5.293	2.02E-05	9.08E-05	1.833	-1.23
RFX7	0.331	7.777	4.586	1.21E-04	4.35E-04	0.041	1.26
RGD1561797	-0.290	7.190	-3.728	1.05E-03	2.97E-03	-2.103	-1.22
RGD1565775	0.268	6.526	5.698	7.37E-06	3.72E-05	2.851	1.20
RGS17	1.049	7.346	10.807	1.14E-10	2.11E-09	14.147	2.07
RHOQ	-0.413	7.966	-6.842	4.63E-07	3.25E-06	5.658	-1.33
RIOK3	0.533	7.168	7.482	1.06E-07	9.05E-07	7.164	1.45
RNASEH2C	-1.005	6.445	-16.058	2.77E-14	1.91E-12	22.655	-2.01
RNASET2	0.605	10.189	14.034	5.11E-13	2.11E-11	19.681	1.52
Rnf114	0.268	6.909	4.288	2.57E-04	8.49E-04	-0.710	1.20
RNF139	0.288	7.550	6.453	1.17E-06	7.32E-06	4.717	1.22
RNFT1	-0.632	6.370	-11.123	6.41E-11	1.29E-09	14.739	-1.55
ROMO1	0.380	9.948	7.605	7.98E-08	7.06E-07	7.449	1.30
RPL10	-0.286	6.263	-3.660	1.25E-03	3.44E-03	-2.268	-1.22
Rpl14	0.307	11.088	6.748	5.78E-07	3.93E-06	5.432	1.24
Rpl14	0.361	11.184	7.458	1.11E-07	9.45E-07	7.111	1.28
RPL17	0.321	11.062	6.743	5.86E-07	3.96E-06	5.419	1.25
RPL31	0.313	7.816	3.450	2.10E-03	5.37E-03	-2.776	1.24
RPL34	0.276	9.992	5.401	1.54E-05	7.11E-05	2.105	1.21
RPL38	0.407	9.372	7.089	2.60E-07	1.99E-06	6.246	1.33
Rpn1	-0.581	9.350	-12.930	2.90E-12	9.47E-11	17.907	-1.50
RPN1	-0.528	7.818	-3.608	1.42E-03	3.82E-03	-2.394	-1.44
RPN2	-0.377	10.428	-8.665	7.88E-09	8.98E-08	9.814	-1.30
RPN2	-0.365	9.821	-4.164	3.52E-04	1.12E-03	-1.022	-1.29
RPP14	-0.568	6.393	-8.418	1.34E-08	1.45E-07	9.275	-1.48
RPS19	0.292	11.192	6.780	5.36E-07	3.66E-06	5.510	1.22
RPS5	0.401	11.111	8.875	5.08E-09	6.18E-08	10.264	1.32
RPS6	0.293	9.161	3.892	6.99E-04	2.05E-03	-1.699	1.22
Rrp1	0.349	6.040	3.541	1.68E-03	4.42E-03	-2.558	1.27
RSL1D1	0.314	7.841	2.588	1.62E-02	3.23E-02	-4.726	1.24
Rsl24d1	0.344	7.715	5.994	3.55E-06	1.92E-05	3.590	1.27
RTN3	-0.393	8.012	-7.630	7.54E-08	6.73E-07	7.507	-1.31
RYK	0.420	6.736	10.187	3.67E-10	5.97E-09	12.952	1.34
S100A11	-0.278	7.784	-4.132	3.81E-04	1.20E-03	-1.101	-1.21
SAT1	-0.513	6.704	-10.591	1.71E-10	3.00E-09	13.736	-1.43
SBSN	-0.553	9.801	-9.344	1.94E-09	2.65E-08	11.250	-1.47
SBSN	-0.491	6.788	-5.497	1.21E-05	5.76E-05	2.348	-1.41
SC4MOL	-0.999	8.194	-19.803	2.62E-16	3.06E-14	27.383	-2.00
SC4MOL	-0.900	6.368	-4.229	2.98E-04	9.68E-04	-0.858	-1.87
Sc5d	-0.492	6.364	-5.214	2.47E-05	1.07E-04	1.633	-1.41
Scarb1	-0.620	6.897	-8.954	4.31E-09	5.36E-08	10.432	-1.54
SCCPDH	-0.279	7.895	-5.868	4.84E-06	2.55E-05	3.276	-1.21
SCD	-0.636	9.542	-15.010	1.20E-13	6.27E-12	21.157	-1.55
SCD	-0.485	7.257	-4.564	1.28E-04	4.54E-04	-0.014	-1.40
Scd1	-0.631	9.240	-11.988	1.40E-11	3.51E-10	16.300	-1.55
Scyl2	-0.266	6.432	-6.384	1.38E-06	8.45E-06	4.550	-1.20
Scyl3	-1.095	5.993	-17.548	3.91E-15	3.35E-13	24.645	-2.14
Sdc1	-0.633	5.513	-10.340	2.74E-10	4.66E-09	13.252	-1.55
Sdc4	0.272	7.039	2.926	7.42E-03	1.63E-02	-3.991	1.21
Sec13	-0.392	7.937	-11.561	2.93E-11	6.80E-10	15.540	-1.31
Sec13	-0.343	6.597	-2.557	1.73E-02	3.43E-02	-4.790	-1.27
SEC23B	-0.688	6.844	-9.242	2.38E-09	3.18E-08	11.038	-1.61
SEC24D	-0.717	7.004	-9.932	6.02E-10	9.28E-09	12.446	-1.64
SEC62	-0.320	7.883	-7.941	3.78E-08	3.61E-07	8.213	-1.25
SEL1L	-0.566	7.016	-5.989	3.60E-06	1.94E-05	3.576	-1.48
SELK	0.376	7.749	7.850	4.61E-08	4.34E-07	8.009	1.30
SELS	-0.566	6.598	-12.151	1.06E-11	2.79E-10	16.585	-1.48
SERPINB1	-0.766	8.041	-10.654	1.52E-10	2.71E-09	13.857	-1.70
SERPINB6	-0.301	8.276	-5.666	7.97E-06	3.98E-05	2.772	-1.23
SERPINH1	-0.787	9.252	-24.211	2.71E-18	7.49E-16	31.975	-1.73
SERPINH1	-0.736	8.798	-8.019	3.17E-08	3.12E-07	8.391	-1.67
Serpinh1	-0.721	8.949	-11.908	1.60E-11	3.91E-10	16.159	-1.65
SET	0.268	8.500	7.080	2.66E-07	2.02E-06	6.224	1.20
SF3A3	0.323	8.084	6.940	3.68E-07	2.66E-06	5.893	1.25
SF3B2	0.314	7.337	3.883	7.14E-04	2.09E-03	-1.721	1.24
SFRS2	-0.394	8.647	-11.465	3.48E-11	7.67E-10	15.365	-1.31
SFRS2	-0.314	6.996	-4.347	2.21E-04	7.46E-04	-0.562	-1.24

SFRS7	-0.315	8.831	-3.207	3.80E-03	9.00E-03	-3.349	-1.24
SGCE	-1.037	6.005	-8.045	3.00E-08	2.99E-07	8.448	-2.05
Sh2b3	0.289	6.519	5.261	2.19E-05	9.69E-05	1.751	1.22
SHMT2	-0.353	6.208	-3.453	2.08E-03	5.34E-03	-2.768	-1.28
SLBP	0.725	6.124	12.449	6.39E-12	1.90E-10	17.099	1.65
Slc12a7	-0.273	6.801	-4.949	4.82E-05	1.93E-04	0.961	-1.21
Slc1a5	-0.409	6.945	-8.148	2.40E-08	2.46E-07	8.678	-1.33
SLC25A1	-0.417	6.910	-4.561	1.29E-04	4.57E-04	-0.022	-1.34
SLC25A12	0.305	7.062	6.805	5.05E-07	3.50E-06	5.570	1.24
SLC25A13	-0.478	6.821	-9.986	5.42E-10	8.47E-09	12.554	-1.39
SLC25A17	0.549	6.764	12.315	8.00E-12	2.26E-10	16.869	1.46
SLC25A28	-0.287	6.288	-6.091	2.81E-06	1.57E-05	3.829	-1.22
Slc25a39	0.325	8.449	3.998	5.35E-04	1.61E-03	-1.437	1.25
SLC35B1	-0.617	7.240	-12.561	5.30E-12	1.60E-10	17.289	-1.53
SLC39A1	-0.353	8.219	-7.041	2.91E-07	2.18E-06	6.132	-1.28
SLC44A1	0.364	7.158	5.514	1.16E-05	5.57E-05	2.390	1.29
Slc4a7	-0.364	6.815	-5.216	2.45E-05	1.07E-04	1.639	-1.29
Slc7a11	-0.539	7.310	-6.407	1.30E-06	8.02E-06	4.607	-1.45
SMC1A	0.272	6.532	5.649	8.32E-06	4.11E-05	2.729	1.21
SMC4	-0.538	6.591	-4.870	5.87E-05	2.30E-04	0.762	-1.45
Smpd1	-0.674	6.110	-7.155	2.23E-07	1.75E-06	6.401	-1.59
SMPD1	-0.664	6.885	-11.036	7.51E-11	1.48E-09	14.577	-1.58
SMS	0.284	7.609	6.546	9.34E-07	5.96E-06	4.945	1.22
SNAP23	0.384	7.105	8.657	8.03E-09	9.10E-08	9.796	1.31
Snn	-1.202	6.495	-15.553	5.56E-14	3.25E-12	21.944	-2.30
Snora28	-0.663	7.421	-12.809	3.53E-12	1.12E-10	17.707	-1.58
SNRPC	0.351	7.891	7.006	3.15E-07	2.35E-06	6.049	1.28
SNRPD3	0.325	8.958	6.983	3.33E-07	2.46E-06	5.995	1.25
SNX12	0.369	6.594	6.893	4.10E-07	2.91E-06	5.781	1.29
SNX2	-0.281	6.773	-6.929	3.77E-07	2.71E-06	5.866	-1.22
SNX21	0.325	6.347	3.882	7.16E-04	2.09E-03	-1.724	1.25
SNX9	0.275	7.033	6.320	1.61E-06	9.68E-06	4.394	1.21
SOD2	-0.499	6.599	-7.935	3.82E-08	3.63E-07	8.202	-1.41
SOD2	-0.330	6.729	-4.867	5.93E-05	2.32E-04	0.753	-1.26
SPATA2	0.583	6.823	11.555	2.97E-11	6.83E-10	15.528	1.50
Spats2	-0.358	6.500	-6.045	3.14E-06	1.73E-05	3.716	-1.28
Spc25	-0.515	6.631	-6.635	7.55E-07	4.89E-06	5.161	-1.43
SPRR1A	0.962	8.643	11.158	6.02E-11	1.24E-09	14.803	1.95
Sptlc1	-0.349	6.938	-3.628	1.35E-03	3.67E-03	-2.345	-1.27
SQLE	-0.621	7.073	-9.322	2.02E-09	2.75E-08	11.205	-1.54
SQLE	-0.435	6.333	-2.828	9.34E-03	1.99E-02	-4.209	-1.35
Srgn	0.542	7.415	6.284	1.75E-06	1.05E-05	4.305	1.46
Srpr	-0.426	8.041	-9.689	9.69E-10	1.43E-08	11.958	-1.34
SRPR	-0.266	6.329	-2.892	8.04E-03	1.76E-02	-4.067	-1.20
Srpr	0.942	10.831	7.841	4.70E-08	4.40E-07	7.989	1.92
Srpr	1.237	10.118	7.439	1.16E-07	9.75E-07	7.066	2.36
SSR4	-0.373	9.057	-4.331	2.31E-04	7.73E-04	-0.603	-1.29
SSU72	-0.732	7.371	-11.519	3.16E-11	7.18E-10	15.463	-1.66
STIM2	0.301	6.458	3.682	1.18E-03	3.29E-03	-2.215	1.23
STK10	-0.289	6.417	-5.046	3.77E-05	1.56E-04	1.207	-1.22
STK39	0.550	6.315	6.050	3.10E-06	1.71E-05	3.727	1.46
STT3A	-0.389	7.885	-6.942	3.67E-07	2.66E-06	5.896	-1.31
SUPT16H	-0.411	10.402	-5.683	7.66E-06	3.84E-05	2.813	-1.33
SUPT4H1	0.465	7.223	8.697	7.37E-09	8.46E-08	9.883	1.38
SUPT6H	0.263	6.509	2.542	1.79E-02	3.53E-02	-4.822	1.20
Surf4	-0.345	7.067	-7.624	7.64E-08	6.80E-07	7.494	-1.27
Syf2	0.317	7.090	5.041	3.82E-05	1.58E-04	1.194	1.25
SYN2	0.356	10.923	9.248	2.35E-09	3.15E-08	11.051	1.28
SYNCRIP	0.965	6.514	5.057	3.66E-05	1.52E-04	1.236	1.95
SYNCRIP	1.221	6.547	18.363	1.42E-15	1.42E-13	25.670	2.33
TAB2	-0.542	7.349	-9.888	6.56E-10	1.01E-08	12.358	-1.46
TACC2	-0.327	6.631	-7.803	5.12E-08	4.77E-07	7.903	-1.25
Taf2	0.431	8.441	9.149	2.88E-09	3.78E-08	10.844	1.35
TAGLN2	-0.287	8.340	-3.959	5.90E-04	1.76E-03	-1.533	-1.22
TARDBP	0.334	7.735	6.712	6.30E-07	4.22E-06	5.345	1.26
Tbc1d20	0.364	7.320	6.456	1.16E-06	7.29E-06	4.726	1.29
Tbl1xr1	0.284	7.703	5.008	4.15E-05	1.70E-04	1.111	1.22
TBPL1	0.361	7.435	5.486	1.25E-05	5.90E-05	2.320	1.28
TCEA1	0.357	8.260	8.146	2.41E-08	2.46E-07	8.673	1.28
Tcf19	0.725	5.912	12.621	4.80E-12	1.48E-10	17.391	1.65
Tdgf1	0.449	6.194	6.658	7.16E-07	4.68E-06	5.215	1.36
TECR	-0.530	8.225	-7.557	8.90E-08	7.82E-07	7.338	-1.44
Tes	-0.873	7.629	-14.629	2.10E-13	9.78E-12	20.591	-1.83
Tes	-0.766	6.550	-15.788	4.01E-14	2.62E-12	22.277	-1.70
TFPI	-0.752	7.291	-9.387	1.78E-09	2.47E-08	11.339	-1.68
Tgfbr2	-0.562	6.364	-7.141	2.30E-07	1.80E-06	6.369	-1.48
Thbd	-1.059	7.746	-12.233	9.19E-12	2.50E-10	16.727	-2.08
THOC4	0.270	8.085	3.353	2.66E-03	6.61E-03	-3.007	1.21
THOC7	0.272	7.681	5.142	2.96E-05	1.27E-04	1.449	1.21

Thumpd3	0.265	6.634	4.927	5.09E-05	2.02E-04	0.906	1.20
TIMM17B	-0.381	6.995	-4.274	2.67E-04	8.76E-04	-0.747	-1.30
TIMM50	-0.601	7.608	-12.204	9.66E-12	2.61E-10	16.676	-1.52
TIMP1	-0.741	6.932	-10.906	9.52E-11	1.83E-09	14.334	-1.67
Timp2	0.389	7.474	5.030	3.92E-05	1.62E-04	1.167	1.31
TIMP2	0.466	8.474	6.749	5.76E-07	3.92E-06	5.435	1.38
TJP2	-0.326	6.785	-6.336	1.55E-06	9.36E-06	4.432	-1.25
TKT	-0.612	6.994	-13.547	1.08E-12	4.19E-11	18.914	-1.53
TKT	-0.552	6.373	-3.948	6.07E-04	1.80E-03	-1.561	-1.47
TMED3	-0.638	6.990	-13.331	1.52E-12	5.42E-11	18.566	-1.56
TMEM101	0.589	6.466	7.074	2.69E-07	2.04E-06	6.210	1.50
Tmem109	-0.373	6.566	-7.167	2.17E-07	1.72E-06	6.429	-1.30
Tmem150a	-0.360	6.896	-5.212	2.48E-05	1.08E-04	1.627	-1.28
TMEM156	0.449	7.139	7.845	4.67E-08	4.38E-07	7.997	1.37
TMEM158	0.398	6.933	4.067	4.50E-04	1.38E-03	-1.264	1.32
Tmem167	0.357	7.216	5.496	1.22E-05	5.77E-05	2.345	1.28
Tmem167b	0.465	6.863	10.418	2.36E-10	4.10E-09	13.403	1.38
TMEM5	-0.332	6.602	-8.256	1.89E-08	2.01E-07	8.919	-1.26
TMSB10	-0.335	10.371	-5.409	1.51E-05	6.98E-05	2.125	-1.26
Tmx1	0.354	8.098	6.437	1.21E-06	7.52E-06	4.680	1.28
Tnfaip3	-1.295	5.235	-14.151	4.28E-13	1.84E-11	19.862	-2.45
Tnfrsf21	-0.627	7.387	-8.841	5.44E-09	6.50E-08	10.193	-1.54
Tnfsf9	-1.105	6.765	-10.354	2.67E-10	4.57E-09	13.278	-2.15
TNIP1	-0.513	6.401	-6.951	3.59E-07	2.62E-06	5.917	-1.43
TNKS2	0.294	6.486	4.934	5.00E-05	1.99E-04	0.924	1.23
TOMM34	0.412	7.388	6.956	3.54E-07	2.59E-06	5.930	1.33
Top2a	-0.451	9.103	-5.717	7.03E-06	3.56E-05	2.899	-1.37
Tpst1	-0.658	6.787	-15.572	5.42E-14	3.24E-12	21.971	-1.58
TPX2	-0.726	6.219	-7.798	5.18E-08	4.78E-07	7.891	-1.65
TRAK2	-0.460	6.593	-5.753	6.43E-06	3.28E-05	2.990	-1.38
TRAP1	0.271	6.783	3.316	2.92E-03	7.13E-03	-3.094	1.21
TRIB1	0.685	6.683	9.848	7.09E-10	1.07E-08	12.279	1.61
TRIM44	0.362	6.784	3.024	5.89E-03	1.33E-02	-3.771	1.29
Trmt6	0.576	6.854	10.530	1.91E-10	3.34E-09	13.619	1.49
Trub1	0.382	7.328	9.472	1.50E-09	2.13E-08	11.514	1.30
TSC22D1	-0.407	10.051	-5.655	8.20E-06	4.07E-05	2.743	-1.33
TSEN15	0.323	7.360	8.824	5.65E-09	6.70E-08	10.155	1.25
TTC33	0.267	6.573	3.817	8.44E-04	2.42E-03	-1.884	1.20
TUBA1A	-0.290	8.193	-3.315	2.92E-03	7.13E-03	-3.097	-1.22
TUBA1C	-0.539	9.068	-11.182	5.76E-11	1.20E-09	14.849	-1.45
TUBB2C	-0.579	8.450	-11.544	3.02E-11	6.91E-10	15.510	-1.49
Tubb6	-0.525	7.128	-13.221	1.82E-12	6.32E-11	18.386	-1.44
Twf1	-0.282	6.551	-2.531	1.84E-02	3.61E-02	-4.844	-1.22
TXNRD1	-0.649	7.365	-4.455	1.68E-04	5.86E-04	-0.289	-1.57
TXNRD1	-0.608	9.549	-15.345	7.45E-14	4.25E-12	21.646	-1.52
Tyms	-0.348	8.010	-4.442	1.74E-04	6.03E-04	-0.322	-1.27
UAP1	-0.413	6.881	-6.693	6.59E-07	4.35E-06	5.300	-1.33
UBA5	-0.374	7.149	-7.986	3.42E-08	3.32E-07	8.315	-1.30
UBASH3B	0.280	8.046	6.855	4.50E-07	3.17E-06	5.688	1.21
UBE2D4	0.524	6.665	8.601	9.03E-09	1.01E-07	9.676	1.44
UBE2E3	-0.288	7.271	-4.393	1.97E-04	6.75E-04	-0.447	-1.22
Ube2h	-0.325	6.554	-2.936	7.25E-03	1.60E-02	-3.969	-1.25
UBL3	0.268	7.629	3.719	1.08E-03	3.02E-03	-2.125	1.20
UBL3	0.359	6.206	6.780	5.36E-07	3.66E-06	5.510	1.28
UBQLN1	0.319	7.255	8.099	2.66E-08	2.70E-07	8.570	1.25
UFD1L	0.294	8.108	6.527	9.77E-07	6.21E-06	4.898	1.23
Ufd1l	0.326	6.294	6.930	3.76E-07	2.71E-06	5.869	1.25
UGCG	0.272	6.614	2.721	1.20E-02	2.47E-02	-4.443	1.21
Uggt1	0.288	7.043	6.326	1.58E-06	9.57E-06	4.408	1.22
Ugt1a1	-0.380	10.340	-8.570	9.64E-09	1.08E-07	9.608	-1.30
Uqcr10	0.264	9.233	6.262	1.85E-06	1.11E-05	4.250	1.20
Uqcr10	0.298	8.893	4.257	2.78E-04	9.08E-04	-0.789	1.23
USO1	-0.573	6.539	-8.477	1.18E-08	1.29E-07	9.404	-1.49
USO1	-0.421	7.181	-8.852	5.33E-09	6.42E-08	10.215	-1.34
USP46	0.406	6.354	6.033	3.23E-06	1.77E-05	3.687	1.32
USP47	-0.276	6.270	-3.381	2.49E-03	6.24E-03	-2.940	-1.21
USP9X	-0.514	7.078	-8.501	1.12E-08	1.23E-07	9.457	-1.43
UTP18	-0.279	7.550	-6.803	5.08E-07	3.51E-06	5.563	-1.21
UTP3	0.370	6.703	7.978	3.48E-08	3.37E-07	8.297	1.29
VAMP8	-0.593	7.162	-10.404	2.43E-10	4.20E-09	13.375	-1.51
Vapb	0.274	8.100	3.246	3.45E-03	8.28E-03	-3.258	1.21
VEGFC	-0.333	6.894	-6.941	3.67E-07	2.66E-06	5.895	-1.26
VIM	0.350	8.032	6.075	2.92E-06	1.63E-05	3.789	1.27
VMA21	0.841	7.724	13.029	2.47E-12	8.21E-11	18.072	1.79
VNN1	-1.124	6.056	-20.540	1.15E-16	1.74E-14	28.215	-2.18
VOPP1	-0.442	6.712	-7.929	3.87E-08	3.67E-07	8.188	-1.36
VWA5A	0.320	7.095	6.251	1.90E-06	1.12E-05	4.225	1.25
Wdfy4	0.776	6.002	7.304	1.58E-07	1.29E-06	6.751	1.71
WDR1	-0.297	6.551	-6.279	1.78E-06	1.06E-05	4.292	-1.23

Wsb2	0.732	6.048	14.700	1.89E-13	9.17E-12	20.697	1.66
WWC2	-0.547	6.927	-9.346	1.93E-09	2.64E-08	11.255	-1.46
WWC3	-0.375	6.647	-7.461	1.11E-07	9.42E-07	7.116	-1.30
XBP1	-1.093	7.896	-19.797	2.64E-16	3.06E-14	27.377	-2.13
Xiap	0.430	6.328	7.115	2.45E-07	1.90E-06	6.306	1.35
Xpot	-0.288	8.618	-4.913	5.27E-05	2.09E-04	0.870	-1.22
YIF1A	-0.517	6.830	-8.090	2.72E-08	2.74E-07	8.548	-1.43
YPEL5	-0.284	6.204	-4.122	3.91E-04	1.22E-03	-1.126	-1.22
Ywhab	0.263	7.203	4.134	3.80E-04	1.20E-03	-1.098	1.20
Ywhae	-0.309	10.471	-2.888	8.12E-03	1.77E-02	-4.077	-1.24
ZC3HAV1	0.729	7.214	13.502	1.16E-12	4.42E-11	18.841	1.66
Zcchc24	0.330	6.916	5.383	1.62E-05	7.37E-05	2.060	1.26
ZFP36L1	-0.645	8.280	-10.842	1.07E-10	2.03E-09	14.213	-1.56
ZMYND11	0.397	7.803	11.170	5.89E-11	1.22E-09	14.826	1.32
ZNF639	-0.267	6.621	-6.199	2.16E-06	1.25E-05	4.095	-1.20

5.2.10 T(S)1 v pcDNA(S) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40607	0.404	7.921	9.875	6.72E-10	7.60E-09	12.303	1.32
40790	-0.641	5.984	-7.270	1.71E-07	9.88E-07	6.640	-1.56
0610031J06Rik	-0.355	7.340	-7.947	3.73E-08	2.53E-07	8.195	-1.28
0610039K10Rik	-0.400	8.116	-8.855	5.29E-09	4.71E-08	10.192	-1.32
1110003E01Rik	-0.396	7.572	-3.812	8.55E-04	2.08E-03	-1.932	-1.32
1110004F10Rik	0.317	6.588	5.781	5.99E-06	2.42E-05	3.026	1.25
1110008F13Rik	-0.268	9.165	-7.595	8.17E-08	5.13E-07	7.394	-1.20
1190005F20Rik	0.811	6.127	17.720	3.15E-15	1.95E-13	24.847	1.75
1500011H22Rik	0.431	6.765	7.838	4.73E-08	3.10E-07	7.950	1.35
1700025O18Rik	0.485	7.116	7.914	4.00E-08	2.68E-07	8.123	1.40
1700052N19Rik	-0.614	6.422	-12.793	3.62E-12	7.84E-11	17.654	-1.53
1700123N01Rik	0.328	8.426	5.948	3.98E-06	1.67E-05	3.441	1.26
1700123N01Rik	0.357	8.639	6.590	8.42E-07	4.07E-06	5.017	1.28
1810022C23Rik	-0.314	6.867	-6.030	3.25E-06	1.39E-05	3.645	-1.24
1810031K17Rik	0.524	6.408	4.597	1.17E-04	3.49E-04	0.035	1.44
1810037I17Rik	-0.425	6.983	-7.950	3.70E-08	2.52E-07	8.204	-1.34
2010107E04Rik	-0.895	9.453	-18.104	1.95E-15	1.35E-13	25.332	-1.86
2310001A20Rik	-0.361	7.257	-6.437	1.21E-06	5.70E-06	4.647	-1.28
2310003F16Rik	-0.354	7.742	-9.142	2.92E-09	2.75E-08	10.799	-1.28
2310014H01Rik	0.373	7.462	6.912	3.93E-07	2.07E-06	5.792	1.29
2310035C23Rik	0.567	6.446	11.312	4.56E-11	7.07E-10	15.059	1.48
2410015M20Rik	-0.627	8.054	-10.551	1.84E-10	2.41E-09	13.630	-1.54
2410017P09Rik	0.279	6.838	6.962	3.49E-07	1.87E-06	5.912	1.21
2410129H14Rik	0.435	6.698	6.481	1.09E-06	5.18E-06	4.753	1.35
2610002M06Rik	-0.289	6.504	-4.939	4.94E-05	1.59E-04	0.901	-1.22
2610030H06Rik	0.435	7.724	6.738	5.91E-07	2.98E-06	5.376	1.35
2700029M09Rik	1.144	6.966	23.071	8.17E-18	1.63E-15	30.866	2.21
2810025M15Rik	0.520	6.900	9.413	1.68E-09	1.70E-08	11.364	1.43
2810482I07Rik	-0.478	6.348	-8.031	3.09E-08	2.16E-07	8.386	-1.39
2900097C17Rik	-0.341	9.676	-7.006	3.15E-07	1.71E-06	6.017	-1.27
3110062M04Rik	-0.382	7.493	-7.924	3.91E-08	2.63E-07	8.145	-1.30
4833417J20Rik	-0.592	7.312	-8.447	1.25E-08	1.01E-07	9.309	-1.51
4930404A10Rik	-0.506	6.588	-10.151	3.94E-10	4.58E-09	12.852	-1.42
4930535I16Rik	0.353	8.352	8.061	2.89E-08	2.05E-07	8.454	1.28
4930539H15Rik	0.422	6.597	9.543	1.30E-09	1.35E-08	11.630	1.34
4932427H20Rik	-0.396	6.583	-6.723	6.14E-07	3.08E-06	5.339	-1.32
4932441K18Rik	-0.318	8.460	-8.166	2.30E-08	1.70E-07	8.687	-1.25
4932703K07Rik	-0.292	9.835	-6.411	1.29E-06	6.05E-06	4.582	-1.22
4933424B01Rik	-0.333	6.952	-3.934	6.29E-04	1.58E-03	-1.631	-1.26
5830485P09Rik	0.297	7.290	3.709	1.11E-03	2.60E-03	-2.184	1.23
5930416I19Rik	0.398	6.436	7.227	1.89E-07	1.08E-06	6.538	1.32
6330406I15Rik	-0.309	8.024	-5.730	6.81E-06	2.71E-05	2.897	-1.24
6430548M08Rik	0.585	5.381	8.347	1.55E-08	1.21E-07	9.089	1.50
9030411K21Rik	0.272	6.758	5.654	8.22E-06	3.20E-05	2.707	1.21
9330115C17Rik	0.525	8.429	6.680	6.78E-07	3.35E-06	5.237	1.44
9430034N14Rik	0.413	6.625	8.144	2.41E-08	1.77E-07	8.639	1.33
9530053H05Rik	-1.117	10.002	-19.356	4.38E-16	4.14E-14	26.850	-2.17
A330042M18Rik	0.753	7.205	11.802	1.92E-11	3.34E-10	15.944	1.69
A330042M18Rik	0.885	6.806	16.790	1.04E-14	5.26E-13	23.632	1.85
A730085K08Rik	-0.460	6.637	-4.573	1.25E-04	3.68E-04	-0.026	-1.38
A930005N03Rik	-0.346	6.933	-4.914	5.25E-05	1.68E-04	0.840	-1.27
Abcc5	-0.331	7.507	-3.774	9.39E-04	2.26E-03	-2.025	-1.26
Abce1	0.273	7.808	5.227	2.39E-05	8.27E-05	1.632	1.21
ABHD2	-0.315	6.655	-6.743	5.85E-07	2.96E-06	5.387	-1.24
Abhd6	-0.402	6.783	-5.121	3.12E-05	1.05E-04	1.363	-1.32
Acadl	-0.272	7.741	-5.428	1.44E-05	5.29E-05	2.139	-1.21
Acat2	-1.308	6.811	-19.781	2.69E-16	2.93E-14	27.345	-2.48
Acbd6	0.625	6.747	11.178	5.80E-11	8.80E-10	14.813	1.54
ACLY	-0.557	8.199	-9.953	5.78E-10	6.57E-09	12.458	-1.47
Aco1	-0.533	6.759	-16.555	1.42E-14	6.80E-13	23.316	-1.45
Acot9	-0.357	6.777	-7.999	3.32E-08	2.31E-07	8.313	-1.28
Acp2	-0.564	6.448	-8.418	1.34E-08	1.07E-07	9.243	-1.48
Actr3	0.316	10.274	5.494	1.23E-05	4.57E-05	2.304	1.25
Actr3	0.398	8.697	6.720	6.17E-07	3.10E-06	5.333	1.32
Actr3	0.427	8.121	2.558	1.73E-02	3.03E-02	-4.823	1.34
Adam10	-0.574	8.078	-8.850	5.34E-09	4.75E-08	10.181	-1.49
Adam9	-0.298	8.339	-3.949	6.05E-04	1.52E-03	-1.592	-1.23
ADD3	0.730	7.763	15.232	8.76E-14	3.24E-12	21.459	1.66
Add3	0.733	6.120	6.214	2.08E-06	9.31E-06	4.100	1.66
Adh5	-0.382	9.619	-5.230	2.37E-05	8.23E-05	1.638	-1.30
Adh5	-0.351	8.532	-2.589	1.62E-02	2.85E-02	-4.759	-1.28
Adipor1	0.494	7.755	10.694	1.41E-10	1.93E-09	13.903	1.41
Adss	0.485	7.645	9.554	1.27E-09	1.33E-08	11.653	1.40
Adss	0.574	8.642	8.860	5.24E-09	4.68E-08	10.201	1.49
AF085738	-0.448	10.908	-10.180	3.72E-10	4.39E-09	12.908	-1.36

Aftph	0.599	7.139	8.346	1.56E-08	1.21E-07	9.085	1.52
Aga	-0.296	9.240	-2.738	1.15E-02	2.10E-02	-4.440	-1.23
Aga	-0.296	10.321	-5.413	1.50E-05	5.46E-05	2.103	-1.23
Agap1	0.302	7.787	3.112	4.77E-03	9.61E-03	-3.604	1.23
Agap1	0.377	7.472	7.519	9.71E-08	5.92E-07	7.218	1.30
Agbl5	-0.325	8.349	-2.793	1.01E-02	1.88E-02	-4.321	-1.25
Ahctf1	0.604	6.293	7.224	1.90E-07	1.09E-06	6.530	1.52
Ahcy	-0.394	6.717	-8.130	2.49E-08	1.82E-07	8.608	-1.31
Ahnak	-1.036	8.479	-13.810	7.21E-13	1.92E-11	19.306	-2.05
Ahsa1	0.268	7.553	4.755	7.86E-05	2.43E-04	0.436	1.20
AI428898	-0.551	7.749	-11.880	1.68E-11	2.96E-10	16.082	-1.47
Aida	0.444	6.309	2.745	1.13E-02	2.07E-02	-4.425	1.36
Aig1	-0.427	6.487	-5.631	8.69E-06	3.37E-05	2.651	-1.34
Aimp1	0.491	8.513	11.904	1.61E-11	2.85E-10	16.125	1.41
Airn	0.343	6.764	6.118	2.62E-06	1.14E-05	3.864	1.27
Ak2	0.357	7.391	2.727	1.18E-02	2.15E-02	-4.465	1.28
Ak2	0.635	9.209	11.773	2.02E-11	3.44E-10	15.892	1.55
Akap12	0.442	7.889	6.628	7.69E-07	3.76E-06	5.109	1.36
Akirin2	0.446	6.069	5.857	4.98E-06	2.06E-05	3.215	1.36
Akr1b8	-0.604	9.869	-18.780	8.62E-16	6.59E-14	26.164	-1.52
Akr7a5	-0.350	6.496	-4.037	4.85E-04	1.25E-03	-1.374	-1.27
Akt3	1.023	5.980	17.058	7.33E-15	3.93E-13	23.989	2.03
Aktip	0.383	7.013	7.946	3.73E-08	2.53E-07	8.193	1.30
AL024054	0.646	6.117	10.976	8.38E-11	1.22E-09	14.436	1.57
Aldh2	-0.426	8.281	-6.858	4.46E-07	2.32E-06	5.663	-1.34
Amacr	-0.333	6.539	-4.940	4.93E-05	1.59E-04	0.904	-1.26
Amd1	1.444	7.422	14.391	2.98E-13	9.09E-12	20.207	2.72
Amd2	1.629	8.027	18.955	7.01E-16	5.85E-14	26.374	3.09
Anapc11	-0.297	7.726	-3.005	6.16E-03	1.20E-02	-3.849	-1.23
Ankrd13c	0.362	7.255	8.726	6.93E-09	5.97E-08	9.914	1.28
Anp32e	0.361	8.064	5.187	2.64E-05	9.02E-05	1.531	1.28
Anp32e	0.440	7.906	9.716	9.20E-10	1.00E-08	11.982	1.36
Anxa2	-0.475	9.836	-3.114	4.75E-03	9.58E-03	-3.599	-1.39
Anxa2	-0.393	10.669	-5.622	8.89E-06	3.42E-05	2.628	-1.31
Ap1ar	0.300	7.345	6.351	1.49E-06	6.93E-06	4.436	1.23
Ap2a2	0.338	7.233	6.984	3.32E-07	1.79E-06	5.964	1.26
Ap2m1	0.267	9.164	3.096	4.96E-03	9.95E-03	-3.641	1.20
Apeh	-0.425	6.410	-6.773	5.44E-07	2.79E-06	5.461	-1.34
Arhgap18	-0.289	6.623	-2.880	8.26E-03	1.57E-02	-4.128	-1.22
Arhgap24	-0.378	7.747	-5.039	3.84E-05	1.27E-04	1.156	-1.30
Arhgap25	-0.469	6.665	-5.630	8.72E-06	3.37E-05	2.648	-1.38
Arhgef6	-0.342	7.955	-5.334	1.83E-05	6.54E-05	1.902	-1.27
Arl10	0.272	6.690	6.310	1.65E-06	7.59E-06	4.337	1.21
Arl15	-0.331	6.329	-5.704	7.27E-06	2.86E-05	2.832	-1.26
Arl2bp	0.292	7.421	4.888	5.62E-05	1.79E-04	0.772	1.22
Arpc3	-0.280	7.652	-3.043	5.63E-03	1.11E-02	-3.762	-1.21
Arpc5	0.750	8.452	11.101	6.67E-11	9.91E-10	14.670	1.68
Arpc5	0.775	8.740	16.633	1.28E-14	6.25E-13	23.422	1.71
Asap1	-0.324	5.925	-6.069	2.96E-06	1.28E-05	3.742	-1.25
Asf1b	-0.407	5.939	-4.860	6.04E-05	1.90E-04	0.701	-1.33
Asns	-0.618	9.296	-12.616	4.85E-12	1.01E-10	17.355	-1.53
Asns	-0.610	9.701	-14.811	1.61E-13	5.43E-12	20.840	-1.53
Asnsd1	0.450	8.235	6.950	3.59E-07	1.91E-06	5.883	1.37
Aspm	0.509	6.112	7.773	5.48E-08	3.56E-07	7.801	1.42
Atf6	0.853	8.404	18.056	2.08E-15	1.38E-13	25.272	1.81
Atg3	0.320	6.477	5.140	2.98E-05	1.01E-04	1.411	1.25
Atp10a	-0.599	7.352	-14.024	5.19E-13	1.49E-11	19.641	-1.51
Atp13a3	-0.491	6.608	-2.534	1.82E-02	3.18E-02	-4.873	-1.41
Atp2a2	0.413	6.749	6.911	3.94E-07	2.07E-06	5.791	1.33
Atp5a1	-0.359	7.427	-3.360	2.62E-03	5.69E-03	-3.025	-1.28
Atp5a1	-0.314	8.837	-7.487	1.04E-07	6.33E-07	7.145	-1.24
Atp5h	0.266	9.664	5.911	4.35E-06	1.82E-05	3.350	1.20
Atp5o	0.480	9.052	10.113	4.24E-10	4.89E-09	12.776	1.39
ATP6V1B2	0.435	7.365	7.290	1.63E-07	9.51E-07	6.687	1.35
Atpif1	-0.436	9.759	-10.180	3.72E-10	4.39E-09	12.909	-1.35
ATXN7L3B	0.419	6.370	8.399	1.39E-08	1.10E-07	9.204	1.34
AU015740	0.383	6.605	5.772	6.14E-06	2.47E-05	3.002	1.30
AU019300	0.456	7.174	9.118	3.07E-09	2.86E-08	10.749	1.37
AW549877	0.317	6.308	4.117	3.97E-04	1.04E-03	-1.175	1.25
AW742525	-0.325	7.372	-8.370	1.48E-08	1.17E-07	9.140	-1.25
AW743318	0.305	8.635	5.711	7.14E-06	2.82E-05	2.850	1.24
Azin1	0.889	6.442	10.722	1.34E-10	1.85E-09	13.957	1.85
Azin1	1.187	6.431	16.392	1.76E-14	8.13E-13	23.094	2.28
B230219D22Rik	0.419	8.128	7.951	3.69E-08	2.52E-07	8.205	1.34
B230220B15Rik	-2.249	7.506	-47.005	5.11E-25	6.39E-22	46.890	-4.75
B3galnt2	0.273	6.763	3.637	1.32E-03	3.05E-03	-2.359	1.21
B4galt3	0.762	6.562	12.727	4.04E-12	8.69E-11	17.543	1.70
Bat2	0.320	6.201	2.916	7.60E-03	1.46E-02	-4.049	1.25
BC029722	-0.603	6.162	-9.257	2.31E-09	2.24E-08	11.040	-1.52

BC046331	0.443	6.418	6.542	9.43E-07	4.52E-06	4.902	1.36
Bcap31	-0.317	7.616	-7.953	3.67E-08	2.51E-07	8.211	-1.25
Bcl2l2	0.469	6.311	10.337	2.76E-10	3.32E-09	13.216	1.38
Bclaf1	0.340	6.690	4.761	7.75E-05	2.40E-04	0.451	1.27
Bclaf1	0.411	7.984	6.598	8.25E-07	4.00E-06	5.038	1.33
Birc5	0.350	7.818	4.335	2.28E-04	6.40E-04	-0.627	1.27
Bloc1s2	0.515	6.230	6.766	5.53E-07	2.83E-06	5.444	1.43
Bmi1	1.229	6.386	14.811	1.61E-13	5.43E-12	20.841	2.34
Bms1	-0.407	6.641	-6.832	4.74E-07	2.46E-06	5.601	-1.33
Bre	-0.323	6.906	-5.739	6.65E-06	2.66E-05	2.921	-1.25
Brp44	0.901	7.735	18.592	1.08E-15	7.92E-14	25.935	1.87
Brp44l	0.416	7.208	2.604	1.56E-02	2.77E-02	-4.727	1.33
Btbd1	0.299	7.455	2.620	1.51E-02	2.68E-02	-4.694	1.23
Btbd1	0.388	8.023	3.588	1.49E-03	3.41E-03	-2.479	1.31
Btg1	-0.300	7.972	-7.899	4.14E-08	2.76E-07	8.087	-1.23
Btg3	0.309	7.374	3.426	2.23E-03	4.91E-03	-2.867	1.24
Bzw1	0.317	6.951	5.688	7.55E-06	2.95E-05	2.794	1.25
C130057N11Rik	0.413	6.393	4.406	1.91E-04	5.44E-04	-0.448	1.33
C1qbp	0.304	7.278	3.730	1.05E-03	2.48E-03	-2.132	1.23
C1rb	-0.527	9.482	-6.676	6.86E-07	3.38E-06	5.225	-1.44
C630016N16Rik	-0.669	9.801	-11.292	4.73E-11	7.29E-10	15.023	-1.59
C630016N16Rik	-0.616	6.788	-6.900	4.04E-07	2.12E-06	5.765	-1.53
C78376	-0.654	7.959	-10.457	2.20E-10	2.78E-09	13.450	-1.57
C79646	-0.385	7.168	-7.972	3.53E-08	2.43E-07	8.252	-1.31
Cab39	0.362	7.933	6.983	3.33E-07	1.79E-06	5.962	1.29
Cab39	0.386	6.216	3.375	2.52E-03	5.52E-03	-2.988	1.31
Cab39	0.418	7.377	6.970	3.43E-07	1.83E-06	5.930	1.34
Cacybp	0.489	8.731	5.534	1.11E-05	4.16E-05	2.406	1.40
Cacybp	0.590	9.038	14.520	2.46E-13	7.83E-12	20.404	1.51
Cald1	-0.507	7.225	-5.721	6.96E-06	2.76E-05	2.875	-1.42
Cald1	-0.494	7.673	-3.868	7.42E-04	1.83E-03	-1.793	-1.41
Calm1	0.458	6.188	4.087	4.27E-04	1.11E-03	-1.248	1.37
Camsap1l1	0.807	6.300	19.029	6.42E-16	5.65E-14	26.463	1.75
Cap1	-0.353	6.658	-3.186	4.00E-03	8.24E-03	-3.434	-1.28
Capg	-0.437	7.250	-4.115	3.98E-04	1.05E-03	-1.179	-1.35
Capg	-0.433	7.270	-5.458	1.34E-05	4.93E-05	2.215	-1.35
Capn2	-0.280	6.868	-3.630	1.34E-03	3.10E-03	-2.375	-1.21
Capn7	0.279	6.641	4.708	8.86E-05	2.70E-04	0.316	1.21
Capns1	0.321	7.079	4.983	4.42E-05	1.44E-04	1.014	1.25
Capza2	0.342	8.594	2.840	9.08E-03	1.70E-02	-4.218	1.27
Capza2	0.358	9.291	5.371	1.66E-05	6.03E-05	1.996	1.28
Capza2	0.385	10.363	8.708	7.21E-09	6.18E-08	9.874	1.31
Cat	-0.788	8.131	-7.906	4.08E-08	2.72E-07	8.103	-1.73
Cat	-0.709	6.892	-3.563	1.59E-03	3.60E-03	-2.538	-1.63
Cav2	-0.668	6.234	-9.228	2.45E-09	2.36E-08	10.978	-1.59
Cbara1	0.267	6.904	3.701	1.13E-03	2.64E-03	-2.204	1.20
Cbfb	0.356	7.994	6.217	2.06E-06	9.30E-06	4.106	1.28
Cbl	0.295	6.841	2.817	9.58E-03	1.79E-02	-4.268	1.23
Ccdc43	0.585	6.315	11.675	2.40E-11	4.03E-10	15.717	1.50
Ccdc50	0.292	7.337	5.139	2.98E-05	1.01E-04	1.410	1.22
Ccdc53	-0.514	7.195	-10.492	2.05E-10	2.64E-09	13.517	-1.43
Ccdc85b	-1.080	7.032	-12.245	9.01E-12	1.75E-10	16.721	-2.11
Ccdc85b	-1.016	6.897	-13.826	7.03E-13	1.89E-11	19.331	-2.02
Ccdc90b	-0.365	7.006	-4.792	7.17E-05	2.24E-04	0.528	-1.29
Ccl2	-2.891	7.806	-21.727	3.21E-17	5.45E-15	29.490	-7.42
Ccl25	0.480	6.699	8.179	2.24E-08	1.66E-07	8.717	1.39
Ccndbp1	0.308	7.359	4.842	6.31E-05	1.99E-04	0.656	1.24
Cct8	-0.264	7.783	-2.420	2.35E-02	4.02E-02	-5.108	-1.20
Cd109	-0.270	6.836	-2.945	7.10E-03	1.37E-02	-3.984	-1.21
Cd151	-0.268	7.704	-4.187	3.32E-04	8.91E-04	-0.998	-1.20
Cd36	1.954	8.162	9.796	7.85E-10	8.76E-09	12.145	3.87
Cdc16	-0.432	7.096	-6.205	2.12E-06	9.50E-06	4.077	-1.35
Cdc42se1	0.283	6.268	4.992	4.32E-05	1.41E-04	1.037	1.22
Cdipt	0.460	7.020	7.088	2.61E-07	1.45E-06	6.210	1.38
Cdk1	0.342	8.673	5.342	1.79E-05	6.42E-05	1.924	1.27
Cdk6	0.522	8.613	8.249	1.92E-08	1.47E-07	8.872	1.44
Cdk6	0.575	6.642	5.170	2.76E-05	9.39E-05	1.487	1.49
Cdk6	0.593	7.547	5.492	1.23E-05	4.57E-05	2.302	1.51
Cdv3	0.530	8.233	9.126	3.02E-09	2.83E-08	10.766	1.44
Cebpb	-0.493	9.362	-12.241	9.07E-12	1.75E-10	16.714	-1.41
Cenpl	0.644	6.032	9.275	2.23E-09	2.18E-08	11.077	1.56
Cetn3	-0.486	8.092	-6.481	1.09E-06	5.18E-06	4.753	-1.40
Cfdp1	-0.273	8.313	-5.434	1.42E-05	5.21E-05	2.155	-1.21
Cflar	-0.355	7.205	-4.507	1.48E-04	4.30E-04	-0.193	-1.28
Chchd3	0.268	6.879	6.055	3.06E-06	1.31E-05	3.706	1.20
Chd2	0.466	6.581	8.070	2.84E-08	2.02E-07	8.472	1.38
Chordc1	0.463	6.453	6.460	1.15E-06	5.44E-06	4.701	1.38
Chpf	0.357	6.081	3.080	5.15E-03	1.03E-02	-3.678	1.28
CHUK	0.348	7.119	6.882	4.21E-07	2.21E-06	5.722	1.27

Cirbp	-0.680	7.470	-10.022	5.05E-10	5.82E-09	12.596	-1.60
Cisd3	-0.659	6.884	-10.583	1.73E-10	2.30E-09	13.692	-1.58
Cited2	-0.471	6.419	-4.991	4.33E-05	1.41E-04	1.033	-1.39
Clec2e	-0.854	5.756	-8.093	2.70E-08	1.95E-07	8.524	-1.81
Clic4	-0.586	8.809	-9.743	8.72E-10	9.61E-09	12.037	-1.50
Clns1a	0.453	7.669	10.907	9.50E-11	1.37E-09	14.307	1.37
Clstn1	0.700	7.732	10.160	3.87E-10	4.51E-09	12.869	1.62
Cltc	-0.298	7.897	-3.335	2.78E-03	6.00E-03	-3.084	-1.23
Cnbp	-0.342	9.436	-3.109	4.81E-03	9.67E-03	-3.611	-1.27
Cnbp	-0.272	10.434	-5.710	7.15E-06	2.82E-05	2.848	-1.21
Cnn2	-0.405	7.200	-3.988	5.48E-04	1.40E-03	-1.495	-1.32
Cnot7	0.283	7.521	6.235	1.98E-06	8.93E-06	4.150	1.22
Cobll1	-0.384	6.463	-7.172	2.15E-07	1.21E-06	6.408	-1.30
Col5a2	0.462	7.606	6.343	1.52E-06	7.05E-06	4.417	1.38
Commd3	0.730	7.861	17.407	4.68E-15	2.67E-13	24.444	1.66
Commd7	0.579	6.618	12.272	8.60E-12	1.69E-10	16.768	1.49
Copa	0.284	6.314	5.782	5.99E-06	2.42E-05	3.027	1.22
Copa	0.285	7.525	3.072	5.25E-03	1.05E-02	-3.696	1.22
Copb2	-0.281	7.048	-4.951	4.79E-05	1.55E-04	0.931	-1.22
Copg	-0.465	6.669	-7.562	8.80E-08	5.43E-07	7.318	-1.38
Copg2	-0.405	6.694	-3.331	2.81E-03	6.04E-03	-3.093	-1.32
Cops4	0.304	7.810	5.201	2.55E-05	8.74E-05	1.567	1.23
Cops8	0.306	7.245	6.186	2.22E-06	9.84E-06	4.031	1.24
Coro1b	-0.529	8.179	-8.369	1.48E-08	1.17E-07	9.137	-1.44
Coro1b	-0.474	7.585	-6.557	9.09E-07	4.37E-06	4.939	-1.39
Cox18	0.323	6.621	8.995	3.95E-09	3.62E-08	10.490	1.25
Cox4i1	0.285	10.414	6.991	3.26E-07	1.76E-06	5.982	1.22
Cox4nb	0.730	7.112	13.267	1.69E-12	3.93E-11	18.436	1.66
Cox7a2l	0.543	8.364	13.253	1.72E-12	4.00E-11	18.413	1.46
Cpn2	-0.300	6.648	-3.796	8.88E-04	2.15E-03	-1.969	-1.23
Cpsf2	0.367	7.072	4.667	9.83E-05	2.97E-04	0.212	1.29
Cpt2	-0.428	6.454	-7.565	8.73E-08	5.42E-07	7.326	-1.35
Crkl	0.455	6.976	10.801	1.15E-10	1.63E-09	14.108	1.37
Crot	-0.373	6.877	-8.193	2.17E-08	1.62E-07	8.748	-1.30
Csda	0.344	7.938	4.875	5.81E-05	1.84E-04	0.739	1.27
Csda	0.420	10.028	8.225	2.02E-08	1.53E-07	8.818	1.34
Cse1l	-0.794	7.389	-15.049	1.14E-13	4.12E-12	21.192	-1.73
Csnk1d	0.429	7.166	7.376	1.34E-07	7.94E-07	6.887	1.35
Csnk1e	0.347	6.379	6.807	5.03E-07	2.59E-06	5.540	1.27
Csnk1e	0.472	6.600	7.302	1.59E-07	9.27E-07	6.714	1.39
Csnk2a1	1.151	6.763	10.459	2.19E-10	2.78E-09	13.453	2.22
Csnk2a2	0.425	7.248	6.140	2.49E-06	1.09E-05	3.918	1.34
Ctage5	-0.311	8.036	-5.354	1.74E-05	6.29E-05	1.952	-1.24
Ctla2b	-1.054	7.178	-12.318	7.96E-12	1.60E-10	16.848	-2.08
Ctsf	-0.364	6.525	-4.893	5.55E-05	1.77E-04	0.785	-1.29
Ctsh	-0.372	6.886	-6.186	2.22E-06	9.84E-06	4.031	-1.29
Ctsz	-0.669	9.672	-14.595	2.21E-13	7.14E-12	20.516	-1.59
Cul5	0.346	7.214	6.938	3.70E-07	1.96E-06	5.854	1.27
Cxcl1	-1.818	6.634	-21.246	5.34E-17	7.99E-15	28.978	-3.53
Cyb5r1	0.700	6.816	10.911	9.44E-11	1.37E-09	14.314	1.62
Cyp51	-0.432	9.012	-7.613	7.84E-08	4.94E-07	7.435	-1.35
Cyp51	-0.379	9.644	-7.049	2.85E-07	1.56E-06	6.119	-1.30
D10Jhu81e	-0.497	6.385	-7.783	5.36E-08	3.49E-07	7.824	-1.41
D15Ertd621e	0.403	7.356	9.568	1.23E-09	1.29E-08	11.681	1.32
D17Wsu104e	-0.553	6.741	-8.226	2.02E-08	1.53E-07	8.821	-1.47
D230049E03Rik	0.290	7.384	5.416	1.49E-05	5.43E-05	2.109	1.22
D2Ertd63e	-0.372	8.866	-8.609	8.88E-09	7.51E-08	9.661	-1.29
D2Ertd93e	-0.321	7.851	-3.340	2.75E-03	5.93E-03	-3.071	-1.25
D2Wsu85e	-0.310	6.977	-4.552	1.32E-04	3.86E-04	-0.078	-1.24
D4Ucla1	0.515	9.740	15.043	1.15E-13	4.12E-12	21.183	1.43
D4Ucla1	0.537	8.647	13.528	1.12E-12	2.77E-11	18.858	1.45
D5Ertd579e	-0.374	7.211	-9.309	2.08E-09	2.06E-08	11.147	-1.30
D6Wsu116e	-0.278	6.601	-5.225	2.40E-05	8.28E-05	1.628	-1.21
D830024F11Rik	-0.336	7.236	-5.314	1.92E-05	6.80E-05	1.852	-1.26
D9Ertd815e	0.490	6.918	9.292	2.15E-09	2.11E-08	11.112	1.40
Dag1	-0.467	7.183	-7.052	2.83E-07	1.55E-06	6.126	-1.38
Dag1	-0.429	8.711	-6.620	7.84E-07	3.81E-06	5.090	-1.35
Dars	0.374	9.109	6.198	2.16E-06	9.62E-06	4.061	1.30
Dcaf13	0.344	7.411	8.156	2.35E-08	1.73E-07	8.664	1.27
Dcakd	-0.299	6.772	-3.840	7.96E-04	1.95E-03	-1.862	-1.23
Dctpp1	-0.339	7.125	-5.532	1.11E-05	4.17E-05	2.402	-1.26
Ddhd2	0.554	7.529	12.308	8.11E-12	1.62E-10	16.829	1.47
Ddit3	0.722	6.496	7.564	8.75E-08	5.42E-07	7.323	1.65
Ddr2	0.601	6.558	2.654	1.39E-02	2.50E-02	-4.621	1.52
Ddx18	0.459	7.916	10.782	1.20E-10	1.67E-09	14.072	1.37
Ddx27	-0.431	6.735	-5.485	1.25E-05	4.64E-05	2.284	-1.35
Ddx39	0.467	7.395	7.303	1.59E-07	9.26E-07	6.717	1.38
Ddx5	-0.267	9.635	-3.259	3.35E-03	7.06E-03	-3.263	-1.20
Ddx54	-0.522	7.085	-7.410	1.24E-07	7.44E-07	6.965	-1.44

Degs1	0.437	8.459	5.857	4.98E-06	2.06E-05	3.215	1.35
Degs1	0.527	6.778	11.928	1.55E-11	2.75E-10	16.166	1.44
Dennd4a	0.841	6.236	16.863	9.45E-15	4.85E-13	23.730	1.79
Derl1	0.549	7.959	7.676	6.81E-08	4.35E-07	7.580	1.46
Dhrs7	-0.609	7.060	-14.760	1.73E-13	5.75E-12	20.765	-1.53
Dlst	0.393	6.415	5.843	5.15E-06	2.11E-05	3.180	1.31
Dnaja2	-0.315	6.750	-4.598	1.17E-04	3.48E-04	0.038	-1.24
Dnajb2	0.437	6.280	4.121	3.93E-04	1.04E-03	-1.165	1.35
Dnajb6	0.447	6.727	4.743	8.11E-05	2.50E-04	0.405	1.36
Dnajc15	0.308	7.484	5.637	8.57E-06	3.32E-05	2.665	1.24
DNAJC8	0.322	7.300	6.204	2.13E-06	9.50E-06	4.075	1.25
Dock9	-0.576	6.569	-8.898	4.84E-09	4.37E-08	10.283	-1.49
Dpf2	0.358	6.623	5.778	6.04E-06	2.43E-05	3.018	1.28
Dpp3	-0.618	6.723	-8.644	8.24E-09	6.99E-08	9.737	-1.53
Dr1	0.286	8.097	3.291	3.10E-03	6.57E-03	-3.187	1.22
Drg1	0.594	6.182	3.459	2.05E-03	4.57E-03	-2.788	1.51
Dus1l	-0.428	7.044	-8.329	1.62E-08	1.25E-07	9.049	-1.35
Dusp1	-0.526	6.221	-8.148	2.39E-08	1.76E-07	8.647	-1.44
Dusp12	0.520	6.981	14.474	2.64E-13	8.25E-12	20.333	1.43
Dusp3	0.399	5.641	4.336	2.28E-04	6.39E-04	-0.624	1.32
Dync1h1	-0.506	6.945	-5.722	6.95E-06	2.76E-05	2.878	-1.42
E130203B14Rik	-0.717	8.135	-8.789	6.08E-09	5.33E-08	10.048	-1.64
E330037I15Rik	0.308	8.259	4.703	8.98E-05	2.74E-04	0.303	1.24
Ebp	-1.112	5.933	-18.438	1.30E-15	9.34E-14	25.746	-2.16
Ect2	0.346	7.232	3.578	1.53E-03	3.48E-03	-2.503	1.27
Edem3	0.881	7.296	14.507	2.51E-13	7.92E-12	20.383	1.84
Efemp2	-0.553	6.616	-6.619	7.84E-07	3.81E-06	5.089	-1.47
Ehd4	-0.627	6.254	-17.336	5.12E-15	2.88E-13	24.353	-1.54
Eif1	0.375	9.538	8.092	2.71E-08	1.95E-07	8.521	1.30
Eif1ad	-0.382	7.437	-9.820	7.49E-10	8.43E-09	12.193	-1.30
Eif3d	0.394	9.026	6.291	1.73E-06	7.92E-06	4.288	1.31
Eif3d	0.438	7.558	3.748	1.00E-03	2.39E-03	-2.088	1.36
Eif3g	0.264	9.161	5.164	2.80E-05	9.51E-05	1.473	1.20
Eif4a1	-0.747	8.429	-4.323	2.35E-04	6.56E-04	-0.656	-1.68
Eif4a1	-0.699	9.235	-16.305	1.98E-14	8.90E-13	22.975	-1.62
Eif4b	-0.301	6.944	-2.614	1.53E-02	2.72E-02	-4.707	-1.23
Eif4b	-0.274	7.807	-5.588	9.69E-06	3.70E-05	2.541	-1.21
Eif4e2	-0.315	7.044	-8.402	1.38E-08	1.10E-07	9.209	-1.24
Eif4g2	-0.344	8.282	-6.406	1.31E-06	6.12E-06	4.570	-1.27
Eif5a	0.503	6.847	10.387	2.51E-10	3.09E-09	13.314	1.42
Eif5a	0.677	9.394	5.480	1.27E-05	4.69E-05	2.271	1.60
Eif6	0.528	8.152	6.300	1.69E-06	7.77E-06	4.311	1.44
Elk3	-0.494	7.108	-6.155	2.40E-06	1.05E-05	3.954	-1.41
Elovl1	-0.337	6.439	-6.501	1.04E-06	4.96E-06	4.802	-1.26
Emb	0.474	8.004	13.229	1.79E-12	4.13E-11	18.375	1.39
Enah	0.687	8.200	12.642	4.64E-12	9.85E-11	17.400	1.61
Enah	0.700	7.328	18.392	1.38E-15	9.69E-14	25.689	1.62
Endod1	-0.341	6.475	-5.572	1.01E-05	3.83E-05	2.501	-1.27
Eng	0.320	7.236	2.738	1.15E-02	2.10E-02	-4.441	1.25
Eprs	0.328	8.814	8.827	5.61E-09	4.94E-08	10.131	1.26
Eprs	0.348	6.781	2.807	9.80E-03	1.82E-02	-4.290	1.27
Erap1	-0.406	6.611	-8.598	9.10E-09	7.67E-08	9.637	-1.32
Ercc1	-0.325	6.529	-4.257	2.78E-04	7.63E-04	-0.824	-1.25
Ergic3	-0.565	7.853	-11.269	4.93E-11	7.53E-10	14.981	-1.48
Ergic3	-0.555	7.529	-5.882	4.67E-06	1.94E-05	3.278	-1.47
Erlin1	0.524	6.942	10.455	2.20E-10	2.78E-09	13.446	1.44
Ermp1	-0.301	7.114	-4.832	6.47E-05	2.03E-04	0.631	-1.23
Ern1	0.498	6.120	7.995	3.35E-08	2.32E-07	8.303	1.41
Erp29	-0.795	7.835	-14.334	3.25E-13	9.73E-12	20.120	-1.74
Erp29	-0.675	6.775	-8.054	2.94E-08	2.08E-07	8.437	-1.60
Esd	-0.710	8.915	-17.231	5.87E-15	3.19E-13	24.215	-1.64
Esyt2	-0.493	7.084	-10.458	2.19E-10	2.78E-09	13.451	-1.41
Ethe1	0.411	7.237	5.892	4.57E-06	1.90E-05	3.302	1.33
EWSR1	0.446	7.376	12.138	1.08E-11	2.02E-10	16.534	1.36
Exosc1	0.657	7.548	8.823	5.65E-09	4.97E-08	10.123	1.58
F2r	-0.316	8.207	-3.501	1.85E-03	4.16E-03	-2.689	-1.24
Faf1	0.518	6.458	3.724	1.06E-03	2.51E-03	-2.148	1.43
Fam100b	0.586	6.914	8.231	2.00E-08	1.52E-07	8.831	1.50
Fam107b	0.563	6.765	10.975	8.40E-11	1.22E-09	14.434	1.48
Fam113a	-0.539	6.737	-8.394	1.40E-08	1.11E-07	9.192	-1.45
Fam125b	0.737	6.716	14.275	3.55E-13	1.05E-11	20.029	1.67
Fam129c	0.323	7.400	4.050	4.70E-04	1.22E-03	-1.342	1.25
Fam20b	0.550	6.715	11.701	2.30E-11	3.87E-10	15.763	1.46
Fam32a	0.271	7.017	5.543	1.08E-05	4.08E-05	2.429	1.21
Fam40a	0.282	6.463	5.904	4.43E-06	1.85E-05	3.333	1.22
Fam54b	0.328	6.657	4.743	8.12E-05	2.50E-04	0.404	1.25
Fam82a2	0.423	6.323	10.138	4.03E-10	4.68E-09	12.826	1.34
Fam92a	0.348	6.361	6.244	1.93E-06	8.76E-06	4.173	1.27
Fam98a	-0.502	6.939	-12.132	1.09E-11	2.03E-10	16.525	-1.42

Fasn	-0.755	6.330	-7.557	8.89E-08	5.47E-07	7.307	-1.69
Fbxo28	0.998	7.050	19.415	4.09E-16	3.97E-14	26.919	2.00
Fbxo6	0.332	7.635	3.991	5.45E-04	1.39E-03	-1.489	1.26
Fbxo8	1.334	6.542	26.968	2.26E-19	8.21E-17	34.448	2.52
Fbxw11	0.306	7.396	5.756	6.38E-06	2.56E-05	2.964	1.24
Fcgrt	0.549	6.491	8.030	3.10E-08	2.16E-07	8.382	1.46
Fdft1	-0.710	6.917	-15.959	3.17E-14	1.32E-12	22.496	-1.64
Fdps	-0.670	8.124	-11.443	3.61E-11	5.72E-10	15.299	-1.59
Fem1b	0.585	7.723	11.615	2.67E-11	4.43E-10	15.610	1.50
Fh1	0.618	8.282	12.126	1.10E-11	2.04E-10	16.515	1.54
Fhl1	1.129	7.505	25.861	5.94E-19	1.94E-16	33.487	2.19
Fhl2	-0.290	6.258	-6.595	8.30E-07	4.02E-06	5.031	-1.22
Fkbp3	-0.371	9.016	-11.461	3.50E-11	5.59E-10	15.330	-1.29
Flnb	-0.398	6.896	-7.393	1.29E-07	7.72E-07	6.925	-1.32
Fmc1	0.439	9.383	5.963	3.83E-06	1.61E-05	3.479	1.36
FMNL2	0.363	7.362	5.504	1.19E-05	4.46E-05	2.330	1.29
Foxj2	0.608	5.870	5.841	5.18E-06	2.12E-05	3.175	1.52
Foxj3	0.504	7.777	9.109	3.13E-09	2.91E-08	10.729	1.42
Fry	-0.561	7.685	-8.043	3.01E-08	2.12E-07	8.412	-1.48
Gabarap	0.427	9.365	9.214	2.52E-09	2.42E-08	10.950	1.34
Gadd45a	0.420	8.855	6.291	1.73E-06	7.92E-06	4.288	1.34
Gadd45b	-1.067	6.746	-15.699	4.54E-14	1.81E-12	22.131	-2.09
Galnt7	0.867	7.466	8.547	1.01E-08	8.32E-08	9.525	1.82
Gamt	-0.397	6.280	-4.942	4.90E-05	1.58E-04	0.910	-1.32
Gapvd1	0.341	6.971	5.037	3.86E-05	1.28E-04	1.150	1.27
Gas1	-0.415	6.643	-4.388	1.99E-04	5.66E-04	-0.492	-1.33
Gclm	-0.437	9.225	-8.395	1.40E-08	1.11E-07	9.194	-1.35
Gemin4	-0.326	6.950	-3.715	1.09E-03	2.56E-03	-2.169	-1.25
Gga2	-0.394	8.255	-10.366	2.61E-10	3.20E-09	13.273	-1.31
Gga2	-0.345	6.153	-4.355	2.17E-04	6.12E-04	-0.577	-1.27
Gla	-0.283	6.688	-5.193	2.60E-05	8.90E-05	1.545	-1.22
Glrx	-0.336	7.177	-6.706	6.39E-07	3.18E-06	5.298	-1.26
Glul	0.481	6.564	7.258	1.76E-07	1.01E-06	6.612	1.40
Gm10031	1.031	6.970	19.898	2.35E-16	2.64E-14	27.479	2.04
Gm10079	0.282	11.797	7.944	3.75E-08	2.53E-07	8.189	1.22
Gm10094	0.333	8.656	5.845	5.12E-06	2.11E-05	3.185	1.26
Gm10725	-0.385	6.956	-7.014	3.10E-07	1.68E-06	6.034	-1.31
Gm10845	-0.413	8.522	-4.235	2.94E-04	7.99E-04	-0.878	-1.33
Gm12231	0.294	8.659	4.404	1.91E-04	5.45E-04	-0.452	1.23
Gm13552	0.289	7.064	3.654	1.27E-03	2.94E-03	-2.318	1.22
Gm13910	-1.033	8.394	-20.981	7.10E-17	9.81E-15	28.690	-2.05
Gm14121	0.485	8.357	7.709	6.32E-08	4.05E-07	7.656	1.40
Gm2260	-0.634	8.341	-10.161	3.86E-10	4.51E-09	12.871	-1.55
Gm3650	0.386	6.961	3.747	1.01E-03	2.40E-03	-2.091	1.31
Gm3650	0.543	7.214	10.503	2.02E-10	2.60E-09	13.537	1.46
Gm5578	0.809	6.726	15.109	1.04E-13	3.83E-12	21.279	1.75
Gm6206	0.667	7.911	11.854	1.76E-11	3.08E-10	16.036	1.59
Gm6304	-0.285	6.832	-4.472	1.61E-04	4.66E-04	-0.282	-1.22
Gm6969	0.555	7.153	5.832	5.30E-06	2.16E-05	3.152	1.47
Gm70	-0.416	7.750	-9.005	3.88E-09	3.57E-08	10.509	-1.33
Gm70	-0.294	7.287	-2.673	1.33E-02	2.40E-02	-4.580	-1.23
Gm7859	0.449	6.920	12.640	4.66E-12	9.85E-11	17.396	1.37
Gm8730	0.269	11.286	3.093	5.00E-03	1.00E-02	-3.648	1.20
Gm9178	0.381	6.970	6.402	1.32E-06	6.17E-06	4.560	1.30
Gm9905	-0.517	6.724	-8.536	1.04E-08	8.48E-08	9.503	-1.43
Gm9993	0.391	8.157	7.551	9.02E-08	5.54E-07	7.292	1.31
Gmfb	0.491	7.391	3.411	2.31E-03	5.09E-03	-2.904	1.41
Gmfb	0.604	8.592	10.559	1.81E-10	2.38E-09	13.646	1.52
Gnai1	0.537	7.278	8.125	2.52E-08	1.84E-07	8.596	1.45
Gnai2	-0.433	6.846	-5.619	8.96E-06	3.45E-05	2.620	-1.35
Gnpat	-0.414	6.582	-7.558	8.89E-08	5.47E-07	7.308	-1.33
GNPTAB	-0.355	7.124	-6.647	7.34E-07	3.60E-06	5.157	-1.28
Gpc6	-1.331	3.584	-15.888	3.49E-14	1.44E-12	22.398	-2.52
Gpd2	-0.536	6.879	-7.037	2.94E-07	1.61E-06	6.089	-1.45
Gpnmb	1.955	5.966	31.010	8.85E-21	4.54E-18	37.642	3.88
Gpr124	0.327	7.400	6.876	4.28E-07	2.24E-06	5.705	1.25
Gpr56	1.280	6.194	16.295	2.01E-14	8.91E-13	22.961	2.43
Gpsm2	-0.400	6.902	-6.057	3.05E-06	1.31E-05	3.712	-1.32
Gpx1	-0.549	7.884	-7.896	4.16E-08	2.77E-07	8.082	-1.46
Gpx4	-0.994	10.579	-19.026	6.45E-16	5.65E-14	26.458	-1.99
Grsf1	0.274	7.133	5.342	1.79E-05	6.42E-05	1.923	1.21
Gsr	-0.303	6.891	-3.373	2.54E-03	5.53E-03	-2.994	-1.23
Gss	-0.412	7.023	-7.579	8.47E-08	5.27E-07	7.357	-1.33
Gss	-0.390	8.655	-8.348	1.55E-08	1.21E-07	9.091	-1.31
Gss	-0.360	6.247	-3.904	6.77E-04	1.69E-03	-1.703	-1.28
Gss	-0.293	6.709	-4.429	1.80E-04	5.15E-04	-0.389	-1.22
Gsta4	0.395	8.552	7.832	4.80E-08	3.14E-07	7.937	1.31
Gstm1	-0.271	9.649	-3.918	6.55E-04	1.64E-03	-1.670	-1.21
Gstm5	-0.410	10.418	-7.161	2.20E-07	1.24E-06	6.384	-1.33

Gstm6	-0.418	7.960	-5.670	7.90E-06	3.08E-05	2.747	-1.34
Gstm7	-0.773	8.876	-11.124	6.40E-11	9.54E-10	14.713	-1.71
Gt(ROSA)26Sor	0.458	6.526	7.921	3.94E-08	2.65E-07	8.138	1.37
GTF2A1	0.320	6.434	4.731	8.37E-05	2.56E-04	0.374	1.25
Gtf2a2	0.308	8.193	8.562	9.82E-09	8.14E-08	9.559	1.24
Gtf2i	0.370	6.845	7.854	4.57E-08	3.00E-07	7.986	1.29
Gtl3	0.290	7.631	3.066	5.33E-03	1.06E-02	-3.710	1.22
Gtl3	0.382	7.181	2.851	8.85E-03	1.67E-02	-4.193	1.30
Guk1	0.302	7.485	6.033	3.23E-06	1.38E-05	3.654	1.23
Gulp1	0.468	6.746	5.402	1.54E-05	5.62E-05	2.073	1.38
Gusb	-0.533	6.935	-10.604	1.67E-10	2.22E-09	13.733	-1.45
Gusb	-0.516	7.029	-8.760	6.45E-09	5.62E-08	9.988	-1.43
Gypc	-0.409	7.466	-7.032	2.97E-07	1.62E-06	6.078	-1.33
H1f0	0.566	8.525	8.983	4.05E-09	3.70E-08	10.464	1.48
H2-T23	-0.582	6.932	-6.978	3.37E-07	1.81E-06	5.949	-1.50
H3f3a	0.441	10.382	10.815	1.13E-10	1.59E-09	14.134	1.36
H3f3a	0.631	10.194	8.322	1.64E-08	1.27E-07	9.034	1.55
H3f3b	0.288	10.004	5.808	5.62E-06	2.28E-05	3.092	1.22
H3f3b	0.350	9.428	8.269	1.84E-08	1.41E-07	8.916	1.27
Hadh	-0.286	7.777	-3.960	5.89E-04	1.49E-03	-1.565	-1.22
Hadha	-0.681	7.157	-5.608	9.22E-06	3.54E-05	2.592	-1.60
Hadha	-0.602	7.683	-8.110	2.60E-08	1.88E-07	8.562	-1.52
Hars	-0.411	6.414	-5.984	3.64E-06	1.54E-05	3.532	-1.33
Hat1	-0.359	6.594	-5.135	3.01E-05	1.01E-04	1.398	-1.28
Hax1	0.268	7.314	5.326	1.86E-05	6.65E-05	1.882	1.20
Hbp1	-0.298	6.407	-2.870	8.46E-03	1.60E-02	-4.151	-1.23
Hbs1l	0.280	6.390	5.966	3.81E-06	1.61E-05	3.485	1.21
Hbxip	0.480	8.226	9.138	2.95E-09	2.77E-08	10.791	1.39
Hdgf	0.281	6.281	3.472	1.99E-03	4.45E-03	-2.758	1.21
Heg1	-0.469	8.653	-7.584	8.37E-08	5.24E-07	7.369	-1.38
Herpud1	-0.509	6.811	-11.480	3.38E-11	5.45E-10	15.366	-1.42
Hint1	0.379	9.534	8.785	6.13E-09	5.36E-08	10.040	1.30
Hip1	-0.415	6.987	-8.227	2.02E-08	1.53E-07	8.822	-1.33
Hirip3	-0.351	6.715	-7.379	1.33E-07	7.91E-07	6.893	-1.28
Hmbs	-0.442	7.118	-9.456	1.54E-09	1.57E-08	11.452	-1.36
HMGCR	-1.004	6.572	-14.971	1.27E-13	4.48E-12	21.078	-2.00
Hmgcs1	-0.988	6.887	-17.916	2.47E-15	1.58E-13	25.096	-1.98
Hmox1	-0.788	7.105	-9.121	3.05E-09	2.85E-08	10.756	-1.73
Hnrpdl	0.297	6.768	4.536	1.37E-04	4.01E-04	-0.120	1.23
Hoxd9	0.284	5.751	2.653	1.40E-02	2.50E-02	-4.623	1.22
Hsd17b12	-0.296	7.796	-6.196	2.17E-06	9.67E-06	4.055	-1.23
Hsp90b1	-0.481	8.375	-2.514	1.91E-02	3.31E-02	-4.915	-1.40
Hspb1	-0.419	9.142	-6.437	1.21E-06	5.70E-06	4.647	-1.34
Iars2	0.269	7.066	6.795	5.17E-07	2.65E-06	5.513	1.20
Iars2	0.347	7.976	9.291	2.15E-09	2.11E-08	11.111	1.27
Idh1	-1.066	6.178	-4.738	8.21E-05	2.52E-04	0.392	-2.09
IDH1	-1.038	8.313	-13.790	7.43E-13	1.95E-11	19.275	-2.05
Idi1	-1.160	6.124	-19.434	4.00E-16	3.97E-14	26.941	-2.23
Ifngr2	-0.266	6.377	-5.826	5.37E-06	2.19E-05	3.137	-1.20
Ifrd1	0.761	9.381	11.162	5.97E-11	9.02E-10	14.783	1.69
Ifrd1	1.074	6.993	5.575	9.99E-06	3.81E-05	2.510	2.11
Ifrd2	0.267	6.584	4.036	4.86E-04	1.26E-03	-1.377	1.20
Ift52	-0.440	7.441	-12.257	8.83E-12	1.72E-10	16.741	-1.36
IGFBP4	-0.585	6.718	-5.589	9.65E-06	3.69E-05	2.545	-1.50
Ildr2	1.057	6.552	20.616	1.06E-16	1.35E-14	28.288	2.08
IMMP1L	0.515	6.798	7.767	5.55E-08	3.60E-07	7.789	1.43
Insig1	-1.033	8.024	-21.127	6.07E-17	8.72E-15	28.848	-2.05
Insig2	0.592	6.483	12.058	1.24E-11	2.22E-10	16.395	1.51
Irak2	-1.429	6.582	-20.053	1.98E-16	2.29E-14	27.656	-2.69
Irs1	0.500	6.303	7.571	8.62E-08	5.36E-07	7.339	1.41
Itgb1bp1	0.276	7.656	7.002	3.19E-07	1.72E-06	6.006	1.21
Itgb5	-0.587	8.275	-5.980	3.68E-06	1.56E-05	3.520	-1.50
Itgb5	-0.539	7.557	-6.041	3.17E-06	1.36E-05	3.671	-1.45
Itih5	-0.695	6.180	-6.261	1.85E-06	8.45E-06	4.215	-1.62
Jak2	0.427	6.679	5.957	3.89E-06	1.64E-05	3.465	1.34
Jun	0.371	7.869	5.225	2.40E-05	8.28E-05	1.628	1.29
Kcmf1	0.507	8.700	11.745	2.12E-11	3.60E-10	15.842	1.42
Kdelr3	-0.266	6.846	-3.982	5.57E-04	1.42E-03	-1.510	-1.20
KIAA0174	0.376	7.533	8.739	6.74E-09	5.83E-08	9.943	1.30
Kifap3	0.518	6.899	8.057	2.92E-08	2.07E-07	8.444	1.43
Klc1	-0.692	7.277	-12.544	5.46E-12	1.13E-10	17.233	-1.62
Klf6	0.373	6.300	4.811	6.82E-05	2.14E-04	0.578	1.29
Klhdc10	0.523	5.617	3.879	7.22E-04	1.79E-03	-1.766	1.44
Kpna4	0.996	7.353	18.079	2.02E-15	1.37E-13	25.301	1.99
Kpna6	0.300	6.350	4.333	2.29E-04	6.42E-04	-0.631	1.23
Kpnb1	0.322	8.159	5.828	5.34E-06	2.18E-05	3.144	1.25
Krr1	0.293	6.347	5.349	1.76E-05	6.34E-05	1.941	1.22
Lamp2	-0.306	6.487	-4.731	8.36E-05	2.56E-04	0.375	-1.24
Lamp3	-0.651	7.710	-12.222	9.36E-12	1.80E-10	16.681	-1.57

Larp1	0.462	7.726	7.588	8.29E-08	5.20E-07	7.379	1.38
Lasp1	-0.326	6.790	-7.459	1.11E-07	6.71E-07	7.080	-1.25
Layn	-1.261	6.973	-17.419	4.61E-15	2.67E-13	24.461	-2.40
LBR	0.642	7.626	14.926	1.36E-13	4.74E-12	21.011	1.56
Ldlr	-0.603	6.729	-9.623	1.11E-09	1.17E-08	11.793	-1.52
Lemd2	0.305	6.690	5.063	3.61E-05	1.20E-04	1.216	1.24
Lemd3	0.313	7.077	3.292	3.09E-03	6.57E-03	-3.186	1.24
Leprotl1	0.332	7.192	8.183	2.22E-08	1.65E-07	8.724	1.26
Lgals1	-0.656	10.053	-7.820	4.93E-08	3.22E-07	7.908	-1.58
Lgals3	-0.452	10.366	-7.174	2.14E-07	1.21E-06	6.413	-1.37
Lgtn	0.531	7.503	13.198	1.88E-12	4.28E-11	18.324	1.44
Lims1	0.317	7.351	2.473	2.09E-02	3.61E-02	-5.000	1.25
LOC100043810	-0.659	7.540	-7.946	3.73E-08	2.53E-07	8.193	-1.58
LOC100131826	-0.711	7.320	-8.358	1.52E-08	1.19E-07	9.112	-1.64
LOC294154	0.503	5.851	4.043	4.78E-04	1.24E-03	-1.359	1.42
LOC434391	-0.327	7.088	-6.489	1.07E-06	5.09E-06	4.774	-1.25
LOC544737	0.571	7.040	7.958	3.63E-08	2.49E-07	8.221	1.49
LOC547349	0.290	7.669	3.697	1.14E-03	2.66E-03	-2.213	1.22
LOC553150	0.289	8.437	4.791	7.18E-05	2.24E-04	0.527	1.22
LOC624853	-0.468	9.491	-3.524	1.75E-03	3.94E-03	-2.634	-1.38
LOC624853	-0.367	10.561	-7.649	7.24E-08	4.60E-07	7.518	-1.29
LOC684894	0.359	8.353	6.345	1.51E-06	7.02E-06	4.422	1.28
LOC685953	1.972	9.130	18.899	7.48E-16	6.11E-14	26.307	3.92
LOC75771	0.283	6.778	6.692	6.60E-07	3.27E-06	5.265	1.22
LOC768253	0.288	6.480	5.784	5.96E-06	2.41E-05	3.033	1.22
Lpcat3	-0.902	6.684	-9.763	8.38E-10	9.30E-09	12.077	-1.87
Lpcat3	-0.803	6.785	-13.883	6.44E-13	1.75E-11	19.422	-1.74
Lpp	0.298	6.385	3.368	2.57E-03	5.60E-03	-3.007	1.23
Lpp	0.399	7.853	7.173	2.14E-07	1.21E-06	6.412	1.32
Lrig1	-1.035	8.856	-10.614	1.63E-10	2.19E-09	13.752	-2.05
Lrp10	-0.977	7.803	-13.668	8.98E-13	2.29E-11	19.081	-1.97
Lrpap1	-0.324	7.109	-5.063	3.61E-05	1.20E-04	1.216	-1.25
Lrrc59	0.459	8.298	9.717	9.17E-10	1.00E-08	11.985	1.37
Lrrc8d	-0.632	6.697	-7.539	9.26E-08	5.68E-07	7.265	-1.55
Lrrfip1	0.714	6.403	8.408	1.36E-08	1.09E-07	9.223	1.64
Lsm3	-0.379	7.307	-6.844	4.61E-07	2.40E-06	5.629	-1.30
Lta4h	-0.267	7.861	-5.663	8.03E-06	3.13E-05	2.730	-1.20
Ly6e	-1.055	7.105	-14.154	4.26E-13	1.25E-11	19.843	-2.08
Lyplal1	0.635	7.767	11.605	2.72E-11	4.50E-10	15.591	1.55
Maged1	-0.264	7.518	-4.335	2.28E-04	6.40E-04	-0.627	-1.20
Magoh	0.374	7.716	7.512	9.85E-08	6.00E-07	7.202	1.30
MAMDC2	-0.767	7.429	-6.639	7.48E-07	3.66E-06	5.138	-1.70
Man2a1	-0.439	6.504	-6.075	2.91E-06	1.26E-05	3.757	-1.36
Man2a1	-0.263	6.683	-3.576	1.54E-03	3.49E-03	-2.506	-1.20
Man2c1	-0.648	6.482	-8.739	6.75E-09	5.83E-08	9.941	-1.57
MAP2K1	0.273	8.294	5.315	1.92E-05	6.80E-05	1.854	1.21
Map4k3	0.498	7.727	9.386	1.78E-09	1.79E-08	11.306	1.41
MAPK1	-0.281	6.595	-5.353	1.74E-05	6.30E-05	1.950	-1.21
Mapkapk2	0.931	6.739	10.365	2.61E-10	3.20E-09	13.272	1.91
Mapkapk5	0.466	6.646	7.564	8.76E-08	5.42E-07	7.322	1.38
Mark3	-0.889	6.635	-19.494	3.73E-16	3.83E-14	27.011	-1.85
Mbnl2	0.290	8.281	6.810	5.00E-07	2.58E-06	5.548	1.22
Mcm6	0.285	7.043	4.285	2.59E-04	7.14E-04	-0.751	1.22
Mdh1	-0.354	9.418	-8.272	1.83E-08	1.40E-07	8.923	-1.28
Mdh1	-0.301	8.427	-2.379	2.57E-02	4.35E-02	-5.190	-1.23
Med24	-0.360	6.772	-5.221	2.43E-05	8.35E-05	1.616	-1.28
Memo1	0.289	7.534	7.052	2.83E-07	1.55E-06	6.126	1.22
METTL10	-0.273	8.998	-4.431	1.79E-04	5.13E-04	-0.385	-1.21
Mettl6	0.344	6.319	7.251	1.79E-07	1.03E-06	6.595	1.27
Mfn2	0.343	7.176	7.708	6.33E-08	4.06E-07	7.653	1.27
Mgst1	-0.365	11.519	-6.173	2.29E-06	1.01E-05	3.999	-1.29
Mgst1	-0.277	11.656	-6.743	5.85E-07	2.96E-06	5.388	-1.21
Mitf	-0.936	7.306	-13.083	2.26E-12	5.02E-11	18.135	-1.91
Mki67ip	0.606	7.731	15.682	4.65E-14	1.84E-12	22.106	1.52
Mmd	-1.180	6.497	-14.530	2.43E-13	7.79E-12	20.418	-2.27
Mmp9	-0.498	6.731	-7.881	4.31E-08	2.85E-07	8.047	-1.41
Mobkl1b	0.269	7.614	4.365	2.11E-04	5.97E-04	-0.551	1.20
Mobkl3	0.400	6.352	8.559	9.88E-09	8.18E-08	9.552	1.32
Mosc2	0.702	7.334	16.886	9.17E-15	4.77E-13	23.760	1.63
Mosc2	0.713	8.107	11.492	3.31E-11	5.36E-10	15.387	1.64
Mpst	-0.293	6.645	-5.089	3.38E-05	1.13E-04	1.281	-1.23
Mrpl1	0.268	7.634	5.360	1.71E-05	6.20E-05	1.967	1.20
Mrpl18	-0.334	7.610	-7.536	9.34E-08	5.72E-07	7.257	-1.26
Mrpl20	0.328	8.473	6.747	5.80E-07	2.95E-06	5.397	1.26
Mrpl34	-0.316	6.826	-4.302	2.48E-04	6.89E-04	-0.710	-1.24
Mrpl37	0.556	7.536	7.614	7.83E-08	4.94E-07	7.437	1.47
Mrpl37	0.582	6.243	4.175	3.42E-04	9.16E-04	-1.028	1.50
Mrpl41	-0.359	7.421	-6.938	3.70E-07	1.96E-06	5.854	-1.28
Mrpl53	0.395	6.945	5.851	5.05E-06	2.08E-05	3.199	1.31

Mrps10	0.481	6.973	5.868	4.84E-06	2.00E-05	3.243	1.40
Mrps14	0.780	7.595	21.807	2.95E-17	5.31E-15	29.574	1.72
Mrps18a	0.362	9.934	5.754	6.42E-06	2.57E-05	2.957	1.29
Mrps22	0.498	6.359	10.624	1.61E-10	2.16E-09	13.770	1.41
Mrps35	0.556	8.616	11.335	4.38E-11	6.81E-10	15.101	1.47
MSL1	0.337	6.484	4.789	7.21E-05	2.25E-04	0.522	1.26
Mta3	0.451	6.286	8.083	2.76E-08	1.97E-07	8.502	1.37
Mtdh	0.311	7.693	6.076	2.90E-06	1.26E-05	3.760	1.24
Mthfd1l	-0.346	7.267	-8.109	2.61E-08	1.89E-07	8.560	-1.27
Mthfd2l	-0.876	6.000	-13.727	8.19E-13	2.10E-11	19.175	-1.83
Myc	0.300	9.167	6.939	3.69E-07	1.96E-06	5.856	1.23
Myg1	0.808	6.223	11.589	2.79E-11	4.60E-10	15.562	1.75
Myof	0.358	9.190	6.174	2.29E-06	1.01E-05	4.001	1.28
N6amt2	-0.327	6.228	-5.482	1.26E-05	4.67E-05	2.276	-1.25
Nadk	0.268	7.568	6.090	2.81E-06	1.22E-05	3.793	1.20
Nap1l4	0.571	6.668	9.169	2.77E-09	2.63E-08	10.855	1.49
Ncam1	1.186	5.358	13.187	1.92E-12	4.33E-11	18.305	2.28
Ncapd3	-0.854	5.993	-12.109	1.13E-11	2.09E-10	16.485	-1.81
Nck2	-0.738	6.349	-9.323	2.02E-09	2.01E-08	11.176	-1.67
Ndc80	0.369	6.736	4.494	1.53E-04	4.42E-04	-0.226	1.29
Ndfip2	0.512	6.868	3.787	9.09E-04	2.19E-03	-1.992	1.43
Ndufa6	0.442	7.661	6.264	1.84E-06	8.41E-06	4.222	1.36
Ndufab1	0.635	6.612	10.977	8.36E-11	1.22E-09	14.439	1.55
Ndufaf2	0.423	7.178	7.775	5.45E-08	3.55E-07	7.806	1.34
Ndufb8	0.317	7.719	3.589	1.49E-03	3.40E-03	-2.476	1.25
Ndufb9	0.379	10.546	10.820	1.12E-10	1.59E-09	14.143	1.30
Ndufs2	0.789	7.740	18.776	8.66E-16	6.59E-14	26.159	1.73
Ndufs7	0.485	9.159	6.716	6.24E-07	3.12E-06	5.321	1.40
Ndufv1	-0.443	7.817	-8.440	1.27E-08	1.03E-07	9.293	-1.36
Neat1	-0.681	8.207	-10.395	2.47E-10	3.06E-09	13.330	-1.60
Neil3	0.292	7.798	4.130	3.83E-04	1.02E-03	-1.141	1.22
Nek2	0.775	8.036	11.777	2.01E-11	3.44E-10	15.898	1.71
Nek7	0.517	9.354	9.574	1.22E-09	1.29E-08	11.693	1.43
Neu1	-0.367	6.374	-6.711	6.30E-07	3.15E-06	5.311	-1.29
Neurl3	0.357	6.664	5.139	2.98E-05	1.01E-04	1.408	1.28
Neurl3	0.487	6.003	3.570	1.56E-03	3.54E-03	-2.522	1.40
Nfib	-0.440	6.638	-5.274	2.12E-05	7.41E-05	1.752	-1.36
Nfic	-0.269	6.666	-3.790	9.03E-04	2.18E-03	-1.986	-1.20
Nfkbia	-1.086	8.243	-17.539	3.96E-15	2.37E-13	24.615	-2.12
Nfkbia	-1.015	8.519	-14.423	2.84E-13	8.74E-12	20.256	-2.02
Nfs1	-0.752	7.421	-17.256	5.67E-15	3.14E-13	24.249	-1.68
Ngdn	0.513	7.269	10.363	2.62E-10	3.21E-09	13.267	1.43
Ngfrap1	-0.492	8.673	-12.510	5.78E-12	1.19E-10	17.176	-1.41
Ngrn	0.392	7.748	10.421	2.35E-10	2.93E-09	13.379	1.31
Nid1	-0.531	8.249	-7.359	1.40E-07	8.25E-07	6.847	-1.44
Nid1	-0.372	8.359	-3.828	8.20E-04	2.00E-03	-1.892	-1.29
Ninj1	-0.446	6.177	-7.023	3.03E-07	1.65E-06	6.057	-1.36
Nit1	-0.350	6.656	-4.428	1.80E-04	5.17E-04	-0.393	-1.27
Nme7	0.471	7.081	8.223	2.04E-08	1.53E-07	8.813	1.39
Nop58	0.352	8.700	4.348	2.21E-04	6.21E-04	-0.594	1.28
Nploc4	0.272	6.036	3.150	4.36E-03	8.89E-03	-3.518	1.21
Nr1h3	-1.088	6.661	-12.853	3.28E-12	7.15E-11	17.754	-2.13
Nr2c1	0.547	6.434	10.753	1.26E-10	1.75E-09	14.016	1.46
Nsmce2	0.442	8.368	6.658	7.16E-07	3.52E-06	5.182	1.36
Nucb2	-0.808	7.514	-15.295	8.01E-14	3.03E-12	21.551	-1.75
Nucks1	0.528	6.595	7.136	2.33E-07	1.31E-06	6.325	1.44
Nudc	0.346	8.891	5.458	1.34E-05	4.93E-05	2.215	1.27
Nudt3	0.500	6.423	5.914	4.33E-06	1.81E-05	3.357	1.41
Nudt5	0.335	7.630	6.220	2.05E-06	9.23E-06	4.114	1.26
NUP205	-0.264	6.845	-5.985	3.64E-06	1.54E-05	3.533	-1.20
Nusap1	0.390	6.996	4.891	5.57E-05	1.78E-04	0.781	1.31
Nvl	0.873	6.210	16.258	2.11E-14	9.24E-13	22.912	1.83
Oaf	-0.268	7.439	-5.785	5.94E-06	2.40E-05	3.035	-1.20
Oaz1	-0.918	7.862	-12.108	1.14E-11	2.09E-10	16.482	-1.89
Oaz1	-0.811	7.602	-14.807	1.62E-13	5.43E-12	20.834	-1.75
Oaz2	-0.772	6.880	-13.345	1.49E-12	3.59E-11	18.563	-1.71
Ormdl1	0.343	6.318	7.746	5.82E-08	3.76E-07	7.739	1.27
Osbpl9	-0.403	6.512	-3.952	6.01E-04	1.51E-03	-1.586	-1.32
Osmr	-0.722	7.659	-11.273	4.89E-11	7.51E-10	14.987	-1.65
Ostf1	-0.269	9.588	-7.485	1.05E-07	6.34E-07	7.141	-1.20
Oxr1	-0.349	6.713	-5.004	4.19E-05	1.37E-04	1.066	-1.27
Pabpc4	0.372	7.915	5.027	3.95E-05	1.30E-04	1.126	1.29
Pabpn1	-0.306	8.440	-6.214	2.08E-06	9.31E-06	4.101	-1.24
Pacsin2	0.282	6.564	4.880	5.73E-05	1.82E-04	0.753	1.22
Palld	-0.931	7.134	-18.998	6.66E-16	5.70E-14	26.425	-1.91
Papd7	0.500	6.772	6.825	4.82E-07	2.49E-06	5.584	1.41
Papola	-0.965	8.682	-9.460	1.53E-09	1.57E-08	11.460	-1.95
Papola	-0.850	8.026	-18.014	2.19E-15	1.43E-13	25.219	-1.80
Papola	-0.812	6.381	-6.740	5.90E-07	2.98E-06	5.379	-1.76

PAPSS1	-0.457	6.702	-7.005	3.16E-07	1.71E-06	6.015	-1.37
Paqr3	0.330	6.379	5.012	4.11E-05	1.35E-04	1.086	1.26
Parp1	0.644	6.304	10.561	1.81E-10	2.38E-09	13.650	1.56
Parp3	-0.929	6.322	-15.347	7.43E-14	2.87E-12	21.627	-1.90
Pbk	0.286	6.261	3.267	3.28E-03	6.93E-03	-3.244	1.22
Pck2	-0.711	6.594	-10.796	1.17E-10	1.64E-09	14.098	-1.64
Pcolce	-0.595	8.526	-7.890	4.22E-08	2.80E-07	8.067	-1.51
Pcp4l1	0.429	8.567	12.697	4.24E-12	9.07E-11	17.493	1.35
Pdap1	0.291	7.806	4.981	4.45E-05	1.45E-04	1.007	1.22
Pdia3	-0.285	9.730	-4.586	1.21E-04	3.58E-04	0.007	-1.22
Pdlim1	-0.469	7.184	-9.961	5.68E-10	6.50E-09	12.476	-1.38
Pdrg1	0.907	7.434	16.327	1.92E-14	8.75E-13	23.006	1.87
Pebp1	-0.353	9.686	-9.378	1.81E-09	1.81E-08	11.290	-1.28
Pes1	0.275	7.542	3.700	1.13E-03	2.65E-03	-2.207	1.21
Pex19	0.911	6.163	18.763	8.80E-16	6.59E-14	26.142	1.88
Pfdn5	-0.358	8.087	-7.456	1.12E-07	6.74E-07	7.073	-1.28
Pgk1	-0.466	9.403	-13.138	2.07E-12	4.65E-11	18.226	-1.38
Pgrmc2	-0.264	7.361	-7.976	3.49E-08	2.41E-07	8.261	-1.20
Pgs1	-0.353	6.290	-5.223	2.41E-05	8.32E-05	1.621	-1.28
Phf20	-0.499	6.196	-10.696	1.40E-10	1.93E-09	13.907	-1.41
Phf20	-0.494	6.211	-7.355	1.41E-07	8.32E-07	6.837	-1.41
PHF5A	0.504	7.050	9.572	1.22E-09	1.29E-08	11.689	1.42
PHGDH	-0.649	7.601	-9.060	3.46E-09	3.20E-08	10.626	-1.57
PIGT	-0.607	6.318	-6.055	3.06E-06	1.31E-05	3.707	-1.52
Pigx	0.443	8.703	12.207	9.62E-12	1.84E-10	16.654	1.36
Pin4	0.270	6.767	5.399	1.55E-05	5.65E-05	2.066	1.21
Pir	-0.780	6.603	-11.436	3.66E-11	5.74E-10	15.286	-1.72
Pja2	0.275	7.451	5.874	4.77E-06	1.98E-05	3.258	1.21
Pkn2	0.310	6.309	3.831	8.14E-04	1.99E-03	-1.884	1.24
Pkn2	0.382	7.422	9.174	2.74E-09	2.62E-08	10.865	1.30
Plaa	-0.394	6.833	-8.044	3.01E-08	2.12E-07	8.415	-1.31
Plac8	0.738	5.438	7.386	1.31E-07	7.81E-07	6.910	1.67
Plbd2	0.423	7.151	6.193	2.19E-06	9.71E-06	4.047	1.34
Plcg1	-0.609	7.243	-13.309	1.58E-12	3.75E-11	18.505	-1.53
Plekha5	-0.325	7.039	-8.021	3.16E-08	2.20E-07	8.363	-1.25
Plin2	-0.392	9.393	-9.437	1.60E-09	1.62E-08	11.413	-1.31
Plin2	-0.297	9.911	-2.693	1.28E-02	2.31E-02	-4.539	-1.23
PLK2	-0.470	7.605	-9.227	2.45E-09	2.36E-08	10.977	-1.39
Pltp	-2.846	7.226	-21.691	3.34E-17	5.45E-15	29.451	-7.19
Plxna1	-0.450	6.084	-8.906	4.76E-09	4.31E-08	10.300	-1.37
Pnn	0.393	7.266	7.387	1.31E-07	7.80E-07	6.913	1.31
Pnp2	-0.452	6.523	-6.761	5.61E-07	2.86E-06	5.430	-1.37
Polr1c	0.267	7.898	5.477	1.28E-05	4.72E-05	2.263	1.20
Polr1c	0.291	6.856	2.590	1.61E-02	2.84E-02	-4.756	1.22
Polr2b	-0.380	8.094	-7.666	6.96E-08	4.44E-07	7.556	-1.30
Polr2g	0.437	8.219	10.778	1.21E-10	1.68E-09	14.064	1.35
Polr3e	0.762	5.962	13.885	6.42E-13	1.75E-11	19.424	1.70
Pomt2	-0.324	7.750	-3.093	5.00E-03	1.00E-02	-3.648	-1.25
Pop7	-0.283	6.953	-4.613	1.13E-04	3.36E-04	0.075	-1.22
Ppap2a	-0.654	6.987	-8.662	7.93E-09	6.77E-08	9.777	-1.57
Ppcs	-0.300	7.062	-5.322	1.88E-05	6.70E-05	1.872	-1.23
Ppif	0.316	7.174	6.255	1.88E-06	8.56E-06	4.200	1.24
Ppp1cb	0.554	9.069	8.462	1.21E-08	9.84E-08	9.342	1.47
Ppp2cb	0.311	9.586	7.092	2.58E-07	1.43E-06	6.220	1.24
PPP2R5A	0.847	6.010	13.904	6.23E-13	1.75E-11	19.454	1.80
Ppp2r5c	-1.004	7.119	-15.309	7.85E-14	3.00E-12	21.571	-2.01
Ppp2r5c	-0.483	7.078	-10.190	3.65E-10	4.33E-09	12.928	-1.40
Ppp3cb	0.390	6.496	6.504	1.03E-06	4.94E-06	4.809	1.31
Pppde1	0.761	6.727	15.976	3.10E-14	1.31E-12	22.520	1.69
Pppde1	1.189	6.277	12.392	7.04E-12	1.42E-10	16.974	2.28
Pqlc3	-0.527	6.550	-4.541	1.35E-04	3.97E-04	-0.105	-1.44
Prc1	0.393	7.462	4.100	4.14E-04	1.08E-03	-1.217	1.31
Prcp	-0.394	7.535	-9.447	1.57E-09	1.60E-08	11.433	-1.31
Prdx6	0.529	9.310	8.322	1.64E-08	1.27E-07	9.033	1.44
Prdx6	0.625	7.805	16.445	1.64E-14	7.77E-13	23.167	1.54
Prkacb	0.318	8.518	6.023	3.31E-06	1.41E-05	3.628	1.25
Prkcdbp	-0.824	6.389	-7.662	7.02E-08	4.47E-07	7.548	-1.77
Prkcsh	-0.310	6.754	-2.790	1.02E-02	1.89E-02	-4.329	-1.24
Prkrir	0.498	7.275	11.464	3.48E-11	5.58E-10	15.337	1.41
Prmt3	0.340	7.986	10.171	3.78E-10	4.44E-09	12.892	1.27
Prmt5	-0.635	7.197	-8.547	1.01E-08	8.32E-08	9.526	-1.55
Procr	-0.316	6.220	-4.738	8.22E-05	2.52E-04	0.392	-1.24
Prpf6	-0.302	7.446	-5.299	2.00E-05	7.03E-05	1.813	-1.23
Prps2	-0.326	7.860	-4.266	2.72E-04	7.46E-04	-0.800	-1.25
Prps2	-0.284	9.468	-5.119	3.13E-05	1.05E-04	1.359	-1.22
Prune2	-0.654	7.588	-11.790	1.97E-11	3.40E-10	15.922	-1.57
Psat1	-0.691	8.652	-10.547	1.85E-10	2.41E-09	13.623	-1.61
Psat1	-0.668	9.538	-14.615	2.14E-13	7.00E-12	20.546	-1.59
Psma7	-0.376	10.089	-11.233	5.26E-11	8.01E-10	14.913	-1.30

PSMB5	-0.484	9.145	-5.196	2.58E-05	8.83E-05	1.554	-1.40
Psmb6	0.367	8.403	6.330	1.57E-06	7.26E-06	4.385	1.29
Psmc2	0.283	9.287	8.118	2.56E-08	1.85E-07	8.580	1.22
Psmc4	0.422	7.500	6.019	3.35E-06	1.42E-05	3.617	1.34
Psmc4	0.465	6.706	4.125	3.88E-04	1.03E-03	-1.153	1.38
Psmc5	0.516	7.329	5.845	5.13E-06	2.11E-05	3.185	1.43
Psmc5	0.542	7.802	13.587	1.02E-12	2.56E-11	18.953	1.46
Psmd1	-0.339	6.492	-5.225	2.40E-05	8.28E-05	1.627	-1.26
Psmd14	0.280	8.374	8.403	1.38E-08	1.10E-07	9.211	1.21
Psme4	-0.388	7.404	-6.524	9.85E-07	4.71E-06	4.858	-1.31
Psmg1	0.389	7.725	9.966	5.63E-10	6.46E-09	12.485	1.31
Ptdss1	-0.341	6.929	-6.102	2.73E-06	1.18E-05	3.824	-1.27
Ptgr1	-0.483	8.581	-9.910	6.28E-10	7.12E-09	12.373	-1.40
Ptgr1	-0.431	7.564	-3.773	9.41E-04	2.26E-03	-2.027	-1.35
Ptp4a2	0.291	9.890	3.884	7.13E-04	1.76E-03	-1.753	1.22
Ptp4a2	0.424	9.223	3.155	4.31E-03	8.79E-03	-3.506	1.34
Ptpn11	0.270	8.669	5.795	5.79E-06	2.35E-05	3.062	1.21
Ptprg	-2.362	5.545	-46.919	5.34E-25	6.39E-22	46.851	-5.14
Ptrf	-0.365	7.830	-4.639	1.06E-04	3.17E-04	0.142	-1.29
Pum2	0.338	7.024	8.093	2.70E-08	1.95E-07	8.524	1.26
Pwp1	0.314	7.363	5.699	7.35E-06	2.89E-05	2.821	1.24
Pxdn	-0.338	7.848	-5.876	4.75E-06	1.97E-05	3.262	-1.26
Qars	-0.514	9.208	-12.618	4.83E-12	1.01E-10	17.358	-1.43
Qsox1	0.334	7.440	4.310	2.43E-04	6.77E-04	-0.691	1.26
R3hdm1	0.312	6.525	6.162	2.36E-06	1.04E-05	3.972	1.24
Rab1	-0.368	9.110	-8.898	4.84E-09	4.37E-08	10.283	-1.29
Rab11a	-0.309	5.948	-2.953	6.97E-03	1.35E-02	-3.966	-1.24
Rab11a	-0.268	7.923	-5.349	1.76E-05	6.34E-05	1.940	-1.20
Rab14	0.358	8.540	8.553	1.00E-08	8.25E-08	9.538	1.28
Rab24	-0.578	6.607	-11.001	8.00E-11	1.18E-09	14.484	-1.49
Rab28	0.333	7.987	4.865	5.95E-05	1.88E-04	0.715	1.26
Rab31	0.308	6.713	3.807	8.64E-04	2.10E-03	-1.943	1.24
Rab32	-0.314	6.424	-4.034	4.89E-04	1.26E-03	-1.382	-1.24
Rab5a	0.303	7.672	5.278	2.10E-05	7.36E-05	1.761	1.23
Rab6	0.501	6.620	7.582	8.41E-08	5.25E-07	7.364	1.42
Rab7	0.323	7.596	5.093	3.35E-05	1.12E-04	1.293	1.25
Ralb	0.314	6.456	6.128	2.56E-06	1.12E-05	3.888	1.24
Ralgapb	-0.589	6.409	-13.734	8.10E-13	2.09E-11	19.186	-1.50
Rapgef1	0.469	9.228	9.674	9.99E-10	1.07E-08	11.898	1.38
Rarres2	-0.812	9.322	-11.439	3.64E-11	5.74E-10	15.290	-1.76
Rbck1	0.812	6.528	9.698	9.53E-10	1.03E-08	11.945	1.76
RBM3	-0.646	7.415	-6.420	1.27E-06	5.94E-06	4.603	-1.56
Rbm39	-0.418	8.443	-8.292	1.75E-08	1.35E-07	8.968	-1.34
Rbm8a	-0.294	8.097	-7.614	7.82E-08	4.94E-07	7.439	-1.23
Rcbtb2	-0.516	7.042	-8.760	6.46E-09	5.62E-08	9.987	-1.43
Rcc2	0.295	7.768	5.666	7.98E-06	3.11E-05	2.738	1.23
Rcn3	0.355	8.011	5.375	1.65E-05	5.98E-05	2.007	1.28
Rdx	0.264	8.988	5.149	2.91E-05	9.85E-05	1.435	1.20
Rer1	0.451	7.708	9.798	7.82E-10	8.75E-09	12.149	1.37
Rer1	0.457	8.619	7.396	1.28E-07	7.67E-07	6.933	1.37
Retsat	-0.960	5.458	-10.234	3.36E-10	4.01E-09	13.014	-1.94
Rev3l	-0.360	7.281	-5.473	1.29E-05	4.77E-05	2.251	-1.28
Rexo2	0.308	9.534	8.042	3.02E-08	2.12E-07	8.409	1.24
Rfc1	-0.297	7.032	-5.345	1.78E-05	6.39E-05	1.930	-1.23
Rfx5	-0.354	6.821	-6.310	1.65E-06	7.60E-06	4.334	-1.28
RGD1560010	0.466	6.651	6.719	6.19E-07	3.10E-06	5.330	1.38
RGD1561797	-0.407	7.190	-5.239	2.32E-05	8.06E-05	1.661	-1.33
Rgs17	0.784	7.346	8.084	2.76E-08	1.97E-07	8.503	1.72
Riok3	0.583	7.168	8.179	2.24E-08	1.66E-07	8.716	1.50
Rnaseh2c	-1.175	6.445	-18.782	8.60E-16	6.59E-14	26.165	-2.26
Rnaset2a	0.570	10.189	13.225	1.80E-12	4.13E-11	18.367	1.48
Rnf10	0.316	7.472	5.733	6.76E-06	2.70E-05	2.904	1.25
Rnf11	0.281	7.884	6.059	3.03E-06	1.30E-05	3.717	1.22
Rnft1	-0.348	6.370	-6.124	2.59E-06	1.13E-05	3.877	-1.27
ROCK2	0.292	7.129	3.139	4.47E-03	9.08E-03	-3.542	1.22
Romo1	-0.381	9.948	-7.619	7.74E-08	4.90E-07	7.449	-1.30
rp9	0.444	6.933	7.385	1.32E-07	7.82E-07	6.907	1.36
Rpia	-0.273	7.906	-4.619	1.11E-04	3.31E-04	0.091	-1.21
Rpn2	-0.458	9.821	-5.217	2.45E-05	8.43E-05	1.606	-1.37
Rpn2	-0.291	10.428	-6.702	6.44E-07	3.20E-06	5.289	-1.22
Rpp21	0.327	8.060	5.300	1.99E-05	7.02E-05	1.815	1.25
Rpp30	0.309	7.292	5.056	3.68E-05	1.22E-04	1.198	1.24
Rps28	-0.819	8.402	-8.579	9.47E-09	7.93E-08	9.596	-1.76
Rps5	0.284	11.111	6.288	1.74E-06	7.97E-06	4.281	1.22
Rps6kb1	0.325	6.763	5.698	7.38E-06	2.90E-05	2.817	1.25
Rqcd1	0.308	6.141	4.628	1.09E-04	3.25E-04	0.113	1.24
Rras	-0.353	6.545	-5.018	4.05E-05	1.33E-04	1.102	-1.28
Rrp1	0.460	6.040	4.666	9.85E-05	2.98E-04	0.211	1.38
Rrp15	0.682	7.873	20.847	8.21E-17	1.09E-14	28.543	1.60

Rsl24d1	0.429	7.715	7.483	1.05E-07	6.37E-07	7.134	1.35
Rsrc1	0.347	6.889	7.985	3.42E-08	2.36E-07	8.282	1.27
Rsu1	-0.290	8.196	-3.449	2.10E-03	4.67E-03	-2.813	-1.22
Ryk	0.342	6.736	8.298	1.73E-08	1.33E-07	8.980	1.27
S100a11	-0.290	7.784	-4.316	2.40E-04	6.68E-04	-0.676	-1.22
SAMD4B	0.460	8.703	9.524	1.35E-09	1.39E-08	11.592	1.38
Samhd1	-0.472	6.716	-9.811	7.63E-10	8.56E-09	12.174	-1.39
Samm50	-0.288	7.616	-6.465	1.13E-06	5.37E-06	4.715	-1.22
Sars	-0.272	7.477	-2.797	1.00E-02	1.87E-02	-4.313	-1.21
Sat1	-1.197	6.704	-24.727	1.67E-18	4.28E-16	32.457	-2.29
SC4MOL	-1.137	8.194	-22.532	1.40E-17	2.65E-15	30.324	-2.20
Sc4mol	-1.056	6.368	-4.963	4.64E-05	1.50E-04	0.964	-2.08
Sc5d	-0.921	6.364	-9.758	8.46E-10	9.35E-09	12.068	-1.89
Scarb1	0.277	6.897	4.001	5.31E-04	1.36E-03	-1.463	1.21
Sccpdh	0.339	7.895	7.122	2.41E-07	1.34E-06	6.292	1.26
Sccpdh	0.477	7.248	13.569	1.05E-12	2.61E-11	18.924	1.39
SCD	-0.528	9.542	-12.449	6.39E-12	1.30E-10	17.072	-1.44
Scd1	-0.541	7.257	-5.086	3.41E-05	1.14E-04	1.274	-1.45
Scd1	-0.472	9.240	-8.957	4.28E-09	3.89E-08	10.409	-1.39
Scd3	-0.537	7.564	-5.749	6.50E-06	2.60E-05	2.945	-1.45
Scd3	-0.401	8.588	-9.629	1.09E-09	1.16E-08	11.807	-1.32
Scfd1	0.323	6.868	5.991	3.58E-06	1.52E-05	3.549	1.25
Scoc	0.300	7.007	4.642	1.05E-04	3.15E-04	0.149	1.23
Sdc1	-0.433	5.513	-7.079	2.66E-07	1.47E-06	6.190	-1.35
Sdc4	-0.491	8.779	-9.741	8.75E-10	9.62E-09	12.033	-1.41
Sdc4	-0.416	7.039	-4.467	1.63E-04	4.72E-04	-0.294	-1.33
Sdf2	-0.333	6.838	-8.192	2.17E-08	1.62E-07	8.746	-1.26
Sdhb	0.341	8.786	3.190	3.96E-03	8.18E-03	-3.425	1.27
Sdhd	-0.303	9.423	-4.752	7.92E-05	2.44E-04	0.429	-1.23
Sec13	-0.319	7.937	-9.412	1.69E-09	1.70E-08	11.361	-1.25
Sec23b	-0.500	6.844	-6.708	6.35E-07	3.17E-06	5.304	-1.41
Sec24d	-0.418	7.004	-5.794	5.81E-06	2.36E-05	3.058	-1.34
Sec63	0.271	6.781	5.565	1.02E-05	3.89E-05	2.485	1.21
Sel1l	-0.355	7.016	-3.751	9.95E-04	2.38E-03	-2.081	-1.28
Selk	0.344	7.749	7.189	2.06E-07	1.17E-06	6.449	1.27
Sema3c	1.004	6.612	17.408	4.67E-15	2.67E-13	24.446	2.01
Senp6	0.293	7.501	3.349	2.69E-03	5.81E-03	-3.051	1.23
SERINC3	-0.583	9.592	-13.387	1.40E-12	3.41E-11	18.630	-1.50
Serpinb1a	-0.663	8.041	-9.229	2.45E-09	2.36E-08	10.981	-1.58
Serpinb6a	-0.291	8.276	-5.493	1.23E-05	4.57E-05	2.302	-1.22
Serpinf1	-1.188	8.278	-13.766	7.71E-13	2.01E-11	19.237	-2.28
Serpinh1	-0.431	8.949	-7.130	2.37E-07	1.32E-06	6.309	-1.35
Serpinh1	-0.389	9.252	-11.963	1.46E-11	2.60E-10	16.229	-1.31
Serpinh1	-0.376	8.798	-4.095	4.19E-04	1.10E-03	-1.228	-1.30
SET	0.522	8.500	13.792	7.40E-13	1.95E-11	19.278	1.44
Sf3b3	-0.688	7.166	-9.003	3.89E-09	3.57E-08	10.506	-1.61
Sfrs1	0.406	7.768	7.944	3.74E-08	2.53E-07	8.190	1.32
Sfrs7	0.265	8.831	2.703	1.25E-02	2.25E-02	-4.516	1.20
Sgce	0.642	6.005	4.977	4.48E-05	1.45E-04	1.000	1.56
Sgk1	0.665	7.327	10.698	1.40E-10	1.93E-09	13.911	1.59
Sh2b3	-0.503	6.519	-9.170	2.76E-09	2.63E-08	10.858	-1.42
Shmt2	-0.835	6.208	-8.173	2.26E-08	1.67E-07	8.704	-1.78
Sin3b	0.475	6.811	5.540	1.09E-05	4.11E-05	2.421	1.39
Slain2	0.344	7.905	6.262	1.85E-06	8.43E-06	4.219	1.27
Slc1a5	-0.465	6.945	-9.273	2.24E-09	2.18E-08	11.073	-1.38
Slc25a1	-0.886	6.910	-9.685	9.78E-10	1.05E-08	11.920	-1.85
Slc25a17	0.317	6.764	7.103	2.52E-07	1.40E-06	6.246	1.25
Slc25a20	-0.354	8.555	-4.978	4.47E-05	1.45E-04	1.002	-1.28
Slc35b1	-0.287	7.240	-5.844	5.14E-06	2.11E-05	3.182	-1.22
Slc44a1	0.287	7.158	4.356	2.16E-04	6.09E-04	-0.573	1.22
Slc6a6	-0.637	8.040	-13.317	1.56E-12	3.73E-11	18.518	-1.56
Slc7a11	-1.284	7.310	-15.281	8.17E-14	3.06E-12	21.531	-2.44
Smpd1	-0.475	6.885	-7.897	4.16E-08	2.77E-07	8.082	-1.39
Smpd1	-0.428	6.110	-4.543	1.35E-04	3.95E-04	-0.100	-1.35
Smyd2	0.949	6.489	17.733	3.10E-15	1.95E-13	24.864	1.93
Snai2	-0.758	6.399	-11.536	3.07E-11	5.01E-10	15.466	-1.69
Snn	-0.814	6.495	-10.534	1.90E-10	2.47E-09	13.597	-1.76
Snora28	-1.612	6.761	-15.853	3.67E-14	1.50E-12	22.347	-3.06
Snora28	-1.327	8.217	-26.955	2.29E-19	8.21E-17	34.437	-2.51
Snora28	-1.268	7.421	-24.486	2.09E-18	5.01E-16	32.232	-2.41
Snora75	-0.307	8.619	-5.712	7.11E-06	2.81E-05	2.853	-1.24
Snrnp27	0.366	7.046	3.364	2.59E-03	5.64E-03	-3.015	1.29
Snrpc	0.522	7.891	10.439	2.27E-10	2.84E-09	13.414	1.44
Snrpe	0.274	9.000	4.695	9.15E-05	2.78E-04	0.284	1.21
SNX21	-0.605	6.347	-7.234	1.86E-07	1.07E-06	6.556	-1.52
Snx4	0.324	8.191	6.679	6.80E-07	3.36E-06	5.234	1.25
Sod1	-0.363	8.506	-6.489	1.07E-06	5.09E-06	4.773	-1.29
Sod2	-0.743	6.599	-11.810	1.90E-11	3.31E-10	15.958	-1.67
SOD2	-0.620	6.729	-9.144	2.91E-09	2.75E-08	10.804	-1.54

Sox12	0.576	5.451	4.274	2.66E-04	7.34E-04	-0.780	1.49
Sprr1a	1.051	8.643	12.199	9.74E-12	1.85E-10	16.641	2.07
Sptlc1	-0.344	6.938	-3.586	1.50E-03	3.42E-03	-2.483	-1.27
Sqle	-0.829	7.073	-12.459	6.29E-12	1.28E-10	17.088	-1.78
Sqle	-0.775	9.031	-12.296	8.27E-12	1.63E-10	16.809	-1.71
Sqle	-0.717	6.333	-4.660	1.00E-04	3.02E-04	0.194	-1.64
Srgn	-0.375	7.415	-4.349	2.20E-04	6.20E-04	-0.592	-1.30
Srp54c	-0.307	6.551	-6.383	1.38E-06	6.44E-06	4.514	-1.24
Srp68	0.303	6.373	3.157	4.28E-03	8.75E-03	-3.500	1.23
Srpr	-0.430	8.041	-9.768	8.29E-10	9.23E-09	12.088	-1.35
Srpr	-0.366	6.329	-3.986	5.51E-04	1.40E-03	-1.500	-1.29
Ssu72	-1.080	7.371	-16.992	7.98E-15	4.22E-13	23.902	-2.11
Stim2	0.434	6.458	5.311	1.94E-05	6.85E-05	1.844	1.35
Stk39	0.387	6.315	4.253	2.81E-04	7.67E-04	-0.833	1.31
Stk4	-0.445	6.572	-10.312	2.89E-10	3.48E-09	13.167	-1.36
Stt3a	-0.624	6.388	-2.803	9.90E-03	1.84E-02	-4.300	-1.54
STT3A	-0.482	7.885	-8.596	9.13E-09	7.68E-08	9.633	-1.40
Stx12	0.384	7.584	7.621	7.70E-08	4.89E-07	7.454	1.30
Stx12	0.399	7.514	7.605	7.99E-08	5.03E-07	7.416	1.32
Stx6	0.471	6.481	7.964	3.59E-08	2.47E-07	8.233	1.39
Sumf2	0.401	7.010	7.324	1.51E-07	8.91E-07	6.766	1.32
SUPT16H	-0.973	10.402	-13.447	1.27E-12	3.12E-11	18.727	-1.96
Supt4h1	0.338	7.223	6.323	1.60E-06	7.38E-06	4.367	1.26
Supt6h	0.492	6.509	4.750	7.97E-05	2.46E-04	0.423	1.41
Syf2	0.420	7.090	6.686	6.69E-07	3.31E-06	5.251	1.34
Syncrip	0.570	6.547	8.564	9.77E-09	8.13E-08	9.563	1.48
Syncrip	0.576	6.514	3.016	6.00E-03	1.17E-02	-3.823	1.49
Sypl	-0.267	7.000	-2.909	7.73E-03	1.48E-02	-4.065	-1.20
Taldo1	-0.282	8.276	-5.254	2.23E-05	7.76E-05	1.701	-1.22
Tbc1d13	0.426	6.269	6.912	3.93E-07	2.07E-06	5.793	1.34
Tbc1d20	1.103	7.320	19.587	3.36E-16	3.55E-14	27.120	2.15
Tbpl1	0.951	7.435	14.437	2.79E-13	8.63E-12	20.278	1.93
Tcea1	0.530	8.260	12.080	1.19E-11	2.16E-10	16.433	1.44
Tceal8	-0.419	6.586	-9.549	1.28E-09	1.33E-08	11.643	-1.34
Tcf19	-0.464	5.912	-8.070	2.84E-08	2.02E-07	8.473	-1.38
Tecr	-0.435	8.225	-6.194	2.18E-06	9.71E-06	4.049	-1.35
Tes	-0.323	7.629	-5.424	1.46E-05	5.34E-05	2.128	-1.25
Tes	-0.276	6.550	-5.693	7.46E-06	2.93E-05	2.805	-1.21
Tfb2m	0.844	7.005	16.628	1.29E-14	6.25E-13	23.415	1.80
TFPI	-0.453	7.291	-5.650	8.31E-06	3.23E-05	2.697	-1.37
Tgm2	-0.565	7.080	-9.326	2.01E-09	2.00E-08	11.182	-1.48
Tgoln2	-0.532	6.450	-6.375	1.41E-06	6.55E-06	4.496	-1.45
Tgoln2	-0.493	7.676	-7.228	1.88E-07	1.08E-06	6.542	-1.41
Thbd	-0.988	7.746	-11.413	3.81E-11	5.95E-10	15.243	-1.98
Thoc7	0.287	7.681	5.420	1.47E-05	5.39E-05	2.118	1.22
Timm10	0.600	7.656	10.848	1.06E-10	1.52E-09	14.195	1.52
Timm17a	0.868	7.349	19.160	5.50E-16	5.07E-14	26.618	1.82
Timm50	0.431	7.608	8.751	6.57E-09	5.70E-08	9.969	1.35
Timp1	-0.438	6.932	-6.440	1.20E-06	5.68E-06	4.654	-1.35
Timp2	0.320	8.474	4.629	1.08E-04	3.24E-04	0.117	1.25
Timp2	0.399	7.474	5.164	2.80E-05	9.51E-05	1.472	1.32
Tinagl1	-1.504	6.534	-13.304	1.59E-12	3.76E-11	18.496	-2.84
Tiprl	0.983	7.485	14.908	1.40E-13	4.82E-12	20.984	1.98
Tjp2	-0.448	6.785	-8.720	7.03E-09	6.04E-08	9.900	-1.36
Tkt	-0.737	6.373	-5.275	2.12E-05	7.41E-05	1.753	-1.67
Tkt	-0.725	6.994	-16.050	2.80E-14	1.21E-12	22.623	-1.65
Tm9sf4	0.394	5.793	6.807	5.03E-07	2.59E-06	5.542	1.31
Tmbim6	-0.344	8.960	-3.078	5.17E-03	1.03E-02	-3.681	-1.27
Tmco1	0.900	7.160	20.586	1.09E-16	1.35E-14	28.256	1.87
Tmed3	-0.621	6.990	-12.967	2.73E-12	5.98E-11	17.943	-1.54
Tmed5	0.341	9.161	8.367	1.49E-08	1.17E-07	9.131	1.27
Tmem101	0.737	6.466	8.848	5.36E-09	4.75E-08	10.177	1.67
Tmem109	-0.502	6.566	-9.636	1.08E-09	1.15E-08	11.819	-1.42
Tmem111	0.312	7.013	5.628	8.76E-06	3.38E-05	2.643	1.24
Tmem14c	-0.289	9.909	-6.625	7.74E-07	3.77E-06	5.103	-1.22
Tmem150a	-0.585	6.896	-8.471	1.19E-08	9.68E-08	9.361	-1.50
Tmem156	0.661	7.139	11.548	3.00E-11	4.93E-10	15.488	1.58
Tmem158	-0.673	6.933	-6.880	4.24E-07	2.22E-06	5.716	-1.59
Tmem167	0.419	7.216	6.438	1.21E-06	5.70E-06	4.649	1.34
Tmem167b	0.382	6.863	8.553	1.00E-08	8.25E-08	9.540	1.30
Tmem183a	0.503	6.350	8.888	4.94E-09	4.42E-08	10.262	1.42
Tmem183a	0.701	6.182	13.378	1.41E-12	3.43E-11	18.616	1.63
Tmem189	-0.405	6.827	-5.572	1.01E-05	3.83E-05	2.501	-1.32
Tmem223	-0.411	7.260	-6.039	3.18E-06	1.36E-05	3.668	-1.33
Tmem30a	-0.336	6.995	-3.224	3.64E-03	7.61E-03	-3.345	-1.26
Tmem38b	0.391	6.934	6.744	5.83E-07	2.96E-06	5.390	1.31
Tmem49	-0.325	6.244	-4.073	4.42E-04	1.15E-03	-1.283	-1.25
Tmem55b	-0.364	7.431	-6.726	6.09E-07	3.06E-06	5.347	-1.29
Tmem60	0.465	6.952	8.059	2.91E-08	2.06E-07	8.448	1.38

TMEM66	0.391	8.790	10.826	1.10E-10	1.57E-09	14.155	1.31
Tnfaip3	-0.814	5.235	-8.895	4.87E-09	4.38E-08	10.276	-1.76
Tnfrsf21	-1.115	7.387	-15.724	4.38E-14	1.77E-12	22.166	-2.17
Tnfsf14	-0.880	7.330	-16.040	2.84E-14	1.21E-12	22.609	-1.84
Tnip1	-0.622	6.401	-8.427	1.31E-08	1.05E-07	9.265	-1.54
Tnks2	0.320	6.639	6.319	1.61E-06	7.44E-06	4.359	1.25
Tollip	0.323	6.477	4.865	5.96E-05	1.88E-04	0.714	1.25
Tomm20l	0.520	7.636	10.638	1.56E-10	2.11E-09	13.797	1.43
Top1	-0.303	7.654	-4.434	1.78E-04	5.11E-04	-0.378	-1.23
Tpm1	-0.296	7.136	-6.606	8.09E-07	3.93E-06	5.058	-1.23
Tpp1	-0.481	7.300	-10.559	1.81E-10	2.38E-09	13.647	-1.40
Tpr	0.565	6.127	8.208	2.10E-08	1.57E-07	8.780	1.48
Tprgl	-0.353	6.781	-5.823	5.41E-06	2.20E-05	3.131	-1.28
Tpst1	-0.470	6.787	-11.131	6.32E-11	9.46E-10	14.726	-1.39
Tpx2	0.732	6.219	7.859	4.53E-08	2.98E-07	7.997	1.66
Tram1	-0.342	6.401	-4.314	2.41E-04	6.70E-04	-0.679	-1.27
Tram1	-0.293	7.603	-7.064	2.76E-07	1.52E-06	6.154	-1.23
Trap1	0.335	6.783	4.095	4.19E-04	1.10E-03	-1.229	1.26
Trappc4	0.566	7.791	10.196	3.61E-10	4.30E-09	12.940	1.48
Trib3	0.847	5.604	5.968	3.79E-06	1.60E-05	3.491	1.80
Trib3	1.125	8.754	24.110	2.98E-18	6.70E-16	31.877	2.18
Trib3	1.202	5.753	8.844	5.42E-09	4.78E-08	10.167	2.30
Trim44	0.368	6.784	3.070	5.27E-03	1.05E-02	-3.700	1.29
Trim44	0.390	6.505	8.570	9.64E-09	8.04E-08	9.577	1.31
Trmt6	0.773	6.854	14.144	4.33E-13	1.25E-11	19.828	1.71
Trub1	0.662	7.328	16.419	1.70E-14	7.94E-13	23.131	1.58
Tsc22d1	-0.317	10.051	-4.409	1.89E-04	5.40E-04	-0.440	-1.25
Tsc22d3	0.490	6.584	9.457	1.54E-09	1.57E-08	11.454	1.40
Tsen15	0.926	7.360	25.323	9.65E-19	2.89E-16	33.004	1.90
Tspan31	-0.416	7.093	-4.213	3.11E-04	8.41E-04	-0.934	-1.33
Tspan4	-0.413	6.391	-7.281	1.67E-07	9.70E-07	6.665	-1.33
TSPYL1	-0.270	8.138	-4.104	4.09E-04	1.07E-03	-1.205	-1.21
Tuba1c	-0.511	9.068	-10.598	1.68E-10	2.24E-09	13.721	-1.42
Tubb2c	-0.610	8.450	-12.177	1.01E-11	1.90E-10	16.602	-1.53
Tubb5	-0.713	7.745	-11.131	6.32E-11	9.46E-10	14.726	-1.64
Tubb6	-0.570	7.128	-14.343	3.21E-13	9.68E-12	20.134	-1.48
Twistnb	0.266	7.652	3.291	3.10E-03	6.57E-03	-3.188	1.20
Txndc15	0.329	6.914	8.509	1.10E-08	8.98E-08	9.443	1.26
Txnip	0.558	8.976	7.926	3.90E-08	2.63E-07	8.148	1.47
Txnl4a	0.266	8.328	6.247	1.92E-06	8.71E-06	4.180	1.20
Tyms	-0.910	8.010	-11.629	2.60E-11	4.35E-10	15.634	-1.88
Uap1	0.280	6.881	4.536	1.37E-04	4.01E-04	-0.118	1.21
Uap1l1	-1.163	6.485	-12.979	2.68E-12	5.90E-11	17.964	-2.24
Uba5	-0.334	7.149	-7.140	2.31E-07	1.30E-06	6.334	-1.26
Ubash3b	-0.544	8.046	-13.292	1.62E-12	3.81E-11	18.476	-1.46
Ube2a	0.316	7.184	2.917	7.58E-03	1.46E-02	-4.046	1.24
UBE2D4	0.316	6.665	5.181	2.68E-05	9.15E-05	1.516	1.24
Ube2f	0.276	6.835	6.701	6.47E-07	3.21E-06	5.285	1.21
Ube2i	0.313	7.595	6.764	5.57E-07	2.84E-06	5.438	1.24
Ube2j2	0.320	7.348	7.134	2.34E-07	1.31E-06	6.320	1.25
Ube2s	0.499	7.516	6.063	3.00E-06	1.29E-05	3.726	1.41
Ube4a	-0.365	7.131	-6.957	3.54E-07	1.89E-06	5.900	-1.29
Ubiad1	0.568	6.641	10.423	2.34E-10	2.92E-09	13.384	1.48
Ubl3	0.526	7.629	7.303	1.59E-07	9.26E-07	6.717	1.44
Ubl3	0.623	6.206	11.780	2.00E-11	3.44E-10	15.905	1.54
Ubqln4	0.267	6.593	2.840	9.08E-03	1.70E-02	-4.218	1.20
UBXN4	0.442	7.191	6.648	7.32E-07	3.59E-06	5.159	1.36
Ubxn4	0.700	7.247	12.587	5.09E-12	1.06E-10	17.306	1.62
Uchl5	0.695	6.364	7.417	1.22E-07	7.33E-07	6.982	1.62
Uck2	0.892	6.008	13.899	6.28E-13	1.75E-11	19.447	1.86
Uck2	1.034	6.716	9.662	1.02E-09	1.10E-08	11.873	2.05
Ufd1l	0.318	8.108	7.057	2.80E-07	1.54E-06	6.138	1.25
Ugcg	-0.598	6.257	-6.860	4.44E-07	2.32E-06	5.667	-1.51
Uggt1	0.265	7.043	5.832	5.29E-06	2.16E-05	3.153	1.20
Ugt1a1	-0.364	10.340	-8.226	2.02E-08	1.53E-07	8.821	-1.29
Unc13a	0.329	8.035	3.923	6.46E-04	1.62E-03	-1.656	1.26
Uqcc	-0.300	7.071	-6.586	8.49E-07	4.10E-06	5.009	-1.23
Uso1	-0.399	6.539	-5.910	4.36E-06	1.82E-05	3.348	-1.32
Usp19	0.291	6.555	5.715	7.06E-06	2.79E-05	2.861	1.22
Usp24	0.379	6.789	8.042	3.02E-08	2.12E-07	8.410	1.30
Usp4	-0.389	6.466	-5.628	8.77E-06	3.38E-05	2.642	-1.31
Usp46	0.362	6.354	5.387	1.60E-05	5.82E-05	2.037	1.29
Vamp3	0.321	8.124	8.438	1.28E-08	1.03E-07	9.287	1.25
Vamp3	0.373	7.446	7.131	2.36E-07	1.32E-06	6.313	1.30
Vamp8	-0.313	7.162	-5.490	1.23E-05	4.59E-05	2.296	-1.24
VAPA	0.366	8.901	10.361	2.63E-10	3.21E-09	13.264	1.29
Vapb	-0.321	8.100	-3.811	8.56E-04	2.08E-03	-1.934	-1.25
Vasn	0.310	6.546	2.744	1.13E-02	2.08E-02	-4.427	1.24
Vasn	0.567	7.151	14.651	2.03E-13	6.69E-12	20.601	1.48

Vdac2	-0.282	8.821	-6.549	9.28E-07	4.45E-06	4.918	-1.22
Vldlr	-0.441	6.836	-6.411	1.29E-06	6.05E-06	4.582	-1.36
Vnn1	-1.275	6.056	-23.297	6.54E-18	1.38E-15	31.090	-2.42
VPS4B	0.514	7.605	10.985	8.25E-11	1.21E-09	14.453	1.43
Vwa5a	-0.531	7.095	-10.359	2.65E-10	3.21E-09	13.259	-1.44
Wasl	0.300	6.738	5.018	4.05E-05	1.33E-04	1.102	1.23
Wdr1	-0.702	8.251	-7.181	2.10E-07	1.19E-06	6.431	-1.63
Wdr1	-0.620	6.551	-13.091	2.23E-12	4.98E-11	18.149	-1.54
Wdr26	0.918	6.370	14.980	1.26E-13	4.47E-12	21.090	1.89
Wdr74	0.273	6.571	4.999	4.25E-05	1.39E-04	1.053	1.21
Wdyhv1	-0.490	7.392	-9.733	8.90E-10	9.75E-09	12.016	-1.40
Wdyhv1	-0.369	6.805	-3.728	1.05E-03	2.50E-03	-2.138	-1.29
Whsc1	0.597	6.747	7.150	2.26E-07	1.27E-06	6.357	1.51
Wnk1	0.307	7.241	2.390	2.51E-02	4.26E-02	-5.168	1.24
Wsb1	0.349	6.023	5.282	2.08E-05	7.30E-05	1.771	1.27
Xbp1	-0.383	7.896	-6.930	3.77E-07	1.99E-06	5.835	-1.30
Xiap	-0.293	6.328	-4.841	6.32E-05	1.99E-04	0.655	-1.23
Xpo6	0.422	6.739	5.349	1.76E-05	6.34E-05	1.941	1.34
Xpot	-0.453	8.618	-7.732	6.00E-08	3.87E-07	7.708	-1.37
Xrcc5	-0.263	6.430	-5.278	2.10E-05	7.36E-05	1.760	-1.20
Ybx1	0.287	11.304	6.253	1.89E-06	8.58E-06	4.196	1.22
Yif1a	-0.396	6.830	-6.204	2.13E-06	9.50E-06	4.075	-1.32
Yipf1	0.445	6.174	7.988	3.40E-08	2.36E-07	8.288	1.36
Ykt6	0.419	6.652	9.199	2.60E-09	2.49E-08	10.918	1.34
Yme1l1	-0.431	8.144	-4.925	5.12E-05	1.64E-04	0.867	-1.35
Ypel5	0.288	6.204	4.176	3.41E-04	9.16E-04	-1.027	1.22
Ywhab	-0.429	6.224	-3.111	4.79E-03	9.64E-03	-3.607	-1.35
Zc3hav1	0.511	7.214	9.468	1.51E-09	1.55E-08	11.477	1.43
Zfand5	0.337	7.866	5.946	3.99E-06	1.67E-05	3.437	1.26
Zfp207	-0.464	7.869	-5.172	2.74E-05	9.33E-05	1.493	-1.38
Zfp207	-0.364	7.955	-8.123	2.53E-08	1.84E-07	8.591	-1.29
Zfp259	0.467	6.408	7.750	5.77E-08	3.74E-07	7.748	1.38
ZFP36L1	-0.276	8.280	-4.644	1.04E-04	3.14E-04	0.154	-1.21
Zfp36l2	-0.497	7.753	-8.363	1.50E-08	1.18E-07	9.124	-1.41
ZMYND11	0.494	7.803	13.883	6.44E-13	1.75E-11	19.420	1.41
Zmynd19	0.264	7.078	6.583	8.55E-07	4.12E-06	5.001	1.20
Zranb1	0.334	7.102	6.207	2.12E-06	9.48E-06	4.082	1.26
Zyx	-0.435	7.962	-6.799	5.13E-07	2.63E-06	5.521	-1.35

5.2.11 T(S)2 v pcDNA(S) gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
40788	-0.739	8.571	-7.012	3.11E-07	2.30E-06	6.074	-1.67
40790	0.537	5.984	6.086	2.84E-06	1.67E-05	3.828	1.45
0610037P05Rik	0.268	6.611	5.629	8.75E-06	4.68E-05	2.689	1.20
0610039K10Rik	0.372	8.116	8.228	2.01E-08	1.97E-07	8.868	1.29
1110005A03Rik	0.339	6.854	5.607	9.24E-06	4.88E-05	2.634	1.26
1700020I14Rik	0.281	8.162	6.601	8.19E-07	5.59E-06	5.089	1.21
1810022C23Rik	0.415	6.867	7.963	3.59E-08	3.20E-07	8.275	1.33
1810031K17Rik	-0.650	6.408	-5.704	7.27E-06	3.97E-05	2.877	-1.57
2010106G01Rik	0.574	8.362	6.690	6.63E-07	4.61E-06	5.305	1.49
2010106G01Rik	0.655	7.487	2.937	7.24E-03	1.71E-02	-3.955	1.58
2310001A20Rik	-0.360	7.257	-6.422	1.26E-06	8.19E-06	4.653	-1.28
2310035C23Rik	-0.266	6.446	-5.309	1.95E-05	9.41E-05	1.883	-1.20
2610030H06Rik	0.487	7.724	7.547	9.11E-08	7.47E-07	7.325	1.40
2700029M09Rik	1.042	6.966	21.010	6.88E-17	1.03E-14	28.735	2.06
2700060E02Rik	0.472	9.852	12.365	7.36E-12	1.77E-10	16.964	1.39
2700094K13Rik	-0.395	6.301	-7.734	5.98E-08	5.11E-07	7.755	-1.31
2810004N23Rik	0.273	6.302	4.029	4.95E-04	1.64E-03	-1.347	1.21
2810025M15Rik	-0.804	6.900	-14.566	2.30E-13	7.95E-12	20.503	-1.75
2810482I07Rik	-0.558	6.348	-9.379	1.80E-09	2.26E-08	11.334	-1.47
2900064A13Rik	0.404	8.255	9.464	1.52E-09	1.95E-08	11.508	1.32
2900097C17Rik	0.327	9.676	6.726	6.08E-07	4.25E-06	5.391	1.25
4930539H15Rik	0.284	6.597	6.419	1.27E-06	8.21E-06	4.647	1.22
5430437P03Rik	0.272	6.759	4.263	2.74E-04	9.82E-04	-0.763	1.21
5830485P09Rik	-0.290	7.542	-5.583	9.82E-06	5.13E-05	2.573	-1.22
5930416I19Rik	0.268	6.436	4.860	6.03E-05	2.54E-04	0.747	1.20
6330406I15Rik	0.299	8.622	4.707	8.88E-05	3.61E-04	0.359	1.23
6430548M08Rik	2.243	5.381	31.992	4.28E-21	2.56E-18	38.322	4.73
9130221J17Rik	-0.374	9.549	-9.432	1.62E-09	2.06E-08	11.443	-1.30
9530053H05Rik	-1.185	10.002	-20.524	1.17E-16	1.50E-14	28.201	-2.27
A330042M18Rik	0.833	7.205	13.045	2.41E-12	6.60E-11	18.107	1.78
A330042M18Rik	0.945	6.806	17.922	2.45E-15	1.69E-13	25.126	1.93
A430057M04Rik	0.307	6.280	4.568	1.26E-04	4.91E-04	0.007	1.24
A830039H05Rik	-0.350	8.012	-6.800	5.12E-07	3.62E-06	5.567	-1.27
Abhd6	-0.878	6.783	-11.178	5.80E-11	1.11E-09	14.850	-1.84
Acadl	-0.741	7.741	-14.817	1.59E-13	5.72E-12	20.879	-1.67
Acat2	-0.990	6.811	-14.983	1.25E-13	4.59E-12	21.125	-1.99
Aco1	-0.349	6.759	-10.839	1.08E-10	1.92E-09	14.217	-1.27
Acot9	-0.416	6.777	-9.313	2.06E-09	2.55E-08	11.197	-1.33
Adam23	-0.810	7.260	-13.033	2.45E-12	6.67E-11	18.087	-1.75
Adam23	-0.771	7.575	-12.121	1.11E-11	2.51E-10	16.540	-1.71
Adh5	0.360	9.619	4.923	5.14E-05	2.20E-04	0.908	1.28
Adh5	0.464	8.532	3.417	2.28E-03	6.28E-03	-2.844	1.38
Adi1	0.356	6.996	5.108	3.22E-05	1.47E-04	1.377	1.28
Ado	0.431	6.943	7.455	1.12E-07	9.08E-07	7.113	1.35
Ado	0.519	7.572	12.920	2.94E-12	7.84E-11	17.900	1.43
Adss	0.825	8.642	12.739	3.96E-12	1.02E-10	17.597	1.77
Adss	0.851	7.645	16.783	1.05E-14	5.10E-13	23.648	1.80
Aftph	0.293	7.139	4.079	4.37E-04	1.48E-03	-1.224	1.23
Agrn	-0.574	7.203	-12.067	1.22E-11	2.72E-10	16.447	-1.49
Ahctf1	0.455	6.293	5.444	1.39E-05	6.93E-05	2.225	1.37
Ahnak	-0.479	8.479	-6.384	1.38E-06	8.86E-06	4.561	-1.39
Aida	0.498	6.309	3.083	5.12E-03	1.27E-02	-3.625	1.41
Aig1	-0.673	6.487	-8.862	5.22E-09	5.91E-08	10.246	-1.59
Airn	0.533	6.764	9.512	1.38E-09	1.78E-08	11.607	1.45
Ak2	0.424	7.391	3.241	3.49E-03	9.11E-03	-3.257	1.34
Ak2	0.452	9.209	8.387	1.43E-08	1.45E-07	9.218	1.37
Akap12	0.548	7.889	8.218	2.06E-08	2.00E-07	8.845	1.46
Akt3	0.352	5.980	5.867	4.86E-06	2.74E-05	3.284	1.28
Amd1	0.977	7.422	9.738	8.81E-10	1.20E-08	12.066	1.97
Amd2	0.910	8.027	10.592	1.70E-10	2.92E-09	13.748	1.88
Anapc11	-0.666	7.726	-6.744	5.83E-07	4.09E-06	5.434	-1.59
Ankrd10	0.405	6.403	5.717	7.03E-06	3.86E-05	2.910	1.32
Ankrd13c	0.390	7.255	9.402	1.72E-09	2.18E-08	11.381	1.31
Anln	-1.184	6.950	-10.261	3.19E-10	4.94E-09	13.106	-2.27
Anxa1	-0.365	10.378	-5.063	3.61E-05	1.61E-04	1.262	-1.29
Anxa2	-0.810	9.836	-5.305	1.96E-05	9.47E-05	1.874	-1.75
Anxa2	-0.793	10.669	-11.332	4.40E-11	8.69E-10	15.133	-1.73
Anxa4	-0.306	7.155	-5.337	1.81E-05	8.80E-05	1.954	-1.24
Apeh	0.271	6.410	4.317	2.39E-04	8.65E-04	-0.626	1.21
App	0.384	10.319	8.945	4.39E-09	5.09E-08	10.424	1.30
App	0.493	8.605	5.099	3.30E-05	1.50E-04	1.353	1.41
App	0.553	6.325	2.420	2.35E-02	4.75E-02	-5.060	1.47
Arf2	-0.594	6.235	-8.331	1.61E-08	1.61E-07	9.094	-1.51
Arhgap18	0.504	6.623	5.029	3.93E-05	1.73E-04	1.176	1.42
Arhgap18	0.508	7.448	6.550	9.27E-07	6.25E-06	4.964	1.42

Arhgap18	0.667	7.541	7.666	6.96E-08	5.83E-07	7.600	1.59
Arhgap18	0.703	6.699	3.102	4.88E-03	1.22E-02	-3.580	1.63
Arhgap21	-0.757	7.070	-11.983	1.41E-11	3.10E-10	16.300	-1.69
Arhgap25	-1.459	6.665	-17.528	4.01E-15	2.40E-13	24.625	-2.75
Arhgdia	-0.617	7.280	-4.309	2.44E-04	8.81E-04	-0.647	-1.53
Arl2bp	0.298	7.421	5.000	4.23E-05	1.85E-04	1.102	1.23
Arl6ip1	-0.423	8.692	-7.364	1.38E-07	1.10E-06	6.903	-1.34
Arl6ip4	0.364	6.141	5.449	1.37E-05	6.86E-05	2.237	1.29
Arl6ip5	0.289	7.904	6.874	4.30E-07	3.09E-06	5.745	1.22
Arpc2	-0.852	10.069	-18.651	1.01E-15	8.21E-14	26.027	-1.81
Arpp19	-0.443	6.929	-7.887	4.25E-08	3.71E-07	8.103	-1.36
Asf1b	-0.782	5.939	-9.324	2.02E-09	2.51E-08	11.218	-1.72
Aspm	-1.165	6.112	-17.770	2.96E-15	1.85E-13	24.934	-2.24
Atad2	-0.347	6.552	-3.903	6.79E-04	2.19E-03	-1.659	-1.27
Atf5	0.325	9.270	2.795	1.01E-02	2.27E-02	-4.269	1.25
Atic	-0.838	6.808	-14.512	2.49E-13	8.43E-12	20.422	-1.79
Atp1a1	0.270	6.924	3.116	4.72E-03	1.18E-02	-3.547	1.21
Atp5o	0.289	9.052	6.082	2.86E-06	1.68E-05	3.819	1.22
Atp6v0e	0.288	6.614	2.764	1.08E-02	2.42E-02	-4.338	1.22
ATXN7L3B	0.342	6.370	6.852	4.52E-07	3.24E-06	5.694	1.27
AU014716	0.450	7.565	8.360	1.51E-08	1.53E-07	9.158	1.37
AU022840	0.339	8.064	6.413	1.28E-06	8.30E-06	4.633	1.27
Aurka	-0.678	6.331	-8.626	8.57E-09	9.25E-08	9.739	-1.60
Aurkaip1	0.419	8.159	5.637	8.57E-06	4.60E-05	2.710	1.34
Azin1	0.453	6.442	5.460	1.33E-05	6.69E-05	2.266	1.37
Azin1	0.543	6.431	7.496	1.02E-07	8.31E-07	7.208	1.46
B230220B15Rik	0.983	7.506	20.553	1.13E-16	1.50E-14	28.234	1.98
B3galnt2	0.330	6.763	4.394	1.96E-04	7.28E-04	-0.432	1.26
B4galt3	0.269	6.562	4.499	1.50E-04	5.74E-04	-0.166	1.21
Baz1a	-0.270	6.516	-3.150	4.35E-03	1.10E-02	-3.470	-1.21
BC005624	0.336	6.428	3.769	9.50E-04	2.93E-03	-1.990	1.26
BC017643	-0.691	6.869	-16.368	1.82E-14	8.39E-13	23.088	-1.61
BCL10	-0.451	8.488	-8.909	4.72E-09	5.44E-08	10.348	-1.37
Bdnf	-0.826	6.480	-15.868	3.59E-14	1.52E-12	22.397	-1.77
Birc5	-0.932	7.818	-11.548	3.00E-11	6.20E-10	15.525	-1.91
Bmi1	1.014	6.386	12.226	9.31E-12	2.19E-10	16.723	2.02
Brp44l	0.531	8.068	11.867	1.72E-11	3.68E-10	16.095	1.45
Brp44l	0.750	7.208	4.700	9.04E-05	3.66E-04	0.342	1.68
Btbd1	0.322	7.455	2.827	9.36E-03	2.13E-02	-4.199	1.25
BTF3L4	0.308	6.571	5.519	1.15E-05	5.92E-05	2.414	1.24
Bud31	0.277	7.762	4.709	8.85E-05	3.60E-04	0.364	1.21
C78226	-0.359	7.237	-7.984	3.43E-08	3.08E-07	8.322	-1.28
C78997	-0.961	6.861	-18.085	2.00E-15	1.47E-13	25.329	-1.95
Cab39	-0.891	7.933	-17.186	6.21E-15	3.49E-13	24.181	-1.85
Cab39	-0.778	6.216	-6.797	5.15E-07	3.63E-06	5.562	-1.71
Cab39	-0.761	7.377	-12.686	4.32E-12	1.08E-10	17.509	-1.69
Cald1	0.363	7.225	4.093	4.21E-04	1.43E-03	-1.187	1.29
CAMK2N1	0.539	6.214	7.194	2.04E-07	1.55E-06	6.503	1.45
Capns1	0.301	7.079	4.671	9.74E-05	3.89E-04	0.267	1.23
Cat	-0.343	8.131	-3.443	2.14E-03	5.92E-03	-2.781	-1.27
Cav1	-0.581	9.812	-4.686	9.37E-05	3.77E-04	0.307	-1.50
Cav2	-0.862	7.077	-11.180	5.79E-11	1.11E-09	14.853	-1.82
Cav2	-0.600	6.234	-8.296	1.74E-08	1.73E-07	9.018	-1.52
Cbfb	0.340	7.994	5.931	4.14E-06	2.36E-05	3.445	1.27
Cbx5	-0.441	7.118	-4.261	2.75E-04	9.84E-04	-0.766	-1.36
Cbx6-Nptxr	0.325	6.780	4.333	2.29E-04	8.33E-04	-0.586	1.25
Ccdc75	0.336	6.959	4.787	7.24E-05	2.98E-04	0.563	1.26
Ccl2	-1.741	7.806	-13.088	2.25E-12	6.21E-11	18.177	-3.34
Ccl25	0.465	6.699	7.925	3.90E-08	3.44E-07	8.190	1.38
Ccna2	-0.937	6.788	-7.328	1.50E-07	1.18E-06	6.817	-1.91
Ccnb1	-1.272	7.434	-15.136	1.01E-13	3.84E-12	21.348	-2.41
Ccnb2	-0.827	6.467	-12.452	6.36E-12	1.57E-10	17.112	-1.77
Ccnd1	1.098	6.627	12.924	2.92E-12	7.84E-11	17.907	2.14
Cd109	-0.815	6.836	-8.902	4.80E-09	5.51E-08	10.332	-1.76
Cd36	2.789	8.162	13.982	5.53E-13	1.71E-11	19.609	6.91
Cdc20	-0.696	7.231	-7.222	1.91E-07	1.46E-06	6.570	-1.62
Cdipt	0.412	7.020	6.349	1.50E-06	9.53E-06	4.476	1.33
Cdk1	-0.275	8.673	-4.292	2.54E-04	9.15E-04	-0.689	-1.21
Cdk17	-0.367	7.214	-6.004	3.47E-06	1.99E-05	3.625	-1.29
Cdkn3	-1.109	8.297	-12.828	3.42E-12	8.90E-11	17.747	-2.16
Cenpa	-1.251	7.615	-13.299	1.60E-12	4.61E-11	18.522	-2.38
Cenpf	-0.751	6.632	-9.134	2.97E-09	3.59E-08	10.823	-1.68
Cenpl	-1.161	6.032	-16.722	1.14E-14	5.45E-13	23.567	-2.24
Cep55	-0.901	6.066	-10.668	1.48E-10	2.59E-09	13.893	-1.87
Cfl2	0.304	6.863	6.420	1.26E-06	8.20E-06	4.650	1.23
Cflar	-0.404	7.205	-5.128	3.06E-05	1.40E-04	1.427	-1.32
Chordc1	0.314	6.453	4.386	2.00E-04	7.40E-04	-0.451	1.24
Cidec	0.457	6.285	9.075	3.36E-09	3.98E-08	10.698	1.37
Cited2	-0.428	6.419	-4.531	1.39E-04	5.35E-04	-0.087	-1.34

Ckap2	-1.028	5.818	-16.800	1.03E-14	5.05E-13	23.671	-2.04
Ckap5	-0.681	6.097	-8.733	6.84E-09	7.56E-08	9.970	-1.60
Cks1b	-0.489	7.858	-9.806	7.70E-10	1.08E-08	12.204	-1.40
Clec2e	0.479	5.756	4.540	1.36E-04	5.24E-04	-0.064	1.39
Clic1	-0.385	8.811	-8.171	2.28E-08	2.19E-07	8.740	-1.31
Clstn1	0.555	7.732	8.050	2.97E-08	2.71E-07	8.469	1.47
Clu	0.365	10.477	4.318	2.38E-04	8.63E-04	-0.623	1.29
Cnn3	-0.450	8.306	-7.989	3.39E-08	3.05E-07	8.333	-1.37
Cnn3	-0.359	8.547	-9.444	1.58E-09	2.02E-08	11.467	-1.28
Col12a1	-1.221	6.218	-25.471	8.44E-19	2.76E-16	33.135	-2.33
Col16a1	0.368	6.726	5.192	2.61E-05	1.22E-04	1.587	1.29
Col4a3bp	-0.329	6.224	-8.273	1.83E-08	1.81E-07	8.966	-1.26
Col5a1	-0.678	6.209	-10.906	9.52E-11	1.71E-09	14.343	-1.60
Col5a2	-0.999	7.606	-13.722	8.25E-13	2.49E-11	19.200	-2.00
Commd3	0.532	7.861	12.690	4.29E-12	1.08E-10	17.514	1.45
Commd7	0.363	6.618	7.686	6.66E-08	5.60E-07	7.645	1.29
Copa	0.306	7.525	3.306	2.99E-03	7.97E-03	-3.107	1.24
Copa	0.398	6.314	8.105	2.63E-08	2.45E-07	8.594	1.32
Copg	-0.282	6.669	-4.586	1.21E-04	4.70E-04	0.053	-1.22
Cops2	0.370	8.102	5.385	1.61E-05	7.90E-05	2.077	1.29
Cops8	-0.541	7.245	-10.952	8.75E-11	1.60E-09	14.430	-1.46
Cpd	0.572	7.815	10.374	2.57E-10	4.16E-09	13.326	1.49
Creg1	0.471	6.806	4.999	4.24E-05	1.85E-04	1.100	1.39
Creld2	-0.684	7.396	-9.528	1.34E-09	1.73E-08	11.639	-1.61
Csda	0.291	10.028	5.700	7.34E-06	3.99E-05	2.867	1.22
Cse1l	-0.676	7.389	-12.816	3.49E-12	9.02E-11	17.727	-1.60
Csf1	0.376	7.153	6.610	8.01E-07	5.47E-06	5.111	1.30
Csnk1d	-0.355	7.166	-6.118	2.62E-06	1.56E-05	3.908	-1.28
Csnk2a1	0.387	6.763	3.518	1.78E-03	5.06E-03	-2.601	1.31
Ctage5	-0.329	8.036	-5.664	8.01E-06	4.33E-05	2.779	-1.26
Ctbp1	0.351	8.025	6.999	3.21E-07	2.35E-06	6.042	1.28
Cuedc2	0.412	6.975	6.574	8.74E-07	5.95E-06	5.023	1.33
Cul3	-0.841	8.127	-18.093	1.98E-15	1.47E-13	25.339	-1.79
Cul3	-0.829	7.260	-11.250	5.10E-11	9.96E-10	14.982	-1.78
Cul3	-0.781	6.424	-4.255	2.80E-04	1.00E-03	-0.782	-1.72
Cxcl1	-1.236	6.634	-14.442	2.77E-13	9.03E-12	20.316	-2.36
Cyp51	-0.564	9.644	-10.491	2.06E-10	3.46E-09	13.553	-1.48
Cyp51	-0.518	9.012	-9.125	3.03E-09	3.64E-08	10.803	-1.43
D5Ertd579e	0.426	6.408	8.853	5.31E-09	6.00E-08	10.228	1.34
D6Ertd439e	-0.524	6.821	-10.951	8.77E-11	1.60E-09	14.428	-1.44
D830024F11Rik	-0.618	7.236	-9.779	8.12E-10	1.13E-08	12.150	-1.54
Dazap2	0.400	7.914	7.659	7.07E-08	5.88E-07	7.583	1.32
Dbf4	-0.795	6.260	-10.290	3.02E-10	4.73E-09	13.163	-1.74
Dbi	-0.316	8.412	-5.088	3.39E-05	1.53E-04	1.326	-1.24
Dcaf13	0.313	7.411	7.426	1.20E-07	9.67E-07	7.046	1.24
Dctpp1	-0.468	7.125	-7.651	7.20E-08	5.98E-07	7.565	-1.38
Ddhd2	0.399	7.529	8.875	5.08E-09	5.77E-08	10.275	1.32
DDHD2	1.084	9.554	4.615	1.12E-04	4.41E-04	0.126	2.12
Ddit3	-0.616	6.496	-6.457	1.16E-06	7.60E-06	4.738	-1.53
Ddx3x	0.375	6.627	2.562	1.72E-02	3.62E-02	-4.769	1.30
Ddx41	0.377	6.481	4.308	2.45E-04	8.83E-04	-0.650	1.30
Ddx50	-0.361	7.623	-8.250	1.92E-08	1.89E-07	8.916	-1.28
Ddx6	0.291	6.386	4.113	4.00E-04	1.38E-03	-1.137	1.22
Degs1	-0.372	6.778	-8.425	1.32E-08	1.36E-07	9.301	-1.29
Degs1	0.417	8.459	5.589	9.66E-06	5.06E-05	2.590	1.33
Dek	-0.292	7.745	-4.717	8.65E-05	3.53E-04	0.386	-1.22
DLGAP5	-1.225	6.521	-13.266	1.69E-12	4.82E-11	18.467	-2.34
Dnajb2	-1.036	6.280	-9.761	8.41E-10	1.16E-08	12.114	-2.05
Dnajc15	0.298	7.484	5.459	1.33E-05	6.70E-05	2.263	1.23
Dnajc3	-0.423	6.786	-8.243	1.95E-08	1.92E-07	8.901	-1.34
Dnajc3	-0.398	6.788	-5.198	2.57E-05	1.20E-04	1.602	-1.32
Dnpep	-0.997	7.288	-12.685	4.33E-12	1.08E-10	17.506	-2.00
Dnpep	-0.891	6.145	-10.651	1.53E-10	2.65E-09	13.861	-1.85
Dtymk	-0.319	7.027	-5.846	5.12E-06	2.88E-05	3.232	-1.25
Dus1l	-0.334	7.044	-6.500	1.04E-06	6.95E-06	4.843	-1.26
Dusp1	0.587	6.221	9.101	3.18E-09	3.78E-08	10.754	1.50
Ebna1bp2	0.296	7.513	5.765	6.24E-06	3.45E-05	3.031	1.23
Ebna1bp2	0.303	8.826	5.292	2.03E-05	9.75E-05	1.840	1.23
Ebp	-0.875	5.933	-14.507	2.51E-13	8.43E-12	20.415	-1.83
Ect2	-1.076	7.232	-11.133	6.30E-11	1.19E-09	14.766	-2.11
Edf1	0.306	8.060	5.133	3.02E-05	1.39E-04	1.440	1.24
Efnb2	0.342	6.492	5.623	8.87E-06	4.72E-05	2.675	1.27
Eif4a1	-0.442	9.235	-10.317	2.87E-10	4.58E-09	13.215	-1.36
Eif4a1	-0.434	8.429	-2.509	1.93E-02	4.02E-02	-4.878	-1.35
Eif4e2	-0.829	7.044	-22.093	2.20E-17	3.95E-15	29.882	-1.78
Eif4ebp2	0.365	6.811	5.115	3.17E-05	1.45E-04	1.392	1.29
Eif4g3	0.399	7.477	5.195	2.59E-05	1.21E-04	1.596	1.32
Eif5a	0.419	6.847	8.655	8.05E-09	8.74E-08	9.803	1.34
Eif5a	0.547	9.394	4.432	1.79E-04	6.69E-04	-0.337	1.46

Emb	0.331	8.004	9.241	2.39E-09	2.94E-08	11.047	1.26
Emp1	-0.928	7.772	-18.586	1.09E-15	8.69E-14	25.948	-1.90
Eng	0.380	7.236	3.254	3.39E-03	8.87E-03	-3.228	1.30
Ephx1	0.624	8.644	8.401	1.38E-08	1.42E-07	9.249	1.54
Ephx1	0.783	7.673	5.051	3.72E-05	1.65E-04	1.232	1.72
Eprs	0.401	8.814	10.770	1.22E-10	2.16E-09	14.087	1.32
Eprs	0.552	6.781	4.453	1.69E-04	6.40E-04	-0.282	1.47
Eps8	-0.359	7.562	-4.466	1.64E-04	6.21E-04	-0.250	-1.28
Ercc1	0.588	6.529	7.709	6.32E-08	5.35E-07	7.698	1.50
Erlec1	-0.304	6.948	-7.789	5.28E-08	4.54E-07	7.881	-1.23
Erp44	-0.294	7.100	-6.131	2.54E-06	1.52E-05	3.939	-1.23
Esd	-0.599	8.915	-14.548	2.36E-13	8.08E-12	20.477	-1.51
Ethe1	0.359	7.237	5.141	2.96E-05	1.37E-04	1.460	1.28
EWSR1	0.526	7.376	14.325	3.30E-13	1.05E-11	20.137	1.44
Ext2	0.468	6.990	9.064	3.43E-09	4.05E-08	10.676	1.38
Fam100b	0.345	6.914	4.836	6.41E-05	2.68E-04	0.686	1.27
Fam107b	0.456	6.765	8.889	4.93E-09	5.63E-08	10.304	1.37
Fam125a	0.390	6.719	6.087	2.83E-06	1.67E-05	3.830	1.31
Fam125b	0.435	6.716	8.428	1.31E-08	1.36E-07	9.308	1.35
Fam129c	0.366	7.400	4.584	1.21E-04	4.72E-04	0.048	1.29
Fam134a	-0.967	7.086	-10.162	3.86E-10	5.85E-09	12.912	-1.96
Fam32a	0.297	6.710	5.446	1.38E-05	6.91E-05	2.229	1.23
Fam32a	0.300	7.017	6.151	2.42E-06	1.46E-05	3.988	1.23
Farp2	-0.647	6.968	-11.914	1.59E-11	3.43E-10	16.178	-1.57
Farsb	-0.991	7.210	-21.287	5.11E-17	7.98E-15	29.034	-1.99
Farsb	-0.938	7.344	-19.105	5.87E-16	5.02E-14	26.571	-1.92
Fasn	-1.002	6.330	-10.031	4.96E-10	7.36E-09	12.654	-2.00
Fat1	-0.476	8.629	-9.397	1.74E-09	2.19E-08	11.369	-1.39
Fbxo28	0.598	7.050	11.624	2.62E-11	5.55E-10	15.662	1.51
Fbxo6	0.485	7.635	5.822	5.42E-06	3.04E-05	3.173	1.40
Fbxo8	1.093	6.542	22.105	2.17E-17	3.95E-15	29.894	2.13
Fcgrt	0.818	6.491	11.962	1.46E-11	3.20E-10	16.263	1.76
Fdps	-0.802	8.124	-13.705	8.48E-13	2.54E-11	19.172	-1.74
Fez2	-0.464	7.112	-10.382	2.53E-10	4.14E-09	13.342	-1.38
Fh1	0.292	8.282	5.721	6.96E-06	3.83E-05	2.921	1.22
Fhl2	-0.288	6.258	-6.555	9.15E-07	6.19E-06	4.977	-1.22
Fkbp9	-0.369	7.631	-7.151	2.25E-07	1.71E-06	6.403	-1.29
Flnb	-0.295	6.896	-5.473	1.29E-05	6.53E-05	2.296	-1.23
Fmc1	0.545	9.383	7.416	1.22E-07	9.84E-07	7.024	1.46
FMNL2	0.478	7.362	7.237	1.85E-07	1.42E-06	6.606	1.39
Fnip2	0.504	7.370	7.667	6.94E-08	5.82E-07	7.603	1.42
Foxj3	0.437	7.777	7.897	4.16E-08	3.63E-07	8.126	1.35
Fry	0.468	7.685	6.712	6.30E-07	4.40E-06	5.356	1.38
FSTL1	-0.376	8.751	-9.009	3.84E-09	4.48E-08	10.560	-1.30
Fubp3	0.360	6.446	6.671	6.93E-07	4.80E-06	5.258	1.28
Fundc1	0.346	6.478	4.070	4.46E-04	1.50E-03	-1.244	1.27
G3bp2	0.301	8.617	5.633	8.67E-06	4.64E-05	2.699	1.23
Gaa	0.313	9.198	4.522	1.42E-04	5.46E-04	-0.109	1.24
Gabarap	0.450	9.365	9.717	9.18E-10	1.24E-08	12.024	1.37
Gadd45a	-0.307	8.855	-4.600	1.16E-04	4.56E-04	0.089	-1.24
Gadd45b	-0.666	6.746	-9.795	7.87E-10	1.10E-08	12.182	-1.59
Galnt7	0.846	7.466	8.342	1.57E-08	1.58E-07	9.118	1.80
Gas1	1.367	6.643	14.464	2.68E-13	8.82E-12	20.349	2.58
Gclm	-0.302	9.225	-5.800	5.73E-06	3.19E-05	3.117	-1.23
Gga2	0.503	8.255	13.232	1.78E-12	5.05E-11	18.412	1.42
Gga2	0.564	6.153	7.122	2.41E-07	1.82E-06	6.333	1.48
Ghr	0.551	6.655	8.752	6.57E-09	7.29E-08	10.011	1.46
Gipc1	0.330	6.525	5.376	1.65E-05	8.05E-05	2.052	1.26
Glud1	0.297	8.163	5.900	4.48E-06	2.54E-05	3.367	1.23
Gm10154	-0.283	12.036	-7.704	6.39E-08	5.41E-07	7.686	-1.22
Gm10259	-0.777	7.512	-12.441	6.49E-12	1.59E-10	17.093	-1.71
Gm10397	0.633	7.405	12.150	1.06E-11	2.42E-10	16.591	1.55
Gm11602	-0.961	7.611	-14.384	3.02E-13	9.67E-12	20.228	-1.95
Gm11602	-0.916	6.581	-6.518	1.00E-06	6.71E-06	4.887	-1.89
Gm15452	-0.798	8.689	-10.204	3.55E-10	5.45E-09	12.996	-1.74
Gm3650	0.295	7.214	5.698	7.36E-06	4.00E-05	2.864	1.23
Gm5506	0.269	9.306	4.847	6.23E-05	2.60E-04	0.715	1.20
Gm5578	0.617	6.726	11.522	3.14E-11	6.45E-10	15.479	1.53
Gm5593	-1.134	5.956	-7.965	3.58E-08	3.20E-07	8.278	-2.19
Gm6158	-0.263	7.472	-5.069	3.56E-05	1.59E-04	1.277	-1.20
Gm6644	-0.268	9.438	-5.299	1.99E-05	9.59E-05	1.860	-1.20
Gm6829	0.327	8.199	6.476	1.10E-06	7.32E-06	4.786	1.25
Gm7859	0.300	6.920	8.440	1.27E-08	1.32E-07	9.335	1.23
Gm9178	0.362	6.970	6.084	2.85E-06	1.68E-05	3.824	1.29
Gm9803	0.331	8.011	6.314	1.63E-06	1.02E-05	4.391	1.26
Gm9816	-0.535	7.596	-7.039	2.92E-07	2.17E-06	6.138	-1.45
Gmcl1	0.292	6.620	4.427	1.81E-04	6.76E-04	-0.350	1.22
Gnpat	-0.435	6.582	-7.942	3.76E-08	3.34E-07	8.228	-1.35
Gpc6	-1.653	3.584	-19.724	2.87E-16	2.64E-14	27.296	-3.14

Gpd2	-0.302	6.879	-3.975	5.67E-04	1.85E-03	-1.483	-1.23
Gpnmb	1.279	5.966	20.289	1.52E-16	1.88E-14	27.938	2.43
Gpr56	1.706	6.194	21.723	3.23E-17	5.27E-15	29.496	3.26
Gstm1	0.685	9.649	9.898	6.43E-10	9.31E-09	12.389	1.61
Gstm6	0.321	7.960	4.344	2.23E-04	8.14E-04	-0.558	1.25
Gstm7	0.316	8.876	4.553	1.31E-04	5.08E-04	-0.030	1.25
Gt(ROSA)26Sor	-0.404	6.526	-6.987	3.30E-07	2.41E-06	6.013	-1.32
Gtf2a2	0.296	8.193	8.237	1.97E-08	1.94E-07	8.888	1.23
Gtf2h5	0.312	6.911	5.206	2.52E-05	1.18E-04	1.625	1.24
Gtf2i	0.296	6.845	6.275	1.79E-06	1.12E-05	4.293	1.23
Guk1	0.393	7.485	7.844	4.67E-08	4.06E-07	8.007	1.31
Gusb	-0.391	6.935	-7.772	5.49E-08	4.71E-07	7.842	-1.31
Gusb	-0.360	7.029	-6.113	2.65E-06	1.58E-05	3.896	-1.28
Gyg	0.796	8.497	9.732	8.91E-10	1.21E-08	12.054	1.74
Gypc	0.316	7.466	5.425	1.45E-05	7.25E-05	2.176	1.24
H2afx	-0.638	6.799	-8.080	2.78E-08	2.56E-07	8.538	-1.56
H2afy	0.479	8.313	11.893	1.64E-11	3.53E-10	16.142	1.39
H3f3a	0.397	10.382	9.740	8.77E-10	1.20E-08	12.070	1.32
H3f3a	0.616	10.194	8.114	2.58E-08	2.43E-07	8.613	1.53
H47	-0.304	6.598	-6.528	9.75E-07	6.56E-06	4.912	-1.23
Hat1	-0.540	6.594	-7.722	6.14E-08	5.23E-07	7.728	-1.45
Hbxip	0.383	8.226	7.298	1.60E-07	1.25E-06	6.748	1.30
Hdac5	0.387	6.214	5.268	2.16E-05	1.03E-04	1.780	1.31
Hdlbp	-0.990	7.276	-21.967	2.50E-17	4.28E-15	29.751	-1.99
Hdlbp	-0.568	6.583	-5.828	5.35E-06	3.01E-05	3.187	-1.48
Hdlbp	-0.464	6.619	-6.914	3.91E-07	2.83E-06	5.840	-1.38
Heg1	0.309	8.653	5.000	4.24E-05	1.85E-04	1.101	1.24
Herc2	-0.360	6.883	-6.660	7.13E-07	4.92E-06	5.230	-1.28
Herpud1	-0.334	6.811	-7.538	9.28E-08	7.60E-07	7.306	-1.26
Hexim1	0.684	7.032	9.899	6.41E-10	9.31E-09	12.391	1.61
Hint1	0.279	9.534	6.454	1.16E-06	7.64E-06	4.731	1.21
Hip1	0.509	6.987	10.089	4.44E-10	6.67E-09	12.768	1.42
Hist1h2bc	0.318	7.314	3.731	1.05E-03	3.19E-03	-2.084	1.25
HMGCR	-0.886	6.572	-13.215	1.83E-12	5.14E-11	18.385	-1.85
Hmgcs1	-0.858	6.887	-15.549	5.60E-14	2.23E-12	21.945	-1.81
Hmgn5	-0.473	8.999	-8.666	7.88E-09	8.60E-08	9.825	-1.39
Hmox1	-0.342	7.105	-3.958	5.91E-04	1.92E-03	-1.523	-1.27
Hnrnpa1	-0.447	6.520	-6.694	6.56E-07	4.57E-06	5.314	-1.36
Hnrnpa3	-0.431	8.503	-4.663	9.94E-05	3.96E-04	0.247	-1.35
Hnrnpa3	-0.421	9.119	-8.659	8.00E-09	8.71E-08	9.810	-1.34
Hnrnpk	-0.273	9.607	-4.993	4.30E-05	1.87E-04	1.085	-1.21
Hnrpdl	-0.352	6.768	-5.377	1.64E-05	8.04E-05	2.056	-1.28
Hnrpdl	-0.273	6.334	-4.277	2.64E-04	9.48E-04	-0.726	-1.21
Hprt	0.541	9.127	10.916	9.35E-11	1.69E-09	14.363	1.45
Hsd17b12	-0.500	7.796	-10.455	2.20E-10	3.65E-09	13.485	-1.41
Hsp90b1	-0.937	8.375	-4.896	5.51E-05	2.34E-04	0.838	-1.91
Hsp90b1	-0.847	7.905	-3.830	8.16E-04	2.58E-03	-1.841	-1.80
Hsp90b1	-0.837	10.245	-13.115	2.15E-12	5.99E-11	18.222	-1.79
Hspa4	0.295	8.370	5.724	6.92E-06	3.81E-05	2.927	1.23
Hspa5	-0.808	11.437	-17.244	5.77E-15	3.34E-13	24.256	-1.75
Hspb8	0.344	7.970	6.999	3.20E-07	2.35E-06	6.044	1.27
Hyou1	-0.678	6.015	-8.306	1.70E-08	1.70E-07	9.040	-1.60
Iars	0.484	6.251	2.467	2.12E-02	4.34E-02	-4.964	1.40
Iars2	0.352	7.066	8.900	4.82E-09	5.51E-08	10.328	1.28
Iars2	0.384	7.976	10.294	2.99E-10	4.71E-09	13.171	1.31
IDH1	-0.487	8.313	-6.473	1.11E-06	7.34E-06	4.777	-1.40
Idi1	-1.011	6.124	-16.937	8.58E-15	4.47E-13	23.853	-2.02
Ifngr2	-0.304	6.377	-6.655	7.20E-07	4.95E-06	5.220	-1.23
Il1r1	0.351	6.416	5.049	3.74E-05	1.66E-04	1.227	1.28
Ildr2	0.315	6.552	6.153	2.41E-06	1.45E-05	3.993	1.24
IMMP1L	0.295	6.798	4.446	1.72E-04	6.49E-04	-0.301	1.23
ING1	-0.396	6.587	-8.394	1.40E-08	1.44E-07	9.234	-1.32
Ing1	-0.329	7.161	-9.195	2.62E-09	3.20E-08	10.950	-1.26
Irs1	-1.267	6.303	-19.195	5.28E-16	4.63E-14	26.678	-2.41
Isy1	0.328	7.407	6.941	3.67E-07	2.66E-06	5.906	1.26
Itgb5	0.339	7.557	3.795	8.91E-04	2.78E-03	-1.927	1.26
Itih5	0.425	6.180	3.827	8.22E-04	2.60E-03	-1.848	1.34
Itm2c	-0.871	8.920	-16.651	1.25E-14	5.90E-13	23.471	-1.83
Jak2	-0.489	6.679	-6.825	4.83E-07	3.44E-06	5.627	-1.40
Jun	-0.848	7.869	-11.943	1.51E-11	3.29E-10	16.229	-1.80
Kif23	-1.123	6.746	-17.364	4.94E-15	2.91E-13	24.413	-2.18
Kifc1	-0.626	6.589	-9.829	7.36E-10	1.04E-08	12.251	-1.54
Klc1	-0.310	7.277	-5.619	8.98E-06	4.76E-05	2.663	-1.24
Klhl5	0.308	7.361	5.771	6.16E-06	3.41E-05	3.044	1.24
Kpnb1	-0.444	8.159	-8.035	3.06E-08	2.79E-07	8.438	-1.36
KPNB1	-0.295	7.476	-2.919	7.54E-03	1.76E-02	-3.995	-1.23
Ktn1	-0.343	7.099	-8.477	1.18E-08	1.23E-07	9.416	-1.27
Lamp2	0.740	6.487	11.450	3.57E-11	7.21E-10	15.347	1.67
Lamp3	-0.401	7.710	-7.522	9.64E-08	7.87E-07	7.268	-1.32

Lass5	0.424	6.341	6.128	2.56E-06	1.53E-05	3.931	1.34
Lats2	0.363	7.475	6.022	3.32E-06	1.92E-05	3.670	1.29
LBR	0.641	7.626	14.911	1.39E-13	5.04E-12	21.018	1.56
Ldlr	-0.661	6.729	-10.559	1.81E-10	3.06E-09	13.684	-1.58
Lgmn	-0.352	9.298	-6.793	5.19E-07	3.66E-06	5.552	-1.28
LOC100043810	0.375	7.540	4.521	1.42E-04	5.46E-04	-0.110	1.30
LOC100131826	-0.477	7.320	-5.615	9.05E-06	4.79E-05	2.655	-1.39
LOC100270747	-0.264	6.813	-4.383	2.02E-04	7.46E-04	-0.460	-1.20
LOC294154	0.300	5.851	2.411	2.40E-02	4.84E-02	-5.079	1.23
LOC544737	0.348	7.040	4.848	6.22E-05	2.60E-04	0.716	1.27
LOC624853	0.330	9.491	2.484	2.04E-02	4.22E-02	-4.931	1.26
LOC684894	0.288	8.353	5.078	3.48E-05	1.56E-04	1.299	1.22
LOC685953	2.644	9.130	25.337	9.53E-19	2.85E-16	33.015	6.25
Lpp	0.274	6.385	3.091	5.01E-03	1.25E-02	-3.605	1.21
Lpp	0.292	7.853	5.247	2.27E-05	1.08E-04	1.726	1.22
Lrp10	-0.568	7.803	-7.954	3.67E-08	3.26E-07	8.254	-1.48
Lrpap1	0.269	7.109	4.215	3.09E-04	1.09E-03	-0.882	1.21
Lrrc8d	-0.355	6.697	-4.236	2.93E-04	1.05E-03	-0.830	-1.28
Lsm3	-0.421	7.307	-7.601	8.06E-08	6.65E-07	7.451	-1.34
Lsmd1	0.346	6.667	6.077	2.90E-06	1.70E-05	3.806	1.27
Ly6e	-0.540	7.105	-7.243	1.82E-07	1.40E-06	6.619	-1.45
Lypla1	0.423	6.729	6.004	3.46E-06	1.99E-05	3.626	1.34
Lyplal1	0.528	7.767	9.657	1.03E-09	1.37E-08	11.902	1.44
Macrod1	0.518	6.433	7.933	3.84E-08	3.40E-07	8.206	1.43
Mad2l1	-0.790	7.100	-12.044	1.27E-11	2.81E-10	16.407	-1.73
Mad2l1	-0.694	6.292	-5.638	8.54E-06	4.59E-05	2.713	-1.62
Mafg	-0.771	6.127	-8.097	2.67E-08	2.48E-07	8.576	-1.71
MAMDC2	0.428	7.429	3.707	1.11E-03	3.34E-03	-2.142	1.35
Man2a1	-0.737	6.504	-10.202	3.57E-10	5.46E-09	12.991	-1.67
Man2a1	-0.601	6.683	-8.155	2.36E-08	2.24E-07	8.705	-1.52
Manf	-0.947	8.744	-17.171	6.34E-15	3.50E-13	24.161	-1.93
Map1lc3b	0.275	8.566	8.380	1.45E-08	1.47E-07	9.203	1.21
Map1lc3b	0.408	8.856	5.660	8.10E-06	4.36E-05	2.767	1.33
Mapk14	0.372	7.353	10.470	2.14E-10	3.57E-09	13.513	1.29
Mapk6	-0.347	7.276	-4.799	7.05E-05	2.91E-04	0.591	-1.27
Mark1	0.941	6.616	20.121	1.83E-16	2.05E-14	27.750	1.92
Mark3	-0.288	6.635	-6.321	1.60E-06	1.01E-05	4.407	-1.22
Mat2a	0.266	7.126	3.925	6.43E-04	2.07E-03	-1.605	1.20
Mbnl2	0.432	8.281	10.127	4.12E-10	6.22E-09	12.844	1.35
Mbnl2	0.470	7.563	8.213	2.08E-08	2.01E-07	8.833	1.39
Mbp	0.804	6.692	12.122	1.11E-11	2.51E-10	16.543	1.75
Mcl1	0.322	7.354	7.660	7.05E-08	5.88E-07	7.587	1.25
MCM7	0.273	7.318	3.863	7.52E-04	2.40E-03	-1.760	1.21
Mdfic	0.310	9.142	5.251	2.25E-05	1.07E-04	1.737	1.24
Mdp1	0.340	6.783	6.482	1.09E-06	7.24E-06	4.799	1.27
Me1	-0.293	7.376	-6.290	1.73E-06	1.08E-05	4.331	-1.23
Me1	-0.291	7.716	-4.428	1.80E-04	6.74E-04	-0.346	-1.22
Mesdc2	-0.398	9.074	-12.293	8.31E-12	1.98E-10	16.839	-1.32
METTL10	-0.327	8.998	-5.307	1.95E-05	9.43E-05	1.880	-1.25
Mettl11a	0.289	6.539	4.554	1.31E-04	5.06E-04	-0.027	1.22
MGC112830	0.530	6.352	9.036	3.64E-09	4.27E-08	10.616	1.44
Mia3	0.362	8.330	6.643	7.42E-07	5.09E-06	5.190	1.29
Mitf	0.450	7.306	6.281	1.77E-06	1.10E-05	4.309	1.37
Mknk2	0.390	6.736	4.373	2.07E-04	7.62E-04	-0.484	1.31
Mknk2	0.457	6.338	6.507	1.02E-06	6.84E-06	4.862	1.37
Mll1	-0.268	6.439	-3.503	1.84E-03	5.23E-03	-2.638	-1.20
Mlycd	0.427	6.291	7.914	4.00E-08	3.52E-07	8.165	1.34
Mmp14	-0.347	6.141	-3.397	2.39E-03	6.54E-03	-2.889	-1.27
Mmp9	-0.708	6.731	-11.211	5.47E-11	1.06E-09	14.910	-1.63
Mosc2	0.814	7.334	19.588	3.35E-16	3.01E-14	27.139	1.76
Mosc2	1.108	8.107	17.873	2.60E-15	1.73E-13	25.063	2.16
Mpv17	0.370	7.533	4.030	4.93E-04	1.64E-03	-1.344	1.29
Mrpl12	-0.291	8.284	-5.907	4.40E-06	2.50E-05	3.384	-1.22
Mrpl53	0.269	6.945	3.988	5.49E-04	1.81E-03	-1.450	1.21
Mrps10	0.327	6.973	3.984	5.54E-04	1.82E-03	-1.460	1.25
Mrps23	0.268	6.974	5.709	7.18E-06	3.92E-05	2.889	1.20
Msrb2	0.548	6.378	8.262	1.87E-08	1.85E-07	8.942	1.46
Mta3	0.405	6.286	7.259	1.76E-07	1.36E-06	6.656	1.32
Mtap1b	-0.321	6.862	-6.218	2.06E-06	1.26E-05	4.154	-1.25
Mtap4	-0.378	6.079	-4.401	1.93E-04	7.17E-04	-0.414	-1.30
Mtbp	-0.335	6.107	-6.346	1.51E-06	9.57E-06	4.468	-1.26
Mtdh	0.392	7.693	7.644	7.31E-08	6.05E-07	7.550	1.31
Mthfd2l	-0.335	6.000	-5.254	2.23E-05	1.06E-04	1.744	-1.26
Myc	-0.319	9.167	-7.368	1.37E-07	1.09E-06	6.912	-1.25
Myd88	0.360	6.590	8.712	7.14E-09	7.87E-08	9.926	1.28
Myl12b	-0.589	10.684	-7.341	1.45E-07	1.15E-06	6.849	-1.50
MYL12B	-0.406	11.165	-12.182	1.00E-11	2.34E-10	16.647	-1.33
Myof	-0.580	9.190	-9.996	5.31E-10	7.86E-09	12.584	-1.49
N6amt2	0.438	6.228	7.339	1.46E-07	1.15E-06	6.844	1.35

Ncam1	0.311	5.358	3.453	2.08E-03	5.80E-03	-2.756	1.24
Ncapd3	-1.396	5.993	-19.804	2.62E-16	2.47E-14	27.388	-2.63
Ncaph	-0.400	6.789	-6.051	3.09E-06	1.80E-05	3.742	-1.32
Nck2	0.404	6.349	5.105	3.25E-05	1.48E-04	1.368	1.32
Ndc80	-1.470	6.736	-17.909	2.49E-15	1.69E-13	25.109	-2.77
Ndufa10	-0.487	6.714	-5.932	4.14E-06	2.36E-05	3.446	-1.40
Ndufa7	0.283	8.951	7.305	1.58E-07	1.24E-06	6.763	1.22
Ndufab1	-0.315	6.612	-5.443	1.39E-05	6.93E-05	2.223	-1.24
Ndufaf2	0.306	7.178	5.619	8.97E-06	4.76E-05	2.664	1.24
Ndufb9	0.401	10.546	11.456	3.53E-11	7.16E-10	15.359	1.32
Ndufv2	-0.813	9.165	-15.286	8.11E-14	3.13E-12	21.566	-1.76
Neat1	-0.531	8.207	-8.099	2.66E-08	2.48E-07	8.580	-1.44
Neil3	-0.582	7.798	-8.220	2.05E-08	1.99E-07	8.849	-1.50
Nek2	-0.332	8.036	-5.049	3.74E-05	1.66E-04	1.225	-1.26
Nfic	-0.349	6.666	-4.919	5.20E-05	2.22E-04	0.896	-1.27
Nfkb2	-0.375	6.057	-3.454	2.08E-03	5.79E-03	-2.755	-1.30
Nfkbia	-1.116	8.243	-18.021	2.17E-15	1.56E-13	25.250	-2.17
Nfkbia	-1.061	8.519	-15.074	1.10E-13	4.15E-12	21.259	-2.09
Nhp2	0.379	7.341	9.107	3.14E-09	3.76E-08	10.767	1.30
Nit1	0.432	6.656	5.462	1.32E-05	6.67E-05	2.271	1.35
Nit2	0.353	7.294	5.238	2.32E-05	1.10E-04	1.706	1.28
Nme1	0.307	9.016	6.881	4.23E-07	3.04E-06	5.762	1.24
Nmt1	0.292	8.067	4.691	9.25E-05	3.73E-04	0.320	1.22
Npc2	-0.276	9.515	-6.056	3.05E-06	1.78E-05	3.754	-1.21
Nr1h3	0.314	6.661	3.706	1.11E-03	3.35E-03	-2.145	1.24
Nr2c1	0.525	6.434	10.325	2.82E-10	4.52E-09	13.232	1.44
Nras	-0.273	7.871	-5.414	1.49E-05	7.39E-05	2.149	-1.21
Nsun5	0.311	6.545	3.578	1.53E-03	4.43E-03	-2.456	1.24
Nucb2	-0.331	7.514	-6.262	1.85E-06	1.15E-05	4.261	-1.26
Nudt3	0.408	6.423	4.834	6.43E-05	2.68E-04	0.682	1.33
Nudt5	0.304	7.630	5.649	8.31E-06	4.47E-05	2.741	1.23
Nusap1	-1.179	6.996	-14.774	1.70E-13	5.97E-12	20.815	-2.26
Nvl	0.947	6.210	17.643	3.47E-15	2.12E-13	24.771	1.93
Oaz1	-0.303	7.862	-4.000	5.32E-04	1.76E-03	-1.419	-1.23
Oaz1	-0.293	7.602	-5.344	1.78E-05	8.68E-05	1.972	-1.22
Oaz2	-0.418	6.880	-7.226	1.89E-07	1.45E-06	6.579	-1.34
Osgep	0.389	6.568	11.245	5.14E-11	9.99E-10	14.974	1.31
Osmr	-0.378	7.659	-5.902	4.45E-06	2.52E-05	3.373	-1.30
Ostc	-0.789	9.604	-17.122	6.75E-15	3.66E-13	24.097	-1.73
Ostc	-0.719	8.595	-10.649	1.53E-10	2.65E-09	13.857	-1.65
Otub1	0.263	6.529	3.964	5.83E-04	1.90E-03	-1.509	1.20
Oxr1	-1.437	6.713	-20.580	1.10E-16	1.50E-14	28.263	-2.71
P4hb	-0.559	8.556	-9.159	2.82E-09	3.43E-08	10.876	-1.47
P4hb	-0.517	7.388	-8.489	1.15E-08	1.20E-07	9.441	-1.43
Palld	-0.991	7.134	-20.224	1.63E-16	1.95E-14	27.866	-1.99
Pam	-0.881	7.027	-12.865	3.22E-12	8.45E-11	17.808	-1.84
PAPSS1	-0.342	6.702	-5.235	2.34E-05	1.11E-04	1.696	-1.27
Parp1	0.688	6.304	11.287	4.77E-11	9.37E-10	15.050	1.61
Parva	0.356	8.144	4.776	7.46E-05	3.07E-04	0.534	1.28
Parva	0.367	6.308	5.627	8.78E-06	4.69E-05	2.685	1.29
Pbk	-0.974	6.261	-11.112	6.55E-11	1.23E-09	14.727	-1.96
Pcgf5	-0.335	6.328	-6.820	4.88E-07	3.47E-06	5.616	-1.26
Pde6d	-1.093	6.781	-8.804	5.89E-09	6.59E-08	10.123	-2.13
Pdia3	-0.640	9.730	-10.295	2.99E-10	4.71E-09	13.173	-1.56
Pdia4	-0.803	7.671	-17.093	7.00E-15	3.70E-13	24.059	-1.74
Pdia4	-0.788	6.027	-3.953	5.99E-04	1.94E-03	-1.535	-1.73
Pdia6	-0.587	10.091	-14.486	2.59E-13	8.63E-12	20.382	-1.50
Pex19	1.237	6.163	25.476	8.40E-19	2.76E-16	33.140	2.36
Pfdn5	-0.326	8.087	-6.789	5.25E-07	3.69E-06	5.542	-1.25
Pgs1	-0.538	6.290	-7.963	3.59E-08	3.20E-07	8.276	-1.45
Phf20	-0.289	6.196	-6.192	2.19E-06	1.33E-05	4.089	-1.22
Phf20	-0.277	6.211	-4.120	3.94E-04	1.37E-03	-1.121	-1.21
Picalm	-0.321	8.359	-5.955	3.91E-06	2.24E-05	3.504	-1.25
PIGT	-0.535	6.318	-5.329	1.85E-05	8.96E-05	1.933	-1.45
Pin4	0.313	6.767	6.251	1.90E-06	1.18E-05	4.236	1.24
Pir	-0.349	6.603	-5.126	3.08E-05	1.41E-04	1.421	-1.27
Pja1	0.488	7.598	11.618	2.65E-11	5.57E-10	15.651	1.40
Pja2	-0.983	7.115	-18.552	1.13E-15	8.85E-14	25.906	-1.98
Pja2	-0.931	7.451	-19.874	2.42E-16	2.47E-14	27.469	-1.91
Plaa	-0.340	6.833	-6.944	3.64E-07	2.65E-06	5.913	-1.27
Plac8	1.246	5.438	12.473	6.15E-12	1.52E-10	17.147	2.37
Pldn	0.409	6.610	9.681	9.86E-10	1.31E-08	11.950	1.33
Plekha1	0.319	6.363	6.370	1.43E-06	9.13E-06	4.526	1.25
Plekhm1	0.352	5.234	3.536	1.70E-03	4.84E-03	-2.557	1.28
Plin2	0.594	8.507	3.089	5.04E-03	1.25E-02	-3.611	1.51
Plin2	0.617	9.911	5.585	9.74E-06	5.10E-05	2.580	1.53
Plin2	0.656	9.393	15.796	3.97E-14	1.66E-12	22.296	1.58
PLK2	-1.419	7.605	-27.860	1.06E-19	4.78E-17	35.182	-2.67
Plp2	-0.413	7.148	-5.699	7.35E-06	4.00E-05	2.865	-1.33

Pls3	0.341	8.295	5.510	1.18E-05	6.02E-05	2.391	1.27
Pltp	0.528	7.226	4.026	4.98E-04	1.65E-03	-1.355	1.44
Pmpca	0.280	7.183	4.568	1.27E-04	4.91E-04	0.006	1.21
Pnn	-0.416	7.266	-7.813	5.01E-08	4.33E-07	7.935	-1.33
Pnn	-0.312	7.913	-4.567	1.27E-04	4.92E-04	0.004	-1.24
Poldip2	0.272	7.267	5.379	1.63E-05	8.00E-05	2.061	1.21
Polr2g	0.400	8.219	9.859	6.94E-10	9.86E-09	12.310	1.32
Ppcs	0.913	7.062	16.209	2.26E-14	1.00E-12	22.870	1.88
Ppic	0.552	7.196	8.550	1.01E-08	1.07E-07	9.575	1.47
Ppip5k2	-0.691	7.088	-10.073	4.57E-10	6.85E-09	12.737	-1.61
Ppp1cb	0.331	9.069	5.048	3.75E-05	1.66E-04	1.225	1.26
Ppp1r7	-0.363	6.619	-6.205	2.12E-06	1.30E-05	4.121	-1.29
Ppp1r7	-0.341	7.153	-4.798	7.06E-05	2.91E-04	0.589	-1.27
Ppp1r7	-0.325	6.514	-2.870	8.47E-03	1.96E-02	-4.105	-1.25
PPP2R5A	1.027	6.010	16.871	9.35E-15	4.75E-13	23.766	2.04
Ppp2r5c	-0.776	7.078	-16.355	1.85E-14	8.43E-13	23.070	-1.71
Ppp2r5c	0.381	7.119	5.805	5.65E-06	3.15E-05	3.130	1.30
Ppp4r1	-1.449	7.146	-22.856	1.01E-17	2.27E-15	30.658	-2.73
Pppde1	0.530	6.727	11.136	6.26E-11	1.18E-09	14.772	1.44
Pppde1	0.695	6.277	7.249	1.80E-07	1.39E-06	6.633	1.62
Prc1	-0.999	7.462	-10.417	2.37E-10	3.90E-09	13.410	-2.00
Prcp	-0.556	7.535	-13.317	1.56E-12	4.51E-11	18.550	-1.47
Prdx3	0.270	8.036	4.017	5.10E-04	1.69E-03	-1.378	1.21
Prdx4	-0.539	8.079	-12.256	8.85E-12	2.09E-10	16.775	-1.45
Prkca	-0.439	5.864	-5.848	5.09E-06	2.87E-05	3.237	-1.36
Prkcdbp	-0.316	6.389	-2.934	7.28E-03	1.72E-02	-3.961	-1.24
Prkcsh	-0.373	6.754	-3.359	2.63E-03	7.13E-03	-2.982	-1.30
Prkrir	0.425	7.275	9.764	8.36E-10	1.16E-08	12.119	1.34
Prmt5	-0.669	7.197	-8.995	3.96E-09	4.60E-08	10.529	-1.59
Pros1	-0.348	7.149	-6.062	3.01E-06	1.76E-05	3.768	-1.27
Prpf38b	-0.448	7.449	-7.402	1.27E-07	1.01E-06	6.990	-1.36
Prune2	-0.454	7.588	-8.187	2.20E-08	2.12E-07	8.776	-1.37
Psap	0.460	8.656	8.521	1.07E-08	1.13E-07	9.511	1.38
Psat1	-0.265	9.538	-5.812	5.56E-06	3.11E-05	3.148	-1.20
Psmb3	0.288	8.872	3.663	1.24E-03	3.67E-03	-2.249	1.22
PSMB5	-0.334	9.145	-3.583	1.51E-03	4.39E-03	-2.444	-1.26
Psmb6	0.285	8.403	4.914	5.26E-05	2.25E-04	0.884	1.22
Psmc4	0.421	7.500	5.995	3.55E-06	2.04E-05	3.602	1.34
Psmc4	0.496	6.706	4.401	1.93E-04	7.17E-04	-0.415	1.41
Psmc5	0.361	7.802	9.048	3.54E-09	4.18E-08	10.642	1.28
Psmc5	0.375	7.329	4.249	2.83E-04	1.01E-03	-0.796	1.30
Psmd1	-0.679	7.960	-10.270	3.13E-10	4.88E-09	13.125	-1.60
Psmd1	-0.647	6.492	-9.971	5.57E-10	8.20E-09	12.535	-1.57
Psmd8	0.311	7.549	5.518	1.15E-05	5.93E-05	2.410	1.24
Psmg1	0.287	7.725	7.363	1.38E-07	1.10E-06	6.899	1.22
Psmg4	0.342	6.705	6.981	3.34E-07	2.44E-06	6.001	1.27
Ptma	-0.721	7.975	-8.114	2.58E-08	2.43E-07	8.613	-1.65
Ptma	-0.464	11.781	-4.817	6.73E-05	2.79E-04	0.637	-1.38
Ptpmt1	-0.292	6.973	-6.016	3.36E-06	1.94E-05	3.656	-1.22
Ptpn12	-0.325	6.870	-6.407	1.30E-06	8.41E-06	4.618	-1.25
Ptprg	-1.752	5.545	-34.805	5.98E-22	5.37E-19	40.217	-3.37
Ptprm	-0.839	7.071	-20.125	1.82E-16	2.05E-14	27.754	-1.79
Ptrf	-0.491	7.830	-6.244	1.93E-06	1.20E-05	4.217	-1.41
Pts	0.515	7.243	6.219	2.05E-06	1.26E-05	4.157	1.43
Pttg1	-0.947	6.979	-11.370	4.11E-11	8.21E-10	15.203	-1.93
Purb	0.447	7.313	7.817	4.97E-08	4.30E-07	7.944	1.36
Pxdn	0.290	7.848	5.043	3.80E-05	1.68E-04	1.211	1.22
Qk	-2.513	5.943	-18.401	1.36E-15	1.04E-13	25.721	-5.71
Qk	-2.382	7.862	-31.366	6.78E-21	3.48E-18	37.875	-5.21
Qsox1	0.764	7.440	9.873	6.75E-10	9.62E-09	12.339	1.70
Rab10	0.288	8.081	3.337	2.77E-03	7.46E-03	-3.032	1.22
Rab11a	-0.387	5.948	-3.699	1.13E-03	3.39E-03	-2.162	-1.31
Rab12	-1.011	7.313	-10.301	2.95E-10	4.69E-09	13.186	-2.02
Rab31	-0.306	6.713	-3.789	9.05E-04	2.81E-03	-1.942	-1.24
Rad21	-0.398	8.291	-5.040	3.83E-05	1.69E-04	1.203	-1.32
Rapgef1	0.618	9.228	12.735	3.98E-12	1.02E-10	17.591	1.53
Rbck1	0.781	6.528	9.334	1.97E-09	2.46E-08	11.241	1.72
Rbm39	-0.323	8.443	-6.406	1.31E-06	8.41E-06	4.615	-1.25
Rbpms	0.329	6.521	5.700	7.34E-06	3.99E-05	2.867	1.26
Rcan1	-0.276	6.276	-4.655	1.01E-04	4.02E-04	0.229	-1.21
Rcc2	0.288	7.768	5.531	1.12E-05	5.76E-05	2.444	1.22
Rcc2	0.452	6.920	9.538	1.31E-09	1.70E-08	11.661	1.37
Rcl1	-0.375	7.522	-9.885	6.59E-10	9.47E-09	12.364	-1.30
Rell1	-0.306	6.441	-7.998	3.33E-08	3.02E-07	8.353	-1.24
Rere	0.470	6.955	5.549	1.07E-05	5.53E-05	2.488	1.39
Retsat	1.665	5.458	17.761	2.99E-15	1.85E-13	24.922	3.17
Rexo2	0.331	9.534	8.630	8.50E-09	9.20E-08	9.748	1.26
Rfx5	-0.295	6.821	-5.259	2.21E-05	1.05E-04	1.757	-1.23
RGD1304567	0.278	6.998	5.272	2.13E-05	1.02E-04	1.792	1.21

RGD1311072	0.267	6.926	6.550	9.26E-07	6.25E-06	4.964	1.20
RGD1560010	-0.357	6.651	-5.139	2.98E-05	1.37E-04	1.454	-1.28
RGD1561797	-0.828	7.190	-10.644	1.55E-10	2.66E-09	13.847	-1.77
Rgs17	0.928	7.346	9.564	1.24E-09	1.62E-08	11.714	1.90
Rnaseh2c	-0.969	6.445	-15.483	6.14E-14	2.40E-12	21.851	-1.96
Rnaset2a	0.677	10.189	15.715	4.44E-14	1.79E-12	22.180	1.60
Rnf10	0.480	7.472	8.692	7.46E-09	8.17E-08	9.881	1.39
Rnf111	-0.341	6.483	-5.085	3.42E-05	1.54E-04	1.317	-1.27
Rnf149	0.276	8.048	3.675	1.20E-03	3.57E-03	-2.221	1.21
Rnft1	-0.279	6.370	-4.913	5.27E-05	2.25E-04	0.883	-1.21
Romo1	0.278	9.948	5.560	1.04E-05	5.41E-05	2.517	1.21
Rpia	0.464	7.906	7.844	4.68E-08	4.06E-07	8.006	1.38
Rpl10	0.401	6.263	5.140	2.97E-05	1.37E-04	1.456	1.32
Rpl34	-0.333	9.992	-6.515	1.01E-06	6.74E-06	4.880	-1.26
Rpl37a	-0.462	12.328	-13.961	5.71E-13	1.75E-11	19.575	-1.38
Rpn1	-0.279	9.350	-6.207	2.11E-06	1.29E-05	4.127	-1.21
Rpn2	-0.281	9.821	-3.206	3.81E-03	9.80E-03	-3.340	-1.22
Rps28	-0.414	8.402	-4.338	2.26E-04	8.26E-04	-0.573	-1.33
Rqcd1	-0.807	6.141	-12.119	1.12E-11	2.51E-10	16.537	-1.75
Rras	-0.294	6.545	-4.173	3.43E-04	1.21E-03	-0.986	-1.23
Rrp15	0.743	7.873	22.714	1.17E-17	2.47E-15	30.516	1.67
Ryk	0.319	6.736	7.732	6.01E-08	5.13E-07	7.750	1.25
S100a11	-0.471	7.784	-7.006	3.15E-07	2.33E-06	6.060	-1.39
SAMD4B	0.416	8.703	8.604	8.98E-09	9.66E-08	9.691	1.33
Sash1	0.303	7.769	5.258	2.21E-05	1.05E-04	1.756	1.23
Sat1	-0.656	6.704	-13.557	1.07E-12	3.17E-11	18.937	-1.58
SC4MOL	-0.947	8.194	-18.761	8.82E-16	7.37E-14	26.160	-1.93
Sc4mol	-0.809	6.368	-3.803	8.74E-04	2.73E-03	-1.907	-1.75
Sc5d	-0.448	6.364	-4.747	8.03E-05	3.28E-04	0.460	-1.36
Scarb1	-0.339	6.897	-4.895	5.52E-05	2.34E-04	0.836	-1.26
Sccpdh	0.525	7.895	11.027	7.63E-11	1.41E-09	14.570	1.44
Sccpdh	0.554	7.248	15.766	4.13E-14	1.71E-12	22.254	1.47
SCD	-0.715	9.542	-16.869	9.38E-15	4.75E-13	23.763	-1.64
Scd1	-0.710	9.240	-13.475	1.21E-12	3.57E-11	18.806	-1.64
Scd1	-0.301	7.257	-2.829	9.33E-03	2.13E-02	-4.196	-1.23
Scd3	-0.351	8.588	-8.420	1.33E-08	1.37E-07	9.291	-1.28
Scd3	-0.319	7.564	-3.416	2.28E-03	6.29E-03	-2.846	-1.25
Scoc	0.265	7.007	4.101	4.12E-04	1.41E-03	-1.167	1.20
Scpep1	0.478	7.152	3.526	1.74E-03	4.96E-03	-2.582	1.39
Scyl2	-0.270	6.432	-6.491	1.07E-06	7.09E-06	4.822	-1.21
Scyl3	-1.051	5.993	-16.836	9.78E-15	4.88E-13	23.720	-2.07
Sec22a	-0.337	6.119	-8.107	2.62E-08	2.45E-07	8.598	-1.26
Sec22b	-0.389	6.590	-7.122	2.41E-07	1.82E-06	6.334	-1.31
Sec23b	-0.470	6.844	-6.317	1.62E-06	1.02E-05	4.397	-1.39
Sec24d	-0.361	7.004	-5.008	4.15E-05	1.82E-04	1.122	-1.28
Sel1l	-0.522	7.016	-5.519	1.15E-05	5.92E-05	2.414	-1.44
Serpinf1	0.389	8.278	4.504	1.49E-04	5.69E-04	-0.155	1.31
Sf3a3	0.454	8.084	9.761	8.42E-10	1.16E-08	12.113	1.37
Sfrs6	0.299	8.208	6.111	2.67E-06	1.58E-05	3.890	1.23
Sfxn1	0.493	7.684	13.871	6.56E-13	2.00E-11	19.434	1.41
Sgce	-1.342	6.005	-10.407	2.42E-10	3.96E-09	13.390	-2.53
Shisa5	-0.379	7.375	-6.325	1.59E-06	1.00E-05	4.417	-1.30
Shmt2	-0.273	6.208	-2.678	1.32E-02	2.88E-02	-4.522	-1.21
Sirt7	-0.845	6.400	-8.575	9.55E-09	1.02E-07	9.628	-1.80
Sirt7	-0.671	5.986	-5.199	2.57E-05	1.20E-04	1.605	-1.59
Slc17a5	-0.375	6.797	-8.193	2.17E-08	2.10E-07	8.789	-1.30
Slc25a17	0.402	6.764	9.015	3.79E-09	4.44E-08	10.572	1.32
Slc25a20	0.314	8.555	4.415	1.87E-04	6.95E-04	-0.380	1.24
Slc30a4	0.350	6.781	7.101	2.53E-07	1.89E-06	6.285	1.27
Slc35b1	-0.311	7.240	-6.323	1.60E-06	1.01E-05	4.410	-1.24
Slc35c2	0.412	6.171	6.131	2.54E-06	1.52E-05	3.939	1.33
Slc4a7	-0.931	6.815	-13.345	1.49E-12	4.35E-11	18.597	-1.91
Slc4a7	-0.747	6.315	-7.595	8.17E-08	6.72E-07	7.437	-1.68
Slc6a6	0.466	8.040	9.727	9.00E-10	1.22E-08	12.044	1.38
Slc7a11	-0.844	7.310	-10.039	4.88E-10	7.28E-09	12.671	-1.79
Smc4	-1.066	6.591	-9.652	1.04E-09	1.38E-08	11.892	-2.09
Smchd1	-0.741	6.819	-4.536	1.37E-04	5.28E-04	-0.073	-1.67
Smpd1	-0.346	6.110	-3.676	1.20E-03	3.57E-03	-2.218	-1.27
Smpd1	-0.289	6.885	-4.811	6.83E-05	2.83E-04	0.623	-1.22
Smyd2	0.920	6.489	17.186	6.21E-15	3.49E-13	24.180	1.89
Snap23	-0.342	7.105	-7.716	6.22E-08	5.29E-07	7.714	-1.27
Snn	-1.113	6.495	-14.400	2.94E-13	9.53E-12	20.252	-2.16
Snora28	-0.618	6.761	-6.078	2.89E-06	1.70E-05	3.808	-1.53
Snora28	-0.539	8.217	-10.946	8.85E-11	1.61E-09	14.419	-1.45
Snora28	-0.442	7.421	-8.540	1.03E-08	1.09E-07	9.553	-1.36
Snora41	-0.589	10.413	-17.985	2.26E-15	1.60E-13	25.205	-1.50
Snora75	-0.521	8.619	-9.684	9.80E-10	1.31E-08	11.957	-1.44
Snrnp200	0.418	7.905	8.400	1.39E-08	1.42E-07	9.247	1.34
Snrpc	0.286	7.891	5.725	6.89E-06	3.80E-05	2.930	1.22

Gene							
Snrpe	-0.263	9.000	-4.515	1.45E-04	5.54E-04	-0.127	-1.20
Snx12	0.412	6.594	7.689	6.62E-08	5.58E-07	7.651	1.33
Snx4	0.313	8.191	6.437	1.21E-06	7.93E-06	4.689	1.24
Snx9	0.305	7.033	7.005	3.16E-07	2.33E-06	6.058	1.24
Sod1	0.374	8.506	6.678	6.82E-07	4.73E-06	5.275	1.30
SOD2	-0.394	6.729	-5.811	5.57E-06	3.11E-05	3.146	-1.31
Spc25	-1.144	6.631	-14.744	1.77E-13	6.18E-12	20.770	-2.21
Sprr1a	0.797	8.643	9.242	2.38E-09	2.94E-08	11.048	1.74
Sqle	-0.548	9.031	-8.701	7.31E-09	8.03E-08	9.901	-1.46
Sqle	-0.444	7.073	-6.667	7.00E-07	4.83E-06	5.249	-1.36
Srpr	0.711	10.831	5.923	4.23E-06	2.41E-05	3.423	1.64
Srpr	0.901	10.118	5.417	1.48E-05	7.37E-05	2.155	1.87
Ssr4	-0.344	9.057	-3.992	5.43E-04	1.79E-03	-1.439	-1.27
Stim2	0.345	6.458	4.223	3.03E-04	1.08E-03	-0.863	1.27
Stmn1	-0.330	7.260	-5.500	1.21E-05	6.16E-05	2.366	-1.26
Strap	-0.269	6.933	-6.175	2.28E-06	1.38E-05	4.048	-1.21
Sumo3	0.383	7.605	9.703	9.43E-10	1.27E-08	11.996	1.30
SUPT16H	-0.537	10.402	-7.420	1.22E-07	9.79E-07	7.031	-1.45
Supt4h1	0.472	7.223	8.838	5.49E-09	6.18E-08	10.195	1.39
Syncrip	0.933	6.514	4.888	5.61E-05	2.38E-04	0.819	1.91
Syncrip	1.061	6.547	15.958	3.18E-14	1.38E-12	22.522	2.09
Tagln2	0.296	7.238	4.943	4.89E-05	2.11E-04	0.958	1.23
Tagln2	0.342	8.340	4.719	8.61E-05	3.52E-04	0.390	1.27
Tbc1d1	0.658	7.646	11.607	2.70E-11	5.62E-10	15.632	1.58
Tbc1d13	0.304	6.269	4.939	4.94E-05	2.12E-04	0.948	1.23
Tbc1d20	0.460	7.320	8.162	2.32E-08	2.22E-07	8.720	1.38
Tbl1xr1	0.448	7.703	7.898	4.15E-08	3.63E-07	8.128	1.36
Tcea1	0.282	8.260	6.425	1.25E-06	8.15E-06	4.660	1.22
Tdgf1	0.546	6.194	8.097	2.68E-08	2.48E-07	8.575	1.46
Tecr	-0.572	8.225	-8.154	2.36E-08	2.24E-07	8.702	-1.49
Tes	-1.241	7.629	-20.812	8.53E-17	1.23E-14	28.518	-2.36
Tes	-1.145	6.550	-23.610	4.82E-18	1.33E-15	31.400	-2.21
Tfb2m	0.654	7.005	12.889	3.10E-12	8.18E-11	17.848	1.57
TFPI	-0.308	7.291	-3.843	7.90E-04	2.51E-03	-1.808	-1.24
Tgfbr2	0.719	6.364	9.134	2.97E-09	3.59E-08	10.822	1.65
Tgm2	0.341	7.080	5.626	8.81E-06	4.70E-05	2.682	1.27
Tgoln2	0.280	7.676	4.110	4.03E-04	1.39E-03	-1.145	1.21
Thoc4	-0.572	8.085	-7.109	2.48E-07	1.87E-06	6.304	-1.49
Thoc7	0.308	7.681	5.813	5.55E-06	3.10E-05	3.150	1.24
Tial1	-0.329	7.699	-9.129	3.00E-09	3.61E-08	10.813	-1.26
Timm8b	0.406	7.727	7.355	1.41E-07	1.11E-06	6.882	1.33
Timp1	-0.495	6.932	-7.275	1.69E-07	1.32E-06	6.694	-1.41
Timp2	0.383	8.376	6.816	4.93E-07	3.50E-06	5.606	1.30
Tinagl1	-0.695	6.534	-6.145	2.45E-06	1.47E-05	3.975	-1.62
Tkt	-0.426	6.994	-9.431	1.62E-09	2.06E-08	11.441	-1.34
Tmbim6	-0.426	8.960	-3.810	8.59E-04	2.70E-03	-1.891	-1.34
Tmed10	0.264	6.968	6.475	1.11E-06	7.33E-06	4.783	1.20
Tmed3	-0.405	6.990	-8.453	1.24E-08	1.29E-07	9.363	-1.32
Tmem101	0.451	6.466	5.417	1.48E-05	7.37E-05	2.156	1.37
Tmem109	-0.379	6.566	-7.278	1.68E-07	1.31E-06	6.701	-1.30
Tmem147	0.447	8.457	8.565	9.74E-09	1.04E-07	9.608	1.36
Tmem150a	-0.563	6.896	-8.159	2.34E-08	2.23E-07	8.713	-1.48
Tmem156	0.405	7.139	7.083	2.64E-07	1.97E-06	6.242	1.32
Tmem158	0.695	6.933	7.112	2.46E-07	1.86E-06	6.312	1.62
Tmem219	0.321	6.567	5.161	2.82E-05	1.31E-04	1.509	1.25
Tmem223	0.317	7.260	4.659	1.00E-04	3.99E-04	0.238	1.25
Tmsb10	0.373	10.371	6.018	3.35E-06	1.94E-05	3.660	1.30
Tnfaip3	-1.282	5.235	-14.012	5.29E-13	1.65E-11	19.655	-2.43
Tnfsf14	-0.875	7.330	-15.943	3.24E-14	1.39E-12	22.502	-1.83
Tnfsf9	-0.731	6.765	-6.850	4.55E-07	3.25E-06	5.688	-1.66
Tnks2	0.573	6.486	9.619	1.11E-09	1.47E-08	11.825	1.49
Top2a	-1.182	9.103	-14.984	1.25E-13	4.59E-12	21.126	-2.27
Tpd52l2	0.300	6.534	3.157	4.28E-03	1.09E-02	-3.454	1.23
Tpx2	-0.903	6.219	-9.699	9.51E-10	1.28E-08	11.988	-1.87
Tram1	0.654	7.603	15.756	4.20E-14	1.71E-12	22.238	1.57
Tram1	0.701	6.401	8.833	5.54E-09	6.22E-08	10.185	1.63
Trap1	0.386	6.783	4.717	8.65E-05	3.53E-04	0.386	1.31
Triap1	0.348	6.780	8.223	2.03E-08	1.99E-07	8.856	1.27
Trib1	0.280	6.683	4.030	4.93E-04	1.64E-03	-1.345	1.21
Trim44	0.395	6.784	3.299	3.04E-03	8.10E-03	-3.123	1.31
Trub1	0.597	7.328	14.802	1.63E-13	5.79E-12	20.856	1.51
Tspo	0.297	8.531	3.961	5.87E-04	1.91E-03	-1.516	1.23
Ttc33	0.357	6.573	5.112	3.19E-05	1.46E-04	1.385	1.28
Ttrap	-0.482	6.488	-11.612	2.68E-11	5.60E-10	15.640	-1.40
Tuba1c	-0.505	9.068	-10.477	2.12E-10	3.54E-09	13.525	-1.42
Tubb2c	-0.276	8.450	-5.498	1.21E-05	6.16E-05	2.362	-1.21
Tubb5	-0.696	7.745	-10.861	1.03E-10	1.85E-09	14.258	-1.62
Tubb6	-0.454	7.128	-11.434	3.67E-11	7.37E-10	15.318	-1.37
Twistnb	0.283	7.652	3.502	1.85E-03	5.23E-03	-2.640	1.22

Txndc15	0.296	6.914	7.664	7.00E-08	5.85E-07	7.594	1.23
TXNDC5	0.400	7.407	7.025	3.02E-07	2.24E-06	6.104	1.32
Tyms	-0.964	8.010	-12.308	8.10E-12	1.94E-10	16.865	-1.95
Uap1	-0.529	6.881	-8.576	9.52E-09	1.02E-07	9.631	-1.44
Uap1l1	0.355	6.485	3.966	5.80E-04	1.89E-03	-1.504	1.28
Uba5	-0.377	7.149	-8.065	2.87E-08	2.64E-07	8.504	-1.30
Ubash3b	0.436	8.046	10.651	1.53E-10	2.65E-09	13.860	1.35
Ube2d3	-0.446	6.574	-11.017	7.78E-11	1.43E-09	14.550	-1.36
UBE2D4	0.599	6.665	9.828	7.37E-10	1.04E-08	12.248	1.51
Ube2f	-0.473	6.835	-11.472	3.43E-11	7.01E-10	15.387	-1.39
Ube2q1	0.321	7.359	4.126	3.87E-04	1.35E-03	-1.106	1.25
Ubl3	0.339	6.206	6.414	1.28E-06	8.29E-06	4.635	1.27
Ugcg	-0.291	6.257	-3.340	2.75E-03	7.41E-03	-3.025	-1.22
Uggt1	0.317	7.043	6.974	3.40E-07	2.47E-06	5.983	1.25
Ugt1a1	-0.362	10.340	-8.166	2.30E-08	2.21E-07	8.730	-1.29
Uqcr10	0.287	8.893	4.106	4.08E-04	1.40E-03	-1.156	1.22
Usp1	-0.304	7.729	-4.901	5.44E-05	2.32E-04	0.851	-1.23
Usp19	0.364	6.555	7.151	2.25E-07	1.71E-06	6.403	1.29
Usp46	0.689	6.354	10.235	3.35E-10	5.16E-09	13.057	1.61
Usp8	0.465	7.823	11.149	6.12E-11	1.16E-09	14.795	1.38
Usp9x	-0.300	7.078	-4.968	4.59E-05	1.99E-04	1.021	-1.23
Vamp7	0.479	6.650	5.934	4.12E-06	2.36E-05	3.450	1.39
VAPA	-0.700	8.901	-19.809	2.60E-16	2.47E-14	27.394	-1.62
Vapb	0.427	8.100	5.067	3.57E-05	1.60E-04	1.272	1.34
Vasn	0.361	6.546	3.201	3.86E-03	9.90E-03	-3.353	1.28
Vasn	0.384	7.151	9.914	6.23E-10	9.10E-09	12.420	1.30
Vcam1	-1.055	6.411	-12.399	6.96E-12	1.69E-10	17.021	-2.08
Vldlr	0.296	6.836	4.303	2.47E-04	8.92E-04	-0.661	1.23
Vnn1	-0.823	6.056	-15.045	1.14E-13	4.28E-12	21.216	-1.77
Vopp1	-0.352	6.712	-6.317	1.62E-06	1.02E-05	4.397	-1.28
Vps25	0.505	7.885	7.925	3.91E-08	3.44E-07	8.188	1.42
Vps29	0.310	8.817	4.295	2.52E-04	9.09E-04	-0.681	1.24
Vps36	0.417	6.947	8.372	1.47E-08	1.49E-07	9.184	1.34
Vwa5a	-0.542	7.095	-10.572	1.77E-10	3.01E-09	13.709	-1.46
Wdfy4	1.501	6.002	14.128	4.43E-13	1.40E-11	19.835	2.83
Wdr26	0.391	6.370	6.379	1.40E-06	8.96E-06	4.548	1.31
Wdr77	-0.271	6.647	-5.163	2.81E-05	1.30E-04	1.514	-1.21
Wdyhv1	0.325	6.805	3.281	3.17E-03	8.38E-03	-3.164	1.25
Whsc1	-0.390	6.747	-4.666	9.86E-05	3.94E-04	0.255	-1.31
Wsb1	0.374	6.023	5.666	7.98E-06	4.32E-05	2.782	1.30
Xbp1	-0.292	7.896	-5.294	2.02E-05	9.71E-05	1.846	-1.22
Xpot	-0.326	8.618	-5.567	1.02E-05	5.32E-05	2.535	-1.25
Xrcc5	-0.890	6.430	-17.835	2.73E-15	1.78E-13	25.015	-1.85
Ybx1	0.348	11.304	7.598	8.11E-08	6.68E-07	7.444	1.27
ZC3H11A	-0.373	9.226	-6.803	5.07E-07	3.60E-06	5.576	-1.29
Zcchc2	0.534	6.640	6.473	1.11E-06	7.34E-06	4.778	1.45
Zcchc24	0.512	6.916	8.358	1.52E-08	1.53E-07	9.154	1.43
Zfp110	0.290	6.538	7.198	2.02E-07	1.54E-06	6.512	1.22
Zfp259	0.354	6.408	5.877	4.74E-06	2.68E-05	3.309	1.28
ZFP36L1	-0.419	8.280	-7.047	2.87E-07	2.13E-06	6.157	-1.34
Zyx	-0.319	7.962	-4.986	4.38E-05	1.90E-04	1.067	-1.25

5.2.12 Stressed v Unstressed gene list

Gene ID	logFC	AveExpr	t	P.Value	adj.P.Val	B	Fold Change
38231	-0.886	5.984	-5.895	1.66E-06	1.53E-04	5.148	-1.85
1810037I17Rik	-0.386	6.983	-6.314	5.04E-07	9.54E-05	6.282	-1.31
2810482I07Rik	-0.274	6.348	-4.338	1.42E-04	2.86E-03	0.917	-1.21
4833417J20Rik	-0.466	7.312	-5.105	1.59E-05	6.73E-04	2.994	-1.38
9530053H05Rik	0.454	10.002	3.729	7.72E-04	7.96E-03	-0.681	1.37
A330042M18Rik	-0.298	6.806	-3.125	3.85E-03	2.22E-02	-2.173	-1.23
Abhd6	0.428	6.783	3.267	2.66E-03	1.77E-02	-1.833	1.34
Acat2	-0.723	6.811	-4.478	9.53E-05	2.18E-03	1.292	-1.65
Actn1	-0.285	8.286	-5.985	1.28E-06	1.52E-04	5.394	-1.22
Add3	-0.281	6.120	-2.801	8.70E-03	3.89E-02	-2.919	-1.22
Adh5	-0.391	9.619	-4.171	2.27E-04	3.80E-03	0.473	-1.31
Aftph	0.284	7.139	4.248	1.83E-04	3.35E-03	0.676	1.22
Ahctf1	0.390	6.293	4.451	1.03E-04	2.28E-03	1.220	1.31
AI646531	-0.430	6.560	-4.352	1.36E-04	2.79E-03	0.955	-1.35
AI646531	-0.299	7.286	-3.499	1.44E-03	1.18E-02	-1.261	-1.23
Aig1	-0.316	6.487	-3.883	5.06E-04	6.20E-03	-0.284	-1.24
Akap12	-0.745	7.889	-3.454	1.62E-03	1.26E-02	-1.373	-1.68
Akirin2	0.349	6.069	3.687	8.66E-04	8.57E-03	-0.788	1.27
Amd1	0.467	7.422	3.240	2.85E-03	1.85E-02	-1.898	1.38
Amd2	0.528	8.027	3.252	2.77E-03	1.81E-02	-1.869	1.44
Anxa4	-0.326	7.155	-5.992	1.25E-06	1.52E-04	5.414	-1.25
Arhgef6	-0.313	7.955	-4.130	2.54E-04	4.06E-03	0.364	-1.24
Arl15	-0.270	6.329	-3.673	8.99E-04	8.74E-03	-0.823	-1.21
Atf6	0.354	8.404	3.979	3.87E-04	5.17E-03	-0.033	1.28
Atp6v0c-ps2	-0.290	7.644	-3.426	1.75E-03	1.33E-02	-1.443	-1.22
AU015740	0.315	6.605	4.377	1.27E-04	2.70E-03	1.021	1.24
AU018778	-1.447	3.649	-4.461	9.99E-05	2.23E-03	1.248	-2.73
AU019300	0.315	7.174	4.517	8.54E-05	2.06E-03	1.396	1.24
B630006N21Rik	0.264	7.660	5.897	1.65E-06	1.53E-04	5.154	1.20
Bdnf	0.331	6.480	3.222	2.99E-03	1.90E-02	-1.940	1.26
BHLHE41	-0.297	6.897	-6.973	7.99E-08	4.63E-05	8.033	-1.23
Bsg	-0.273	8.496	-4.627	6.25E-05	1.68E-03	1.693	-1.21
C1rb	-0.419	9.482	-3.394	1.90E-03	1.41E-02	-1.523	-1.34
C80142	0.358	6.839	5.025	2.00E-05	7.87E-04	2.776	1.28
Cald1	-0.342	7.673	-2.739	1.01E-02	4.32E-02	-3.057	-1.27
Cat	-0.304	8.131	-3.376	2.00E-03	1.46E-02	-1.567	-1.23
Cav1	-0.301	9.812	-2.981	5.54E-03	2.90E-02	-2.509	-1.23
Cav2	-0.282	6.234	-3.342	2.18E-03	1.55E-02	-1.651	-1.22
Chd2	0.273	6.581	3.743	7.43E-04	7.79E-03	-0.645	1.21
Chmp2a	-0.496	7.416	-4.864	3.18E-05	1.04E-03	2.336	-1.41
Chordc1	0.271	6.453	3.124	3.85E-03	2.22E-02	-2.175	1.21
Cidec	0.280	6.285	3.641	9.81E-04	9.14E-03	-0.905	1.21
Cited2	0.506	6.419	3.938	4.34E-04	5.63E-03	-0.140	1.42
Clic4	-0.267	8.809	-3.849	5.55E-04	6.47E-03	-0.371	-1.20
Col5a1	-1.053	6.209	-5.300	9.08E-06	4.59E-04	3.528	-2.07
Commd7	0.269	6.618	2.785	9.04E-03	4.01E-02	-2.955	1.21
Cox4nb	0.270	7.112	3.389	1.93E-03	1.42E-02	-1.535	1.21
Cpn2	-0.600	6.648	-3.074	4.38E-03	2.41E-02	-2.292	-1.52
Csda	0.283	7.938	4.255	1.79E-04	3.32E-03	0.695	1.22
Csnk1e	0.317	6.600	3.664	9.21E-04	8.83E-03	-0.846	1.25
CTBS	-0.270	6.573	-5.212	1.17E-05	5.30E-04	3.288	-1.21
Ctsh	-0.321	6.886	-4.483	9.41E-05	2.17E-03	1.305	-1.25
Ctsz	-0.374	9.672	-3.789	6.55E-04	7.15E-03	-0.526	-1.30
Cyb5r3	-0.310	7.652	-5.587	3.99E-06	2.56E-04	4.310	-1.24
Cyp51	-0.402	9.644	-4.587	6.99E-05	1.76E-03	1.587	-1.32
Cyp51	-0.388	9.012	-4.729	4.66E-05	1.36E-03	1.971	-1.31
D10Jhu81e	-0.287	6.385	-3.195	3.21E-03	1.99E-02	-2.006	-1.22
D17Wsu104e	-0.346	6.741	-4.901	2.86E-05	9.78E-04	2.437	-1.27
Dag1	-0.299	7.183	-3.323	2.30E-03	1.59E-02	-1.697	-1.23
Dag1	-0.294	8.711	-2.898	6.84E-03	3.29E-02	-2.701	-1.23
Dbi	-0.283	8.412	-2.886	7.04E-03	3.34E-02	-2.727	-1.22
Dcaf13	0.270	7.411	5.302	9.03E-06	4.59E-04	3.533	1.21
Ddx39	0.288	7.395	4.011	3.54E-04	4.84E-03	0.052	1.22
Degs1	-0.287	6.778	-2.829	8.12E-03	3.70E-02	-2.857	-1.22
Dram1	-0.359	6.300	-2.681	1.17E-02	4.71E-02	-3.185	-1.28
Drg1	0.320	6.182	3.316	2.33E-03	1.61E-02	-1.712	1.25
Dusp1	-0.470	6.221	-3.587	1.13E-03	1.00E-02	-1.040	-1.38
E130203B14Rik	-0.692	8.135	-5.700	2.89E-06	2.04E-04	4.618	-1.62
Ebp	-0.535	5.933	-3.751	7.25E-04	7.66E-03	-0.622	-1.45

Ehd4	-0.296	6.254	-2.983	5.52E-03	2.89E-02	-2.505	-1.23
Eif4a1	-0.556	8.429	-5.492	5.25E-06	3.20E-04	4.051	-1.47
Eif4a1	-0.456	9.235	-5.706	2.84E-06	2.04E-04	4.635	-1.37
Eif4ebp2	0.290	6.811	6.266	5.77E-07	1.04E-04	6.153	1.22
Eif6	0.306	8.152	3.176	3.37E-03	2.04E-02	-2.052	1.24
Ercc1	-0.421	6.529	-3.354	2.12E-03	1.52E-02	-1.621	-1.34
Erp29	-0.281	7.835	-2.685	1.15E-02	4.68E-02	-3.176	-1.21
Ethe1	-0.340	7.237	-3.496	1.45E-03	1.18E-02	-1.268	-1.27
F2r	-0.445	8.207	-3.524	1.34E-03	1.13E-02	-1.198	-1.36
Faf1	0.398	6.458	4.073	2.98E-04	4.38E-03	0.213	1.32
Fam102b	-0.486	7.655	-5.944	1.44E-06	1.52E-04	5.283	-1.40
Fam40b	0.423	6.679	4.871	3.11E-05	1.04E-03	2.357	1.34
Fasn	-0.511	6.330	-4.364	1.31E-04	2.75E-03	0.988	-1.42
Fcgrt	-0.899	6.491	-10.521	9.41E-12	3.38E-08	16.481	-1.86
Fdft1	-0.331	6.917	-3.842	5.66E-04	6.52E-03	-0.390	-1.26
Fdps	-0.510	8.124	-4.670	5.53E-05	1.54E-03	1.810	-1.42
Foxj2	0.378	5.870	4.712	4.90E-05	1.41E-03	1.924	1.30
FSTL1	-0.376	8.751	-5.864	1.81E-06	1.61E-04	5.064	-1.30
Galnt7	-0.344	7.466	-3.128	3.81E-03	2.21E-02	-2.165	-1.27
Gas1	-0.691	6.643	-3.867	5.27E-04	6.27E-03	-0.323	-1.61
Glul	-0.298	6.564	-3.449	1.64E-03	1.26E-02	-1.386	-1.23
Gm15104	1.096	6.022	2.815	8.41E-03	3.79E-02	-2.889	2.14
Gm5578	0.374	6.726	4.029	3.37E-04	4.71E-03	0.098	1.30
Gm6377	-0.333	8.134	-6.527	2.76E-07	6.64E-05	6.854	-1.26
Gm7859	0.293	6.920	3.960	4.08E-04	5.43E-03	-0.081	1.22
Gm9905	-0.318	6.724	-3.619	1.04E-03	9.45E-03	-0.958	-1.25
Gnpat	-0.286	6.582	-3.559	1.22E-03	1.06E-02	-1.110	-1.22
Gpx1	-0.439	7.884	-3.388	1.93E-03	1.42E-02	-1.537	-1.36
H6pd	-0.307	6.439	-4.588	6.97E-05	1.76E-03	1.590	-1.24
Hadh	-0.294	7.777	-3.175	3.38E-03	2.04E-02	-2.053	-1.23
Hbxip	0.294	8.226	4.293	1.61E-04	3.12E-03	0.797	1.23
HMGCR	-0.622	6.572	-4.923	2.68E-05	9.44E-04	2.498	-1.54
Hmgcs1	-0.863	6.887	-7.209	4.16E-08	3.74E-05	8.651	-1.82
Hnrnpa3	-0.309	8.503	-4.467	9.84E-05	2.21E-03	1.263	-1.24
Hnrnpa3	-0.266	9.119	-3.925	4.50E-04	5.77E-03	-0.173	-1.20
Hnrpdl	-0.264	6.334	-3.250	2.78E-03	1.82E-02	-1.874	-1.20
Hoxd9	0.288	5.751	3.614	1.05E-03	9.52E-03	-0.973	1.22
Hsd17b12	-0.301	7.796	-5.234	1.10E-05	5.13E-04	3.346	-1.23
Htra1	-0.286	10.001	-4.339	1.41E-04	2.86E-03	0.919	-1.22
Idh1	-0.755	6.178	-4.260	1.77E-04	3.30E-03	0.708	-1.69
IDH1	-0.683	8.313	-5.405	6.74E-06	3.78E-04	3.812	-1.61
Idi1	-0.717	6.124	-5.339	8.14E-06	4.30E-04	3.633	-1.64
Ifitm3	-0.408	9.676	-3.261	2.70E-03	1.78E-02	-1.848	-1.33
Ildr2	0.479	6.552	3.275	2.61E-03	1.75E-02	-1.814	1.39
Insig1	-0.394	8.024	-3.337	2.21E-03	1.55E-02	-1.661	-1.31
Itfg3	-0.439	6.192	-4.507	8.78E-05	2.07E-03	1.371	-1.36
Jub	0.516	7.280	4.122	2.60E-04	4.08E-03	0.344	1.43
Kpna4	0.362	7.353	2.832	8.05E-03	3.68E-02	-2.849	1.29
Lamb1-1	-0.284	9.576	-4.224	1.95E-04	3.49E-03	0.614	-1.22
Laptm4a	-0.545	7.312	-3.177	3.36E-03	2.04E-02	-2.049	-1.46
Laptm4a	-0.509	7.522	-3.857	5.42E-04	6.38E-03	-0.349	-1.42
Layn	-0.735	6.973	-4.728	4.68E-05	1.36E-03	1.967	-1.66
Ldlr	-0.346	6.729	-2.844	7.82E-03	3.61E-02	-2.823	-1.27
Lgals1	-0.423	10.053	-3.468	1.56E-03	1.23E-02	-1.338	-1.34
Lrig1	-0.526	8.856	-2.710	1.09E-02	4.49E-02	-3.120	-1.44
Lrrfip1	0.331	6.403	3.216	3.04E-03	1.92E-02	-1.955	1.26
MAMDC2	-0.539	7.429	-3.340	2.19E-03	1.55E-02	-1.654	-1.45
Man2a1	-0.443	6.504	-4.141	2.46E-04	3.99E-03	0.394	-1.36
Man2a1	-0.423	6.683	-4.370	1.29E-04	2.73E-03	1.004	-1.34
Mef2a	-0.264	7.229	-5.919	1.55E-06	1.52E-04	5.214	-1.20
Mgst1	-0.269	11.519	-4.688	5.25E-05	1.47E-03	1.859	-1.20
Mknk2	-0.273	6.736	-3.290	2.50E-03	1.70E-02	-1.777	-1.21
Mmd	-0.543	6.497	-3.747	7.35E-04	7.72E-03	-0.635	-1.46
Mrpl20	0.288	8.473	3.554	1.24E-03	1.07E-02	-1.125	1.22
Mrpl24	0.306	7.227	3.776	6.79E-04	7.32E-03	-0.560	1.24
Mrps35	0.326	8.616	4.926	2.66E-05	9.44E-04	2.506	1.25
Myo1b	0.293	8.879	4.056	3.13E-04	4.52E-03	0.169	1.23
Ncam1	1.141	5.358	3.790	6.53E-04	7.15E-03	-0.524	2.21
Ncapd3	-0.529	5.993	-3.472	1.54E-03	1.22E-02	-1.328	-1.44
Nck2	-0.295	6.349	-2.658	1.23E-02	4.88E-02	-3.234	-1.23
Ndufaf2	0.303	7.178	4.804	3.76E-05	1.17E-03	2.175	1.23
Neil3	0.293	7.798	3.226	2.96E-03	1.89E-02	-1.931	1.23
Ninj1	-0.366	6.177	-4.113	2.67E-04	4.16E-03	0.320	-1.29

Nvl	0.460	6.210	4.071	3.00E-04	4.38E-03	0.208	1.38
Palld	-0.476	7.134	-2.920	6.47E-03	3.17E-02	-2.651	-1.39
Pam	-0.375	7.027	-3.721	7.88E-04	8.00E-03	-0.700	-1.30
Parp1	0.318	6.304	2.773	9.32E-03	4.08E-02	-2.981	1.25
Pcolce	-0.412	8.526	-3.580	1.16E-03	1.02E-02	-1.058	-1.33
Pin4	0.291	6.767	3.661	9.28E-04	8.84E-03	-0.853	1.22
Pja1	-0.379	7.598	-6.154	7.93E-07	1.30E-04	5.851	-1.30
Plxna1	-0.320	6.084	-3.129	3.81E-03	2.21E-02	-2.164	-1.25
Polr3e	0.296	5.962	3.114	3.95E-03	2.26E-02	-2.199	1.23
Ppap2a	-0.571	6.987	-3.872	5.21E-04	6.26E-03	-0.312	-1.49
Ppcs	-0.358	7.062	-2.944	6.09E-03	3.07E-02	-2.595	-1.28
Ppp2r1a	-0.282	6.065	-2.671	1.19E-02	4.78E-02	-3.206	-1.22
PPP2R5A	0.324	6.010	2.671	1.19E-02	4.78E-02	-3.205	1.25
Prkca	-0.410	5.864	-4.607	6.60E-05	1.74E-03	1.641	-1.33
Prkrir	0.263	7.275	3.924	4.51E-04	5.77E-03	-0.176	1.20
Pros1	-0.551	7.149	-4.824	3.55E-05	1.12E-03	2.230	-1.46
Prune2	-0.386	7.588	-5.640	3.43E-06	2.32E-04	4.456	-1.31
Psme4	0.306	7.404	4.824	3.56E-05	1.12E-03	2.228	1.24
Ptma	-0.269	7.975	-2.757	9.68E-03	4.18E-02	-3.016	-1.21
Pxdn	-0.476	7.848	-5.095	1.64E-05	6.83E-04	2.968	-1.39
Rab11a	-0.307	5.948	-2.678	1.17E-02	4.73E-02	-3.190	-1.24
Rab31	-0.426	6.713	-4.727	4.69E-05	1.36E-03	1.966	-1.34
Rarres2	-0.596	9.322	-4.092	2.82E-04	4.27E-03	0.265	-1.51
Rcbtb2	-0.365	7.042	-3.514	1.38E-03	1.15E-02	-1.225	-1.29
Rgs17	-0.288	7.346	-2.682	1.16E-02	4.70E-02	-3.182	-1.22
Rnaseh2c	-0.393	6.445	-2.700	1.11E-02	4.56E-02	-3.142	-1.31
Rnaset2a	0.444	10.189	4.731	4.64E-05	1.36E-03	1.976	1.36
ROCK2	0.273	7.129	3.213	3.06E-03	1.93E-02	-1.964	1.21
Rpf1	0.385	9.587	3.457	1.61E-03	1.25E-02	-1.367	1.31
Rrp15	0.344	7.873	3.832	5.82E-04	6.66E-03	-0.415	1.27
Rsu1	-0.278	8.196	-4.171	2.27E-04	3.80E-03	0.473	-1.21
Sc4mol	-0.709	6.368	-4.067	3.03E-04	4.40E-03	0.199	-1.64
SC4MOL	-0.628	8.194	-4.862	3.19E-05	1.04E-03	2.331	-1.54
Sc5d	-0.604	6.364	-6.525	2.78E-07	6.64E-05	6.847	-1.52
Sccpdh	0.264	7.895	2.891	6.96E-03	3.33E-02	-2.717	1.20
Sccpdh	0.316	7.248	3.872	5.21E-04	6.26E-03	-0.311	1.24
SCD	-1.042	9.542	-3.871	5.22E-04	6.26E-03	-0.314	-2.06
Scd1	-1.110	7.257	-3.628	1.01E-03	9.33E-03	-0.936	-2.16
Scd1	-1.040	9.240	-3.725	7.79E-04	7.96E-03	-0.690	-2.06
Scd3	-0.547	7.564	-5.630	3.53E-06	2.35E-04	4.428	-1.46
Scd3	-0.467	8.588	-6.433	3.60E-07	7.61E-05	6.603	-1.38
Scp2	-0.306	8.021	-6.705	1.68E-07	6.26E-05	7.327	-1.24
Sdc1	-0.724	5.513	-4.759	4.28E-05	1.30E-03	2.053	-1.65
Sepp1	-0.429	9.267	-6.503	2.96E-07	6.64E-05	6.790	-1.35
Serbp1	-0.410	10.563	-4.906	2.82E-05	9.73E-04	2.450	-1.33
Serpinb1a	-0.368	8.041	-2.824	8.23E-03	3.73E-02	-2.869	-1.29
Sf3b1	-0.332	5.926	-4.468	9.82E-05	2.21E-03	1.265	-1.26
Sgce	-1.097	6.005	-5.479	5.43E-06	3.25E-04	4.017	-2.14
Slc25a20	-0.329	8.555	-4.138	2.49E-04	3.99E-03	0.384	-1.26
Slc4a7	-0.363	6.315	-3.194	3.22E-03	1.99E-02	-2.009	-1.29
Slc4a7	-0.358	6.815	-2.734	1.02E-02	4.34E-02	-3.067	-1.28
Smyd2	0.340	6.489	2.826	8.18E-03	3.72E-02	-2.864	1.27
Snhg11	-0.458	6.593	-5.949	1.42E-06	1.52E-04	5.297	-1.37
Snn	-0.627	6.495	-4.640	6.01E-05	1.65E-03	1.731	-1.54
Snx18	0.283	6.521	4.172	2.26E-04	3.80E-03	0.475	1.22
Snx5	-0.276	7.211	-4.506	8.81E-05	2.07E-03	1.367	-1.21
Sod1	-0.289	8.506	-3.639	9.84E-04	9.14E-03	-0.908	-1.22
Sprr1a	1.582	8.643	9.140	2.63E-10	4.72E-07	13.412	2.99
Sqle	-0.498	6.333	-3.540	1.29E-03	1.10E-02	-1.158	-1.41
Sqle	-0.465	7.073	-3.946	4.25E-04	5.55E-03	-0.120	-1.38
Sqle	-0.411	9.031	-3.301	2.43E-03	1.67E-02	-1.749	-1.33
Stk39	0.283	6.315	3.047	4.69E-03	2.53E-02	-2.356	1.22
Stmn1	-0.506	7.260	-6.627	2.09E-07	6.26E-05	7.120	-1.42
SUPT16H	-0.454	10.402	-3.671	9.04E-04	8.74E-03	-0.829	-1.37
Supt4h1	0.282	7.223	4.052	3.16E-04	4.53E-03	0.159	1.22
Tbc1d20	0.277	7.320	2.665	1.21E-02	4.82E-02	-3.218	1.21
Tbpl1	0.380	7.435	3.282	2.56E-03	1.73E-02	-1.797	1.30
Tcf19	-0.382	5.912	-2.787	8.99E-03	4.00E-02	-2.949	-1.30
Tecr	-0.290	8.225	-4.397	1.20E-04	2.58E-03	1.076	-1.22
Tfb2m	0.505	7.005	5.346	7.97E-06	4.30E-04	3.653	1.42
Tgfbr2	-0.368	6.364	-2.640	1.29E-02	5.00E-02	-3.272	-1.29
Tgm2	-0.408	7.080	-2.887	7.02E-03	3.34E-02	-2.725	-1.33
Thbd	-0.876	7.746	-3.476	1.53E-03	1.22E-02	-1.319	-1.84

Thra	-0.282	6.614	-5.818	2.06E-06	1.76E-04	4.941	-1.22
Timm10	0.332	7.656	4.242	1.86E-04	3.37E-03	0.660	1.26
Timp1	-0.489	6.932	-4.698	5.10E-05	1.44E-03	1.886	-1.40
Tinagl1	-0.785	6.534	-3.342	2.18E-03	1.55E-02	-1.650	-1.72
Tkt	-0.333	6.373	-3.095	4.16E-03	2.34E-02	-2.245	-1.26
Tkt	-0.272	6.994	-3.425	1.75E-03	1.33E-02	-1.445	-1.21
Tm9sf3	-0.318	6.640	-6.191	7.14E-07	1.22E-04	5.951	-1.25
Tmed3	-0.388	6.990	-5.354	7.78E-06	4.30E-04	3.675	-1.31
Tmem101	0.378	6.466	4.240	1.87E-04	3.37E-03	0.655	1.30
Tmem109	-0.406	6.566	-5.041	1.91E-05	7.81E-04	2.819	-1.32
Tmem150a	-0.384	6.896	-4.635	6.09E-05	1.65E-03	1.717	-1.31
Tnfrsf21	-0.985	7.387	-5.859	1.84E-06	1.61E-04	5.051	-1.98
Tpm1	-0.323	7.136	-6.087	9.58E-07	1.50E-04	5.671	-1.25
Trappc4	0.329	7.791	4.044	3.24E-04	4.61E-03	0.137	1.26
Trub1	0.304	7.328	3.868	5.26E-04	6.27E-03	-0.321	1.23
Tsc22d1	-0.370	6.481	-3.164	3.47E-03	2.08E-02	-2.079	-1.29
Tsc22d1	-0.307	10.051	-3.929	4.45E-04	5.74E-03	-0.163	-1.24
Ttc13	-0.324	7.694	-6.010	1.19E-06	1.52E-04	5.462	-1.25
Ttll3	-0.343	11.009	-5.010	2.09E-05	7.87E-04	2.734	-1.27
Ttll3	-0.342	11.241	-5.008	2.10E-05	7.87E-04	2.728	-1.27
Ttll3	-0.338	11.078	-4.804	3.77E-05	1.17E-03	2.174	-1.26
Tuba1a	-0.593	8.193	-8.929	4.47E-10	5.35E-07	12.917	-1.51
Tuba1c	-0.351	9.068	-2.845	7.81E-03	3.61E-02	-2.821	-1.28
Tubb2c	-0.529	8.450	-5.434	6.19E-06	3.53E-04	3.893	-1.44
Tubb5	-0.344	7.745	-3.111	3.99E-03	2.27E-02	-2.206	-1.27
Tubb6	-0.302	7.128	-3.504	1.42E-03	1.17E-02	-1.248	-1.23
Tyms	-0.336	8.010	-2.974	5.65E-03	2.93E-02	-2.527	-1.26
Uap1l1	-0.506	6.485	-3.534	1.31E-03	1.11E-02	-1.175	-1.42
Uba52	-0.322	8.310	-2.872	7.29E-03	3.42E-02	-2.759	-1.25
Uba52	-0.303	10.676	-3.201	3.16E-03	1.97E-02	-1.991	-1.23
Uba52	-0.296	10.694	-3.250	2.78E-03	1.82E-02	-1.874	-1.23
Uba52	-0.288	10.442	-3.059	4.55E-03	2.48E-02	-2.328	-1.22
Ubqln4	0.299	6.593	5.999	1.23E-06	1.52E-04	5.431	1.23
Uck2	0.379	6.716	3.053	4.62E-03	2.51E-02	-2.342	1.30
Vcam1	-0.599	6.411	-3.697	8.41E-04	8.42E-03	-0.761	-1.52
Wdr26	0.320	6.370	3.049	4.67E-03	2.52E-02	-2.351	1.25
Wsb1	0.341	6.023	4.081	2.91E-04	4.30E-03	0.236	1.27
Xbp1	-0.432	7.896	-3.784	6.64E-04	7.23E-03	-0.539	-1.35
Ybx1	-0.296	11.304	-5.132	1.47E-05	6.29E-04	3.068	-1.23
Zfp110	-0.294	6.538	-4.174	2.25E-04	3.80E-03	0.480	-1.23
ZFP36L1	-0.473	8.280	-5.059	1.82E-05	7.50E-04	2.868	-1.39
Zfp36l2	-0.481	7.753	-5.946	1.43E-06	1.52E-04	5.288	-1.40