Transcriptional Regulation of Chemically Induced Epithelial Lung Cell Differentiation, *in vitro*

A thesis submitted for the degree of Ph D

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By

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The research work described in this thesis was performed

under the supervision of

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I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph D is entirely of my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my own work

Signed _____ ID No 96023082

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This thesis is dedicated to my parents
and my brother who have been so
supportive throughout my life

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ABBREVATIONS

4E-BP	-	eIF4E-Binding Protein
5-FU	-	5-Flurouracil
5,2 -FdU	-	5-Fluro-2 -deoxyuridine
5,5 -FdU	-	5-Fluro-5 -deoxyuridine
Ab	-	Antibody
ATCC	-	American Tissue Culture Collection
BrdU	-	5-Bromo-2 -deoxyuridine
IdU	-	Iododeoxyuridine
CdU	-	Chlorodeoxyuridine
cDNA	-	complementary Deoxyribonucleic Acid
c-*myc*	-	Refers to c-myc gene or mRNA (italicised *myc*)
c-Myc	-	Refers to c-myc protein (Capital M)
Da	-	Daltons
DEPC	-	Diethyl Pyrocarbonate
DMEM	-	Dublecco's Mininum Essential Medium
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic Acid
EDTA	-	Ethylenediaminetetraacetic Acid
ECM	-	ExtraCellular Matrix
eIF	-	eukaryotic Translation Initiation Factor
ES cells	-	Embryonic Stem Cells
FBS	-	Fetal Bovine Serum
HEPES	-	N-[2-Hydroxyethyl]piperazine-N -[2-ethanesulphonic acid]
MAb	-	Monoclonal Antibody
MEM	-	Minimum Essential Medium
Min	-	Minutes
MMLV-RT	-	Moloney Murine Leukaemia Virus –Reverse Transcriptase
mRNA	-	messenger RNA
NCTCC	-	National Cell and Tissue Culture Centre
NICB	-	National Institute for Cellular Biotechnology

NSCLC	-	Non-Small Cell Lung Carcinoma
PBSA	-	Phosphate Buffered Saline A
rpm	-	Revolution Per Minute
RT	-	Room Temperature
SCLC	-	Small Cell Lung Carcinoma
SDS	-	Sodium Doedecyl Sulphate
sec	-	Seconds
SMGC	-	Small Mucous Granule Cell
TBS	-	Tris Buffered Saline
TDS cells	-	Tissue Determined Stem Cells
Tris	-	Tris(hydroxymethyl)aminomethane
v/v	-	volume/volume
w/v	-	weight/volume
YY1	-	Yin Yang 1

Abstract

Bromodeoxyuridme (BrdU) is a thymidine analogue capable of inducing epitheloid morphology and altering the expression of neuroendocrine markers in SCLC cell lines The ability of BrdU to alter differentiation in neuronal, muscle and haematopoietic lineages has been well documented in the literature Evidence suggests that this incorporation into the DNA alters the DNA's conformation, which in turn may affect interactions with specific transcription factors, leading to either inhibition or induction of differentiation Following on from work previously performed in our laboratory, several pyrimidine analogues were studied to investigate if they possessed similar differentiating properties to BrdU

The DLKP cell line was established at the NICB from a tumour histologically diagnosed as a poorly differentiated lung carcinoma DLKP cells have properties which suggest they could be classified as either SLCL-V or non-small-cell-lung carcinoma with neuroendocrine differentiation (NSCLC-NE) In this study it demonstrate that the DLKP cell line, and the more differentiated adenocarcinoma line, A549, upon treatment with the BrdU and a panel of other pyrimidine analogues, showed increased expression of cytokeratins 8, 18 and 19 proteins Increased protein expression levels of integrin subunits α_2 and β_1, as well as the cellular adhesion molecule Ep-CAM, was demonstrated in both cell lines following exposure to drug

DNA microarray experiments were also performed on DLKP cells exposed to BrdU, IdU and 5,2 -FdU Following gene expression analysis on these microarray experiments, lists of differentially expressed genes were generated From earlier work performed in this thesis, we demonstrate that all three pyrimidine analogues induce a similar pattern of differentiation in DLKP cells Therefore, the three microarray experiments were compared to each other in order to identify a common differentiation pathway We reveal that a total of 93 up-regulated genes were common to all three microarray experiments EASE analysis was performed on these 93 genes and identified 20 genes from this list of 93, which are thought to be involved in cellular development From this list of 20 development genes, we identify in particular, two families of transcription factors that potentially are involved in the regulation of differentiation in our system These transcription factor families are the Id and KLF proteins We propose that the Id family of proteins in play an important part in the regulation of differentiation in pyrimidine-treated DLKP cells We also suggest a role for KLF4 in the regulation of cytokeratin expression, mediated through IFNγ and STAT-1 proteins

The transcription factor YY1 is a 65kDa protein that is ubiquitously expressed and is highly conserved among human, mouse and Xenopus YY1 possesses the unusual property of regulating transcription in three ways, depending on the cellular context YY1 has been shown to activate, repress or initiate transcription

of a number of cellular genes and has previously been shown to associate with c-*myc*, resulting in its activation and up-regulation

We have also shown that BrdU-treated cells show increased levels of c-Myc and eIF-4E protein In order to investigate the role c-Myc and eIF-4E play in the differentiation of the DLKP lung cell line, a clonal variant of DLKP, DLKP-SQ, was transiently and stably transfected with a human YY1 cDNA expression vector It was observed that in stable clones over-expression of YY1 up-regulated c-Myc protein levels The over-expression of YY1 appears to have further effects on other cellular genes such as increased levels of eIF-4E, eIF-2α and Ornithine Decarboxylase proteins We also demonstrate that the transient over-expression of YY1 is capable of inducing genes identified as differentially expressed, namely Id2, Id3, HMOX1 and FHL1, in the DLKP, IdU and 5,2 -FdU microarray experiments

Section 1.0 Introduction

1.1 Cellular differentiation

Cellular differentiation can be defined as the process leading to the expression of phenotype characteristic of the functionally mature cell *in vivo* As the differentiation process progresses there is an associated reduction in cell division and cell proliferation activities are eventually lost (Davila, *et al* , 1990) The principal cells that differentiate are referred to as stem cells, which are capable of rapid cell growth and division These cells are multipotent and have the potential to differentiate into several different cell types In general, stems cells possess unlimited proliferative potential but they can remain quiescent under certain microenvironment conditions (Davila, *et al* , 1990) Differentiated cells are thought to be produced, not directly from stem cells, but rather via a committed progenitor or transit amplifying population (Watt, 1991)

The density of a cell during embryogenesis and development is regulated by gene expression which restricts the number of lineages that stem cells have the potential to form Previous studies (Ham and Veomott, 1980) have proposed that 'determination' is a process whereby a cell becomes committed to differentiate into a specific lineage A determined or committed cell initially may not appear phenotypically different, this only occurs after the genetic blueprint has been implemented (Maclean and Hall, 1987) A cell can differentiate in a manner which results in either the irreversible loss of its proliferative properties, terminally differentiated, or in the retention of some of its proliferative capacity while the cell itself is fully differentiated, non-termmally differentiated A number of differentiation states are also well documented dedifferentiation is the process by which a cell loses its differentiated phenotype and transdifferentiation occurs when a cell dedifferentiates and redifferentiates into a new and distinct phenotype (Davila, *et al* , 1990) It is apparent from this that as a cell undergoes differentiation, its gene expression profile will likewise change

The process of cellular differentiation is often meditated by the tissue type the cell is present in A progenitor stem cell represents the progeny of stem cells which possess more limited proliferation and differentiation potential This cell is usually involved in a single lineage Although stem cells in adult organs are pluripotent, the differentiated daughter cells are not usually expressed beyond the relevant organ in

which the stem cell originates from, i e , the cells are tissue determined stem cells (TDS) and are thus considered separate from embryonic stem cells (ES cells) (Sell, 1994)

Tissue determined stem cells are believed to undergo a slow cell cycle in order to reduce the risk of errors during DNA replication As TDS cells are present throughout the life of an organism, such errors could become amplified in the organism (Lajtha,, 1982) and had been proposed that many tumours contain TDS cell populations (Khan, *et al* , 1991) and that the overlapping expression of differentiation markers (Gazdar, *et al* , 1988) within cancer cells is indicative of a stem cell origin for most lung epithelial carcinomas During the differentiation process of TDS cells it is necessary that they maintain a constant cell number One popular model for this is asymmetrical cell division According to this model, when the stem cell divides one daughter cell remains as a stem cell while the other becomes a transit cell and enters the differentiation process

Although proliferation and differentiation appear to be interlinked processes during stem cell maturation, they are quite often separate events that occur concomitantly This suggests the whole differentiation process may be understood in terms of spiral model (Potten and Loffler, 1990) Some TDS cells appear to be highly pluripotent giving rise to several different cell lineages, e g the haematopoietic system Given its pluripontency, it can be envisaged that depending on the signal, a stem cell will adopt one direction of maturation over another

1.2 Lung Development

The development of the lung requires cell proliferation, branching morphogenesis, alveolar saccule formation and cell differentiation These processes require well co-ordinated events, which are achieved by epithelial-mesenchymal interactions, activation and repression of transcriptional factors and signalling

The existence of principle of stem cells and the *in vitro* cultivation and manipulation has now been well established and demonstrated for tissues such as mammary glands (Rudland and Barraclough, 1998), Liver (Sell, 1994), haematopoietic tissue (Fraser, *et*

al , 1995) and skin (Jones, *et al* , 1995) However, the existence of a similar stem cell model has no yet been identified in lung tissue, though is strongly suspected given the ability of the lung to regenerate when exposed to local damage by atmospheric components Identification of such lung stem cells is hampered by the complexity of the respiratory system and the variety of cell types present (Plopper and Hyde, 1992, Paine and Simon, 1996)

The most predominant hypothesis for stem cells *in vivo* is that different set of progenitor cells exist each destined to give rise to a discrete differentiated cell type (Plopper *et al* 1992) In the case of type II lung cells, these cell proliferate and then differentiate into type I cells (Adamson and Bowden, 1979) and Clara cells can differentiate into ciliated cells (Jutten, 1991) However, other thinking on the existence of lung stem cells suggests the existence of a monotypic stem cell, which gives rise to a transit cell described as a small mucous granule cell (SMGC) This cell is defined as being of a secretory yet premature type containing a few small granules which are periodic acid Schiff reaction positive, and also possess a well developed endoplasmic reticulum, prominent Golgi complex and tonofilmament bundles It is believed that SMGCs are able to give rise through dedifferentiation to any differentiated secretory cell type

To date, very little scientific evidence exists relating to stem cells of the lung, the pathways they follow, their distribution and mechanism of action No markers yet exist for lung stem cells and the idea of dedifferentiation is in contrast to the stem cell models developed in other tissues suck as the skin, liver and intestine, where stem cells pre-exist in the epithelium (Emura, 1997) The lung is susceptible to local damage from a number of different sources including ozone, carbon black particles, lipophilic chemicals absorbed into the blood stream from the gut, or damage induced from bacterial or viral infection Therefore, the lung must posses some form of self regeneration, even if limited to overcome such damage In attempting to identify if a cell is a stem cell, its native state is often altered during the investigation This may result in loss of the stem cell or only a limited spectrum of responses being observed Thus, due to the variety of cell types present and the complexity of the respiratory system, indemnification of a lung stem cell may prove to be a difficult task

1 3 Identification of a stem-like lung cell line, DLKP

All of this has interesting implications with the isolation of a poorly-differentiated lung cell line, DLKP, at the NICB (Law, *et al*, 1992) Clones derived from this cell line exhibit the amazing capacity to regenerate the mixed parental population over time The DLKP novel cell line has been categorised as extremely poorly differentiated and consists of at least three subpopulations, termed SQ (Squamous), I (Intermediate) and M (Mesenchymal) (McBride, *et al*, 1998) These populations have demonstrated the ability to interconvert and eventually, when cultured alone, replenish the parental phenotype This, combined with the lack of expression of a number of differentiation-specific markers, has lead to the speculation that DLKP may represent a stem cell-like population This has afforded a unique opportunity to study the process of lung cancer differentiation *in vitro*, particularly the early stages of this process Such studies will provide insights in the mechanisms of early lung development

1 4 Synthetic agents capable of inducing epithelial lung cell differentiation

1 4 1 Halogenated thymidine analogues - BrdU, IdU and CdU

Bromodeoxyuridine (BrdU) is a halogenated thymidine analogue that is known to influence the differentiation of cells It is best referred to as a differentiation modulating agent since it has been shown to be a potent inducer of differentiation in some cell lines (Yen *et al*, 1987, Sugimoto *et al*, 1988, Valyi-Nagy *et al*, 1993), while it can inhibit the differentiation of others (Seecoff and Dewhurst, 1976, Tapscott *et al*, 1989, Lee *et al*, 1992) BrdU competes with naturally occurring Thymidine for incorporation into DNA during replication and as such it, and other similar compounds, should be ideal candidates for anti-tumour agents, since they require cell division and DNA synthesis to exert their effects (Bick and Devine, 1977) While few clinical trials are based on the differentiation-modulating properties of this drug (Freeman, 1969, Ameye *et al*, 1989), BrdU has been used widely as a radiosensitiser in an attempt to improve radiological treatments (Lawrence *et al*, 1992, McGinn and Kinsella, 1993) Radiosensitisation trials to date include the treatment of malignant glioma (Vander *et al*, 1990), ulcerative herpetic keratitis (van

Bijsterveld *et al.*, 1989), malignant astrocytomas (Greenberg *et al.*, 1988) and malignant brain tumours (Matsutani *et al.*, 1988). More recently, BrdU has entered clinical trials as a radiosensitiser in the treatment of pancreatic cancer (Robertson *et al.*, 1997), colorectal liver metastases (Robertson *et al.*, 1997) and cervical cancer (Eisbruch *et al.*, 1999), while studies in relation to malignant gliomas continue (Prados *et al.*, 1998). Administration of BrdU is normally by controlled perfusion (Doirion *et al.*, 1999), and has been used in combination with radiolabelled monoclonal antibodies (Buchsbaum *et al.*, 1994). While radiolabelled antibody approaches offer the potential of targeted chemotherapy, they are limited by low dose-relate deliverable. As such, the trials of Buchsbaum *et al* (1994) may offer a means of enhancing the efficacy of low dose radiolabelled monoclonal antibody approaches.

BrdU incorporates into DNA in a non-random fashion at sequences termed "fragile sites" (Hecht *et al.*, 1988; Sutherland, 1988; Sutherland, 1991). This explains the reproducibility of the effects observed with BrdU-induced differentiation. O'Neill and Stockdale (1973) developed a model for BrdU-induced modulation of differentiation that assumes that BrdU "sensitivity" resides on a single pair of chromosomes, suggesting the presence of a "master gene" or target through which BrdU exerts its effects. In this model, inhibition of differentiation occurs in a dominant fashion if approximately 30% or more of naturally occurring thymidine is replaced by BrdU in the readout strand of either chromosome. This sort of model agrees with the predicted mechanisms of action of a number of DNA-intercalating agents. BrdU substitution into DNA and intercalation of such agents may have similar effects, thought to be through direct DNA bending at either major or minor grooves, thereby altering promoter structure and availability to transcription factors. Intercalation of the antibiotics, elsamicin A or actinomycin D in the promoter of the c-*myc* gene induced a decrease in the level of transcription from this promoter (Vaquero and Portugal, 1998). However, relatively low levels of elsamicin incorporation actually induced an increase in c-myc transcription through the P1 promoter. Bromodeoxyuridine (BrdU) has been demonstrated to decrease c-*myc* expression at the transcriptional level in the leukaemic cell line, HL60 (Yen and Forbes, 1990) and in human melanoma lines (Valyi-Nagyi *et al.*, 1993). These results would appear to suggest that the c-*myc* promoter regions are particularly susceptible to modulation by agents that disrupt

promoter structure either through Thymidine substitution (BrdU) or intercalation (Elsamicin). Alternatively, BrdU may directly influence the ability of proteins to associate with DNA. In the *lac* operon, BrdU-substitution has been shown to result in increased binding of the *lac* repressor protein (Lin and Riggs, 1972), suggesting that BrdU may be capable of altering the binding of regulatory factors.

The mechanism by which BrdU exerts its differentiation-modulating effects remains unclear, but it appears that incorporation into DNA is essential. This involves BrdU being converted to Bromodeoxyuridine monophosphate, which competes with thymidine for incorporation into DNA (O'Neill and Stockdale, 1974). Experimental evidence for this hypothesis comes from a study by Keoffler *et al.* (1983) which showed that a thymidine kinase-deficient human myeloid cell line, HL60, was unable to incorporate BrdU into its DNA and subsequently failed to respond to the ability of BrdU to modulate its differentiation status.

A number of models exist to explain the ability of BrdU to modulate differentiation:

Model 1:

This model envisages that BrdU induces chromosomal breakages. These breakages and the associated chromosomal aberrations can be associated with stepwise changes in the differentiation status of a cell. These breakages are specific points called 'fragile' sites, 32 of which have been identified in murine chromosomes. It is proposed that BrdU associates with these fragile sites which are known to be recombinogenic (Alexander, *et al.*, 1992).

Model 2:

BrdU alters the affinity of DNA sequences for regulatory proteins. Studies on the *lac* operon with BrdU incorporation demonstrate that the *lac* suppressor was bound with greater affinity (Lin and Riggs, 1972).

Model 3:

In this model BrdU has been found to exert its effects on differentiation by alteration of a key regulatory gene(s) that alters transcription of genes involved in differentiation (Arnold, *et al.*, 1988; Rauth and Davidson, 1993). In BrdU inhibition

of myoblast differentiation, such alterations occurs with the down-regulation or complete inhibition of the key regulatory gene, MyoD1 (Topscott, *et al*, 1989, Nanthakumar and Henning, 1993)

Model 4.

This model envisages that BrdU incorporation causes an alteration in the reading frame of the DNA template resulting in the formation of abnormal mRNA, which is incapable of synthesising the correct differentiation products (Hill, *et al*, 1974)

BrdU is considered by some scientists to be an inducer of pre-commitment to differentiation rather than an actual differentiation inducing agent This was highlighted by the findings that BrdU treatment of HL60s for 24 hours, followed by treatment with Retmoic Acid resulted in a faster response to Retinoic Acid (RA) than the single addition of RA alone (Yen *et al*, 1990) It would appear that BrdU can initiate some of the early changes induced by RA in HL60 differentiation, including early c-myc down-regulation However, the same author reported previously (Yen *et al*, 1987) that pre-commitment to differentiation involves an early increase in c-myc levels in the same Leukaemic line, as induced by RA This suggests that pre-commitment to differentiation in these cells involves increased expression of c-myc It therefore appears that the true mechanisms of induction and commitment to differentiation remain unclear, even in individual cell types

1 4 2 Mode of Action of Thymidine Analogues

The exact mechanism(s), by which BrdU and the various thymidine analogues investigated in these this study, exert their differentiation-modulating effects remains poorly understood In the case of BrdU, it is thought that incorporation into DNA is critical in the process (O'Neill and Stockdale, 1974) Low levels of BrdU have been shown to alter the differentiation status of many different cell types in both inhibitory e g myoblast cells (O'Neill and Stockdale, 1974) and stimulatory e g neuroblastoma cells (Ross A H , *et al*, 1995) Incorporation into DNA involves the conversion of BrdU to Bromodeoxyuridine monophosphate, which competes with thymidine for incorporation into DNA (O'Neill and Stockdale, 1974) A study by Keoffler *et al* (1983) showed that a thymidine kinase-deficient human myeloid cell line, HL-60, was

unable to incorporate BrdU into its DNA and subsequently failed to respond to the ability of BrdU to modulate the differentiation status of HL-60 cells.

Incorporation of BrdU into DNA occurs in a non-random fashion, with incorporation occurring into repeated nucleotide sequences; know as 'fragile sites' (Schwartz and Snead, 1982; Hecht *et al.*, 1988). It is as a result of this consistency of incorporation that may explain the reproducibility of BrdU-induced differentiation. It is thought that breakages in DNA may occur at these fragile sites and these breakages and chromosomal aberrations may be associated with changes in the differentiation status of the cell. It was been also reported that BrdU associates with these fragile sites. (Alexander *et al.*, 1992).

It is also thought BrdU substitution into DNA may also induce effects similar to DNA-intercalating agents, by altering DNA bending at either the major or minor groves, and thus alter the structure of promoter regions and the affinity of DNA binding proteins (Lin and Riggs, 1972). Thus, BrdU is likely to exert is effects at least in part, on differentiation by altering of expression of set of regulatory genes that are involved in the control of another set of differentiation-related genes (Arnold *et al.*, Rauth and Davidson, 1993).

The biological action of CdU and IdU is also thought to be similar to that of BrdU. CdU is converted to chlorodeoxyuridine monophosphate by thymidine kinase, and has been shown to compete with thymidine for incorporate into DNA (Cortès *et al.*, 1987).

1.4.3 Mode of Action of Fluro-pyrimidines

In contrast to BrdU, IdU and CdU, the 5,5`-Fdu, 5-FU and 5,2`-FdU analogues modes of action are slightly different. 5,5`-FdU is cleaved by nucleoside phophorylase enzyme to yield 5-flurouracil (5-FU) (Armstrong and Diasio, 1980). The 5-FU generated is subsequently metabolised via several steps to yield flurodeoxyuridine monophosphate (FdUMP) (Pratt *et al.*, 1994). FdUMP binds to thymidylate synthase, forming an irreversible covalent ternary complex in which enzyme, folate cofactor, and FdUMP are bound, thus inhibiting thymidine monophosphate production and

hence DNA synthesis (Pratt *et al.*, 1994). The inhibition of DNA synthesis causes cells to delay in the S-phase of cell cycle and this stall has previously been shown to induce differentiation of embryonal carcinoma cells, PC13, to endoderm-like cells, following exposure of the cells to Retinoic Acid (Nishimure *et al.* 1983; Mummery *et al.*, 1984).

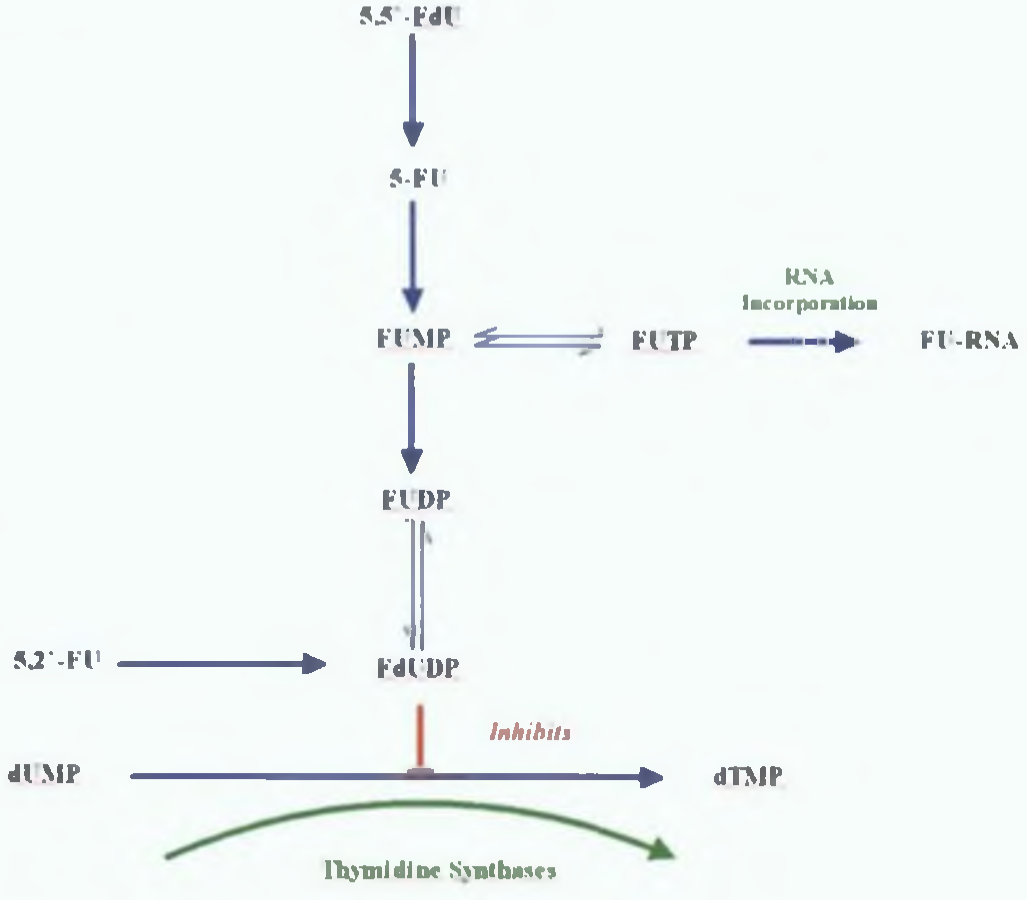


Figure 1.? **Pathway of 5,5`-FdU Metabolism**

1 5 Keratins as markers of epithelial differentiation

A large proportion of the cytoplasm of vertebrate cells, normal and transformed, is occupied by components of the cytoskeleton, including actin, tubulin and the intermediate filaments (Moll *et al*, 1982) They are formed in different cell types from different proteins of a multigene family or from different subunit polypeptides of a class of related proteins By far the most striking differentiation specificity of composition has been observed in the intermediate-sized filaments This class of filaments includes the desmin filaments typical of myogenic cells, the neurofilaments typical of neuronal cells, vimentins occur in mesenchymally derived cells and vascular smooth muscle cells, and the keratins occur in epithelial cells (Moll *et al*, 1982, Hatzfeld and Franke, 1985, Daly *et al*, 1998) Keratin Intermediate Filament (IF) proteins have three domains a central alpha-helical rod domain of constant size that derives from common ancestors, and two end-domains of variable structure thought to be involved in tissue-specific functions (Blumenberg, 1988) The specificity of keratin expression patterns in epithelial cells has been used in prognostic and diagnostic situations as markers of both epithelial origin and state of differentiation in patients with small cell lung cancer (Bepler *et al*, 1987, Broers *et al*, 1988), and other tumour pathologies (Virtanen *et al*, 1984, Trask *et al*, 1990) to distinguish normal and tumour-derived epithelial cells Keratins are thought to serve a structural function to protect the cell against environmental stresses and strains as for other filaments (Daly *et al*, 1998), but their expression in human ovarian adenocarcinoma lines has been associated with altered sensitivity to various chemotherapeutic drugs (Parekh and Simpkins, 1995) Interestingly, in studies using a number of chemical differentiating agents the levels of mdr-1/Pgp (p-glycoprotein) increased and expression appears to correlate with the degree of differentiation (Mickley *et al*, 1989) However, induction of these pumps is not always accompanied by expression of the multidrug-resistance phenotype, which may possibly be explained by changes in keratin expression during the differentiation of these cells The human K8 mRNA encodes a nucleic acid-binding domain, suggesting that keratm filaments may bind to nucleic acid sequences and play a role in regulating DNA replication and gene transcription (Yamamoto *et al*, 1990) It is also possible that they play a role in the regulation of translation of particular mRNAs through their localisation to regions within the cell, in a similar manner to the way in which polar

expression of developmental proteins *nos* and *bicoid* are regulated (Gavis *et al.*, 1992). Genetic disease states associated with loss of keratin regulation include the blistering phenotype of Epidermolysis Bullosa Simplex (EBS) (Oshima, 1992; Fuchs and Byrne, 1994) and development of dwarf phenotypes and diabetes in transgenic mice expressing the K8 gene (Casanova *et al.*, 1995).

The keratins (K) are divided into two categories; the acidic type I keratins are K9-20, while the more basic type II keratins are K1-8. Keratin filament formation is dependent on the pairing of partners from both groups to produce a proteolytically stable hetero-polymer filament (Kulesch *et al.*, 1989). Despite the fact that their function is relatively unknown, the pattern of expression of keratin filaments is specific to both epithelial origin and degree of differentiation (Tseng *et al.*, 1982). As described in "The Catalogue of Human Cytokeratins" (Moll *et al.*, 1982), while K9-K11 is predominant in the epidermis, K12 has only been observed in the cornea. Cytokeratin 8 represents simple epithelia, and its normal partner, K18, shows the same tissue distribution (Trask *et al.*, 1990). K8 and K18 are the first keratins to appear during mouse development (Casanova *et al.*, 1995) and are thought to be the evolutionary ancestors of many of the present keratin forms (Blumenberg, 1988). Cytokeratin 19 is found in a broad range of epithelial tissues and is a major component of simple epithelia. K14 and K19 are known to be "promiscuous" in that they can partner Type II Keratins in the absence of their "usual" Type I partner to form stable filaments (Hatzfeld and Franke, 1985; Darmon, 1985; Lersch *et al.*, 1989). K19 lacks a variable terminal domain. This, combined with its promiscuity, means that K19 is thought to play a critical regulatory role by pairing with any one of the basic keratins without contributing a potentially harmful variable terminal domain, the region in which tissue-specific function of keratins resides (Blumenberg, 1988). It therefore acts to redress keratin imbalances. Keratins 7/8/18/19/20 have been associated with simple epithelia, while K4/5/17 are associated with stratified epithelia (Mobus *et al.*, 1994). Both classic and variant small cell lung cancers express K8 and K18/19, detectable by western blotting when immunocytochemical staining is weak (Elias *et al.*, 1988). Stem cell populations of the lung have been speculated to exist as pluripotent populations residing in tumours and cell lines (Trask *et al.*, 1990; Pfeifer *et al.*, 1991). The almost complete absence of keratin expression in DLKP, a novel

poorly differentiated NSCLC-NE/SCLC-variant cell line isolated at the NCTCC, has led to speculation that this cell line may represent a stem cell-like population

1 5 1 Regulation of Keratin Expression

The regulation of keratin filament formation is complex and is controlled at multiple levels Regulation of keratin expression has been reported at the transcriptional level (Roop *et al*, 1988), involving AP-1 activation of transcription (Neznanov and Oshima, 1993) which is mediated by the ras signalling pathway (Pankov *et al*, 1994) Relatively short sequences in the 5' upstream region of keratin genes can confer tissue-specific transcription (Blessing *et al*, 1989, Neznanov and Oshima, 1993) In addition, histone and chromosomal insulation of keratin genes (Casanova *et al*, 1995), labile inhibitors of transcription (Cremisi and Duprey, 1987), and post-transcriptional proteolysis (Kulesh *et al*, 1989) have all been implicated in the cell-specific and developmental regulation of keratin filament formation An important aspect to the proteolytic regulation of keratin filament formation, in which both partners of the pair are required for proteolytic stability and filament expression, is that it would appear that the expression of a type II keratin is sufficient to induce the expression of a type I partner (Giudice and Fuchs, 1987, Knapp and Franke, 1989, Lersch *et al*, 1989, Rothnagel *et al*, 1993) Type I keratin expression has been suggested to be dependent on accumulation of unpolymerised Type II keratin (Giudice and Fuchs, 1987) for proteolytic stability for overall filament formation Type I proteolysis may form a universal regulatory element while specificity in Type II expression will therefore result in Type I induction and tissue-specific Intermediate Filament formation (Rothnagel *et al*, 1993) Synthesis of both keratin types can be uncoupled and control of cytokeratin Intermediate Filament formation can occur at different levels (Knapp and Franke, 1989), strengthening this suggestion There is substantial evidence for additional post-transcriptional regulatory mechanisms (Blouin *et al*, 1991, Crowe *et al*, 1993), including mRNA degradation (Paine *et al*, 1992) and the suggestion that there is a possible block on the translation of certain keratin mRNAs, such as K8 (Tyner and Fuchs, 1984) This speculatively involves translational repression (Su *et al*, 1994) and even masking of keratin mRNAs in epithelial squamous cell carcinomas (Winter and Schweizer, 1983)

1 6 Integrins

The integrin receptors consist of two heterodimer chains, α and β, both of which form a non-covalently associated complex (Hynes, 1987) The α subunit family of integrins possesses 15 variants, while the β subunit family contain 8 variants In theory these two families could associate to give rise to over 100 integrins However, the actual diversity is much more restricted and in reality the subunits combine into 22 different integrins (Buck and Horwitz, 1987) The integrin family is subdivided on the basis of its β subunit (Newham and Humphires, 1996) For example, the β_1 integrins are involved principally in the adhesion between the ECM and the cellular cytoskeleton (Buck *et al*, 1987), while the β_2 integrins participate in cell-cell interactions (Ruoslahti, 1991) The specificity of binding is not determined solely by integrin pairing, but also by the cell type it is expressed in

Integrins have been implicated in such diverse processes as inflammation, cellular growth and differentiation (Albelda and Buck, 1990) For example, the interaction in developing lung between the ECM and the epithelium is mediated by integrin receptors, and allows normal lung branching to occur (Gumbiner, 1996) As well as functioning as cell adhesion molecules, the integrins have signalling functions that regulate various aspects of cell behaviour and differentiation This signalling is accomplished through focal adhesion proteins In this study we have chosen the increased expression of both α_2 and β_1 integrins as markers of differentiated epithelial lung cell differentiation

1 7 Transcriptional Control of Gene Expression

Transcriptional control of gene expression during both proliferation and differentiation has been widely studied Transcription factors such as MyoD and Myogenin have been shown to play critical roles in the regulation of muscle-specific differentiation (Weintraub, 1993, Buckingham, 1994) On the other hand, factors such as *c-fos*, *c-jun*, and c-*myc* have long been established as playing roles in the regulation of cellular proliferation, differentiation and transformation of a wide variety of cell types Selective transcription of genes such as alcohol dehydrogenase (Adh) during development is known to occur through specific sequences in the promoter regions of genes that bind regulatory factors known as transcriptional enhancers (Novina and Roy, 1996) Despite this, the process of transcription and the mechanisms by which transcription factors regulate differentiation are still not fully understood

1 7 1 The c-*myc* proto-oncogene

First identified as the transforming gene of the avian myelocytomatosis virus (v-*myc*) (reviewed, Evan, 1990), the *myc* family of oncogenes must rank among the most widely studied of all proto-oncogenes Despite this, there is a relative paucity of direct c-*myc* targets that have been identified to explain the capacity of this gene to induce transformation and malignancy (Ryan and Birnie, 1996) While no direct role for c-*myc* was found in some malignant conversions (DeBenedetti *et al*, 1994), c-*myc* expression has been shown to be critical to transformation by both v-abl and BCR-ABL, as evidenced using dominant negative c-*myc* expression (Sawyers *et al*, 1992) Genetic instability and abnormality is associated with lung cancers (Fong *et al*, 1995) and c-*myc* abnormalities are frequently associated with carcinogenesis c-*myc* activation has been shown to occur via gene amplification, chromosomal translocation, proviral insertion and retroviral transduction (Ryan and Birnie, 1997)

1 7.1 1 c-Myc structure and Function

The c-*myc* gene is highly conserved, apart from its first exon, throughout vertebrate evolution It first came to notice because of its homology to the viral oncogene, v-*myc* While deregulated expression of c-*myc* has been associated with a variety of neoplasms, early studies indicated that introduction of the c-*myc* gene into normal fibroblasts was not sufficient to transform cells (reviewed Evan *et al*, 1990) The 5' region of the c-*myc* gene contains four promoters, termed P0-P3 However, the two major promoters, P1 and P2 contribute 75%-90% and 10-25% of the cytoplasmic c-*myc* mRNAs, respectively (Ryan and Birnie, 1996, Nanbru *et al*, 1997) The functional significance of these promoters remains a mystery They may play roles in processes such as proliferation and differentiation, or may simply represent evolutionary redundance of the P0 and P3 promoters The c-Myc protein is a phosphoprotein, phosphorylated by casein kinase II (Hagiwara *et al*, 1992) and DNA-PK (DNA-activated protein kinase) (Iijima *et al*, 1992, Chibazakura *et al*, 1997), and its expression is induced in response to serum and growth factor stimulation c-Myc possesses a short cluster of basic ammo acids that serve as nuclear localisation sequences (NLS) (Saphire *et al*, 1998), in addition to DNA-binding leucine zipper motifs The N-terminal region contains the transcriptional transactivation domain (Ryan and Birnie, 1996) There are two isoforms of the protein, c-Myc1 and c-Myc2, which differ by 20 ammo acids in their N-terminal region (DeBenedetti, personal correspondence)

c-Myc exerts its effects through oligomerisation with other proteins (Figure 1 6), characteristic of other DNA-binding transcription factors (e g Jun and Fos) Originally thought to homodimerize, it is now known that this is untrue Oncogenic activation of c-Myc requires heterodimerization with activating Max proteins (Amati *et al*, 1993), which then bind DNA through basic-hehx-loop-helix-leucine zipper motifs Negative regulation of c-Myc activity occurs through interaction with another factor, termed Mad (Ryan and Birnie, 1996), which has no transactivating function but competes with Max for binding to the same region of the c-Myc protein It is, therefore, a competitive inhibitor of c-Myc activation by Max No initial sequence specificity of c-Myc binding was apparent, but it is now understood that c-Myc binds through a basic amino acid α-helix region (Fisher *et al*, 1993) to what are termed

myc-binding sequences or "E-box elements" (CACGTG). These sites require association of Max in addition to c-*myc* for activation (Ryan and Birnie, 1997). *In-vivo* activation of E-box containing genes by Myc/Max heterodimers, including an RNA helicase gene belonging to the DEAD-box family, has been demonstrated (Grandori *et al.*, 1996). c-Myc/Max complexes, active in transcription, appear to be dependent on the levels of c-Myc available within the cell (Amati *et al.*, 1993), that is, Myc synthesis is rate-limiting for Myc-Max dimerisation and activity. Myc overexpression activates, while Max overexpression represses transcription through E-box sites. This is because Max/Max homodimers do not activate, and so compete with Myc/Max complexes when Max is over-expressed (Somer *et al.*, 1998). Max overexpressing lines show reduced expression of transiently transfected Myc-responsive genes (Zhang *et al.*, 1997), implying a role for Max expression in the regulation of processes such as differentiation.

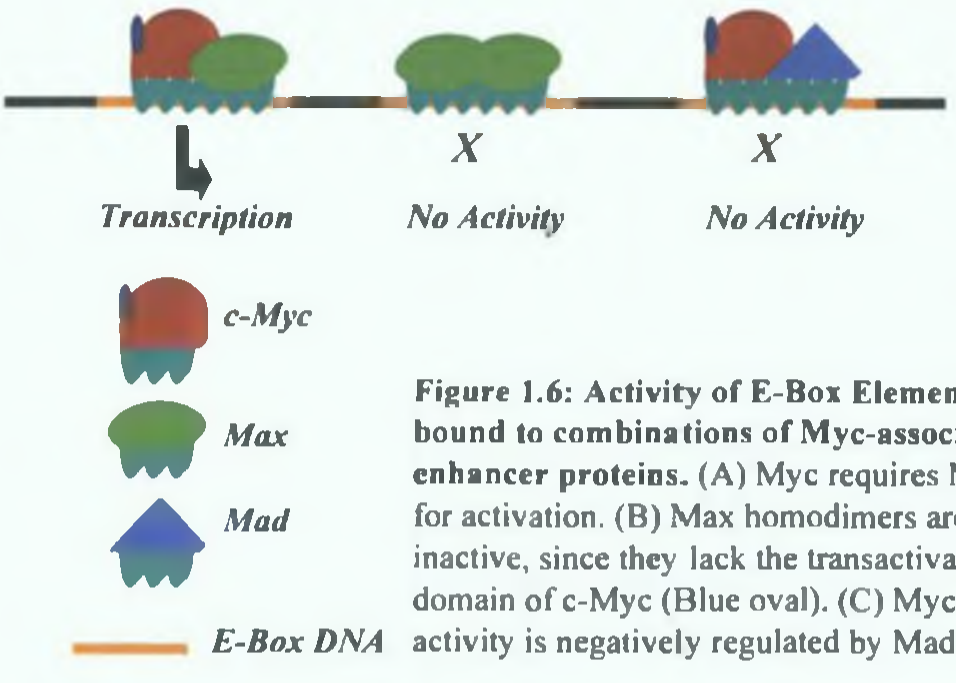


Figure 1.6: Activity of E-Box Elements bound to combinations of Myc-associated enhancer proteins. (A) Myc requires Max for activation. (B) Max homodimers are inactive, since they lack the transactivation domain of c-Myc (Blue oval). (C) Myc activity is negatively regulated by Mad.

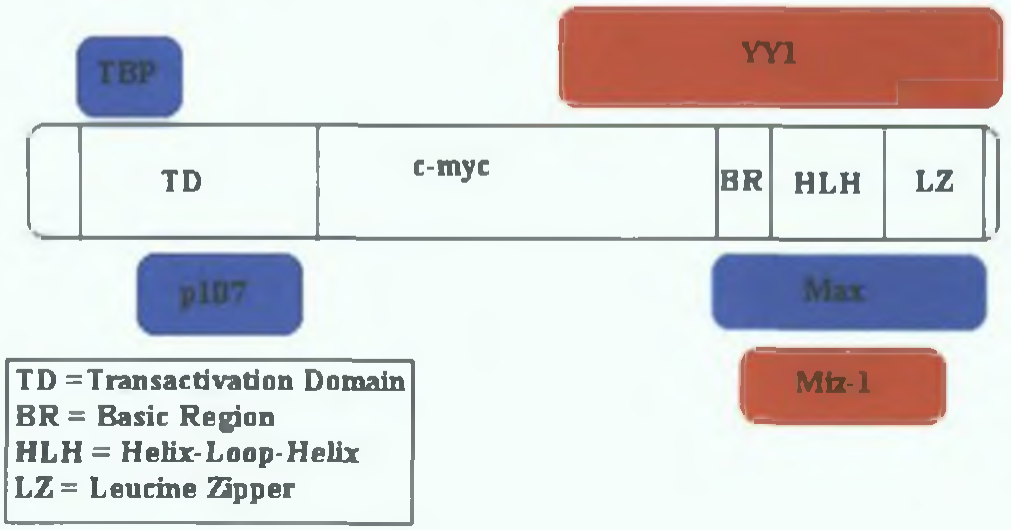
Max appears to be extremely simple and is comprised of only 160 amino acids, 80 of which constitute the DNA-binding/dimerization domain (Cole, 1991), suggesting that

transactivation of basal transcription occurs through the longer N-terminal region of the c-myc portion of the Myc/Max complex. This explains the lack of transactivation by Max homodimers and the findings that myc levels are rate limiting in the transactivation by Myc/Max heterodimeric complexes. Myc/Max, Max/Max and Mad/Myc complexes all bind to the Myc E-box with the same affinity (Somer *et al.*, 1998). Therefore, since the transactivation domain of these complexes lies in the Myc N-terminal, complexes lacking a Myc partner act as competitive inhibitors of Myc/Max transactivation.

However, c-Myc has also been shown to exhibit a degree of "dual functionality" in that it is capable of transcriptional repression, as well as activation (Antonson *et al.*, 1995), depending upon the position of the E-box relative to the transcription start site. As such, the role of c-*myc* in the regulation of cellular growth and proliferation should not be confined to a narrow view of transcriptional enhancement and stimulation of proliferation. Roles for c-myc in apoptosis and differentiation are evident, but as yet unclear. "It would be naïve to assume that the only transcriptional targets of c-*myc* are those involved in transformation" (Ryan and Birnie, 1997). Human bronchial epithelial cells transformed by overexpression of c-*raf*-1 and c-*myc* proto-oncogenes were capable of inducing multi-differentiated carcinomas in nude mice (Pfeifer *et al.*, 1991). This suggests that the role of c-*myc* in regulating differentiation may be cell-specific, and that down-regulation of *myc* expression during differentiation (Yen and Forbes, 1990; Valy-Nagyi *et al.*, 1993) may not be a "universal" requirement of all cell types, as observed for AP-1. In addition, c-*myc* has been shown to play a role in the induction of apoptosis (Harrington *et al.*, 1994; Kohlhuber *et al.*, 1995).

c-Myc has been found to directly interact with a number of additional proteins, many of which are novel transcription factors in themselves (Figure 1.7). These interactions may form another level at which myc exerts its influence over the transcription process. A novel zinc-finger protein, termed Miz-1 (Myc interacting zinc-finger protein-1) has been identified that specifically interacts with Myc, but not with Max (Peukert *et al.*, 1997). Miz-1 is a transcription factor with potent anti-proliferative effects. Binding of Myc to Miz-1 inhibits the promoter activation activity of Miz-1, relieving the anti-proliferative effects of Miz-1 expression. Of note is the interaction between c-myc and the developmental regulator known as Yin-Yang 1 (YY1). YY1

regulates c-Myc levels, while association of c-Myc and YY1 proteins reduces the activity of both proteins. This may form the basis of an auto-regulatory mechanism to control the levels/activity of these two proteins. Such interactions with key transcription factors, regulating their activity, may play a significant role in the activity of c-Myc. This is particularly intriguing in light of the lack of direct transcriptional targets identified for c-Myc to date. A diagram of the known interactions between c-Myc and other enhancer proteins is shown in Figure 1.7



(reproduced from Ryan and Birnie, 1996):

Figure 1.7: Regions of c-Myc interacting with other transcription factors. (Note: p107 is a member of the Retinoblastoma family of negative regulators. Its association with c-Myc inhibits the transactivation activity of c-Myc).

1 7 2 The Yin-Yang Transcription factor, YY1 (NF-E1, NF-δ, UCRBP, CF-1)

YY1 (Yin-Yang 1) is a developmentally important transcription factor, so-named because of its ability to act as both a transcriptional activator and repressor It belongs to the GLI-Kruppel family of negative transcription factors (Licht *et al*, 1990, Shi *et al*, 1991), of which relatively few are known in eukaryotes The YY1 gene was localised to chromosome 14 in humans (Yao *et al*, 1998), although pseudogenes or additional YY1 genes have been suggested to exist (Zhu *et al*, 1994) The promoter region of YY1 lacks consensus TATA or CCAAT boxes, but contains multiple SP-1 binding sites (Yao *et al*, 1998), including a critical promoter region (Safrany and Perry, 1993) Four laboratories working independently cloned the YY1 gene in 1991, perhaps highlighting the universally important role of YY1 in transcriptional regulation

1 Park and Atchison (1991) isolated a factor they termed NF-E1, which was capable of binding to both the immunoglobulin κ 3' enhancer and the immunoglobulin heavy-chain μE1 site, transcriptionally repressing and activating these promoters, respectively The authors also reported that NF-E1 (Common Factor 1, CF1) was capable of binding the c-*myc* promoter The binding of CF1 was shown to be capable of activating transcription through a c-*myc* CF1 site (Riggs *et al*, 1991) Overexpression of YY1 was shown to be a strong activator of murine c-*myc* expression, with mRNAs increasing from both the P1 and P2 promoters of the endogenous c-myc gene (Riggs *et al*, 1993) These promoters account for the vast majority of c-*myc* transcript present in the cytoplasm

2 NF-δ was found to bind to and activate critical downstream promoter elements in the mouse ribosomal protein rpL30 and rpL32 genes (Hariharan *et al*, 1991)

3 Flanagan *et al* (1991) isolated a negative transcription factor, UCRBP (UCR-Binding Protein) that bound to the upstream conserved region (UCR) of MMLV (Moloney Murine Leukaemia Virus), down-regulating promoter activity A negative regulatory region in the HPV-18 (Human Papilloma Virus) was shown to bind YY1 with high affinity (Bauknert *et al*, 1992) and mutation of the YY1

binding site leads to enhanced activity of the HPV-18 promoter Many viruses that cause cancer have been found to have lost YY1 binding sites, which may be a means of escaping this negative regulation (Shrivastava and Calame, 1994)

4 Finally, YY1 was isolated and given its more widely used name by Shi *et al* (1991) when it was found to associate with the Adenovirus P5 promoter, activated by the viral E1A protein In the absence of E1A this promoter is silenced by YY1, and only becomes activated in the presence of E1A Both E1A and YY1 were found to share overlapping binding sites m the P5 promoter, but YY1 binding is not eliminated upon E1A binding, suggesting that competition for binding is not the means by which regulation occurs E1A-mediated activation is speculated to involve unmasking regions of the YY1 N-termmal involved in activation but normally masked in the full-length protein (Lee *et al*, 1994) (Figure 1 8)

Consensus activation and repression sequences for YY1 are shown below, although these are known to vary giving rise to changes in binding capacity of these sites for YY1 (Hyde-DeRuyscher *et al*, 1995)

Activation **CGGCCATCTTGNCTG**
Repression **CCATNTTNNNA**

1.7 2 1 The Structure and Function of YY1

There is evidence that YY1 is a phosphoprotein Eight consensus phosphorylation sites are found in the deduced amino acid sequence and YY1 activity can be abolished through the use of phosphatases (Becker *et al*, 1994) The amino acid sequence of the YY1 protein displays a number of unique properties to date, including acid rich domains similar to transcriptional activators, as well as Ala+Gly-rich and His rich sequences common to transcriptional repressors (Park and Atchison, 1991) The very unusual N-terminal region consists of 11 consecutive negatively charged amino acids and 12 consecutive histidines, thought to form two oppositely charged symmetrical helices separated by a highly flexible glycine-rich loop (Helix-Loop-Helix, HLH) (Hariharan *et al*, 1991) These regions are speculated to be capable of forming an acidic activation domain that could be neutralised or modulated under certain

conditions to allow interaction with polymerase II before and after transcription has commenced The ammo terminal transactivation domain requires ammo acids 16-29 and 80-100 for maximal activity (Bushmeyer *et al*, 1995)

The C-terminal contains four zinc fingers, characteristic of DNA-binding transcription factors, while the central region is largely unstructured, consisting of large loop and helix regions The YY1 repression domain lies near the carboxy terminus and is embedded within the YY1 zinc finger region necessary for DNA-binding (Bushmeyer *et al*, 1995) Particular importance has been placed upon zinc fingers 3 and 4 for repression activity

The functional diversity of YY1 was conceivably attributed to its structural plasticity (Hariharan *et al*, 1991) It is generally thought that repression of gene transcription is the usual function of YY1, with the activating N-terminal region being masked Interaction with activating proteins, such as viral E1A, then releases the N-terminal region and converts YY1 to an activator of transcription through the same promoter (Figure 1 8) However, it has also been suggested that repression is not the intrinsic activity of YY1 Rather, YY1 acts to bend DNA (Natesan and Gilman, 1993) in a way that modulates the interaction of proteins bound to the two flanking regions When the orientation of the YY1 binding site is reversed or the phasing of the sites is changed, YY1 becomes an activator of the same promoter (Natesan and Gilman, 1995) Rather than bending two proteins away from one another, YY1 now bends them towards one another to bring them into closer contact and increase association Therefore, YY1 will have distinct local effects on protein-DNA and protein-protein interactions depending upon the position and orientation of its binding site within the promoter, supporting a general role for YY1 in the building of highly organised promoter complexes This is particularly important in the formation of promoter structures at TATA-less promoters, since YY1 has been shown to bend DNA in a manner suitable to provide a site for transcription initiation (Kim and Shapiro, 1996) Both promoter orientation-dependent and co-factor-dependent activity of YY1 was also suggested in the human Interferon-γ promoter (Ye *et al*, 1994) In this case, DNA-binding is a required function of YY1, while in other cases DNA-binding is not required for YY1 to exert its effects upon promoter formation and activity

Figure 1.8: Diagrammatic Representation of E1A-mediated activation of YY1 Transactivating Potential. YY1 is a repressor of the P5 promoter (A), but in the presence of EIA, the N-terminal Activating Region (Blue) is unmasked and transcription is activated (B).

1.7.2.2 Transcription Factors interact with YY1 to regulate its activity

A YY1 binding site in the *c-fos* promoter is required for adenovirus E1A activation of *c-fos* transcription (Gedrich and Engel, 1995). Rather unusually and almost paradoxically, repression by YY1 was also found to be independent of the presence of YY1 binding sites in *c-fos* reporter constructs (Zhou *et al.*, 1995). It was shown that YY1 repression was mediated through interaction of YY1 with CREB (cyclic AMP Response Element Binding) Proteins. Thus, within the *c-fos* promoter alone YY1 is known to interact with E1A and CREBPs to either increase or decrease transcription, respectively, and these functions can be both dependent and independent of the ability of YY1 to bind DNA. This has lead to claims that YY1 activity is regulated through interactions with other proteins and that it must contain a C-terminal repression domain that is independent of its ability to bend DNA (Hyde-DeRuyscher *et al.*, 1995).

Numerous YY1-associated complexes appear to be targets for E1A activation (Shi *et al.*, 1991; Gedrich and Engel, 1995; Labrie *et al.*, 995). In fact, a major role of viral E1A may be the activation of genes normally repressed by YY1, including viral genes (Shrivastava and Calame, 1994). SP1 frequently acts as a regulator of YY1-associated complexes (Bennett *et al.*, 1999), particularly during TATA-less promoter complex formation. "Bi-functionality" is evident in the ability of YY1 to simultaneously up-

regulate some genes while down-regulating their antagonists. For example, transcription of the LDL (Low-Density Lipoprotein) receptor gene is inhibited by YY1 during high cholesterol (Bennett *et al.*, 1999), while that of Cholesterol Esterase is enhanced (Gauthier *et al.*, 1999). This was attributed to interactions of YY1 with SP1 in the cholesterol esterase promoter and with SRE-BP in the LDL promoter, inhibiting their function. The ability of YY1 to repress numerous SRE-BP (Serum Response Elemement-Binding Proteins) regulated genes has been associated with the displacement of Factor Y, a positive regulator of gene transcription (Ericsson *et al.*, 1999). Similar "bi-functionality" is evident during proliferation, in which YY1 up-regulates c-*myc* gene transcription, correlating with cellular proliferation, and inhibits muscle actin expression, correlating with differentiation (Lee *et al.*, 1994). However, despite being shown to interact both *in-vitro* and *in-vivo* (Lee *et al.*, 1993; Seto eta l, 1993), both YY1 and SP-1 appear to function independently at the surf-1 promoter, where the YY1 binding site has been shown to be both necessary and sufficient to confer growth-factor inducibility in transcription of the Surf-1 gene (Cole and Gatson, 1997). Activation of transcription by YY1 independent of DNA-binding has been shown for the α-1 acid glycoprotein (AGP) promoter through functional interaction with a negative DNA-binding factor, termed Factor B (Lee and Lee, 1994). In the human GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor) promoter co-factors in addition to YY1 were required for activator function and promoter complex formation (Ye *et al.*, 1994), but in this case binding of YY1 to DNA is required (Ye *et al.*, 1996).

Additionally, YY1 has been suggested to participate in the stimulation of autonomously replicating human chromosome fragments through interaction with a replication-enhancing element, REE1 (Obuse *et al.*, 1998) and in the regulation of transposable elements of the genome (Becker *et al.*, 1993; Satyamoorthy *et al.*, 1993; Singer *et al.*, 1993). These elements are thought to be a major source of functional diversity allowing evolution to continue. Overall, YY1 appears to perform a multitude of tasks, many of which are influenced by its ability to bind to DNA and affect promoter structure and formation, as well as interact with numerous transcriptional enhancers to modify its own activity. The complexity of the regulatory effects of YY1 is highlighted by Bushmeyer *et al* (1995); "YY1 can either activate or repress some

promoters depending on either promoter architecture or intracellular milieu" These unique properties suggest an unusual and complex role for YY1 in the regulation of gene expression

1 7.2 3 YY1 and TATA-less Transcription

YY1 is thought to play a central role in the formation of transcription initiation complexes at TATA-less promoters Promotion of TATA-less transcription by YY1 was initially suggested by the *in-vitro* transcription experiments of Seto *et al* (1991) and Hahn *et al* (1992) YY1 has been shown to bend DNA and is thought to play a role in the formation of promoter structures for RNA pol II binding (Natesan and Gilman, 1995, Kim and Shapiro, 1996) In an *in-vitro* transcription reaction, supercoiled DNA templates could be transcribed in the presence of only YY1, TFIIB and RNA Pol II (Usheva and Shenk, 1994) Overall, YY1 is thought to be a key regulator of TATA-less promoter initiation, probably in all TATA-less promoters (Azizkhan *et al*, 1993) Its ubiquitous expression is in agreement with the findings that many universally expressed housekeeping genes appear to lack any discernible TATA recognition sequence, including the YY1 gene itself (Yao *et al*, 1998) In light of this, a report challenging the concept that TBP-mediated association of TFIID with the TATA-box is limiting in the rate of transcription initiation is of interest (Antoniou *et al*, 1995) Altered transcription was only observed when TBP binding was drastically decreased in the promoter of the β-globin gene However, this promoter also contains an active YY1 binding site, the importance of which may have been overlooked by the authors

The ability of YY1 to interact with TFIIB/D is also thought to be a means by which YY1 regulates TATA-less promoter formation, by-passing the requirement for TBP in these systems Recently $TAF_{II}55$ (TATA-Binding Protein-Associated Factor), a subunit of TFIID, has been shown to interact directly with the largest subunit, $TAF_{II}230$ through its central region and with multiple activators – including SP1, YY1 and Adenoviral E1A – through a distinct amino-terminal domain (Chiang *et al*, 1995) This subunit may form the "bridge" between transcriptional enhancers and the actual transcriptional components surrounding RNA polymerase II It is possible that

YY1 is part of, or is actually the "bridging unit", particularly in TATA-less promoters (since TAF55 is a basal unit, while YY1 appears to be "in-limbo" between enhancer and basal transcription factor, depending upon the promoter) The effects of SP1 on YY1-mediated transcription initiation, particularly from TATA-less promoters, may reside in its interaction with $TAF_{II}55/230$ to guide the initiation complex towards the Inr-associated YY1 to begin initiation

Further evidence that YY1 plays a role transcription through TATA-less promoters has been provided by Gatson and Fried (1994), Cole and Gaston (1997), Johansson *et al* (1998) and Karantzoulis *et al* (1999) In addition, YY1 is thought to play a role in the downstream regulation of transcription (Last *et al*, 1999) The majority of known transcriptional enhancers are upstream, since they would interfere with the actual transit of the RNA polymerase II if situated downstream, while YY1 appears to interact with many of the basal factors and may form part of the basal RNA holoenzyme in some circumstances

1 7 2 4 YY1 in Differentiation and Development

The unusual nature of the YY1 protein has led to speculation that it may play a key role in the regulation of differentiation and development Both Chromatin structure and methylation are thought to be key mechanisms by which cells control specific gene transcription during differentiation The Nuclear Matrix Protein-1 (NMP-1), a transcription factor which has been shown to associate with the nuclear matrix to mediate gene-matrix interactions within the nucleus, has been shown to be none other than YY1 (Guo *et al*, 1995) Sequences necessary for nuclear localisation and association with the nuclear matrix have been identified in the C-terminal region of the YY1 peptide (Bushmeyer and Atchison, 1998, McNeil *et al*, 1998) Nuclear-matrix-associated transcription factors may affect gene regulation by mediating transient associations between DNA and the nuclear matrix, locally unravelling chromatin structure to allow the transcriptional machinery to access promoters and begin transcription, implying roles for YY1 in activating repressed genes during development

Binding of YY1 to DNA during globin promoter formation is known to be methylation-sensitive (Satyamoorthy *et al*, 1993, Yost *et al*, 1993), which may imply a role for YY1 in tissue- and developmental-specific transcription of genes A YY1 binding site is thought to function in the stage-specific expression of the fetal (gamma) globin gene (Zhu *et al*, 1999) The human ε-globin gene is transcribed in erythroid cells only during the embryonic stages of development A binding site for YY1, around nucleotide –269, was identified as critical in the formation of the ε-globin repressor complex (Raich *et al*, 1995), forming part of the local regulatory elements suggested to be involved in the regulation of embryonic stage-specific expression of this gene Processes such as these, resulting in the stage-specific switches in gene expression, are thought to be associated with methylation of CpG islands, which silence transcription of developmentally important genes and to which YY1 binding is sensitive

In addition, levels of YY1 have been shown to decrease during differentiation of mouse myoblasts (Lee *et al*, 1992) YY1 contains several peptide regions prone to proteolytic cleavage, raising the possibility that protease-mediated degradation events may contribute to diminished YY1 protein levels during myogenesis (Lee *et al*, 1994) Two proteolytic pathways through which YY1 can be differentially targeted under different cell growth conditions have been identified (Walowitz *et al*, 1998), identifying a role, at least partially, for protease calpain II (m-calpain) However, in serum starvation studies YY1 protein expression was lost only after 24 hours, despite the fact that YY1 transcript expression was lost within hours (Flanagan, 1995), suggesting that the YY1 protein is relatively stable This does not exclude the possibility that proteolytic regulation of YY1 levels may play a role in different processes

Treatment of myoblasts with the differentiation modulating agent, BrdU results in inhibition of myogenesis, resulting in/from an increase in expression of YY1 and decreased α-actin levels (Lee *et al*, 1992) Transfection of SRF (Serum Response Factor), which competes with YY1 for the regulation of α-actin gene transcription, could directly transactivate the actin promoter in BrdU-treated myoblasts Both SRF and YY1 are ubiquitously expressed, suggesting that they may have antagonistic

functions in regulating genes such as *c-fos*, α-actin and cardiac creatine kinase-M (Vincent *et al*, 1993, Liu *et al*, 1995) during development High levels of YY1 in non-differentiated muscle cells down-regulate the dystrophin promoter, at least in part, by interfering with the spatial organisation of the promoter (Galvagni *et al*, 1998) YY1 and a positive regulator of dystrophin, DPBF (dystrophin promoter bending factor), induce opposite bends in the CArG element of this promoter, suggesting that their binding induces alternative promoter structures to regulate muscle development

1.8 eukaryotic Translation Initiation Factor, eIF-4E

eIF4E, otherwise known as eIF4α or the small cap binding protein, binds directly to the 5' 7-Methyl-Gppp cap in an ATP-dependent manner, and is thought to be the first factor to interact with the mRNA to initiate translation eIF-4E is a 25 kDa phospho-protein responsible for Cap-binding specificity in eIF-4F complexes during eukaryotic translation initiation events eIF-4E consists of a single αβ domain which contains 8 anti-parallel β strands forming a curved β sheet (Sonenberg and Gingras, 1998) This sheet is backed by three long α-helices The mRNA cap-structure binds loosely to an hydrophobic pocket in the concave inner surface of eIF-4E, across which salt-bridges form after phosphorylation to "lock" the cap in place (Marcotrigiano *et al*, 1998, CSHL abstracts), while the convex dorsal surface interacts in a mutually exclusive manner with either eIF-4G or the 4E-BPs Phosphorylation of eIF-4E occurs as part of the eIF-4F complex (Tauzon *et al*, 1990) greatly enhancing and stabilising its association with the cap (Minich *et al*, 1994, Joshi *et al*, 1995)

eIF-4E is widely accepted as the limiting factor in translation initiation, particularly for mRNAs with complex 5' UTRs It is present in molar levels significantly lower than that of other initiation factors (DeBenedetti and Rhoads, 1990, Sonenberg, 1996) It is the most specifically targeted mRNA-binding eIF and is an essential component of the cytoplasmic cap-binding complex The cap-binding activity of the eIF-4E

peptide is thought to reside in a highly evolutionarily conserved placement of tryptophan residues in both yeast and mammals (Altmann *et al*, 1988) This factor therefore plays a critical role in the regulation of translation, particularly of specific mRNA species, and the levels and activity of eIF-4E are critical to the control of cellular proliferation and differentiation (Jaramillo *et al*, 1991) A rather novel and as-yet to be proven additional function for eIF-4E has been suggested, namely that it may play some part in the transport of mRNAs from the nucleus The 5' Cap-structure is known to be involved in the process of nucleocytoplasmic transport (Sonenberg and Gingras, 1998), already thought to be the function of the novel eIF-4E homologue protein, eIF-4EHP (Rome *et al*, 1998) In light of the Cap-binding specificity of eIF-4E and recent findings of localisation of a fraction of eIF-4E to the nucleus (Pollard *et al*, 1999), this additional role for eIF-4E is not implausible

Frequently mammalian cells express at least two forms of this factor (Jaramillo *et al*, 1991, Haghighat *et al*, 1995) The gene(s) for eIF-4E is thought to lie on chromosome 4 in humans (Gao *et al*, 1998) Gao *et al* (1998) isolated two genes for eIF-4E from placental genomic libraries, in which case eIF-4E1 contained six introns but the other (eIF-4E2) was intronless Subtle differences between the two genes were identified and both genes were reported to be differentially expressed in four human cell lines A notable difference between the two genes was that the eIF-4E1 promoter contained c-myc-binding elements while that of eIF-4E2 did not, suggesting constitutive expression of the latter and inducible expression of the former In fact, eIF-4E has been identified as one of the few targets for c-Myc induction (Rosenwald *et al*, 1993, Jones *et al*, 1996) The complexity of eIF-4E expression patterns in eukaryotic cells was highlighted by the findings that in Drosophila a single eIF-4E gene could code for three alternatively spliced mRNA transcripts, two of which resulted in expression of the same form of eIF-4E, while the other encoded an isoform differing at the amino-terminal sequence of the protein (Lavoie *et al*, 1996) The three eIF-4E transcripts varied greatly in the lengths of their respective 5' UTRs, suggesting that each was subject to varying degrees of translational regulation themselves This may reflect a means of auto-regulating levels of eIF-4E expression during phases of hyper- and hypo-proliferation of cells

Following on from BrdU work that has previously been performed in the laboratory (McBride S , *et al* , 1999, P Meleady, PhD Thesis, 1997, F O'Sullivan, PhD Thesis, 1999, D Walsh, PhD Thesis 1999, P Doolan, PhD Thesis, 2001) it was decided to investigate the ability of other halogenated thymidine analogues to induce differentiation in DLKP and A549 cells In this study it was decided to utilise the expression of α_2-, β_1-integrin, EpCAM, cytokeratins 8, 18 and 19 as markers of differentiation in the two cell lines The thymidine analogues initially chosen for this work were 5-Iodo-2 -deoxyUridine, 5-Chloro-2 -deoxyUridine, 5-FluroUracil, 5-Fluro-2 -deoxyUridine, 5-Fluro-5 -deoxyUridine, 5-BromoUridine and 5-BromoUracil

Exposure of the both cell lines to BrdU and the other halogenated thymidine analogues investigated also resulted in a morphological change in the treated cells These changes included a flattening and stretching of the cells, with the cells doubling or quadrupling in size, following treatment Cells treated with 5,2 -FdU exhibited the greatest alteration in morphology and the greatest increase in cell size

1 9 Aims of Thesis

- Previous research performed in this laboratory has demonstrated that the halogenated thymidine analogue, Bromodeoxyuridine, induces the *in vitro* differentiation of the lung cell lines DLKP and A549 This differentiation is indicated by the induction of cytokeratins 8, 18 and 19 (McBride, *et al* , 1999, Meleady and Clynes 2001a, Meleady and Clynes, 2001b, O'Sullivan, PhD Thesis, 1999) Also shown to be induced by BrdU are the integrins α_2 and β_1 (Meleady, PhD Thesis, 1997) and Ep-CAM (O'Sullivan, PhD Thesis, 1999)

- The thymidine analogues BrdU has been shown in this laboratory to induce the expression of cytokeratin and integrin proteins The ability of other halogenated pyrimidine analogues, IdU, CdU, 5,5 -FdU, 5,2 -FdU, 5-FU, BromoUracil and Bromouridine, to alter expression of these proteins was also to be investigated It was hoped that such investigation would help us gain a better understanding of the mechanisms by which differentiation is regulated in our *in vitro* model system

- In order to investigate the mechanisms involved in lung cell differentiation in our model system initial work examined the effect of the pyrimidine analogues had on the cytokeratin and integrin proteins in DLKP and A549 cell lines This was principally performed by immunocytochemistry

- To investigate the global transcriptional changes induced in the DLKP cell line following exposure to the pyrimidine analogues, DNA microarray experiments were employed to help to elucidate genes which may be common to the pathway(s) regulating differentiation in our cell system The use of such techniques may yield helpful leads to help us understand the overall processes of differentiation involved

- Work performed by Walsh (PhD Thesis, 1999) in our laboratory suggested that the transcription factors c-*myc* and Yin Yang 1 may be key proteins

involved in the control of differentiation which is induced by BrdU in DLKP cells cDNAs coding for these two proteins were transfected into DLKP and a clonal subpopulation DLKP-SQ, to asses their ability to induce simple differentiation in this poorly differentiated cell line It was hoped that compiling results from BrdU-treated cells and transfections would allow us to develop a model for the regulation of K8 and K18 synthesis in our lung cell line models, with possible implications for understanding the early stages of lung development as well as aspects of de-differentiation in lung cancer Such models are severely lacking in lung biology

Section 2.0 Materials & Methods

2.1 WATER

Ultrapure water was used in the preparation of all media and solutions. This water was purified by a reverse osmosis system (Millipore Milli-RO 10 Plus, Elgastat UHP) to a standard of 12 - 18 MΩ/cm resistance.

2.2 GLASSWARE

Solutions pertaining to cell culture and maintenance were prepared and stored in sterile glass bottles. Bottles (and lids) and 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) for at least 1 hour. Following scrubbing and several rinses 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 ultrapure water and sterilised by autoclaving.

2.3 STERILISATION

Water, glassware and all thermostable solutions were sterilised by autoclaving at 121^{0}C for 20 minutes (min) under pressure of 1bar. Thermolabile solutions were filtered through a 0.22µm sterile filter (Millipore, millex-gv, SLGV-025BS). Low protein-binding filters were used for all protein-containing solutions.

2.4 MEDIA PREPARATION

Medium was routinely prepared and sterility checked by Joe Carey. The basal media used during routine cell culture were prepared according to the formulations shown in Table 2.4.1. 10x media were added to sterile ultrapure water, buffered with HEPES and $NaHCO_3$ and adjusted to a pH of 7.45 - 7.55 using sterile 1.5M NaOH and 1.5M HCl. The media were then filtered through sterile 0.22µm bell filters (Gelman, 121-58) and

stored in 500ml sterile bottles at 4^0C Sterility checks were carried out on each 500ml bottle of medium as described in Section 2 5 6

The basal media were stored at 4^0C up to their expiry dates as specified on each individual 10x medium container Prior to use, 100ml aliquots of basal media were supplemented with 2mM L-glutamine (Gibco, 25030-024) and 6% foetal calf serum (Sigma, F-7524 Batch) and this was used as routine culture medium This was stored for up to 2 weeks at 4^0C, after which time, fresh culture medium was prepared

Table 2 4 1 Preparation of basal media

	DMEM (Gibco, 12501-029)	**Hams F12** (Gibco, 21700-109)
10X Medium	500ml	Powder
Ultrapure H_20	4300ml	4700ml
1M HEPES* Sigma , H-9136	100ml	100ml
7 5% $NaHCO_3$ BDH, 30151	45ml	45ml

* HEPES = N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)

For most cell lines, ATCC (Ham's F12/ DMEM (1 1)) supplemented with 6% FCS, 1% Sodium Pyruvate and 2mM L-glutamine was routinely used

2 5 CELL LINES

All cell culture work was carried out in a class II down-flow re-circulating laminar flow cabinet (Nuaire Biological Cabinet) and any work which involved toxic compounds was carried out in a cytoguard (Gelman) Strict aseptic techniques were adhered to at all times The laminar flow cabinet was swabbed with 70% industrial methylated spirits (IMS) before and after use, as were all items used in the cabinet Each cell line was assigned specific media and waste bottles Only one cell line was worked with at a time in the cabinet which was allowed to clear for 15min between different cell lines The cabinet itself was cleaned each week with industrial detergents (Virkon, Antec International, TEGO, TH Goldschmidt Ltd), as were the incubators The cell lines used during the course of this study, their sources and their basal media requirements are listed in Table 2 5 1 Lines were maintained in 25cm^2 flasks (Costar, 3050), 75cm^2 (Costar, 3075) or 175cm^2 flasks (Corning, 431079) at 37^{0}C and fed every two to three days

2.5.1 Subculture of Adherent Lines

During routine subculturing or harvesting of adherent lines, cells were removed from their flasks by enzymatic detachment

Waste medium was removed from the flasks and rinsed with a pre-warmed (37^{0}C) trypsin/EDTA (TV) solution (0 25% trypsin (Gibco, 25090-028), 0 01% EDTA (Sigma, EDS) solution in PBS A (Oxoid, BR14a)) The purpose of this was to remove any naturally occurring trypsin inhibitor which would be present in residual serum Fresh TV was then placed on the cells (2ml/25cm^2 flask or 4ml/75cm^2 flask) and the flasks incubated at 37^{0}C until the cells were seen to have detached (5-10 min) The trypsin was deactivated by addition of a equal volume of growth medium (*i e* containing 6% serum) The entire solution was transferred to a 30ml sterile universal tube (Sterilin, 128a) and centrifuged at 1,000 rpm for 5 mm The resulting cell pellet was resuspended in pre-warmed (37^{0}C) fresh growth medium, counted (Section 2 5 3) and used to re-seed a flask at the required cell density or to set up an assay

Table 2 5.1 Cell lines used during the course of this study

Cell line	Basal medium	Cell type	Source
DLKP (and subpopulation SQ)	ATCC[2]	Human poorly-differentiated lung carcinoma	Dr Geraldine Grant, NCTCC
A549	ATCC[2]	Human lung adenocarcinoma	ATCC[2]

* These cells grow in suspension

[1] ATCC = American Type Culture Collection

2 5 3 Cell Counting

Cell counting and viability determinations were carried out using a trypan blue (Gibco, 15250-012) dye exclusion technique

An aliquot of trypan blue was added to a sample from a single cell suspension in a ratio of 1 5 After 3 min incubation at room temperature, a sample of this mixture was applied to the chamber of a haemocytometer over which a glass coverslip had been placed Cells in the 16 squares of the four outer corner grids of the chamber were counted microscopically, an average per corner grid was calculated with the dilution factor being taken into account, and final cell numbers were multiplied by 10^4 to determine the number of cells per ml The volume occupied by the chamber is 0 1cm x 0 1cm x 0 01cm *i e* 0 0001cm^3 Therefore cell number x 10^4 is equivalent to cells per ml Non-viable cells were those that stained blue while viable cells excluded the trypan blue dye and remained unstained

2 5 4 Cell Freezing

To allow long-term storage of cell stocks, cells were frozen and cryo-preserved in liquid nitrogen at temperatures below -180^{0}C Once frozen properly, such stocks should last indefinitely

Cells to be frozen were harvested in the log phase of growth (*i e* actively growing and approximately 50 - 70% confluent) and counted as described in Sections 2 5 3 Pelleted cells were re-suspended in serum An equal volume of a DMSO/serum (1 9, v/v) was slowly added dropwise to the cell suspension to give a final concentration of at least $5x10^6$ cells/ml This step was very important as DMSO is toxic to cells When added slowly the cells had a period of time to adapt to the presence of the DMSO, otherwise cells may have lysed The suspension was then aliquoted into cryovials (Greiner, 122 278) which were then quickly placed in the vapour phase of liquid nitrogen containers (approximately -80^0C) After 2 5 to 3 5 hours, the cryovials were lowered down into the liquid nitrogen where they were stored until required

2 5.5 Cell Thawing

Immediately prior to the removal of a cryovial from the liquid nitrogen stores for thawing, a sterile universal tube containing growth medium was prepared for the rapid transfer and dilution of thawed cells to reduce their exposure time to the DMSO freezing solution which is toxic at room temperature The suspension was centrifuged at 1,000 rpm for 5 min, the DMSO-containing supernatant removed and the pellet re-suspended in fresh growth medium A viability count was carried out (Section 2 5 3) to determine the efficacy of the freezing/ thawing procedures Thawed cells were placed into tissue culture flasks with the appropriate volume of medium (5ml/25cm^2 flask and 10ml/75cm^2 flask) and allowed to attach overnight After 24 hours, the cells were re-fed with fresh medium to remove any residual traces of DMSO

2.5 6 Sterility Checks

Sterility checks were routinely carried out on all media, supplements and trypsin used for cell culture Samples of basal media were inoculated into Columbia (Oxoid, CM331) blood agar plates, Sabauraud (Oxoid, CM217) dextrose and Thioglycollate (Oxoid, CM173) broths which detect most contaminants including bacteria, fungus and yeast Growth media (*i e* supplemented with serum and L-glutamine) were sterility checked at least 2 days prior to use by incubating samples at 37^0C, which were subsequently examined for turbidity and other indications of contamination

2.6 *MYCOPLASMA* ANALYSIS

Mycoplasma examinations were carried out routinely (at least every 3 months) on all cell lines used in this study These analyses were performed by Aine Adams and Michael Henry

2 6 1 Indirect Staining Procedure

In this procedure, *Mycoplasma*-negative NRK cells (a normal rat kidney fibroblast line) were used as indicator cells These cells were incubated with supernatant from test cell lines and then examined for *Mycoplasma* contamination NRK cells were used for this procedure because cell integrity is well maintained during fixation A fluorescent Hoechst stain was utilised which binds specifically to DNA and so will stain the nucleus of the cell in addition to any *Mycoplasma* DNA present A *Mycoplasma* infection would thus be seen as small fluorescent bodies in the cytoplasm of the NRK cells and sometimes outside the cells

NRK cells were seeded onto sterile coverslips in sterile Petri dishes at a cell density of $2x10^3$ cells per ml and allowed to attach over night at 37^0C in a 5% CO_2, humidified incubator 1ml of cell-free (cleared by centrifugation at 1,000 rpm for 5 mm) supernatant from each test cell line was then inoculated onto a NRK Petri dish and incubated as before until the cells reached 20 - 50% confluency (4 - 5 days) After this time, the waste medium was removed from the Petri dishes, the coverslips washed twice with sterile PBS A, once with a cold PBS/Carnoys (50/50) solution and fixed with 2ml of Carnoys solution (acetic acid methanol-1 3) for 10 min The fixative was then removed and after air drying, the coverslips were washed twice in deionised water and stained with 2ml of Hoechst 33258 stain (BDH)(50ng/ml) for 10 min

From this point on, work was carried out in the dark to limit quenching of the fluorescent stain

The coverslips were rinsed three times in PBS They were then mounted in 50% (v/v) glycerol in 0 05M citric acid and 0 1M disodium phosphate and examined using a

fluorescent microscope with a UV filter

2.6.2 Direct Staining

The direct stain for *Mycoplasma* involved a culture method where test samples were inoculated onto an enriched *Mycoplasma* culture broth (Oxoid, CM403) - supplemented with 16% serum, 0 002% DNA (BDH, 42026), 2mg/ml fungizone (Gibco,15290-026), $2x10^3$ units penicillin (Sigma, Pen-3) and 10ml of a 25% (w/v) yeast extract solution - to optimise growth of any contaminants and incubated at 37^0C for 48 hours Samples of this broth were then streaked onto plates of *Mycoplasma* agar base (Oxoid, CM401) which had also been supplemented as above and the plates incubated for 3 weeks at 37^0C in a CO_2 environment The plates were viewed microscopically at least every 7 days, the appearance of small, "fried egg" -shaped colonies is indicative of a Mycoplasma infection

2 7 DIFFERENTIATION STUDIES

Differentiation studies were carried out using 5-bromodeoxyuridine (BrdU) (Sigma, B5002), 5-Chloro, 2-deoxyuridine (Sigma, C6891) and 5-Iodo, 2-deoxyuridine (Simga, I7125), 5,2 -FdU (Sigma, B5002), 5,5 -FdU (Sigma,F8791), 5-FU (Sigma, F8423), 5-bromouracil (Sigma, 852473) and 5-bromouridine (Sigma, B9752) For each of the analogues, the powder was reconstituted in UHP water to a stock concentration of 10mM and the resultant solution was filter sterilised through a sterile 0 22μm filter, aliquoted into sterile Eppendorfs and stored at -20^{0}C for up to 1 year

2.7.1 Differentiation Assays

For immunocytochemical analysis (Section 2 8), cells were plated onto 6-well plates (Costar, 3516) at densities of 1x10^4 cells per well 1 ml of medium was sufficient for each well The cells were allowed to attach and form colonies by incubating at 37^{0}C, 5% CO_2 for 24 hours The plates were covered with parafilm to prevent contamination After this time the media was replaced with fresh medium containing 10μM the appropriate thymidine Plates were wrapped in parafilm and incubated for up to 7 days Medium was replaced every 3-4 days over the course of the assay All waste medium was retained for disposal by incineration At the end of the assay the cells were fixed with methanol as described in Section 2 8 1 Immunocytochemistry/fluorescence was then carried out using a range of antibodies as described in Section 2 8 2

For additional analytical techniques (Western blotting, immunoprecipitation, iso-electric focusing, PCR and DNA Microarrays), cells were inoculated into 75cm^2 flasks at a density of 1x10^5 cells per flask or into 175 cm^2 flasks at a density of 5x10^5 cells per flask, and were incubated for two days at 37^{0}C Analogue containing medium, at a concentration of 10μM, was then added to the cells The medium was replaced with fresh, drug-containing media every 3-4 days The cells were then harvested by trypsinisation, washed in sterile PBS A, counted, pelleted and stored at -80^{0}C until required For RNA extraction (section 2 14), pellets were lysed in tri-reagent and stored at -80^{0}C

2 8 IMMUNOCYTOCHEMISTRY

2 8 1 Fixation of cells

For fixation, medium was removed from 6-wells plates, cells were rinsed 3 times with PBS A and then incubated at -20^{0}C for 7 minutes using ice-cold methanol The methanol was then removed from the cells, which were allowed to dry at 37^{0}C for a few minutes and then stored at -20^{0}C until required

2 8 2 Immunocytochemical procedure

The avidin-biotin-peroxidase complex (ABC) immunoperoxidase technique combined with the diaminobenzidine (DAB) visualisation procedure was employed to indicate primary antibody binding The ABC method involves application of a biotin-labelled secondary antibody, followed by the addition of avidin-biotin-peroxidase complex which results in a high staining intensity due to the formation of an avidin-biotin lattice which contains several peroxidase molecules The peroxidase enzyme reacts with DAB solution to give an insoluble, brown-colour precipitate Therefore, observation of a brown precipitate following this procedure is indicative of primary antibody reactivity

The procedure used is as follows

Cell preparations (6-well tissue culture plates) which had been previously fixed in methanol and frozen at -20^{0}C were allowed to thaw and equilibrate at room temperature A grease pen (DAKO, S2002) was used to encircle cells in tissue culture plates to retain the various solutions involved The cells were equilibrated in Tris-buffered saline (TBS) (0 05M Tris/HCl, 0 15M NaCl, pH 7 6) for 5 minutes The slides were then incubated for 20 minutes at room temperature (RT) with either normal rabbit (DAKO, X092) or goat (DAKO, X0907) serum diluted 1 5 in TBS to block non-specific binding, depending upon the host source of the primary antibody in question This was then removed and 25-30µl of optimally diluted primary antibody (Table 2 8 1) was placed on the cells The slides and tissue-culture plates were placed on a tray containing moistened tissue paper and incubated at 37^{0}C for 2 hours or 4^{0}C

overnight The primary antibodies used in the study are listed in Table 2 8 1 The slides were then rinsed in TBS/ 0 1% Tween (Sigma, P-1379) for 5min x3 times, and then incubated for 30 min with a suitable biotinylated secondary antibody (rabbit anti-mouse immunoglobulins (DAKO, E354), goat anti-rabbit (DAKO, E0432) diluted 1 300 in TBS The slides were rinsed as before and incubated with strepABComplex/Horse Radish Peroxidase (HRP) (DAKO, K377) for 30 mm at RT, after which they were rinsed again in TBS/ 0 1% Tween for 5min x3 times The cells were then incubated with a DAB solution (DAKO, S3000) for 10-15 min The plates were then rinsed off with UHP water and counterstained with 2% methyl green solution, and samples mounted using a commercial mounting solution (DAKO, S3023)

2 8.3 Immunofluorescence

Immunofluorescence was performed using a similar approach to that described in 2 8 above Cell preparations (6-well tissue culture plates) which had been previously fixed in methanol and frozen at -20^{0}C were allowed to thaw and equilibrate at room temperature A grease pen (DAKO, S2002) was used to encircle cells in tissue culture plates to contain the various solutions involved The cells were equilibrated in Tris-buffered saline (TBS) (0 05M Tris/HCl, 0 15M NaCl, pH 7 6) for 5 minutes The slides were then incubated for 20 minutes at room temperature (RT) with normal rabbit/goat serum (DAKO, X092/Dako, X0907) (depending upon the primary in question) diluted 1 5 in TBS to block non-specific binding This was then removed and 25-30µl of optimally-diluted primary antibody was placed on the cells and incubated on a tray containing moistened tissue paper at 4^{0}C overnight The following day the slides were then rinsed in TBS/ 0 1% Tween (Sigma, P-1379) for 5min x3 times All subsequent manipulations were performed in a darkened room, and incubations were performed in trays covered in tinfoil as a precaution to minimise "quenching" of fluorescence by exposure to light for extended periods Cells were incubated for 60 min with FITC-labelled goat anti-rabbit immunoglobulin (Sigma, F-6005) diluted 1 160 in TBS/0 1%Tween The slides were then rinsed in TBS/ 0 1% Tween (Sigma, P-1379), x3 in 15 min , air-dried and mounted in fluorescent mounting medium (DAKO, S3023) Antibody reactivity was determined by examination under a fluorescent microscope

Antibody	Dilution/ Concentration	Supplier	Catalogue no
Cytokeratin 8 (M)	1/200	Sigma	C-5301
Cytokeratin 18 (M)	1/800	Sigma	C-8541
Cytokeratin 19 (M)	1/50	Sigma	C5301
eIF-4E (M)	1/250	Transduction Laboratories	E27620
323/A3 (Anti-EpCAM)*	1/150	NeoMarkers	MS-181-P1
Ep-CAM Ab1 (VU-1D9)*	1/150	NeoMarkers	MS-144-P1
β_1-integrin	1/100	Serotech	MCA1188
α_2-integrin	1/250	Serotech	MCA1186

Nomenclature (M) = Mouse-anti-human IgG

(R) = Rabbit-anti- human IgG

* These antibodies were used in combination to improve sensitivity

Table 2 8 1 Primary antibodies used for immunocytochemistry/Immunofluorescence

2 9 WESTERN BLOT ANALYSIS

Proteins for western blot analysis were separated by SDS-polyacylamide gel electrophoresis (SDS-PAGE)

2.9 1 Sample preparation

Cell pellets (Section 2 7 1) were lysed in TG lysis buffer (20mM Tris-HCl pH 8, 10% glycerol, 1% TritonX-100, 1 5mM $MgCl_2$, 2mM EDTA, 137mM NaCl, 1mM Na_3VO_4, 1mM Pefabloc (Boehringer, 84500920-22), and 1X Protease inhibitor cocktail (Boehringer, 1697498) for 20 min on ice The extracts were either used immediately for western blot analysis or snap frozen in liquid nitrogen and stored at -80^0C Alternatively, cells were lysed by resuspension in boiling loading buffer (2 5ml

1 25M-Tris/HCl, 1 0g SDS, 5 8ml glycerol and 0 1% bromophenol blue (Sigma, B8026) made up to 25ml with distilled water) and incubated at 100^0C for 2-3 min , cooled to room temperature and used immediately for western blot analysis

2 9.2 Gel electrophoresis

Resolving and stacking gels were prepared as outlined in Table 2 9 1 and poured into clean 10cm x 8cm gel cassettes which consisted of 1 glass and 1 aluminium plate, separated by 0 75cm plastic spacers The resolving gel was poured first and allowed to set The stacking gel was then poured and a comb was placed into the stacking gel in order to create wells for sample loading Once set, the gels could be used immediately or wrapped in aluminium foil and stored at 4^0C for 24 hours

Before samples were loaded onto the stacking gels, equal cell numbers ($2x10^4$ cells per lane) were lysed in 2x loading buffer (Section 2 9 1) The samples were then loaded alongside molecular weight colour protein markers (Sigma, C-3437) The gels were run at 250V, 45mA for approximately 1 5 hours (until the protein was run at least half way into the gel as judged by the migration of colour markers during the electrophoretic process All gels were made from a stock of Acrylamide (details below) Sample calculations for two different percentage gels are shown in table 2 9 1

Components	Resolving gel (7 5%)	Resolving gel (12%)	Stacking gel
Acrylamide stock*	3 75ml	6ml	0 8ml
Ultrapure water	8 0ml	5 75ml	3 6ml
1 875M-Tris/HCl, pH 8 8	3 0ml	3 0ml	-
1 25M-Tris/HCl, pH 6 8	-	-	0 5ml
10% SDS (Sigma, L-4509)	150µl	150µl	50µl
10% APS (Sigma, A-1433)	60µl	60µl	17µl
TEMED (Sigma, T-8133)	10µl	10µl	6µl

*Acrylamide stock – Sigma Cat No - 148660

Table 2 9.1 Preparation of electrophoresis gels

2.9.3 Western blotting

Following electrophoresis, the acrylamide gels were equilibrated in transfer buffer (25mM Tris, 192mM glycine (Sigma, G-7126) pH 8.3-8.5 without adjusting) for 20 min. Proteins in gels were transferred onto Hybond ECL nitrocellulose (Amersham, RPN 2020D) or PVDF (Polyvinyl diflouride) (Boehringer, 1722026) membranes by semi-dry electroblotting. Six sheets of Whatman 3mm filter paper (Whatman, 1001824) were soaked in transfer buffer and placed on the cathode plate of a semi-dry blotting apparatus. Excess air was removed from between the filters by moving a glass pipette over the filter paper. Nitrocellulose or PVDF (pre-activated in methanol for 1-2 min. and washed in UHP for 5 min), cut to the same size of the gel, was soaked in transfer buffer and placed over the filter paper, making sure there were no air bubbles. The acrylamide gel was placed over the nitrocellulose and six more sheets of presoaked filter paper were placed on top of the gel. Excess air was again removed by rolling the pipette over the filter paper. The proteins were transferred from the gel to the nitrocellulose/PVDF at a current of 0.34mA at 15V for 20-30 min. depending upon the size of the protein.

1. Anode buffer 1: (4 sheets of filter paper; squeeze dry)
   33.35g Tris, 200ml Methanol in 1 L.
2. Anode buffer 2: (2 sheets of filter paper; squeeze dry)
   3.03g Tris, 200ml Methanol in 1 L.
3. PVDF membrane (pre-activated in methanol as before).
4. Polyacrylamide gel.
5. Cathode Buffer: (4 sheets of filter paper; squeeze dry)
   3.03g Tris, 5.25g 6-amino-n-hexanoic acid (Sigma, A-2504), 200ml Methanol in 1 L.

All incubation steps from now on, including the blocking step, were carried out on a revolving apparatus to ensure even exposure of the membrane blot to all reagents.

The nitrocellulose/PVDF membranes were blocked for 2 hours at room temperature with fresh filtered 5% non-fat dried milk (Cadburys; Marvel skimmed milk) in TBS/ 0.1% Tween, pH 7.4.

After blocking, the membranes were rinsed with TBS/0 1% Tween and incubated with primary antibody overnight at 4^0C Primary antibodies used are listed in table 2 9 2 The following day the primary antibody was removed and the membranes rinsed 3 times with TBS/ 0 1% Tween The membranes were incubated in 1/1000 dilution of a suitable HRP-labelled secondary antibody (Mouse, Sigma, A-6782 or Rabbit, Sigma, A-4914) in TBS/0 1% Tween for 1 hour at room temperature (R T) The secondary was then removed and blots were washed for 15 min in TBS/0 1%Tween Bound antibody was detected using enhanced chemiluminescence (ECL) (Section 2 9 4)

Antibody	Dilution/ Concentration	Supplier	Catalogue no.
Cytokeratin 8 (M)	1/400	Sigma	C-5301
Cytokeratin 18 (M)	1/800	Sigma	C-8541
Cytokeratin 19 (M)	1/50	Sigma	C5301
eIF-4E (M)	1/500	Transduction Laboratories	E27620
c-myc (M)	1/500	Santa Cruz	SC-040
YY1 (R)	1/250	Santa Cruz	SC-281
β_1-integrin	1/250	Chemicon	AB1937
Mad1	1/250	Santa Cruz	SC-222

Nomenclature (M) = Mouse anti-human IgG

(R) = Rabbit anti-human IgG

Table 2 9 2 Antibodies used for western blot analysis

2 9.4 Enhanced chemiluminescence detection

Protein bands were developed using the Enhanced Chemiluminescence Kit (ECL) (Amersham, RPN2109) according to the manufacturer's instructions

After blots were washed in TBS/0 1% Tween x3 times for 5 mm, a sheet of parafilm was flattened over a smooth surface, *e g* a glass plate, making sure all air bubbles were removed The membrane was then placed on the parafilm, and excess fluid removed 1 5ml of ECL detection reagent 1 and 1 5ml of reagent 2 were mixed and covered over

the membrane Charges on the parafilm ensured the fluid stayed on the membrane The reagent was removed after one minute and the membrane wrapped in cling film The membrane was exposed to autoradiographic film (Kodak, X-OMAT S, 500 9907) in an autoradiographic cassette for various times, depending upon the strength of the signal obtained The autoradiographic film was then developed The exposed film was developed for 5min in developer (Kodak, LX24), diluted 1 6 5 in water The film was briefly immersed in water and transferred to a Fixer solution (Kodak, FX-40) diluted 1 5 in water, for 5min The film was transferred to water for 5 min and then air-dried

2 10 RNA EXTRACTION

RNA was extracted from cells as follows

Cells were trypsinised, washed once with PBS A and the sample was counted Approximately 10^8 cells were pelleted and lysed using 1ml of TRI REAGENT™ (Sigma, T-9424) The samples were allowed to stand for 5 min at RT to allow complete dissociation of nucleoprotein complexes and then snap-frozen in liq N_2 and stored at -80^0C

When thawed, samples were allowed to stand for 5 min before 0 2ml of chlorofom was added per ml of TRI REAGENT™ used Samples were then shaken vigorously for 15 sec and allowed to stand for 15 min at RT Samples were then centrifuged at 13000rpm in a microfuge for 15 min at 4^0C This step separated the mixture into 3 phases, the RNA was contained in the colourless upper aqueous layer This layer was then transferred to a fresh Eppendorf and 0 5ml of isopropanol was added The sample was mixed and allowed to stand at RT for 10 min before being centrifuged at 13000rpm in a microfuge for 10 min at 4^0C The RNA formed a precipitate at the bottom of the tube The supernatant was removed and the pellet was washed with 1ml of 75% ethanol and centrifuged at 4^0C for 5-10 mm at 8500rpm The supernatant was removed and the pellet was briefly allowed to air-dry 20-30µl of DEPC-treated water was then added to the RNA to resuspend the pellet

Concentrations of RNA in samples were calculated by determining OD at 260nm and 280nm and using the following formula -

OD_{260nm} x Dilution factor x 40 = µg/µl RNA

The purity of the RNA extraction was calculated by determining its OD at 260nm and 280nm An A_{260nm} A_{280nm} ratio of 2 is indicative of pure RNA Only those samples with ratios between 1 7 and 2 1 were used

2 10.1 Qiagen Kit RNA Isolation

Alternatively, high quality RNA was isolated from cells using the Rneasy mini kit (Qiagen, 74104) The Rneasy extraction is based on guanidine thiocyanate method of extraction The procedure was performed according to the manufacturer's instructions

2.11 REVERSE TRANSCRIPTASE REACTION

Reverse transcriptase (RT) reactions were carried out in laminar flow cabinets using micropipettes which were specifically allocated to this work

cDNA was formed using the following procedure -

- 1µl oligo $(dT)^{12-18}$ primers (1µg/µl) (Promega, C1101)
- 1µl total RNA (1µg/µl) (section 2 14)
- 3µl water

were mixed in a 0 5ml Eppendorf (Eppendorf, 0030 121 023), heated to 70^0C for 10 mm and then chilled on ice To this, the following were added -

- 4µl of a 5x buffer (250mM-Tris/HCl pH 8 3, 375mM-KCl and 15mM-$MgCl_2$)
- 2µl DTT (100mM) (Gibco, 510-8025 SA)
- 1µl RNasin (40U/µl) (Promega, N2511)
- 1µl dNTPs (10mM of each dNTP)
- 6µl water
- 1µl Moloney murine leukaemia virus-reverse transcriptase (MMLV-RT) (40,000U/µl) (Gibco, 510-8025 SA)

The solutions were mixed and the RT reaction was carried out by incubating the Eppendorfs at 37^0C for 1 hour The MMLV-RT enzyme was then inactivated by heating to 95^0C for 2 min The cDNA was stored at -20^0C until required for use in PCR reactions as outlined m Section 2 16

2 12 1 POLYMERASE CHAIN REACTION

A standardised polymerase chain reaction (PCR) procedure was followed in this study The Eppendorf tubes used (Eppendorf, 0030 121 023) and the sterile water were DEPC-treated All reagents had been aliquoted and were stored at -20^0C and all reactions were carried out in a laminar flow cabinet

Each PCR tube contained the following -

- 24 5µl water
- 5µl 10x buffer* (100mM-Tris/HCl, pH 9 0, 50mM-KCl, 1% Triton X-100)
- 3µl 25mM-$MgCl_2$*
- 8µl dNTPs (1 25mM each of dATP, dCTP, dGTP and dTTP) (Promega, U1240)
- 1µl each of first and second strand target primers (250ng/µl)
- 1µl each of first and second strand endogenous control primer (250ng/µl) (β-actin)
- 0 5µl of 5U/µl *Taq* DNA polymerase enzyme*
- 5µl cDNA

*(Promega, N1862)

DNA was amplified by PCR as follows

95^0C for 5 mm - to denature double-stranded DNA

30 cycles 95^0C for 30 sec - denature

*^{0}C for 30 sec - anneal

72^0C for 30 sec - extend

72^0C for 7 min – extend

* the annealing temperature varied with the primer set used See Table 2 appropriate annealing temperatures

The reaction tubes were then stored at 4^0C until analysed by gel electrophoresis as described in Section 2 17

Primers were K8/18 (McBride *et al*, 1999), K19 (Meleady, PhD Thesis 1997) c-myc (NicAomhlimh, R , PhD thesis, 1997) and β-actin (NicAomhlimh, R , PhD thesis, 1997)

Gene	Sense Sequence	Anti-Sense Sequence
Id2	gacccgatgagcctctatc	cgcttattcagccacagtgc
Id3	gtggaaatcctacagcgcgtc	gcaccaggtttagtctccagg
FSTL1	gaggcacagaccatgtgtctgg	cctgctgacagatgcagtaaa
Spd/Spn	tggagagcacccctttttaccac	aaccctcttcactggacagatc
TNFSF7	gtcacttgggtgggacgtagc	ggcgctgggaggcaatggta
FHL2	acaagcagcaacttctctgtgt	cacaaggagtgcttcgtgtgc
HMOX1	cttcttcaccttccccaacatt	ctttccagagagaggggcaca
Zyxin	ccactccattcafttccaagtc	gggctccaggactgaacttgg
GPX3	gtggagggctttgtccctaatttt	Atgagacggccttcagttactt
LOXL2	gcaccgtgtgcgatgacga	aatccgaatgtgcctccaccgg
eIF2-associated p67	aaacagaccctccctcagttcc	aattccaggccttgcattaatc
p21	cctggcacctcacctgctctgc	gcagaagatgtagaggggcc

Table 2 12 1 Primer Sequences for PCR amplification

2 12 2 Real Time-PCR

RNA was isolated (Section 2 14) cell and cDNA synthesised as per Section 2 15 The Taqman® Real time PCR analysis was preformed using the Applied BioSystems Assays on Demand PCR Kits, and experiments were preformed in triplicate, following per manufacturer's instructions

2 13 ELECTROPHORESIS OF PCR PRODUCTS

A 2% agarose gel (NuSieve, GTG) was prepared in TBE buffer (5 4g Tris, 2 75g boric acid, 2ml 0 5M-EDTA pH 8 0 in 500ml water) and melted in a microwave oven After allowing to cool, 0 003% (v/v) of a 10mg/ml ethidium bromide solution was added to the gel which was then poured into an electrophoresis apparatus (BioRad) Combs were placed in the gel to form wells and the gel was allowed to set

10µl loading buffer (50% glycerol, 1mg/ml xylene cyanol, 1mg/ml bromophenol blue, 1mM EDTA) was added to 50µl PCR samples and 20µl was run on the gel at 80-90mV for approximately 2 hours When the dye front was seen to have migrated the required distance, the gel was removed from the apparatus and examined on a UV-transilluminator and photographed

2 14 OVEREXPRESSION STUDIES

2 14 1 Plasmid Preparation

Cultures were streaked on LB agar containing 50µg/ml AMP (Sigma, G9516) and 50µg/ml Ampicillin and incubated at 37^{0}C overnight From these, a single colony was inoculated into 10ml of LB AMP (50µg/ml each) and grown overnight A 2ml sample of this suspension was then added to 200ml of TB AMP 50µg/ml and left to grow overnight at 37^{0}C for large-scale isolation of plasmid from transformed cells The following day the cells were pelleted, 15 minutes at 5000 rpm The plasmid DNA was then isolated from the cells using the Maxi-Mini Qiagen Plasmid DNA Extraction kit (Qiagen, 12143) The DNA concentration was determined by measuring the OD_{260nm}

2 14 2 Lipofectin Transfection of attached mammalian cells

On the day prior to transfections, cells to be transfected were plated from a single cell suspension and seeded into 25cm^2 flasks at 3x10^5 cells per flask On the day of the transfection, the plasmids to be transfected were prepared along with the lipid

transfection reagents according to the manufacturers protocols (Lipofectin - GibcoBRL , 18292-011) The cells were transfected for four hours in the absence of serum after which the media was supplemented with 10% serum overnight The following morning flasks were washed with serum-containing medium and re-fed Selection began 12-24 hours after re-feeding For all transfections the cells were incubated at 37^0C

2 14.3 Selection of Transfected cells

After transfection, cells that had taken up the plasmid were selected by feeding the cells with media containing geneticin (Sigma, G9516) - the plasmids used had a geneticin-resistance marker, therefore, only those cells containing the plasmid will survive treatment with geneticin 2 days after transfection the flask of cells was fed with 200μg/ml geneticin in complete media The concentration of geneticin was increased step-wise evey 2 days to a final concentration of 800μg/ml Untransfected control flasks were killed after 4-5 days From surviving cells, frozen stocks were made and cells were prepared for immunocytochemical (Section 2 8) and western blot (Section 2 9) analysis

2 14 4 Transient Transfection of DNA using Fugene 6 Reagent

Cells were seeded into 25cm^2 flasks at a cell density of 1 5 x 10^5 cells/ml (in 4 mL medium) Fugene 6 reagent DNA complex was used at a 3 2 ratio which was found to be an optimal ratio The Fugene DNA complex was made up according to manufacturer's recommendations and with 100μL of the complex mixture was added to the cells in a drop-wise fashion Cells were returned to the 37^0C incubator Cells were harvested for RNA and protein at 24, 48 and 72 hours

2.15 Affymetrix GeneChips®

The microarray gene expression experiments which were performed in this body of work were performed using Affymetrix® Human Genome U133A GeneChips® Affymetrix GeneChip probe microarrays are manufactured using technology that combines photolithography and combinatorial chemistry Tens to hundreds to thousands of different oligonucleotide probes are synthesised and each of these oligonucleotides is located in a specific area on the microarray slide, called a probe cell Each probe cell contains millions of copies of a given oligonucleotide and each feature size on the Affymetrix U133A GeneChip is 18 microns Due to advances in microarray design Affymetrix have since launched a new GeneChip, U133 Plus 2, which has decreased the feature size of the probes from 18 microns to 11 microns The new U133 Plus 2 GeneChips are now comprised of the old Affymetrix U133A and U133B GeneChips on a single slide The reduction in feature size to 11 microns has resulted in an increase in feature definition, with improved sharpness and signal uniformity

The most important aspect in efficient probe design is the quality of the sequence information used Probe selection and array design are two major factors in reliability, sensitivity, specificity and versatility of expression probe arrays Probes selected for gene expression arrays by Affymetrix are generated from sequence and annotation data obtained from multiple databases such as GenBank, RefSeq and dbEST Sequences from these databases are collected and clustered into groups of similar sequences Using clusters provided by UniGene database as a starting point, sequences are further subdivided into subclusters representing distinct transcripts

This categorisation process involves alignment to the human genome, which reveals splicing and polyadenylation variants The alignment also extends the annotation information supplied by the databases pinpointing low quality sequences These areas are usually trimmed for subsequent generation of high quality consensus sequences or alternatively Affymetrix employ quality ranking to select representative sequences, called exemplars, for probe design

In general, Affymetrix use 11 to 16 probes which are 25 bases in length for each transcript The probe selection method used by Affymetrix for their U133 GeneChips

takes into account probe uniqueness and the hybridisation characteristics of the probes which allow probes to be selected based on probe behaviour Affymetrix use a multiple linear regression (MLR) model in the probe design that was derived from thermodynamic model of nucleic acid duplex formation This model predicts probe binding affinity and linearity of signal changes in response to varying target concentrations An advantage of this type of model-based probe selection system is that it provides a physical and mathematical foundation for systematic and large-scale probe selection Also, an essential criterion of probe selection by Affymetrix for quantitative expression analysis is that hybridisation intensities of the selected probes must be linearly related to target concentrations

A core element of Affymetrix microarray design is the Perfect/Mismatch probe strategy For each probe that is designed to be perfectly complimentary to a given target sequence, a partner probe is also generated that is identical except for a single base mismatch in its center These probe pairs, called the Perfect Match probe (PM) and the Mismatch probes (MM), allow the quantitation and subtraction of signals caused by non-specific cross-hybridisation The differences in hybridisation signals between the partners, as well as their intensity ratios, serve as indicators of specific target abundance

2 15 1 Sample and Array Processing

After RNA isolation, quantification and purification using the Qiagen Rneasy isolation method (Section 2 14 1), cDNA was synthesised using the GeneChip T7-Oligo (dT) Promoter Primer Kit (Affymetrix, 900375) from 10μg total RNA First strand cDNA synthesis was then performed using the SuperScript Choice Kit (BioSciences, 11917-010) First strand cDNA synthesis involved 'primer hybridisation' where the T7-Oligo (dT) primer was incubated with the RNA and DEPC-treated H_2O at 70^0C for 10 mins , followed by a short incubation in ice, 'temperature adjustment' where 5X first strand buffer, DTT and dNTP mix were added to the RNA mix and incubated at 42^0C for 2 mins , and 'First Strand synthesis' where SuperScript II RT was added to the mix and incubated at 42^0C for 1 hour Second strand cDNA synthesis was performed and purified using GeneChip Sample Cleanup module (Affymetrix, 900371) as recommended by the manufacturers instructions

cRNA was then synthesised and biotin-labelled using the Enzo BioArray HighYield RNA Transcript Labelling Kit (Affymetrix, 900182) Biotin-labelled cRNA was purified using the GeneChip Cleanup Module Kit (Affymetrix, 900371) and quantified The value obtained was adjusted to reflect carryover of unlabelled total RNA A sample of biotin-labelled cRNA was taken for gel electrophoresis analysis The labelled cRNA was then fragmented before hybridisation onto the Affymetrix GeneChip probe microarrays The aliquot of fragmented sample RNA was stored at -20^{0}C until ready to perform the hybridisation step

Hybridisation of cRNA onto the Affymetrix GeneChip probe human microarrays (Affymetrix, HU133A and HU133 Plus 2) was performed in the Conway Institute, University College Dublin, where the Affymetrix Hybridisation Oven and Fluidics Station is set up along with the Affymetrix GeneChip Scanner, which exported the data directly into the Affymetrix analysis software, MicroArray Suite 5 1 (MAS 5 1)

2.15 2 Microarray Data Normalisation

The purpose of data normalisation is to minimise the effects of experimental and technical variation between microarray experiments so that meaningful biological comparisons can be drawn from the data sets and that real biological changes can be identified among multiple microarray experiments Several approaches have been demonstrated to be effective and beneficial However, most biologists use data scaling as the method of choice despite the presence of other alternatives In order to compare gene expression results from experiments performed using microarrays, it is necessary to normalise the data obtained following scanning the microarray chips There are two main ways in which this type of normalisation is performed, the first of which is 'Per-chip' normalisation This type of normalisation helps to reduce minor differences in probe preparation and hybridisation conditions which may potentially result in high intensity of certain probe sets These adjustments in probe intensity are made to set the average fluorescence intensity to some standard value, so that all the intensities on a given microarray chip go up or down to a similar degree However, this type of normalisation should only be performed on microarrays using similar cell or tissue types One drawback from this of normalisation is that some aspects of the microarray

data may potentially be obscured, such as whether the RNA samples or the probe preparation steps were equivalent for each sample.

The second way in which most biologist normalise their data sets is by employing 'per gene' normalisation method. The main aim of microarray experiments is to identify genes whose expression changes in different conditions, be that tracking gene changes across a temporal experiment or when comparing gene expression between normal and diseased tissue. Therefore, it is necessary to normalise microarray data sets using 'per gene' normalisation. In 'Per gene' normalisation is necessary to find genes that have similar expression pattern across an experiment. Analysis of raw data from microarray experiments is useful for identifying genes that are expressed at the same level, for example, genes that are highly abundant in the samples.

2.15.3 Probe Logarithmic Intensity ERror estimation (PLIER)

The PLIER algorithm (http://www.affymetrix.com) is a new tool introduced by Affymetrix for the use in data analysis of their GeneChips and has replaced the need to normalise microarray data by using the 'per chip' and 'per gene' normalisation methods. This algorithm incorporates model-based expression analysis and non-linear normalisation techniques. PLIER accounts for differences in probes by means of a parameter termed "probe affinity". Probe affinity is a measure of how likely a probe is to bind to a complimentary sequence, as all probes have different thermodynamic properties and binding efficiencies. Probe affinities determine the signal intensities produced at a specific target concentration for a given probe, and are calculated using experimental data across multiple arrays. By accounting for these observed differences, all the probes within a set can be easily compared. An example of how the PIER algorithm works is if one probe is consistently twice as bright as other probes with in a set, PLIER appropriately scales the probe intensities. In the case of a probe set, this enables all set numbers to be compared and combined accurately.

PLIER also employs an error model that assumes error is proportional to the probe intensity rather that of the target concentration. At high concentrations, error is approximately proportional to target concentration, since most of the intensity is due to target hybridisation signal. However, at the low end, error is approximately

proportional to background hybridisation intensity, which is the largest component of the observed intensity. Due to this effect, it is inaccurate to treat errors as a proportion of target concentration in all circumstances. The PLIER error model smoothly transitions between the low end, where error is dependent upon background, and the high end, where error is dependent on signal.

The PLIER algorithm supports a multi-array approach that enables replicate sample analysis. PLIER ensures consistent probe behaviour across experiments to improve the quality of results in any one given experiment and helps to discount outliers. Benefits of this algorithm include an improved coefficient of variation of signals from probe sets while retaining accuracy. Also higher differential sensitivity for low expressors maybe achieved using PLIER.

2.16 Genomatix Software Suite

One company that is providing software that allows users to explore textual data as well as combine sequence analysis, and genome annotation in order to help researchers to discover new contexts from biological data; is Genomatix (www.genomatix.de). The analysis offered by Genomatix software is aimed to help researchers gain a better understanding of gene regulation at the molecular level. The Genomatix software suite is comprised of six main tools: ElDorado, Gene2Promoter, BiblioSphere, GEMS Launcher, MatInspector and PromtoerInspector. ElDorado is a gene orientated genome search engine which provides the user with information about functional genomic elements within a specific region of the genome. This piece of software compiles and integrates information from several sources and includes functional information, synonyms and information on gene function and regulatory pathway. In addition, information on mRNAs, their exon/intron structure and coding sequences, single nucleotide polymorphisms (SNPs) and potential promoter regions maybe retrieved using ElDorado.

Since co-regulation of gene transcription often originates from common promoter elements the identification and characterization of these elements provides a more in-depth analysis for expression of microarray clusters. Gene2Promoter allows users to automatically extract groups of promoters for genes that may of interest. This piece of

Genomatix software provides access to promoter sequences of all genes annotated in available genomes. Results from Gene2Promoter are presented in a graphical format and common transcription factor binding sites are high lighted along the gene input sequence.

One powerful member of the Genomatix Software Suite, which illustrates the emerging emphasis on the visual presentation of complex data, is BiblioSphere. BiblioSphere is a data-mining tool for extracting and studying gene relationships from literature databases and genome-wide promoter analysis. The data-mining strategy allows to find direct gene-gene co-citations and even yet unknown gene relations via interlinks. BiblioSphere data is displayed as 3D interactive view (Figure 1.?) of gene relationships. Results can be classified by tissue, Gene Ontology and MeSH. Statistical rating by z-scores indicates over- and under-representation of genes in the referring biological categories.

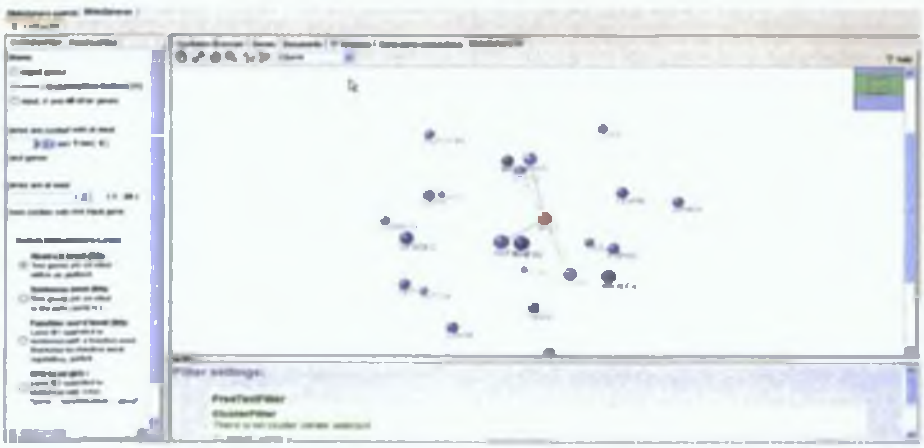
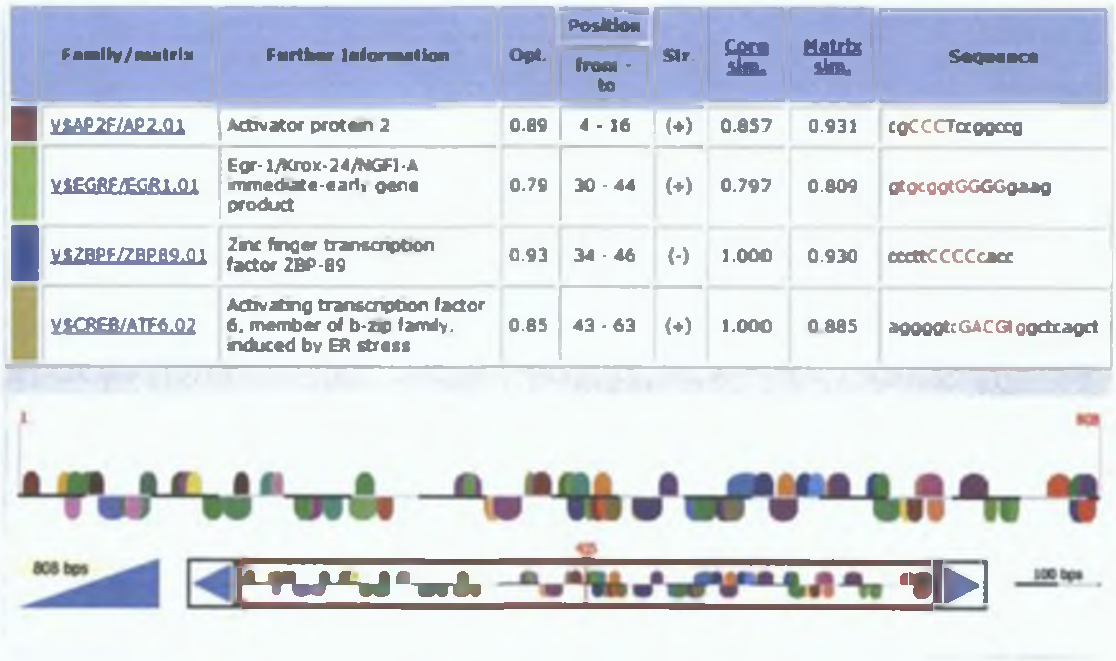


Figure 2.1 Screen shot of BiblioSphere

Although transcription is regulated by a variety of DNA sequences, including enhancer and matrix attachment regions, promoters can be seen as the most important part of the sequence because any activator or repressor has to act on the promoter to influence transcriptional initiation of a particular gene. Promoters are DNA regions of several hundred base pairs that contain the transcription start site of genes. The most important functional elements within promoters are binding sites for specific proteins called transcription factors. The control of gene transcription is a common method used in biological systems to regulate protein expression. Transcription regulation in eukaryotes depends on a series of complex signal transduction networks that control

gene promoter activity. Genomatix have develop a software packages, GEMS Launcher with helps researchers to identify transcription factor binding sites in a given gene promoter. GEMS Launcher is divided up into several parts, the first of which is MatInspector.



Family/matrix	Further information	Opt.	Position from - to	Str.	Core sim.	Matrix sim.	Sequence
V$AP2F/AP2.01	Activator protein 2	0.89	4 - 16	(+)	0.857	0.931	cgCCCTccggccg
V$EGRF/EGR1.01	Egr-1/Krox-24/NGFI-A immediate-early gene product	0.79	30 - 44	(+)	0.797	0.809	gtgcggtGGGGgaag
V$ZBPF/ZBP89.01	Zinc finger transcription factor ZBP-89	0.93	34 - 46	(-)	1.000	0.930	ccctCCCCcacc
V$CREB/ATF6.02	Activating transcription factor 6, member of b-zip family, induced by ER stress	0.85	43 - 63	(+)	1.000	0.885	agggggtcGACGtggctcagct

Figure 2.2 Graphical example of Transcription Factor Binding Sites located in a Promoter sequence.

MatInspector is a tool that employs a library of matrix descriptions for transcription factor binding sites and locates these binding sites on a given promoter sequence. Graphical display of transcription factor binding sites common to a set of inputted promoters is obtained following MatInspector analysis. FrameWoker software tool that allows users to extract a common *framework* of elements from a set of DNA sequences. These elements are usually transcription factor binding sites since this tool is designed for the comparative analysis of promoter sequences. FrameWorker generates the most complex models that are common to the input sequences. These are all elements that occur in the same order and in a certain distance range in all (or a subset of) the input sequences.

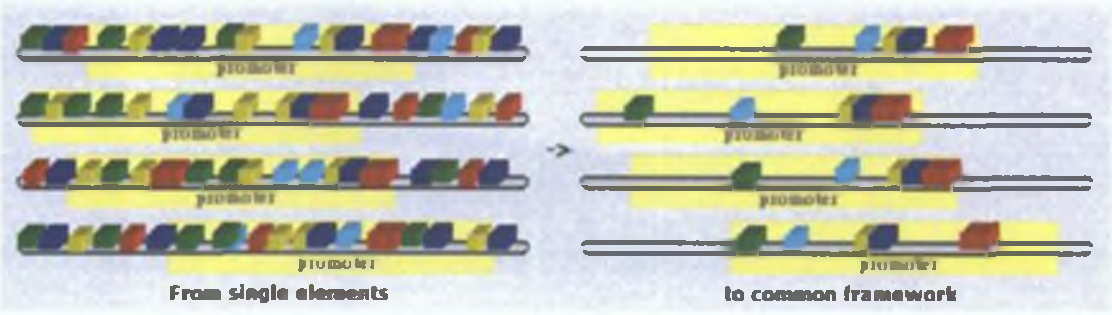


Figure 2.3 Screen shot of FrameWorker Results

Once a model of transcription factor binding sites is generated using FrameWoker software, it is possible using Genomatix ModelInspector program to scan sequence databases for regulatory units that match the model which have been generated using MatInspector. ModelInspector provides a library of experimentally verified promoter models against which transcription factor models maybe scanned.

It is with software packages provided by companies such as Genomatix, that scientists will have to reply on in order to help them make sense of the vast quantities of data that is being generated by DNA microarray experiments, not only carried out in their own laboratories, but also the great wealth of information that is available in public accesses databases. The type information retrieval, visualisation, standardisation and analysis offered by Genomatix, is and will receive a great deal of attention from countless other companies and bioinformatics will undoubtedly remain an extremely important and ever changing area of scientific research in the future.

Section 3.0: Results

Section 3 1 INVESTIGATION OF THE EFFECT OF PRYMIDINE ANALOGUE EXPOSURE IN THE HUMAN LUNG CARCINMOA CELL LINES, DLKP AND A549

Pervious studies in this laboratory have shown that 10μM 5,2 -Bromodeoxyuridine (BrdU) induces cytokeratin 8, 18 and 19 protein expression in the DLKP cell line and enhances their expression in A549 (McBride, *et al*, 1999, O'Sulllivan, PhD Thesis, 1999, Meleady and Clynes, 2001) Induced α_2-, β_1-integrin has been observed in both cell lines (Meleady and Clynes, 2001) as well as the protein expression of Ep-CAM (O'Sullivan, PhD Thesis, 1999)

In this study we expand the range of pyrimidine analogues investigated in our pervious studies in order to determine if all analogues investigated induced a similar pattern of differentiation in both the DLKP and A549 cell lines Immunocytochemistry was the primary analysis carried on treated cells and was used to qualitatively investigate the changes in cytokeratins 8, 18 and 19, α_2-, β_1-integrin and Ep-CAM protein expression in both cell lines following treatment (Section 2 7) with each analogue investigated Western blot analysis was performed on a subset of treatments

Section 3 1 1 Changes in α_2-integrin expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of α_2-integrin protein to be increased in treated cells The intensity of the observed staining in IdU, CdU and 5,2 -FdU treated A549 cells was comparable to that obtained following 10μM BrdU treatment In contrast immunocytochemistry analysis showed that cells treated with Bromouracil, Bromouridine and 5-FU did not exhibit the same intensity as seen with the other analogues

Figure 3.1.1.1 Control

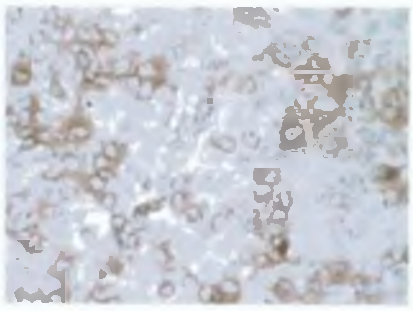


Figure 3.1.1.2 BrdU

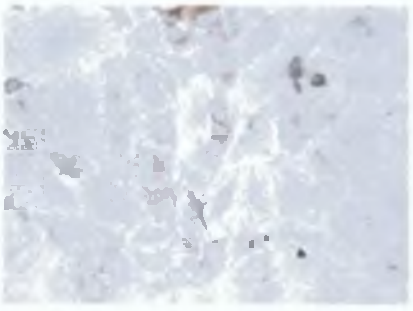


Figure 3.1.1.3CdU

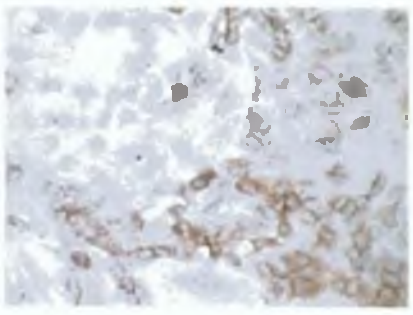


Figure 3.1.1.4 IdU

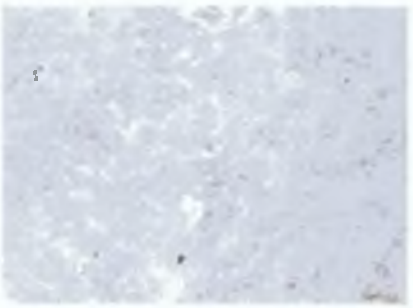


Figure 3.1.1.5 5-FU

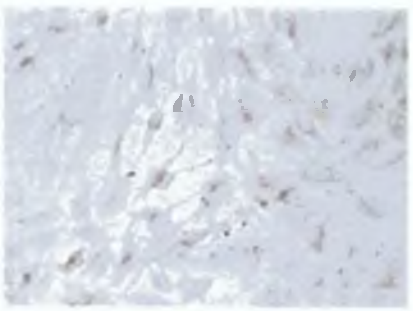


Figure 3.1.1.6 5,2`-FdU

Figure 3.1.1.7 Bromouracil

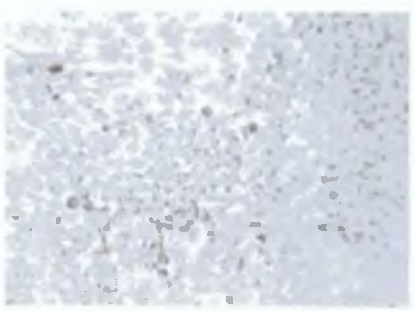


Figure 3.1.1.8 Bromouridine

Magnification - 40X

Section 3 1 2 Changes in β_1-integrin expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of β_1-integrin protein to be increased in treated cells The intensity of the observed staining in CdU, 5-FU and 5,2 -FdU treated A549 cells was comparable to that obtained following 10μM BrdU treatment Immunocytochemical analysis showed that IdU-treated cells to have a greater increase in β_1-integrin protein than BrdU-treated cells In contrast immunocytochemistry revealed that treatment with Bromouracil and Bromouridine did not exhibit the same intensity as seen with the other analogues

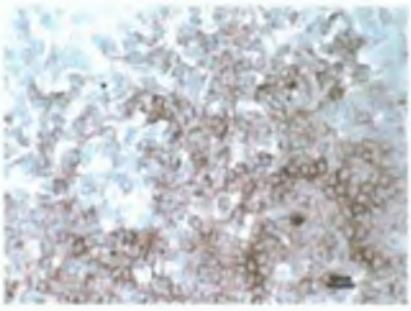


Figure 3.1.2.1 Control

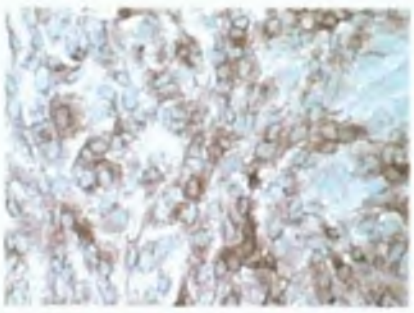


Figure 3.1.2.2 BrdU

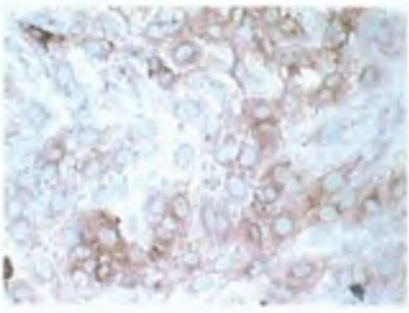
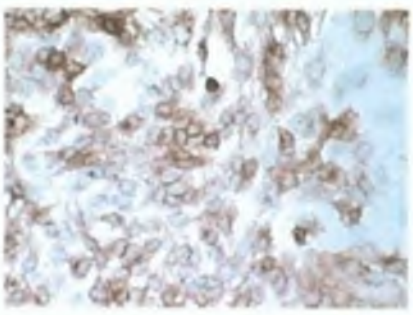


Figure 3.1.2.3 CdU



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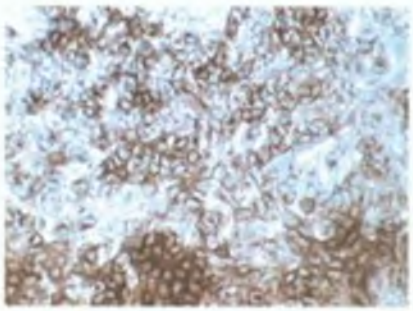


Figure 3.1.2.5 5-FU

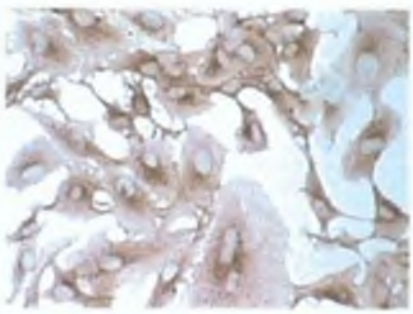


Figure 3.1.2.6 5,2-FdU

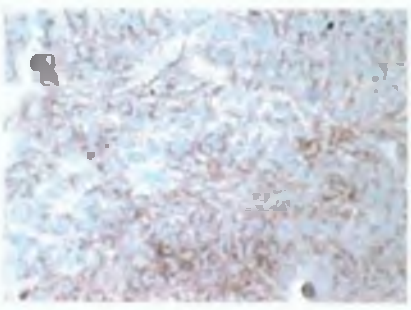


Figure 3.1.2.7 Bromouracil

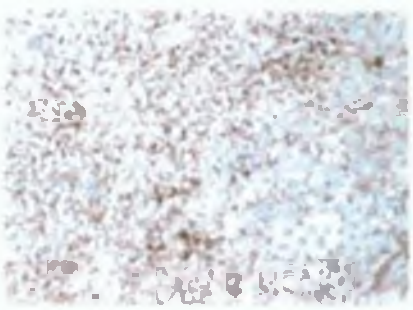


Figure 3.1.2.8 Bromouridine

Magnification – 40X

Section 3 1 3 Changes in EpCAM expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of EpCAM protein to be increased in treated cells The intensity of the observed staining in IdU, 5-FU and 5,2 -FdU treated A549 cells was comparable to that obtained following 10μM BrdU treatment Immunocytochemical analysis showed that CdU-, Bromouracil- and Bromouridine-treated cells to have an only slightly greater level of increase in EpCAM expression than the control A549 cells

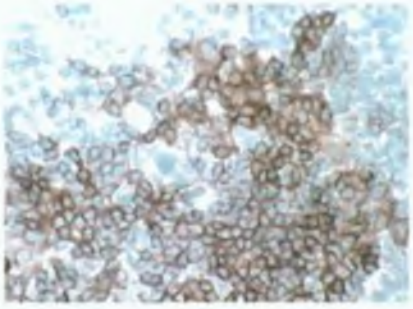


Figure 3.1.3.1 Control

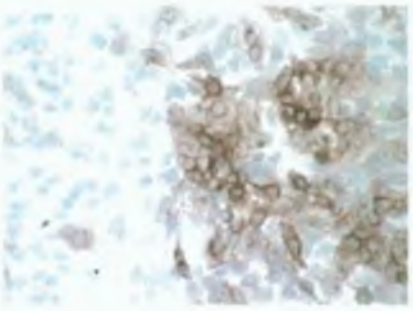


Figure 3.1.3.2 BrdU

Figure 3.1.3.3 CdU

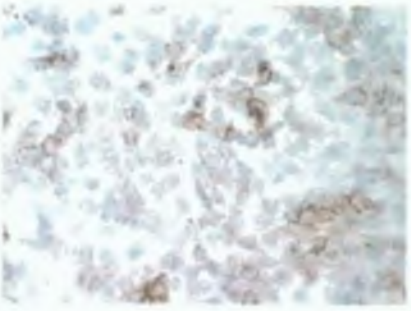


Figure 3.1.3.4 IdU

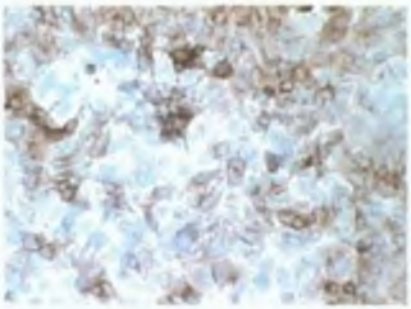


Figure 3.1.3.5 5-FU

Figure 3.1.3.6 5,2'-FdU

Figure 3.1.3.7 Bromouracil

Magnification – 40X

Section 3 1 4 Changes in CK8 expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical and immunofluorescence analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of cytokeratins 8 protein to be increased in treated cells The intensity of the observed staining in all the analogue treatments was increase in comparison to the A549 control cells CdU and 5,2 -FdU treated A549 cells exhibited a greater increase cell size in comparison to the other treatments The morphology of 5,2 -FdU treated A549 cells were greatly altered in comparison to control cells and the other analogue treatments

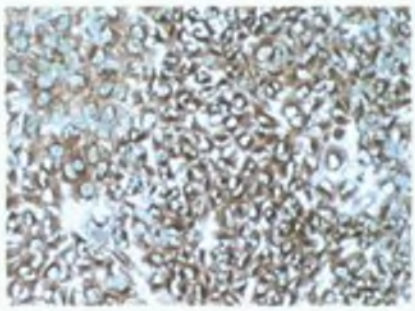


Figure 3.1.4.1 Control

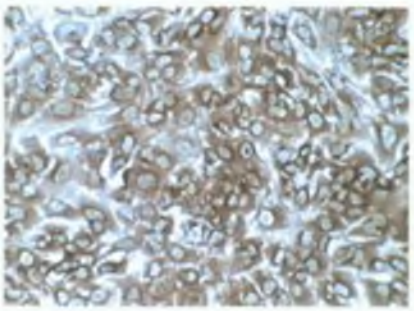


Figure 3.1.4.2 BrdU

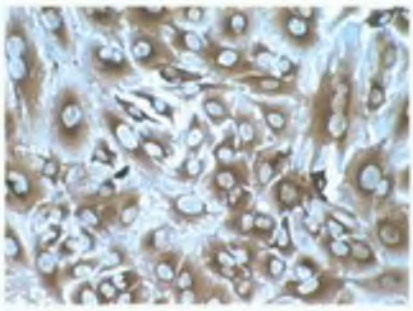


Figure 3.1.4.3 CdU

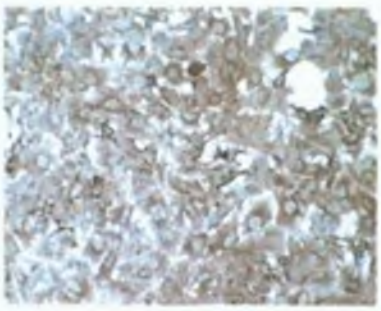


Figure 3.1.4.4 IdU

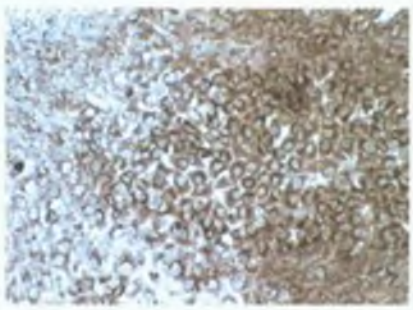


Figure 3.1.4.5 5-FU

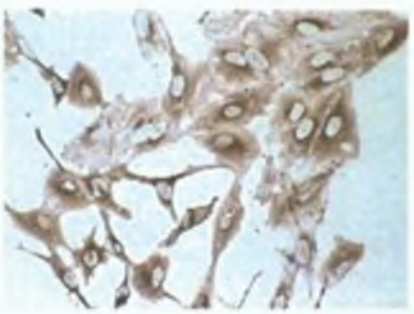


Figure 3.1.4.6 5,2'-FdU

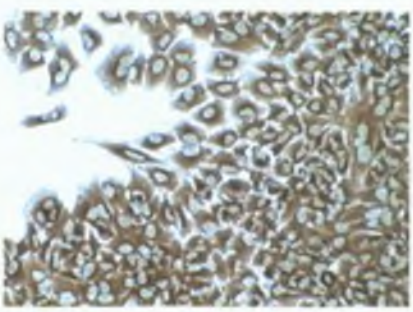


Figure 3.1.4.7 Bromouracil

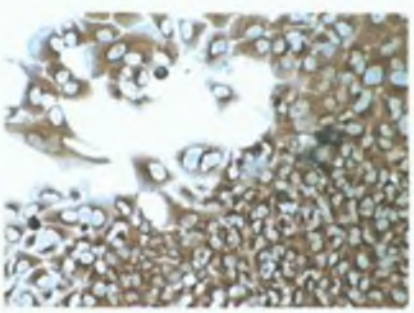


Figure 3.1.4.8 Bromouridine

Magnification – 40X

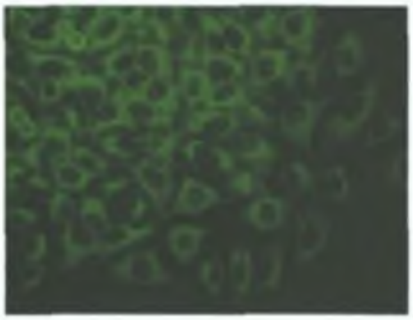


Figure 3.1.4.9 Control

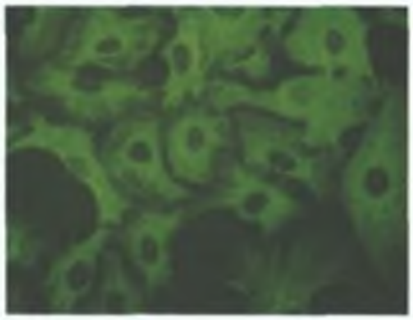


Figure 3.1.4.10 IdU

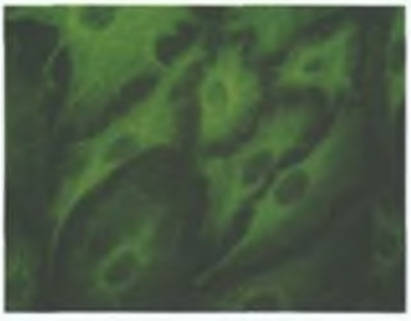


Figure 3.1.4.11 CdU

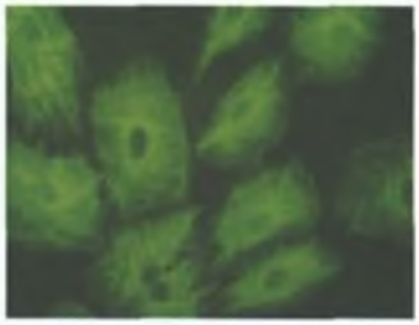


Figure 3.1.4.12 5,2`-FdU

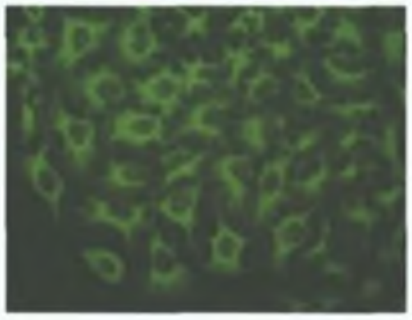


Figure 3.1.4.13 BromoUracil

Magnification – 40X

Section 3 1 5 Changes in CK18 expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of cytokeratin 18 protein to be increased in treated cells The intensity of the observed staining in all the analogue treatments was increase in comparison to the A549 control cells All treatments displayed a similar increase in CK18 comparable to that observed in 10μM BrdU treated cells CdU and 5,2 -FdU treated A549 cells exhibited a greater increase in size of the cells in comparison to the other treatments The morphology of 5,2 -FdU treated A549 cells were greatly altered in comparison to control cells and the other analogue treatments

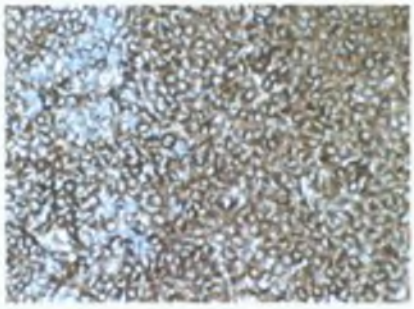


Figure 3.1.5.1 Control

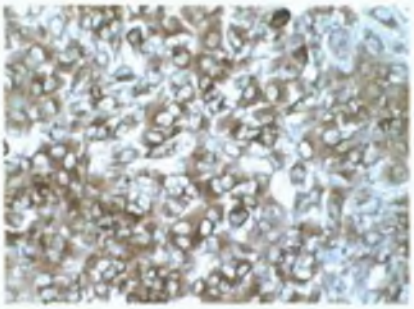


Figure 3.1.5.2 BrdU

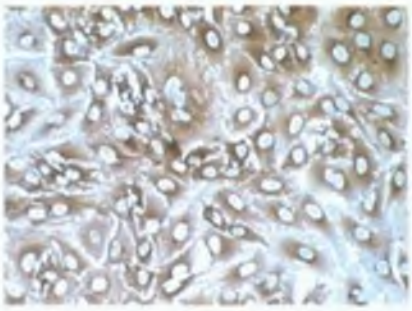


Figure 3.1.5.3 CdU

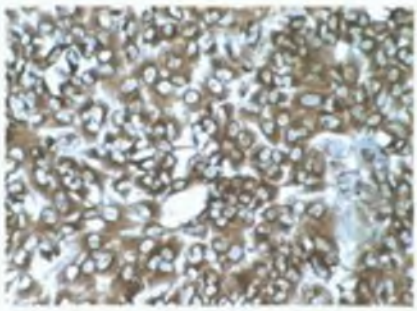


Figure 3.1.5.4 IdU

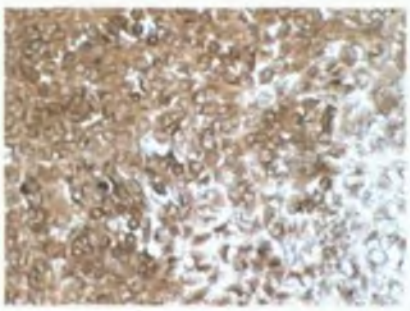


Figure 3.1.5.5 5-FU

Figure 3.1.5.6 5,2`-FdU

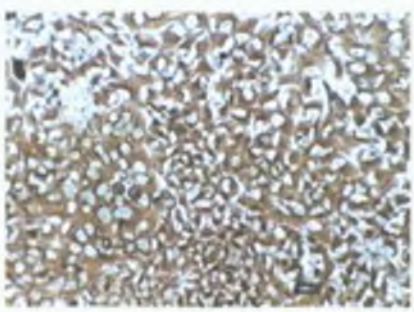


Figure 3.1.5.7 Bromouracil

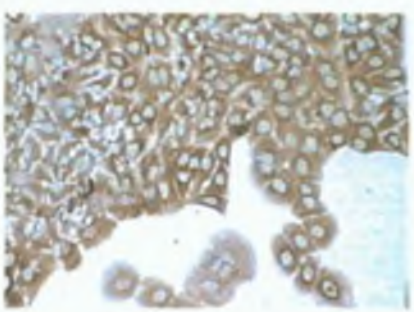


Figure 3.1.5.8 Bromouridine

Magnification – 40X

Figure 3.1.5.9 Control

Figure 3.1.5.10 IdU

Figure 3.1.5.11 CdU

Figure 3.1.5.12 Bromouridine

Figure 3.1.5.13 5,2'FdU

Magnification – 40X

Section 3 1 6 Changes in CK19 expression in A549 cells following treatment with the thymidine analogues

Immunocytochemical analysis of A549 cells treated with 10μM BrdU, IdU, CdU, 5-FU, 5,2 -FdU, and 70μM Bromouracil and Bromouridine showed that expression of cytokeratin 19 protein to be increased in treated cells The intensity of the observed staining in all the analogue treatments was increase in comparison to the A549 control cells All treatments displayed a similar increase in CK18 comparable to that observed in 10μM BrdU treated cells IdU, 5-FU and 5,2 -FdU treated A549 cells exhibited a greater increase in CK19 than the other treatments CdU and 5,2 -FdU displayed a great increase in cell size The morphology of 5,2 -FdU treated A549 cells were greatly altered in comparison to control cells and the other analogue treatments

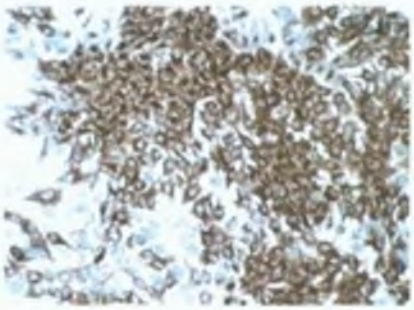


Figure 3.1.6.1 Control

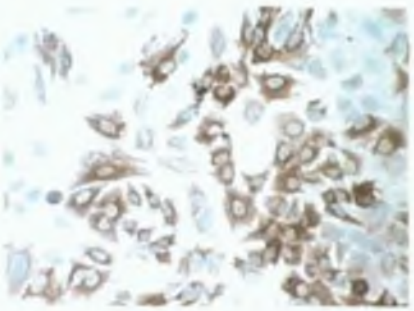


Figure 3.1.6.2 BrdU

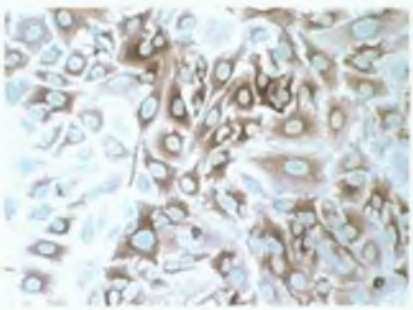


Figure 3.1.6.3 CdU

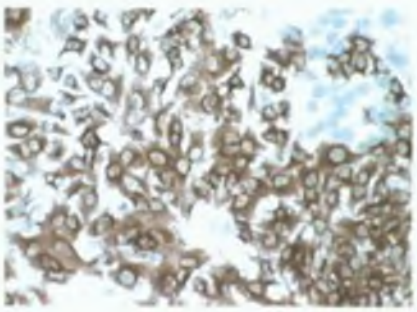


Figure 3.1.6.4 IdU

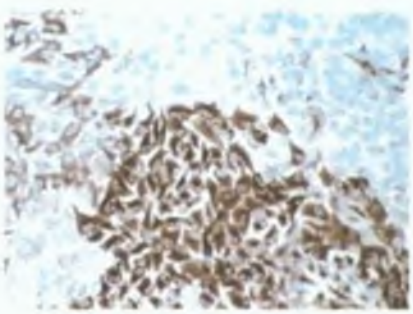


Figure 3.1.6.5 5-FU

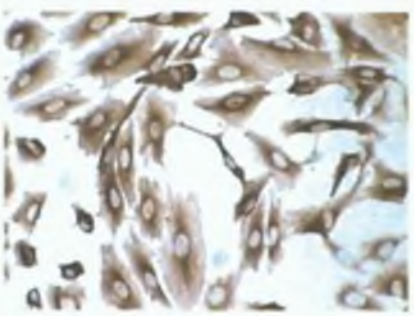


Figure 3.1.6.6 5,2'-FdU

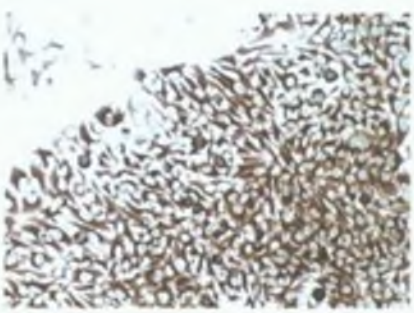


Figure 3.1.6.7 Bromouracil

Figure 3.1.6.8 Bromouridine

Magnification – 40X

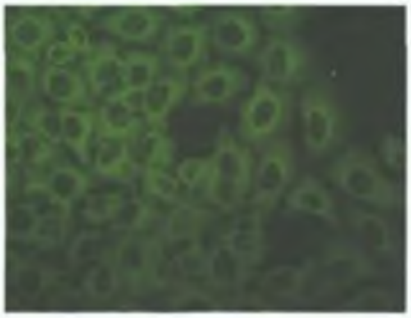


Figure 3.1.6.9 Control

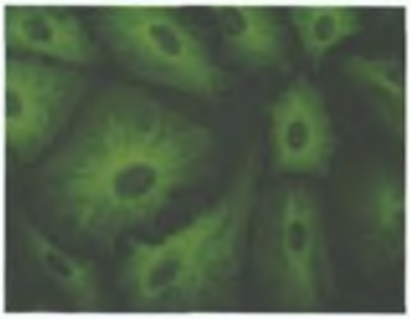


Figure 3.1.6.10 IdU

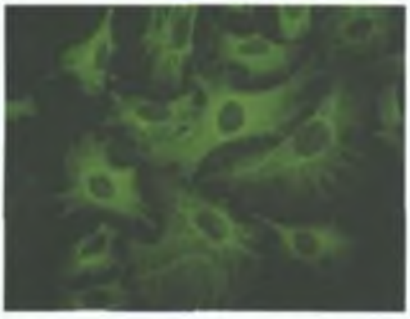


Figure 3.1.6.11 CdU

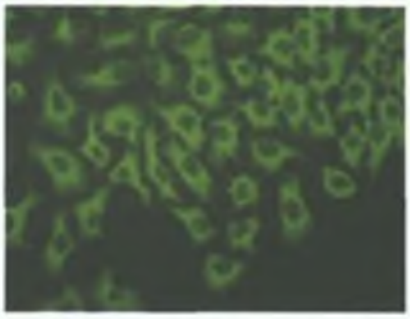


Figure 3.1.6.12 BromoUracil

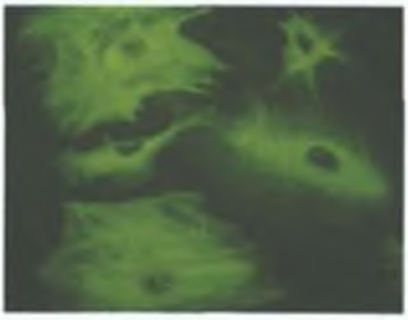


Figure 3.1.6.13 5,2`-FdU

Magnification – 40X

	Control	BrdU	5,2' IdU	5,2'CdU	BromoUracil	Bromouridine	5- FU	5,2'- FdU
α_2 Integrin	+	++	++	++	+	+	+	++
β_1 Integrin	+	++	+++	++	+	++	++	+++
Ep- CAM	+	++	++	+	+	+	++	++
CK-8	+	+++	+++	+++	+++	+++	+++	+++
CK-18	+	+++	+++	+++	++	+++	+++	+++
CK-19	+	+++	+++	+++	+++	++	+++	+++

Table 1 **Summary of results obtained for different marker proteins in A549 treated cells with various thymidine analogues**

+ - Expression, ++ - Strong Expression, +++ - Intense Expression

Section 3 1 7 Changes in α_2-integrin expression in DLKP cells following treatment with the thymidine analogues

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU and 5,5 -FdU (results not shown) showed that expression of α_2-integrin protein to be increased in treated cells The intensity of the observed staining in these analogue treatments was increase comparable to that seen in BrdU treated cells In contrast, immunocytochemical analysis of DLKP cells treated with 70μM Bromouridine and Bromouracil revealed that the cells did not exhibit the same increase in α_2-integrin as seen with the other analogues

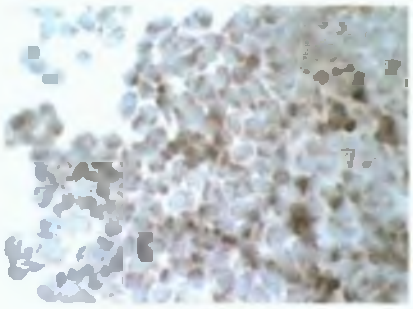


Figure 3.1.7.1 Control

Figure 3.1.7.2 BrdU

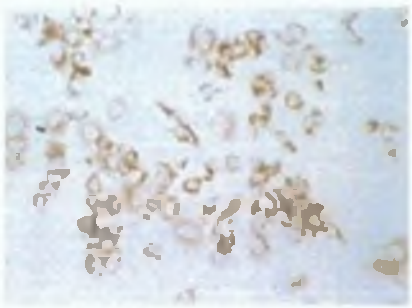


Figure 3.1.7.3 CdU

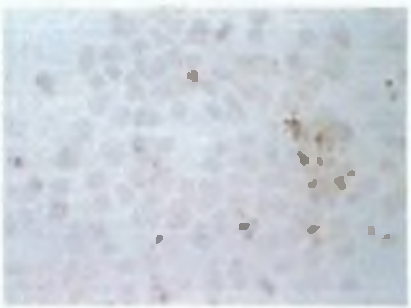


Figure 3.1.7.4 IdU

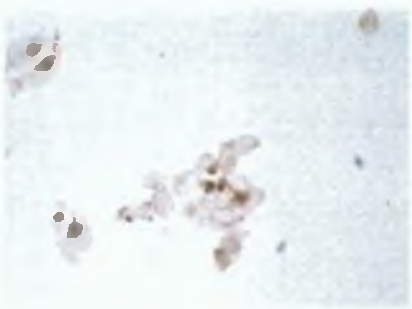


Figure 3.1.7.5 5,2`-FdU

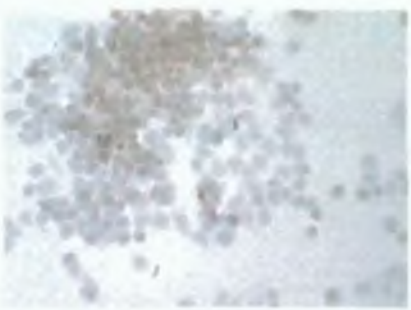


Figure 3.1.7.6 Bromouracil

Figure 3.1.7.7 Bromouridine

Magnification – 40X

Section 3 1 8 Changes in β_1-integrin expression in DLKP cells following treatment with the thymidine analogues

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU and 5,5 -FdU (results not shown) showed that expression of β_1-integrin protein to be increased in treated cells The intensity of the observed staining in IdU, 5,2 -FdU and 5,5 -FdU-treated DLKP cells was comparable to the staining observed in BrdU treated cells Immunocytochemistry analysis revealed that cells treated with 70μM Bromouracil and Bromouridine did not exhibit the same intensity as seen with the other analogues

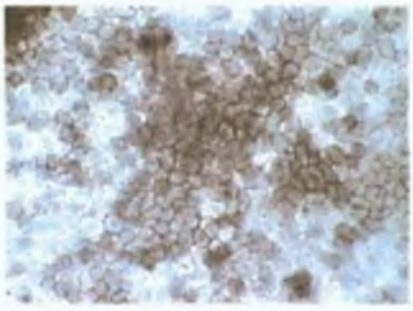


Figure 3.1.8.1 Control

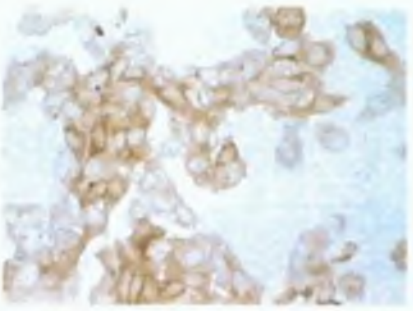


Figure 3.1.8.2 BrdU

Figure 3.1.8.3 CdU

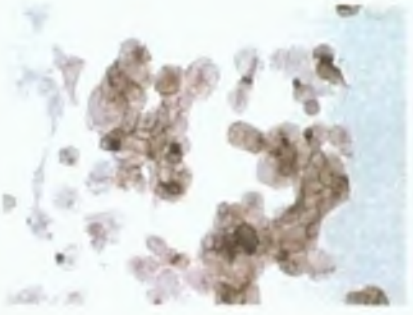


Figure 3.1.8.4 IdU

Figure 3.1.8.5 5,2`-FdU

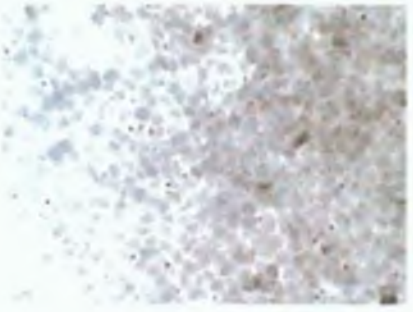


Figure 3.1.8.6 Bromouracil

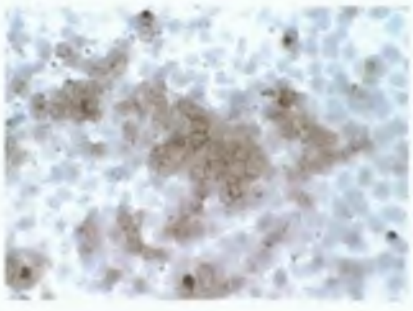


Figure 3.1.8.7 Bromouridine

Magnification – 40X

Section 3 1 9 Changes in EpCAM expression in DLKP cells following treatment with the thymidine analogues

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU, 5,5 -FdU (results not shown), and 70μM Bromouridine and Bromouracil showed that expression of EpCAM protein to be increased in treated cells The intensity of the observed staining in all analogue treatments was only slightly greater than that seen in the DLKP control

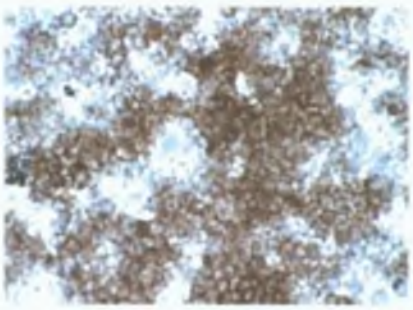


Figure 3.1.9.1 Control

Figure 3.1.9.2 CdU

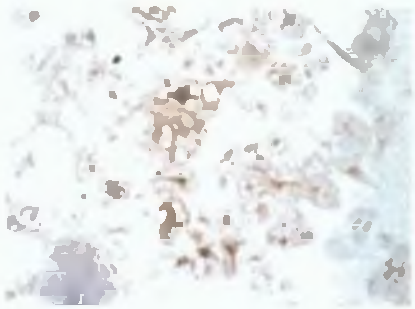


Figure 3.1.9.3 IdU

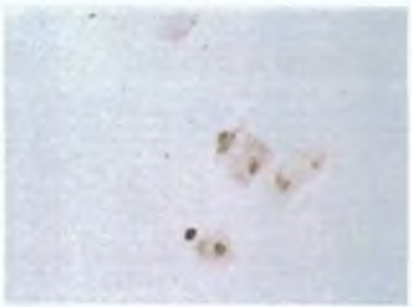


Figure 3.1.9.4 5,2`-FdU

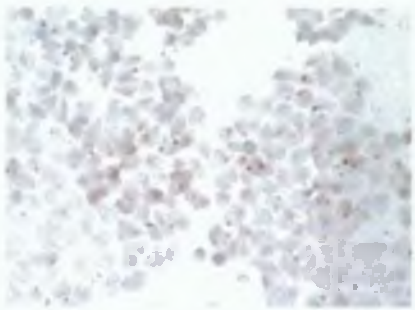


Figure 3.1.9.5 Bromouracil

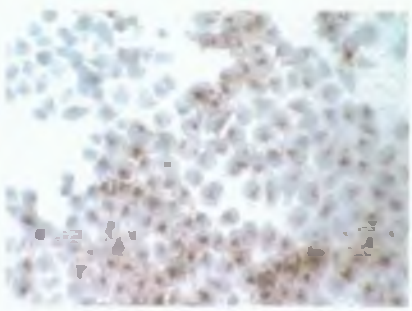


Figure 3.1.9.6 Bromouridine

Magnification – 40X

Section 3 1 10 Changes in CK8 expression in DLKP cells following treatment with the thymidine analogues

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU, 5,5 -FdU (results not shown), and 70μM Bromouracil and Bromouridine showed that expression of cytokeratin 8 protein to be induced in treated cells The intensity of the observed staining 5,2 -FdU-treated DLKP cells to be similar to that of BrdU treated cells

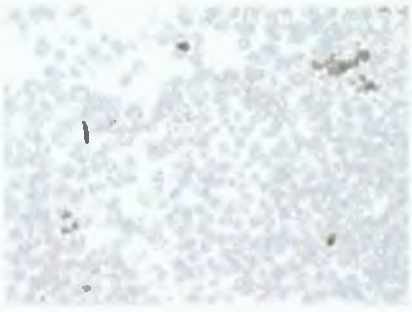


Figure 3.1.10.1 Control

Figure 3.1.10.2 BrdU

Figure 3.1.10.3 CdU

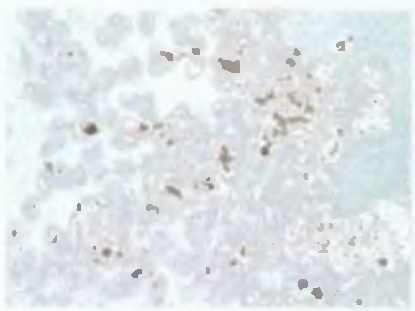


Figure 3.1.10.4 IdU

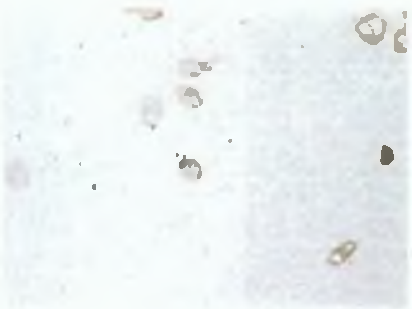


Figure 3.1.10.5 5,2'-FdU

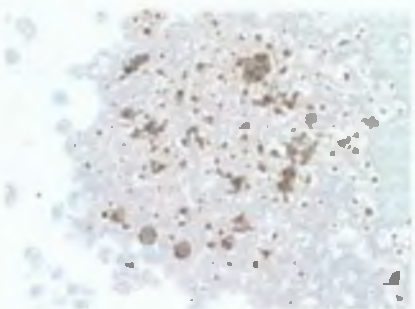


Figure 3.1.10.6 Bromouracil

Figure 3.1.10.7 Bromouridine

Magnification – 40X

Section 3 1 11 Changes in CK18 expression in DLKP cells following treatment with the thymidine analogues.

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU, 5,5 -FdU (results not shown), and 70μM Bromouridine and Bromouracil showed that expression of cytokeratin 18 protein to be induced in treated cells The intensity of the observed staining in IdU-, 5,2 -FdU, and 5,5 -FdU-treated DLKP cells was comparable to the staining observed in BrdU treated cells Immunocytochemistry analysis revealed that cells treated with Bromouracil and Bromouridine did not exhibit the same intensity as seen with the other analogues

Figure 3.1.11.1 Control

Figure 3.1.11.2 BrdU

Figure 3.1.11.3 CdU

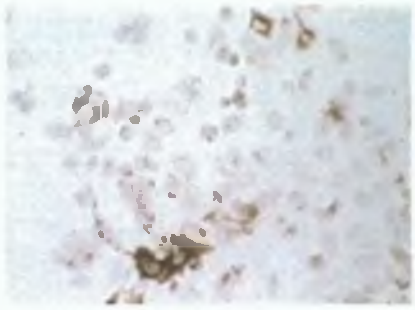


Figure 3.1.11.4 IdU

Figure 3.1.11.5 5,2'-FdU

Figure 3.1.11.6 Bromouracil

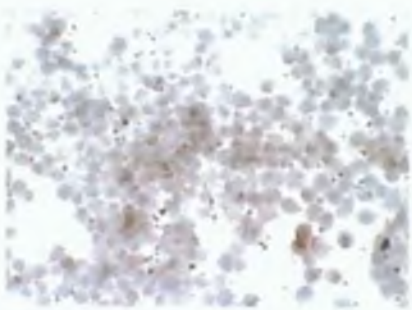


Figure 3.1.11.7 Bromouridine Magnification – 40X

Section 3 1 12 Changes in CK19 expression in DLKP cells following treatment with the thymidine analogues

Immunocytochemical analysis of DLKP cells treated with 10μM BrdU, IdU, CdU, 5,2 -FdU, 5,5 -FdU (results not shown), and 70μM Bromouracil and Bromouridine showed that expression of cytokeratin 19 protein to be induced in treated cells The intensity of the observed staining in IdU, 5,2 -FdU, and 5,5 -FdU-treated cells was comparable to the staining observed in BrdU treated cells Immunocytochemistry analysis revealed that cells treated with CdU, Bromouracil and Bromouridine did not exhibit the same increase intensity as seen with the other analogues

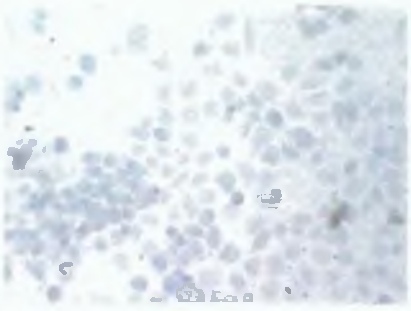


Figure 3.1.12.1 Control

Figure 3.1.12.2 BrdU

Figure 3.1.12.3 CdU

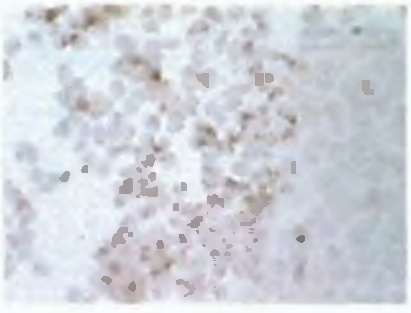


Figure 3.1.12.4 IdU

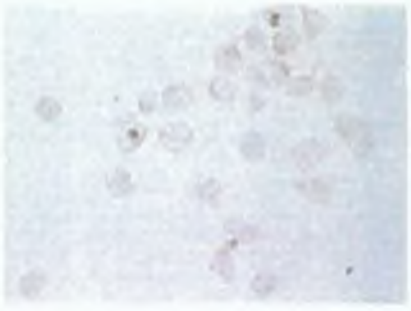


Figure 3.1.12.5 5,2'-FdU

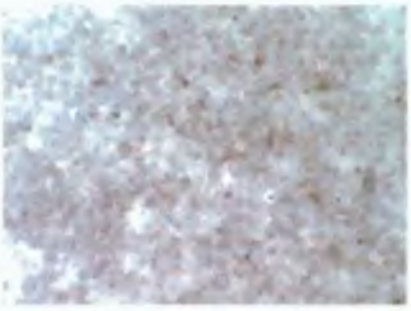


Figure 3.1.12.6 Bromouracil

Figure 3.1.12.7 BromoUridine Magnification – 40X

	Control	BrdU	5,2' IdU	5,2'CdU	BromoUracil	Bromouridine	5,2'-FdU	5,5'- FdU
α_2 Integrin	+	++	++	++	+	+	++	++
β_1 Integrin	+	++	+++	++	+	+	++	++
Ep-CAM	-	+	++	+	+	+	+	+
CK-8	-	++	+	+	+	+	++	+
CK-18	-	++	++	+	++	+	++	++
CK-19	-	+	++	+	+	+	++	++

Table 2 **Summary of results obtained for different marker proteins in DLKP treated cells with various thymidine analogues.**

- No Expression, + Expression, ++ Strong Expression, +++ Intense Expression.

Section 3.1.13 INVESTIGATION OF CHANGES IN CYTOKERATIN 8 AND EIF4E EXPRESSION LEVELS IN TREATED A549 CELLS

To quantify the changes in expression of cytokeratin 8 protein observed in the immunochemical analysis of treated A549 cells, Western blot analysis was performed on cells which had been treated for up to 14 days with a selection of analogues

An increase in cytokeratin 8 protein levels was demonstrated in A549 cells treated with BrdU, IdU, CdU, 5,2 -FdU, 5,5 -FdU and 5-FU An increase in cytokeratin expression was observed at day 7 with a great increased noted in cells exposed to drug for 14 days

The expression level of eIF4E in treated A549 cells was also investigated and we demonstrate an increase of this protein in A549-treated cells Western blot analysis also revealed that only phosphorylated eIF4E was present in treated cells

Figure 3.2.1 Western Blot analysis of Keratin 8 protein in A549 cells following treatment with 10μM IdU for 7- and 14-days.

Figure 3.2.2 Western Blot analysis of Keratin 8 protein in A549 cells following treatment with 10μM CdU for 7- and 14-days.

Figure 3.2.3 Western Blot analysis of Keratin 8 protein in A549 cells following treatment with 10μM 5-FU for 7- and 14-days.

Figure 3.2.4 Western Blot analysis of Keratin 8 protein in A549 cells following treatment with 10μM 5,2`-FdU for 7- and 14-days.

Figure 3.2.5 Western Blot analysis of Keratin 8 protein in A549 cells following treatment with 10μM 5,5`-FdU for 7- and 14-days.

Figure 3.2.6 Western blot analysis for K8 expression in A549 cells treated with 10μM BrdU, 5-FU and 5,2`-FdU for 7-days.

Section 3.1.14 **Summary of the effects of treatment with pyrimidine analogues on eIF4E protein expression in A549 cells.**

A549 cells were treated with IdU, CdU and Bromouracil (Section 2.7) in order to investigate the effect these treatments had on the protein expression levels of eIF4E in the A549 cell line. Western blot analysis revealed an increase in the expression of phosphorylated eIF4E in the three drug treatments.

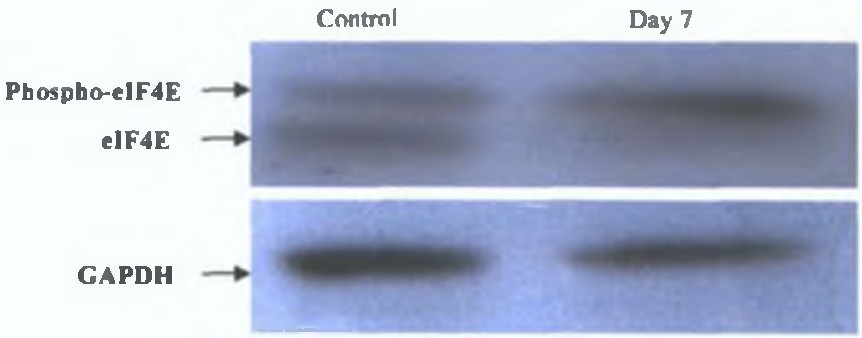


Figure 3.2.7 Western Blot analysis of eIF4E protein in A549 cells following treatment with 10μM IdU for 7 days.

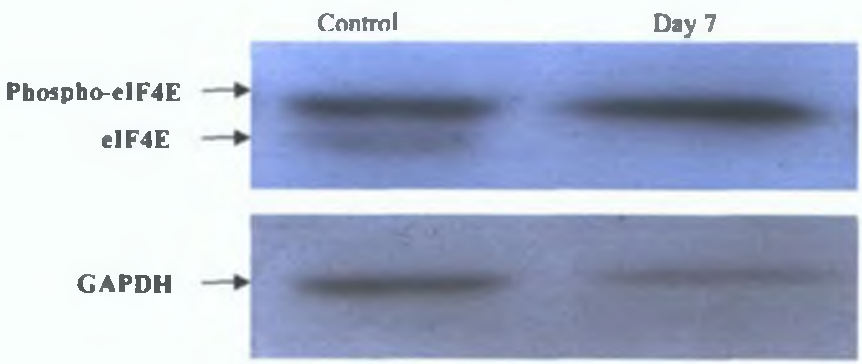


Figure 3.2.8 Western Blot analysis of eIF4E protein in A549 cells following treatment with 10μM CdU for 7 days.

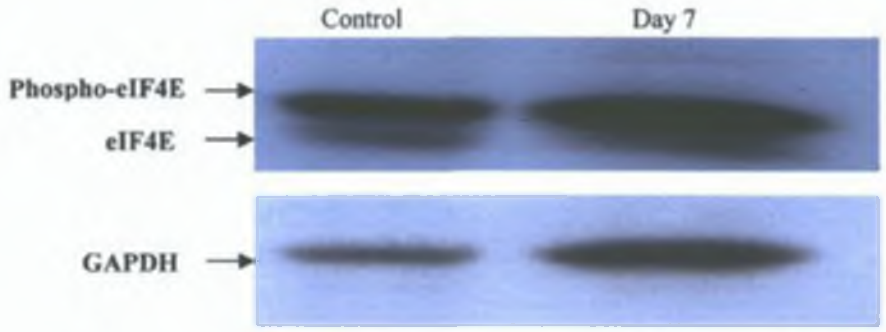


Figure 3.2.9 Western Blot analysis of eIF4E protein in A549 cells following treatment with 70μM BromoUracil for 7 days.

Section 3.1 15 Summary of the effects of 5-Bromo-2`-deoxyUridine (BrdU) on differentiation status of DLKP and A549 cell lines

The change of morphology in both cell lines following 7-days of treatment with 10μM BrdU was investigated Both cell lines an approximate 5- to 10-fold increase in cell surface area occurred, with most of the cells acquiring a stretched, flattened appearance

Immunocytochemical analysis for was conducted on treated DLKP and A549 cells to investigate if the observed changes m morphology were accompanied by changes in marker protein expression CK8, CK18 or CK19 were not detected by immunocytochemistry or western blot analysis m DLKP prior to treatment with BrdU, but following treatment all three cytokeratins were present in approximately 10% of the treated-DLKP cells Increased number of keratin-positive cells and increased intensity of staining was evident in DLKP cells treated for 7 days

CK8, CK18 and CK19 filaments were present in A549 cells prior to BrdU treatment Increased keratin synthesis was detected immunocytochemically following BrdU-treatment and this was further confirmed for CK8 by western blotting where an increase in the cytokeratin proteins where observed after 7-day and 14-day exposure

DLKP and A549 BrdU-treated cells were also examined immunocytochemically to determine any alterations in mtegrin expression Analysis revealed that appeared to be marked increases both α_2- and β_1-mtergrin subunits EpCAM expression levels were also up-regulated in both cell lines following BrdU-treatment

Section 3.1 16 Summary of the effects of 5-Iodo-2`-deoxyUridine (IdU) on the differentiation status of DLKP and A549

The ability of 5-Iodo-2 -deoxyUridine (IdU) to induce differentiation was also investigated DLKP and A549 cells treated with 10μM IdU for 7-days and investigated for morphological changes Both DLKP and A549 treated cells became very flattened and stretched The observed alteration in morphology after IdU treatment was comparable to the observed in BrdU treatments

Analysis of the selected marker proteins by immunocytochemistry revealed that cytokeratins 8, 18 and 19 were all increased in A549-treated cells and that they were induced in the DLKP cell line, when compared to the control untreated DLKP and A549 cells Western blot analysis was performed for CK8 in IdU-treated A549 cells exposed for 7- and 14-days All three cytokeratins were found to be increased after 7-days with a greater increase observed after 14-days by immunocytochemistry

Immunocytochemical analysis for α_2-, β_1-intergrin and EpCAM revealed that expression levels of these markers were increase following exposure to IdU for 7-days in both DLKP and A549 cell lines β_1-intergrin showed a greater increase than the other two markers

Section 3 1 17 Summary of the effects of 5-Chloro-2`-deoxyUridine (CdU) on the differentiation status of DLKP and A549

DLKP and A549 cells exposed to CdU for 7-days were investigated for morphological changes Treated DLKP and A549 cells became very flattened and stretched and an approximately 5- to 10-fold increase in cell surface area occurred The morphological changes seen were comparable to those noted in BrdU treatment of these cell lines

Analysis of the selected differentiation protein markers by immunocytochemistry revealed that cytokeratins 8, 18 and 19 were induced in DLKP and increased in A549 following CdU treatment Increases in the cytokeratin filaments were also observed in A549 treated cells Western blot analysis confirmed that A549 cells treated with CdU for 7- and 14-days that cytokeratins 8 was increased

DLKP and A549 BrdU-treated cells were also examined immunocytochemically to determine any alterations in integrin expression Analysis revealed that appeared to be marked increases both α_2- and β_1-intergrin subunits EpCAM expression levels were also up-regulated in both cell lines following CdU treatment

Section 3 1 18 Summary of the effects of 5-Fluro-deoxyUridine (5-FU) on the differentiation status of A549

Note the affect of 5-FU was not investigated in the differentiation status of DLKP

A549 cells exposed to 5-FU for 7-days were investigated for morphological changes Treated A549 cells became flattened and stretched The increase in cell surface area observed in BrdU treated cells did not occur to the same extent in 5-FU treated cells

Analysis of the selected differentiation protein markers by immunocytochemistry revealed that cytokeratins 8, 18 and 19 were noted A549 cells following treatment with 5-FU Western blot analysis confirmed that A549 cells treated with 5-FU for 7- and 14-days that cytokeratins 8 was increased

A549 5-FU-treated cells were also examined immunocytochemically to determine any alterations in integrin expression Analysis revealed that appeared to be marked increases both α_2- and β_1-intergrin subunits EpCAM expression levels were also up-regulated in both cell lines following 5-FU treatment

Section 3 1 19 Summary of the effects of 5-Fluro-2`-deoxyUridine (5,2`-FdU) on the differentiation status of DLKP and A549.

DLKP and A549 cells exposed to 5,2 -FdU for 7-days were investigated for morphological changes Treated DLKP and A549 cells became very flattened and stretched and an approximately 10-fold increase in cell surface area occurred A549 cells exhibited a much larger increase in cell surface area than seen in 5,2 -FdU treated cells The increase in cell surface area was much greater than that observed in BrdU treated DLKP and A549 cells

Analysis of the selected differentiation protein markers by immunocytochemistry revealed that cytokeratins 8, 18 and 19 were induced in DLKP following 5,2 -FdU treatment Increases in the cytokeratin filaments were also observed m A549 treated cells Western blot analysis confirmed that A549 cells treated with CdU for 7- and 14-days that cytokeratins 8 was increased

DLKP and A549 BrdU-treated cells were also examined immunocytochemically to determine any alterations in integrin expression Analysis revealed that appeared to be marked increases both α_2- and β_1-intergrin subunits EpCAM expression levels were also up-regulated in both cell lines following 5,2 -FdU treatment

Section 3.1 20 Summary of the effects of 5-Fluro-5`-deoxyUridine (5,5`-FdU) on the differentiation status of DLKP.

The ability of 5,5 -FdU to induce differentiation was also investigated DLKP cells treated with 10μM 5,5 -FdU for 7-days and investigated for morphological changes DLKP treated cells became very flattened and stretched The observed alteration in morphology after 5,5 -FdU treatment was comparable to the observed m BrdU treatments

Analysis of the selected differentiation protein markers by immunocytochemistry revealed that cytokeratins 8, 18 and 19 were induced in DLKP following 5,5 -FdU treatment 5,5 -FdU DLKP-treated cells were also examined immunocytochemically to determine any alterations in integrin expression Analysis revealed that appeared to be a moderate increase both α_2- and β_1-intergrin subunits EpCAM expression levels were also induced in DLKP following 5,5 -FdU treatment

Section 3 1 21 Summary of the effects of 5-BromorUridine and 5-BromoUracil on the differentiation status of DLKP and A549.

Toxicity profiles (results not shown) for 5-BromoUridine and BromoUracil in both DLKP and A549 cells revealed that it did not appear to be very toxic A concentration of 70μM was chosen for the differentiation studies Investigation of the morphological changes in DLKP and A549 cells treated with 5-BromoUridine and BromoUracil for 7-days did not reveal any obvious alterations in cell surface area The cells did not acquire a flattened and stretched appearance as was seen in some of the other analogue treatments

Immunocytochemical analysis of DLKP cells treated 5-BromoUridine and BromoUracil for 7-days appeared to show very little induction of the CK8, CK18 and CK19 Assessment of immunocytochemistry of the results revealed no major change in the expression level of α_2-, β_1-integrins or EpCAM

Section 3.2 DNA Microarrays

Section 3.2.1 DNA Microarrays

The success of the human genome project has allowed biologists to identify almost all the genes that are responsible for the genetic makeup of humans. The next important task is to assign function to the nearly 40,000 genes sequenced. The use of microarrays to analyse the gene expression on a global level has recently received a great deal of attention. In order to understand the complex mechanisms and networks involved in the control processes of normal and disease states of eukaryotic cells, it is no longer enough to focus on isolated pathways or single genetic events. Global transcriptional profiling using microarray techniques now offer the chance to develop a more complete understanding of gene function, regulation and interactions. The technique of microarray analysis has the huge advantage over other gene profiling methods in that mRNA isolated from a given cell, tissue or tumour can be used to prepare a labelled sample and hybridised simultaneously to a vast number of DNA sequences. Microarrays now offer great flexibility in the number of target sequences that maybe analysed per microarray. As few as a couple of probe sequences to as many as the nearly 40,000 genes of the human genome can now be analysed on one single microarray slide.

A wide variety of different microarrays platforms have been developed by both academic and commercial companies. The two main groups of microarrays currently in mainstream use; these are complementary DNA (cDNA) and oligonucleotides. Probes for cDNA arrays are generally printed onto glass or nylon slides at exact locations. The probes for cDNA (spotted) microarrays are prepared from products of the polymerase chain reaction (PCR) generated from cDNA libraries. Oligonucleotide microarrays on the other hand, are mostly 20-100mers which are synthesised *in situ*, either by photolithography onto silicon wafers or by ink-jet technology. One advantage oligonucleotide microarrays have over cDNA microarrays is the fact that the sequence used for the oligos can be designed to the most unique part of a given transcript, making it possible to discriminate between closely related genes or splice variants. The microarray gene expression studies which were performed in this body of work were

performed using Affymetrix® Human Genome U133A GeneChips® (Section 2 15)

Section 3 2 2 BrdU Array

Three separate DNA microarray experiments were performed on DLKP cells treated with BrdU over a time course These experiments have been labelled Exp 1, 2 and 3 for simplicity The initial experiments, Exp 1 and Exp 2, were preformed at the same time, therefore, the results from these two experiments will be discussed together Also, these initial microarray experiments were performed using one biological sample from each time point, whereas, the third experiment, Exp 3, was performed in triplicate using biological replicates And the results of Exp 3 will be discussed in a separate subsection

Section 3 2 2 1 BrdU Microarrays - Exp 1 and Exp 2

In the preliminary two BrdU-treated DLKP microarray experiments, Exp 1 and Exp 2, hybridisations were performed using the Affymetrix U133A chips (Section 2 15) Cells were harvested and RNA isolated (as per Section 2 10) at the following time points of 0, 1, 3, 7 and 14 days in Exp 1 and 0, 1 and 7 days for Exp 2 These microarray experiments were performed using single samples from each time point cRNA was prepared (Section 2 15 1) from total RNA which had been checked for quality, and shipped to the Conway Institute at University College Dublin, where it was hybridised to the Affymetrix® Human Genome U133A GeneChips®, and the chips scanned using the Affymetrix Chip scanner This data was then sent back to us

The resulting data sets were scaled and normalised as per analysis carried out by Rushton, J J , *et al* , (2003) In order to compare gene expression results in the microarray experiments performed, it was necessary to normalise the data obtained following scanning the microarray GeneChips There are two main ways in which this type of normalisation is performed 'Per-chip' and 'Per-gene' normalisations (Reviewed in Section 2 15 1) This was performed using

Affymetrix Microarrays Suite v5 0 software, which scales all of the probe sets so that the average is 100 for each GeneChip The average probe signal was calculated for each GeneChip and this was adjusted to 100 by multiplying by a scaling factor By doing this for each GeneChip it makes the data easier to compare across different GeneChips in a given experiment

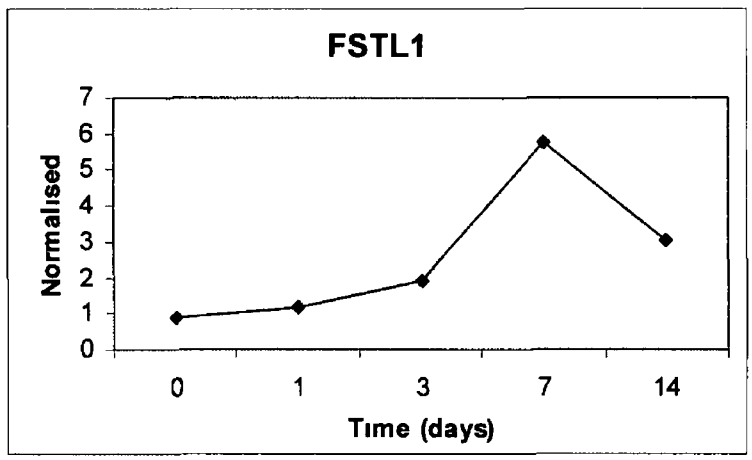
Additional data analysis was preformed using GeneSpring (Silicon Genetics) The data were normalised to the mean of the day 0 samples and then filtering was used to identify genes that were consistently up- or down-regulated in the day 1, 3, 7 or 14 day samples The filter used was a 1 5 fold increase or decrease and the genes were also filtered using a 'Present' or 'Marginal' filter A filter of 1 5-fold up or down was selected in these microarray experiments since it is generally thought that the expression levels of transcription factors do not alter dramatically in cells It is thought that the control of gene expression is tightly controlled by small alterations in transcription factor expression levels Gene lists were then generated for each of the two rounds of microarrays containing the gene name, accession number, Affymetrix Probe Id number and the fold increase/decrease of each gene found to be differentially expressed

Section 3.2.2.2 Validation of Initial DNA Microarrays and Gene expression changes in BrdU-treated DLKP cells

In order to validate the gene lists which were generated from the expression analysis performed using the GeneSpring analysis software package, and to demonstrate that the fold increase/decrease observed were real expression changes, a set of genes was selected from the gene lists generated RT-PCR analysis was performed on these genes In the majority of cases the results confirmed the trends observed in the microarray analysis The following section contains the result for each of the selected genes In each subsection a graph illustrating the fold increase/decrease found following microarray analysis, along with the corresponding RT-PCR result, is shown

Section 3 2 2 2 1 FSTL1

FSTL1 is protein with similarity to follistatin, an activin-binding protein It is known to contain an FS module, a follistatin-like sequence containing 10 conserved cysteine residues FST1 is thought to be an autoantigen associated with rheumatoid arthritis Following gene expression analysis, using GeneSpring analysis software, the FSTL1 gene was demonstrated to increase in BrdU-treated DLKP cells It was noted, that after three days of exposure of the cells to BrdU, the level of expression of the gene started to increase to a maximum at 7-days A decrease in FSTL1 was observed between day 7 and day 14 samples This result was confirmed by RT-PCR analysis (Section 2 11), which showed a similar pattern of expression for the FLST1 gene



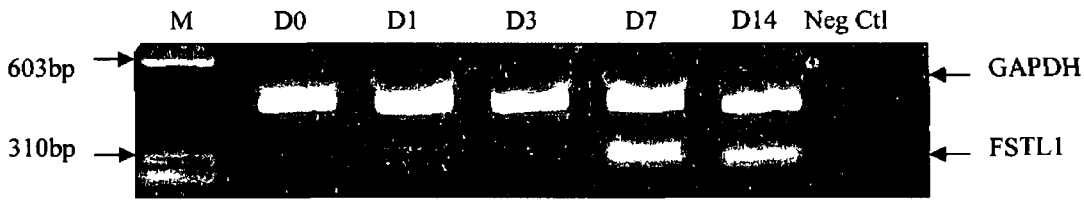


Figure 3 2.1 Microarray analysis (top image) showing fold increase of FSTL1 mRNA RT-RCR analysis (lower image) confirming an increase in FSTL1 mRNA expression levels

Section 3.2.2.2 2 FLH2

FHL2 belongs to the family of LIM proteins, which are known involved in protein-protein interaction and transcriptional regulation Recent evidence has suggested that FHL proteins may act as co-regulators involved in the modulation of tissue-specific gene expression by interacting with different transcription factors Such transcription factors include JUN and FOS and these associations have been shown to result in a powerful activation of AP-1-mediated transcription Following gene expression analysis, an increase in the expression of FHL2 mRNA was observed in BrdU-treated DLKP cells mRNA levels were to dramatically increase at day 7 to approximately 6 5-fold at 7days However, this increase was not maintained at day 14 where they were reduced to 4-fold higher than control cells This result was confirmed by RT-PCR analysis, which showed a similar pattern of expression for the FHL2 gene

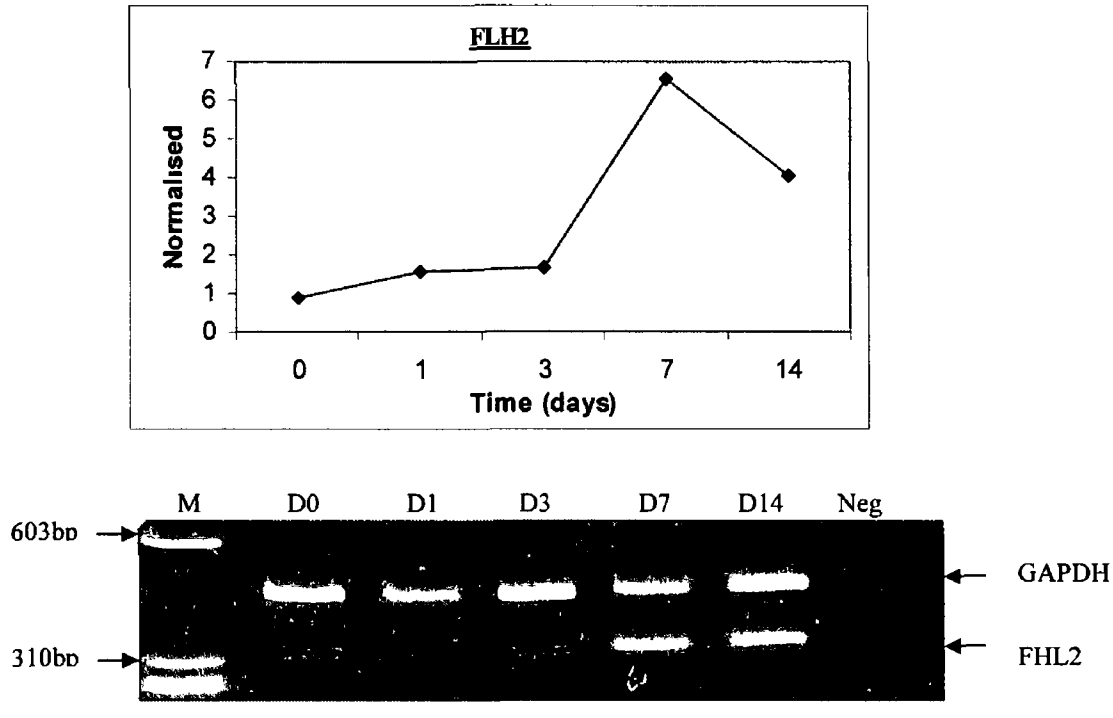


Figure 3.2 2 Microarray analysis (top image) showing fold increase of FHL2 mRNA RT-RCR analysis (lower image) confirming an increase in FHL2 mRNA expression levels

Section 3 2.2.2.3 TNFSF7

TNFSF7 belongs to the tumour necrosis factor (TNF) ligand family and this cytokine has been reported to a play a role in the regulation of B-cell activation and IgG production Microarray analysis revealed an increase in TNFSF7 transcript levels in drug-treated cells It was found that after one day of exposure of the cells to BrdU that the level of expression of TNFSF7 mRNA increased gradually over the course of the experiment, to approximately 6 5-fold at day 14 This result was confirmed by RT-PCR analysis, which showed a similar pattern of expression for the TNFSSF7 gene

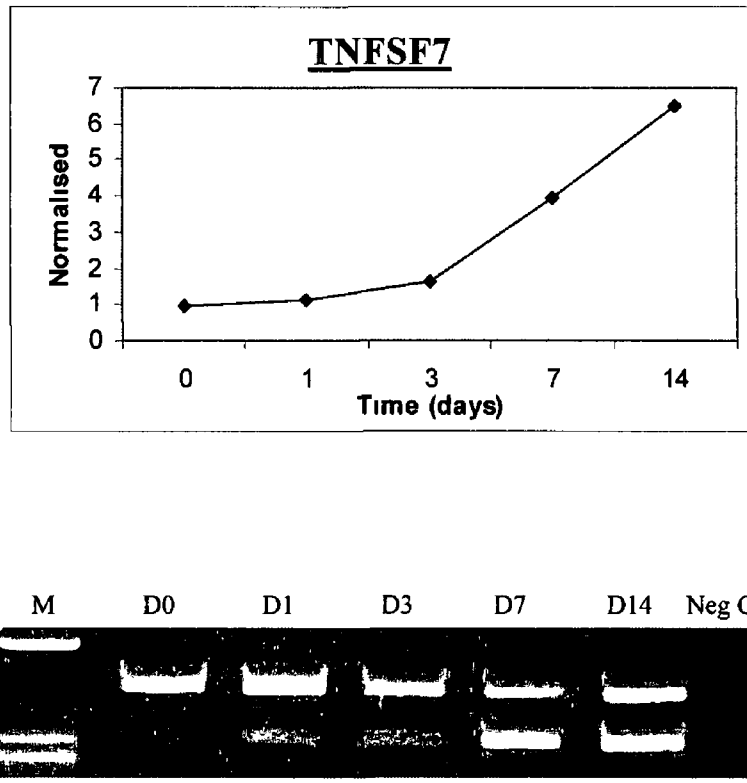


Figure 3 2 3 Gene expression analysis (top image) showing fold increase of TNFSF7 mRNA RT-RCR analysis (lower image) confirming an increase in TNFSF7 mRNA expression levels

Section 3.2.2 2 4 GPX3

Microarray analysis revealed that an increase in GPX3 mRNA expression levels following exposure to DLKP to BrdU It was demonstrated at day 1 the level of GPX3 mRNA levels increased by 4-fold to a maximum of approximately 7-fold after 3-days of exposure to the drug A decrease in GPX3 mRNA was observed at day 7 and day 14 where mRNA levels had reduced to 5- and 3-fold, respectively These results was confirmed by RT-PCR analysis, which showed a similar pattern of expression for the GPX3 mRNA expression levels

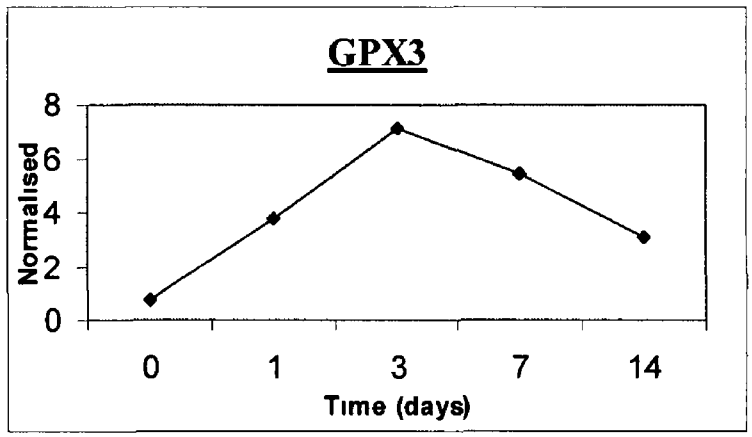


Figure 3 3 4 Gene expression analysis (top image) showing fold increase of GPX3 mRNA RT-RCR analysis (lower image) confirming an increase in GPX3 mRNA expression levels

Section 3 2 2 2 5 Zyxin

Zyxin is a low abundance phosphoprotein that is concentrated at adhesion plaques and along the actin filament bundles near where they insert at adhesion plaques Zyxin has features of an intracellular signal transducer and may function as a scaffold for the assembly of multimeric complexes These protein assemblages could mediate integrin-dependent signalling events that lead to cell differentiation or modulation of cyto-architecture Microarray analysis revealed that an increase in Zyxin transcript levels in BrdU-treated DLKP cells A 3-fold increase in Zyxin mRNA was observed in the day 1 sample and this increase was maintained up to day 7, where mRNA levels had to a maximum of approximately 3 5-fold At day 14 a decrease in Zyxin mRNA expression levels was observed, but did not return to control levels This result was confirmed by RT-PCR analysis, which showed a similar pattern of expression for the Zyxin transcript levels

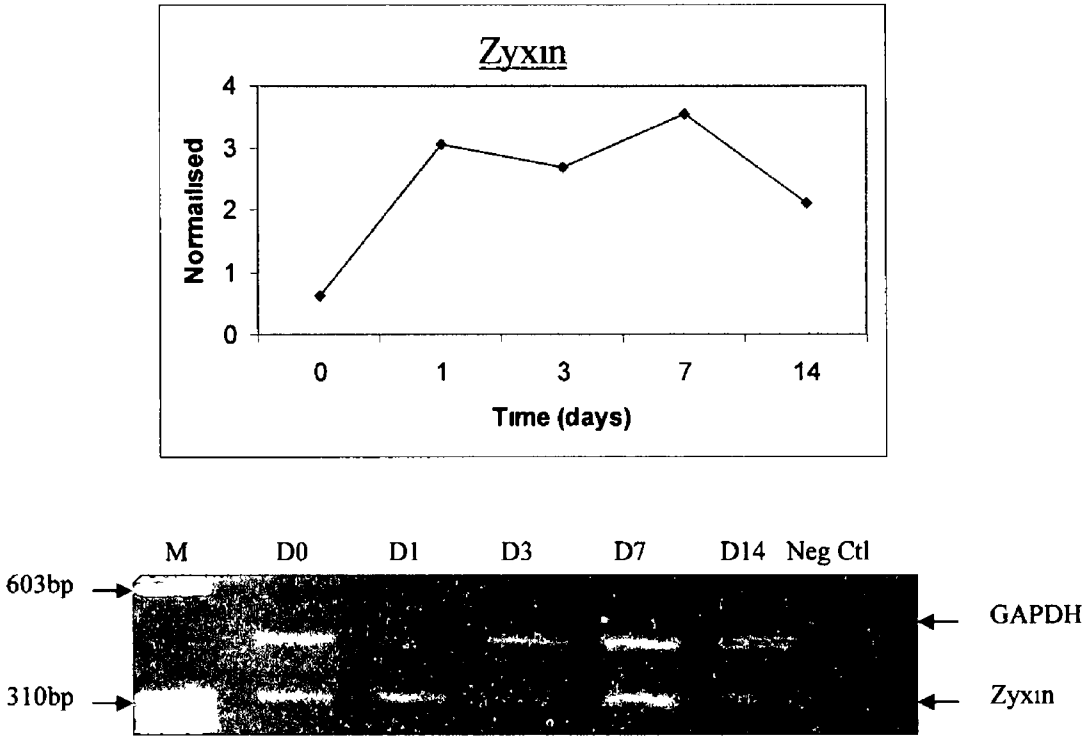


Figure 3 2 5 Gene expression analysis (top image) showing fold increase of Zyxin mRNA RT-RCR analysis (lower image) confirming an increase in Zyxin mRNA expression levels

Section 3.2.2.2.6 Spermidine/spermine N-acetytransferase (Spd/Spn)

Spermidine/spermine N-acetytransferase is a highly regulated enzymatic protein is known induced by a variety of toxic agents, hormones and polyamines. It is also in belongs to a metabolic pathway which involves ornithine decarboxylase and S-adenosylmethionione decarboxylase. The combination of these enzymes fine tune intracellular polyamine concentration, underscoring the important role of these proteins in growth and cell survival. Following microarray analysis, Spd/Spn mRNA levels found to remain at a similar expression level as controls cells up to three days of exposure to BrdU. However, microarray analysis revealed a 4.5-fold increase in Spd/Spn mRNA levels following 7 days exposure to the drug, but this increase in transcript levels was reduced to approximately 3-fold at day 14. RT-PCR analysis confirmed the results generated following microarray expression analysis using GeneSpring software.

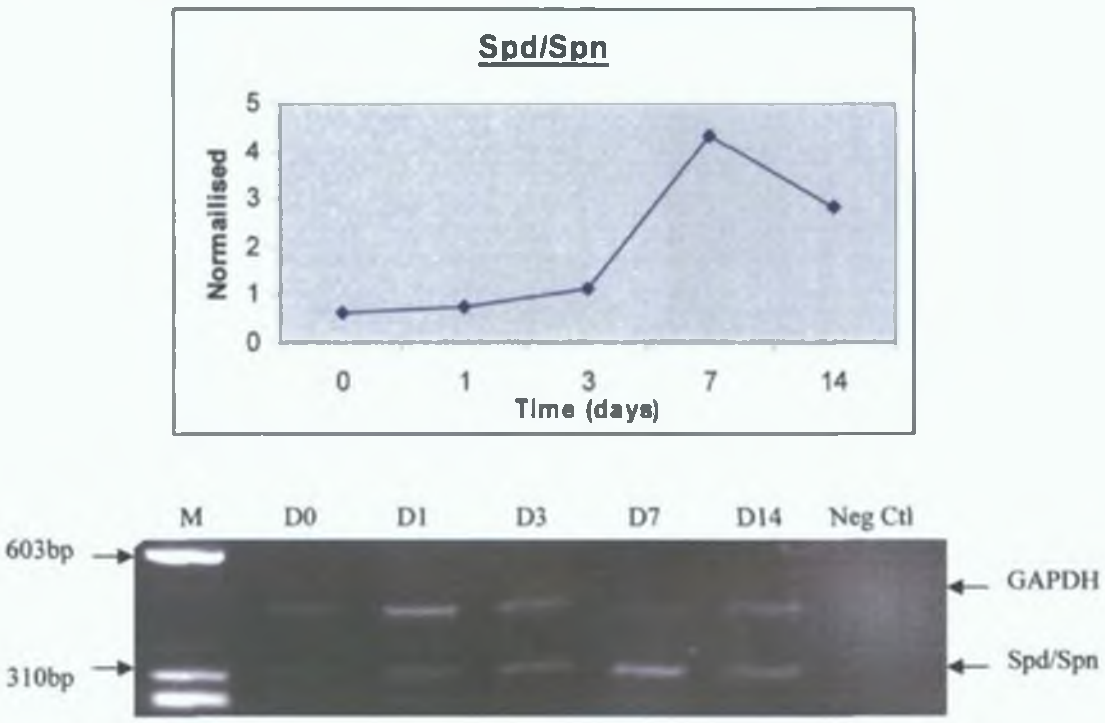


Figure 3.2.6 Gene expression analysis (top image) showing fold increase of Spd/Spn mRNA. RT-RCR analysis (lower image) confirming an increase in Spd/Spn mRNA expression levels.

Section 3.2 2 2.7 Id2

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and they are now thought to be important in development, cell cycle control and tumour biology Microarray analysis revealed that Id2 transcript levels were increased to approximately 5 5-fold following exposure of cells to BrdU for 24 hours However, over the remainder of the experiment Id2 mRNA levels declined to 2 5-fold day 7 and day 7 By day 14 transcript levels had returned to those of control cells A similar pattern of expression was demonstrated following RT-PCR analysis for Id2 in BrdU-treated cells

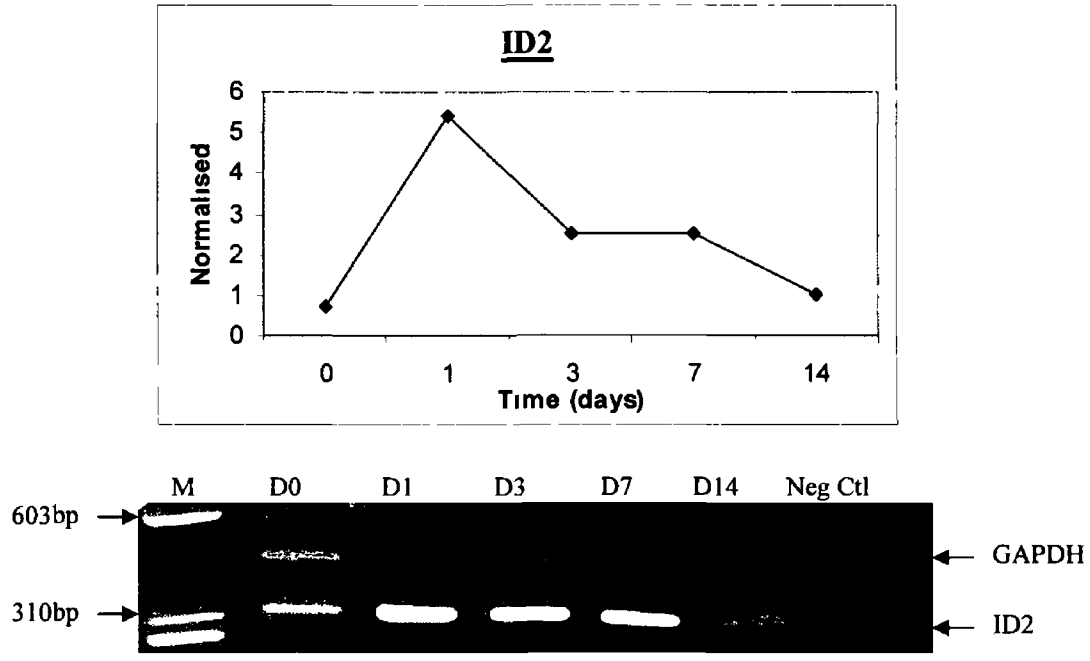
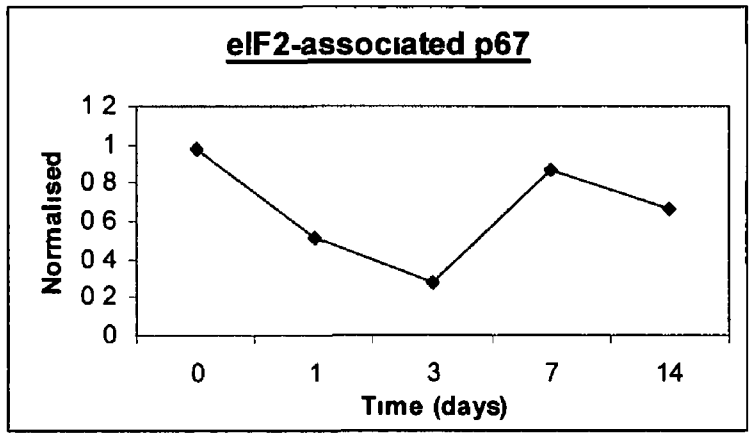


Figure 3 2 7 Gene expression analysis (top image) showing fold increase of Id2 mRNA RT-RCR analysis (lower image) confirming an increase in Id2 mRNA expression levels

Section 3 2 2 2.8 eIF2-associated p67

Initiation of protein synthesis plays a central role in gene expression and is largely regulated at the level of formation of the initiation complex with the eukaryotic initiation factor 2 (eIF2), Met-tRNA, and the 40S ribosome The rate of protein synthesis is regulated at the level of phosphorylation of the α-subunit of eIF2, which is controlled by the cellular glycoprotein, p67 p67 protects the α-subumt from phosphorylation by its kinases Following microarray expression analysis, eIF2-associated p67 mRNA expression levels were shown to decrease at day 1 and day 3 in BrdU-treated DLKP cells and to increase to near control levels at the day 7 However, a decrease at day 14 decrease marginally was observed RT-PCR analysis demonstrated a small decrease in eIF2-associated p67 mRNA in the day 3 sample, with the remaining time points exhibiting the same level of expression as control cells



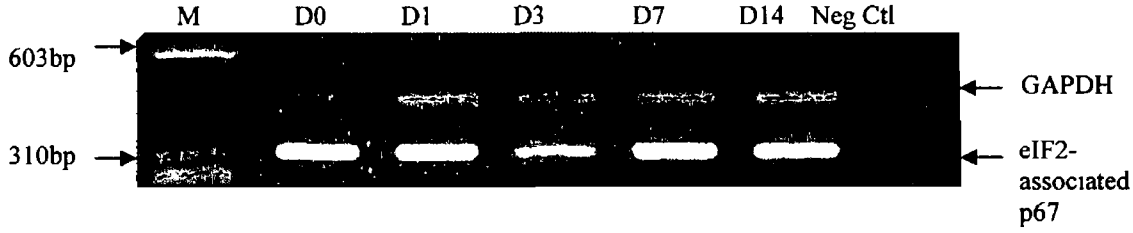
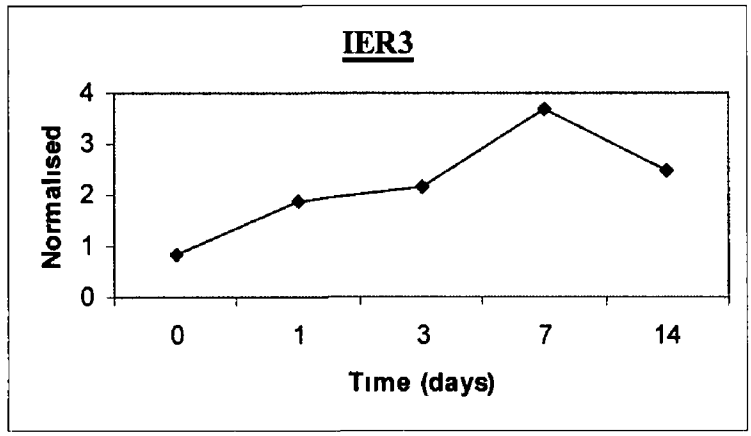


Figure 3 2 8 Gene expression analysis (top image) showing fold increase of eIF2-associated p67 mRNA RT-RCR analysis (lower image) confirming an increase in eIF2-associated p67 mRNA expression levels

Section 3 2 2 2 9 Immediate-early Response factor 3 (IER3)

IER3 is a member of intermediate-early gene family of proteins that are thought to be critical for the control of cell proliferation and apoptosis in several cell types Following gene expression analysis, using GeneSpring analysis software, the IER3 gene was shown to increase in BrdU-treated DLKP cells It was found that after 1 day of exposure of the cells to drug that the mRNA levels of IER3 had increased to approximately 2-fold and increased further to 3 5-fold at day 7 At day 14 a reduction in transcript levels was observed, but did not return to that of control levels RT-PCR analysis confirmed the microarray expression results obtained following GeneSpring analysis, however a increase in IER3 mRNA levels was demonstrated across all samples and no reduction m IER3 signal was found at day 14



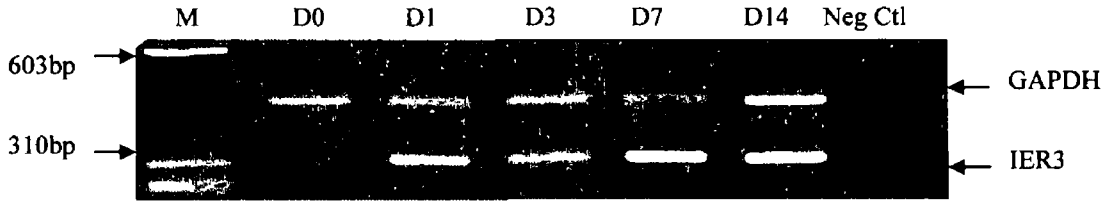


Figure 3 2 9 Gene expression analysis (top image) showing fold increase of IER3 mRNA RT-RCR analysis (lower image) confirming an increase in IER3 mRNA expression levels

Section 3 2.2.2.10 LOXL2

A family of four proteins know as LOXL (LOXL 1-4) were recently identified and it has been suggested that these proteins are located in distinct intracellular and intranuclear locations, each with related but different functions that include cell growth control, tumour suppression, senescence and chemotaxis

Microarray analysis revealed an increase in LOXL2 transcript levels in drug treated DLKP cells It was demonstrated that after 3 days of exposure of DLKP cells to BrdU mRNA expression levels of LOXL2 remained at control levels up to day 3, after which they were observed to increase to approximately 4-fold However, at day 14 mRNA levels were reduced marginally to 3 5-fold This result was confirmed by RT-PCR analysis, which demonstrated that LOXL2 transcript levels increased over the course of the experiment

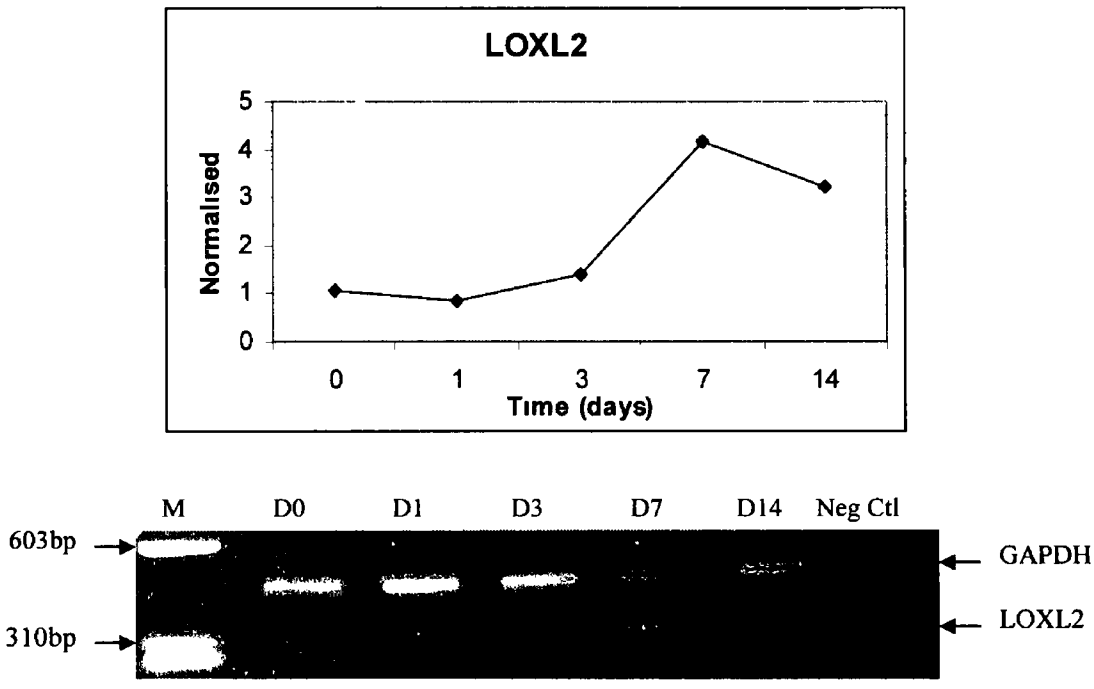


Figure 3 2.10 Gene expression analysis (top image) showing fold increase of LOXL2 mRNA RT-RCR analysis (lower image) confirming an increase in LOXL2 mRNA expression levels

Section 3 2.2.2 11 p21

Following microarray expression analysis mRNA expression levels of CDKN1A were found to increase in drug-treated DLKP cells Gene expression and RT-PCR analysis revealed a marked increase in CDKN1A mRNA expression levels after 24 hours of exposure of cells to BrdU and this increase was maintained over the experiment

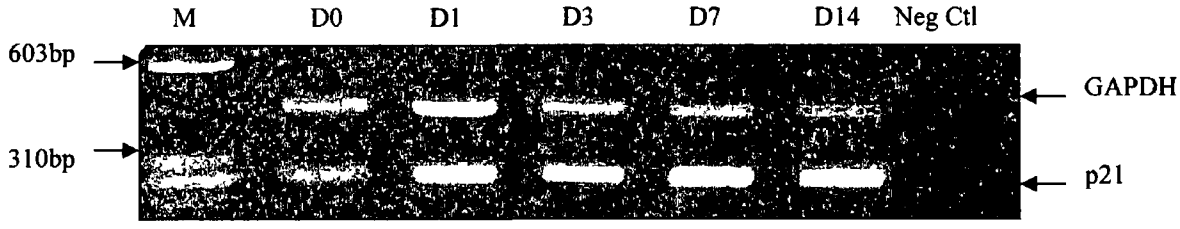


Figure 3 2 11 RT-RCR analysis (bottom image) shows the level of CDKN1A mRNA following exposure of DLKP to BrdU

Section 3 2 2.3 DLKP BrdU Array – Exp 3

The third DLKP BrdU microarray experiment, Exp 3, was performed using Affymetrix U133A GeneChips® RNA was isolated (Section 2 10) from cells harvested after 0, 3 and 7 days exposure to BrdU (Section 2 7) This set of DNA microarrays were performed in triplicate using biological replicates The isolated RNA was shipped to Affymetrix where the cRNA was prepared and hybridised to U133A GeneChips® and the chips scanned using an Affymetrix GeneChip scanner The data was received back, and expression analysis was performed as described below

The data set was normalised using the Affymetrix PLIER (Probe Logarithmic Error intensity Estimate) method in GREX This normalisation method is recommended by Affymetrix for use on their GeneChips™ The PLIER method (Reviewed in Section 2 15 3) produces an improved signal by accounting for experimentally observed patterns in probe behaviour and handling error at the appropriately low and high signal values Some of the advantages of using this method of normalisation included higher reproducibility of signal (lower coefficient of variation) without loss of accuracy and higher differential sensitivity for low expressors

Having subjected the data set to the PLIER normalisation method, the data was then imported into GeneSpring analysis software (Silicon Genetics) and a new experiment was set up As a result of using the PLIER method of normalisation, the data did not need to undergo 'Per Chip' normalisation in GeneSpring In GeneSpring the samples were then normalised to the mean of the day 0 samples

A filter was then applied to the data to select genes that had a 'Present' or 'Marginal' flag in three out of the nine samples The filter also contained a condition that selected for genes that crossed a 1 5 fold threshold, up or down, in either the day 3 or day 7 samples

Section 3 2 2.4 Validation of the DLKP-BrdU Exp 3 Microarrays

In order to validate the gene lists, which were generated from the expression analysis carried out using GeneSpring, and to demonstrate that the fold increase/decrease observed were actual real expression changes, a set of genes were selected from the gene lists generated RT-PCR analysis was performed on these genes The expression patterns which confirmed the results that were obtained from the chip expression analysis

The following section contains the result for each of the genes selected In each subsection a graph illustrating the fold increase/decrease found following expression analysis, along with the confirmation RT-PCR result, is shown

Section 3 2 2.4 1 Spd/Spn

Spermidine/spermine N-acetytransferase is a highly regulated enzymatic protein is known to be inducible by a variety of toxic agents, hormones and polyamines It is also in belongs to a metabolic pathway which involves ornithine decarboxylase and S-adenosylmethiomone decarboxylase The combination of these enzymes fine tune intracellular polyamine concentration, underscoring the important role of these proteins in growth and cell survival Microarray analysis revealed an increase in Spd/Spn mRNA expression levels following treatment of DLKP cell with BrdU Spd/Spn transcript levels were shown to increase at day three to 1 8-fold and to 2 8-fold at day 7 RT-PCR analysis demonstrated an increase of several fold in Spd/Spn mRNA expression levels following three days exposure of DLKP to BrdU A slight reduction in Spd/Spn mRNA was observed in the day 7 sample in comparison to the day 3, however, Spd/Spn mRNA levels were still several fold increased when compared to control untreated cells

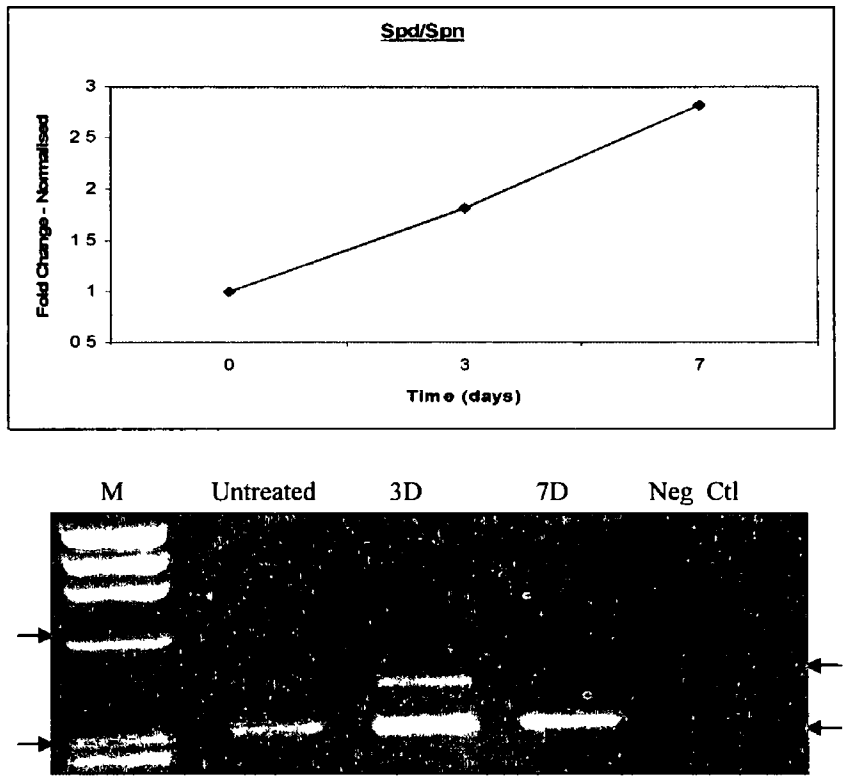


Figure 3.2 12 The top graph illustrates the fold change in Spd/Spn mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of Spd/Spn mRNA following exposure of DLKP to

Section 3.2.2.4.2 HMOX1

HMOX1 is one of two isoforms of heme oxygenase that is involved in catabolizing heme to biliverdin, carbon monoxide and free iron and is thought to be involved in iron homeostasis Following gene expression analysis, HMOX1 mRNA levels were found to increase by approximately 2-fold in the day 3 sample and the expression level of this gene was maintained at this level in the day 7 sample This result was confirmed by RT-PCR analysis which demonstrated a significant increase in HMOX1 mRNA expression levels across the experiment in comparison to untreated control cells

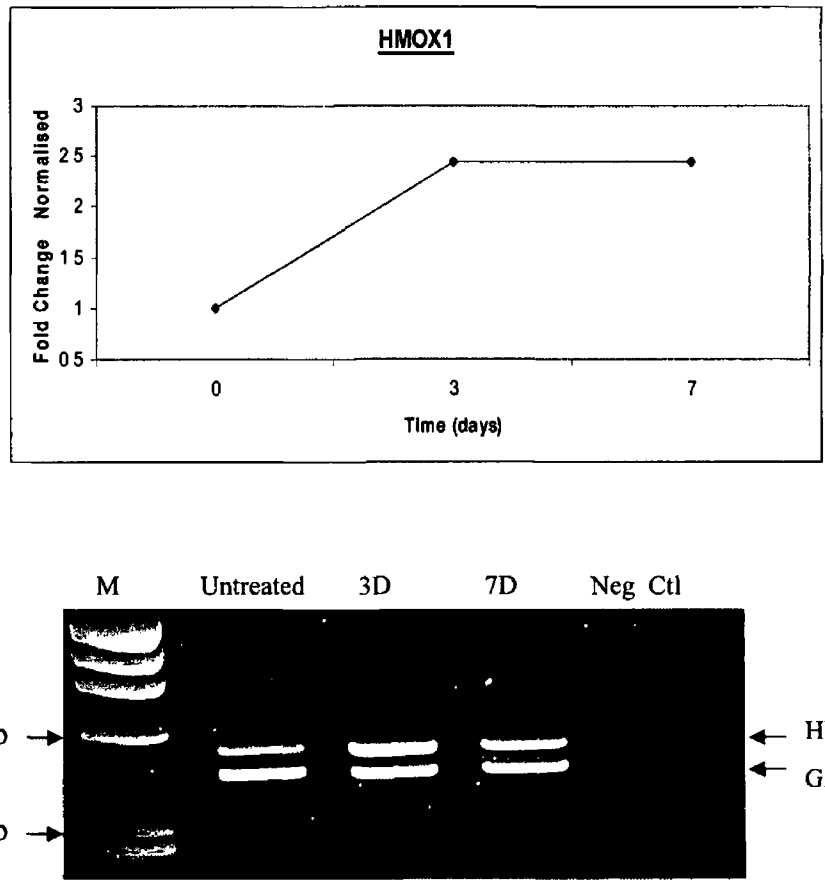


Figure 3.2.13 The top graph illustrates the fold change in HMOX1 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of HMOX1 mRNA following exposure of DLKP to BrdU

Section 3.2.2.4 3 Id2

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and are now thought to be important in development, cell cycle control and tumour biology Microarray analysis revealed a 4-fold increase in Id2 mRNA transcript levels At day 7 Id2 expression levels were found to decrease, but still remained approximately 2-fold higher than control cells These results were confirmed by RT-PCR analysis which demonstrated a significant increase in Id2 mRNA levels in the day 3 and day 7 samples, with the day 7 samples slowing a reduction in Id2 signal in comparison to the day 3 sample

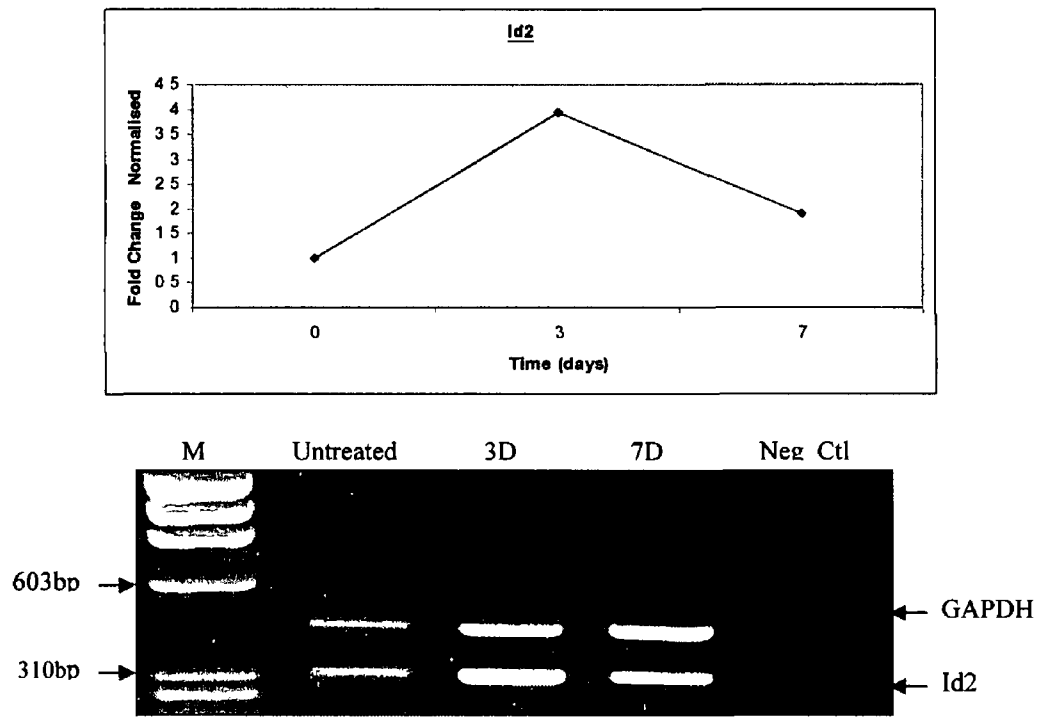


Figure 3.2.14 The top graph illustrates the fold change in Id2 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of Id2 mRNA following exposure of DLKP to BrdU

Section 3 2 2 4 4 Id3

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and are now thought to be important in development, cell cycle control and tumour biology Microarray analysis revealed a dramatic increase in Id3 mRNA transcript levels following exposure of DLKP cells to BrdU A 7 5- and 5 5-fold increase in Id3 mRNA expression level was observed m the day 3 and day 7 samples, respectively RT-PCR analysis demonstrated a similar pattern of expression for Id3

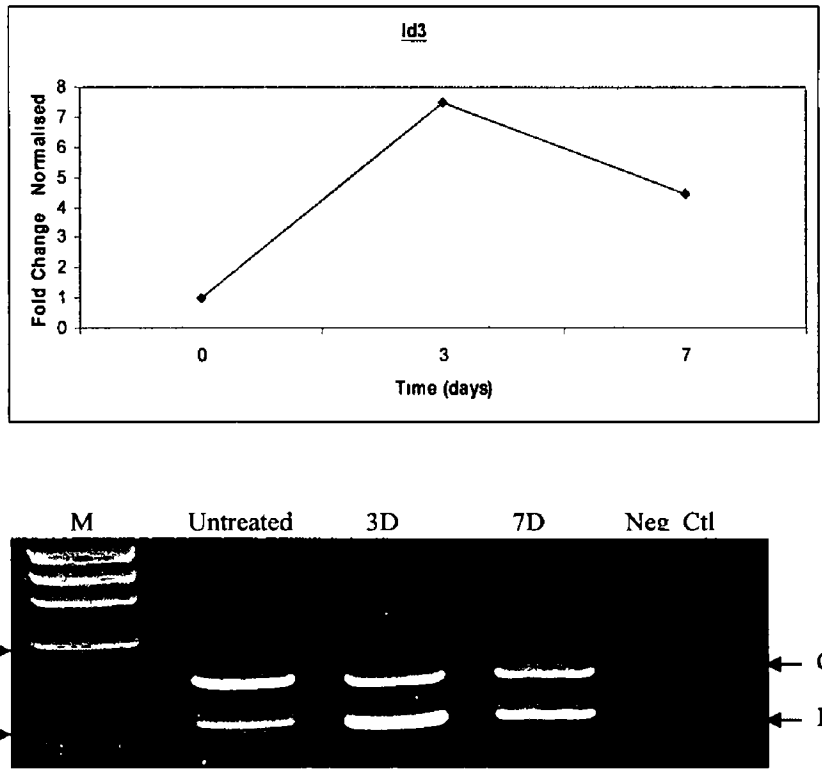


Figure 3 2.15 The top graph illustrates the fold change in Id3 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of Id3 mRNA following exposure of DLKP to BrdU

Section 3 2 2 4 5 FSTL1

FSTL1 encodes a protein with similarity to follistatin, an activin-bindmg protein It contains an FS module, a follistatin-like sequence containing 10 conserved cysteine residues This gene product is thought to be an autoantigen associated with rheumatoid arthritis Following microarray analysis using GeneSpring analysis software, FSTL1 mRNA expression levels were revealed to increase several fold after exposure to BrdU Following three days treatment of DLKP with drug FSTL1 mRNA were shown to increase by 1 5-fold and increased further to approximately 3-fold by day 7 RT-PCR analysis (Section 2 11) confirmed the trend in FSTL1 mRNA expression observed in the microarray analysis

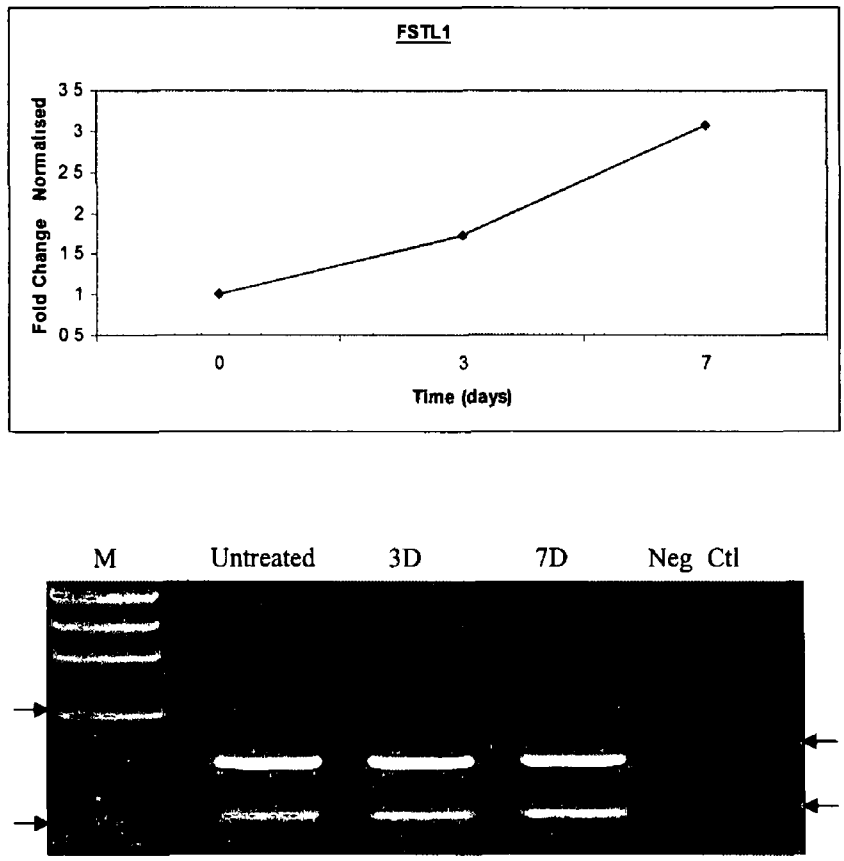


Figure 3 2 16 The top graph illustrates the fold change in FSTL1 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of FSTL1 mRNA following exposure of DLKP to BrdU

Section 3 2 2 4 6 FHL2

FHL2 belongs to the family of LIM proteins, which are involved in protein-protein interaction and transcriptional regulation Recent evidence has suggests that FHL proteins may act as co-regulators involved in the modulation of tissue-specific gene expression by interacting with different transcription factors Such transcription factors include JUN and FOS and these associations have been shown to result in a powerful activation of AP-1-mediated transcription Microarray analysis revealed an increase in FHL2 transcript levels in drug-treated DLKP cells A 3-fold increase in FHL2 mRNA expression levels was observed following three days exposure of cells to BrdU and expression levels of the gene increase further to 5 5-fold in the day 7 sample RT-PCR analysis demonstrated a similar increase in FHL2 transcript levels as was observed in the microarray analysis

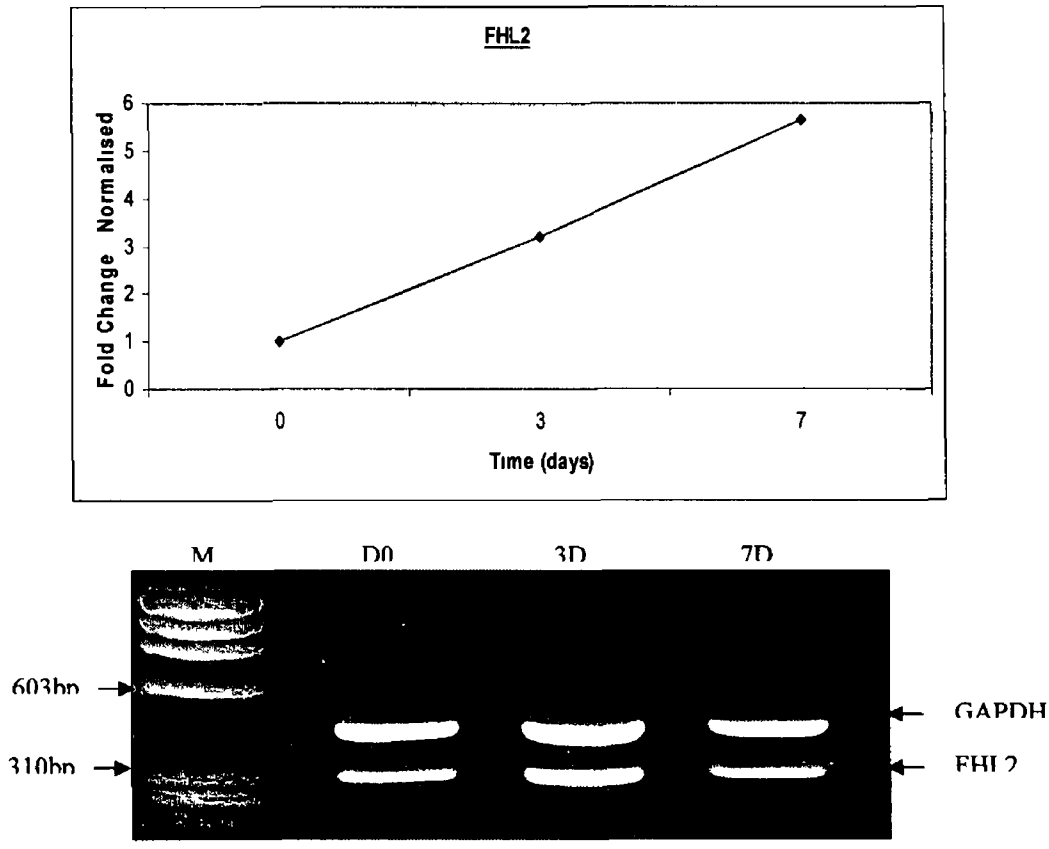
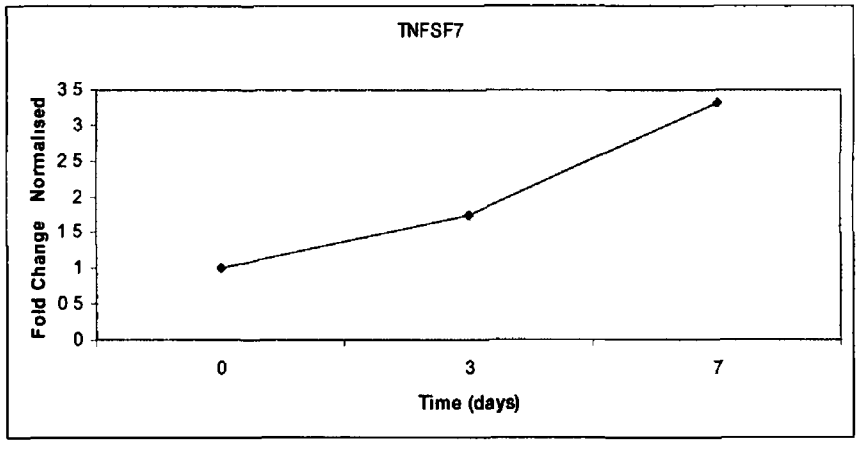


Figure 3 2 17 The top graph illustrates the fold change in FHL2 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of FHL2 mRNA following exposure of DLKP to BrdU

Section 3 2 2.4 7 TNFSF7

TNFSF7 belongs to the tumour necrosis factor (TNF) ligand family and this cytokine has been reported to a play a role in the regulation of B-cell activation and IgG production Following microarray analysis, using GeneSpring analysis software, it was observed that the mRNA expression levels of TNFSF7 increase by approximately 1 5- and 3 3-fold in the day 3 and day 7 samples, respectively This results was confirmed by RT-PCR analysis which demonstrated a similar increase in TNFSF7 transcript levels



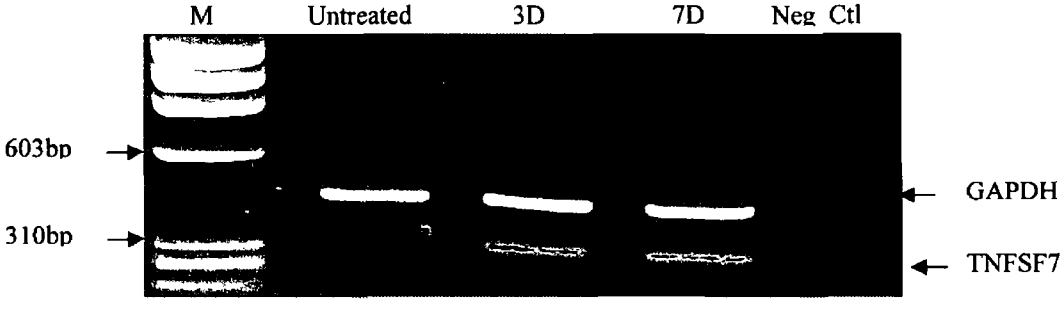


Figure 3.2 18 The top graph illustrates the fold change in TNFSF7 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of TNFSF7 mRNA following exposure of DLKP to BrdU

Section 3.2.2.5 Investigation of potentially co-regulated genes in BrdU-treated DLKP cells as identified using DNA microarrays

Time course microarray experiments reveal information about the temporal transcription profile of spotted genes. Sets of genes with the same expression pattern can be grouped into clusters but the identification of molecular mechanisms responsible for co-expression requires further investigation. By using comparative promoter analysis it is possible to identify genes for which co-expression may be a result of co-regulation. The aim of this analysis is to find promoter features that may be potentially responsible for co-expression of differentiation related genes.

DLKP cells, exposed to the differentiation-modulating agent BrdU, undergo morphological change and the several epithelial markers of lung cell differentiations are induced. In order to analyse global changes the transcriptional profiles of BrdU-treated DLKP cells were examined using Affymetrix UA133 DNA microarrays. Following DNA microarray analysis the data generated was normalised and filtered (Section 2.15.2) and gene lists for up- and down-regulated differentially expressed genes were then generated.

Section 3.2.2.5.1 Gene Clustering

Effective comparative promoter analysis requires tightly clustered gene expression profiles. To generate tight clusters the gene lists were subjected to ANOVA analysis and the data from this microarray experiment was grouped into 13 clusters (Figure 3.2.19). In theory from analysis of clustered data sets it is possible to identify genes that are co-regulated and promoter models that are involved in the regulation of these clustered genes. One software package which is currently available for this type of analysis is the Genomatix Software Suite (www.genomatix.de).

Clustering algorithms may be performed on microarray data sets to help identify genes that have similar patterns of gene expression and the results can be easily visualised. Hierarchical clustering has become one of the most widely used

techniques for the analysis of gene expression data Clustering is an agglomerative approach in which single expression profiles are joined together until the process had been carried to completion Simplified, hierarchical clustering works on the basis where a pair of genes that have the most similar expression pattern are found, these two genes are joined together and then the method identifies the next most similar pair of genes This process continues until all of the genes are joined into one large cluster Clustering of microarray data may only be carried out on data sets that have been normalised and there are several different variations on hierarchical clustering

An alternative to hierarchical cluster is k-means clustering In k-means clustering objects are partitioned into a fixed number of clusters This process may be computationally intensive K-means clustering is particularly useful when used with other techniques such as principal component analysis Principal component analysis allows visual estimation of the number of clusters represented by the data

The hypothesis behind clustering techniques is that genes in a cluster may share some common function or regulatory elements However, classifications based on clustering algorithms are dependent on the particular methods used, the manner in which the data are normalised, and the manner in which similarity is measured All of these factors can have a huge effect on the outcome of clustering analysis Therefore, no single method of clustering is more or less appropriate to use on a given data set Furthermore, use of one of more methods of clustering may in fact highlight differentiation relationships within a data set which consequently may be found to be important Tools and techniques for analysis data sets are under continual development and the ultimate guide to the use of any data analysis method must be our biological understanding of the experiment under investigation

A worked example for the identification of co-regulated genes in the BrdU Exp 3 microarray experiment is detailed in Section 4 6

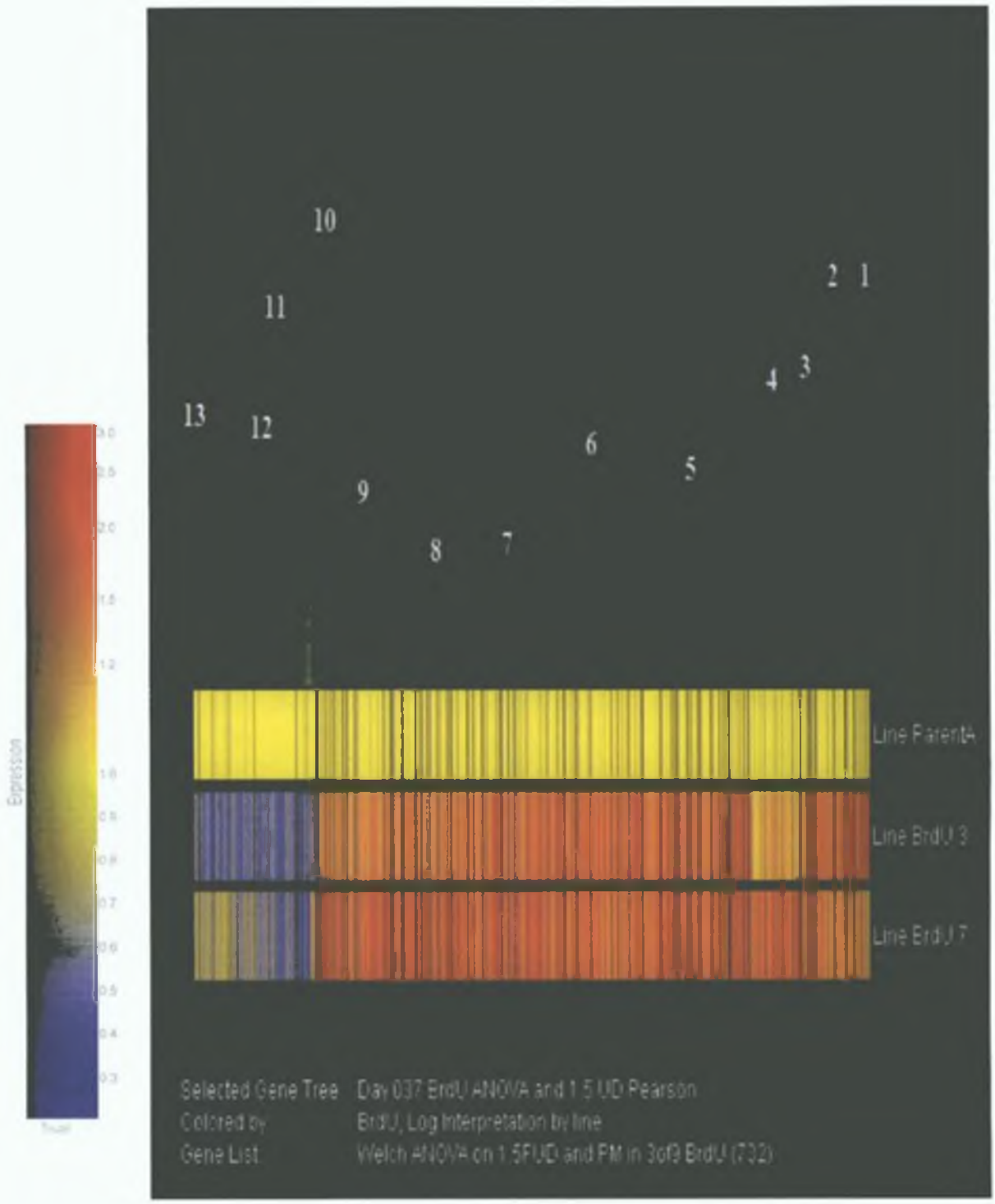


Figure 3.2.19 Heat map of 732 differentially regulated genes in BrdU DNA microarray. Following gene expression analysis of the DNA microarrays, the data set was subjected to statistical analysis and the gene lists then clustered. 13 clusters were then selected for further analysis. Clusters were chosen so that not more than approximately 150 genes were contained in each cluster.

Section 3 2 3 DLKP 5,2-FdU Arrays DNA Microarrays

Affymetrix U133A human expression DNA microarrays were probed with RNA isolated from 5,2 -FdU-treated DLKP cells from a time course experiment Cells were harvested after 0, 3 and 7 days exposure to the modulating agent (Section 2 7) and RNA isolated (Section 2 10) cRNA was then prepared (Section 2 15 1) and transported to the Conway Institute at University College Dublin, where it was hybridised to the U133A GeneChips™, and the chips were scanned using the Affymetrix Gene Chip scanner The raw data was retrieved from the Conway Institute and the following analysis was performed on the data set

The data set was normalised and filtered as has been described for in the BrdU Exp 3 microarray experiment (Section 3 2 2 3) using the Affymetrix PLIER (Probe Logarithmic Error intensity Estimate) method in GREX and using GeneSpring gene expression analysis software

Section 3.2 3 1 Validation of the 5,2`-FdU DNA Microarrays

In order to validate the gene lists, which were generated from the expression analysis carried out using GeneSpring, and to demonstrate that the fold increase/decrease observed were actual real expression changes, a set of genes were selected from the gene lists generated RT-PCR analysis was performed on these genes The expression patterns which confirmed the results that were obtained from the chip expression analysis

The following section contains the result for each of the genes selected In each subsection a graph illustrating the fold increase/decrease found following expression analysis, along with the confirmation RT-PCR result, is shown

Section 3.2 3 1 1 Id2

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and are now thought to be important in development, cell cycle control and tumour biology Following microarray analysis in DLKP cells treated with 5,2 -FdU a significant increase in Id2 mRNA expression levels was observed Id2 transcript levels increased to a maximum of 7 5-fold in the day 3 sample, and declined slightly to 4 8-fold at day 7 RT-PCR analysis confirmed the results of the microarray analysis performed and demonstrated a similar increase in Id2 mRNA levels

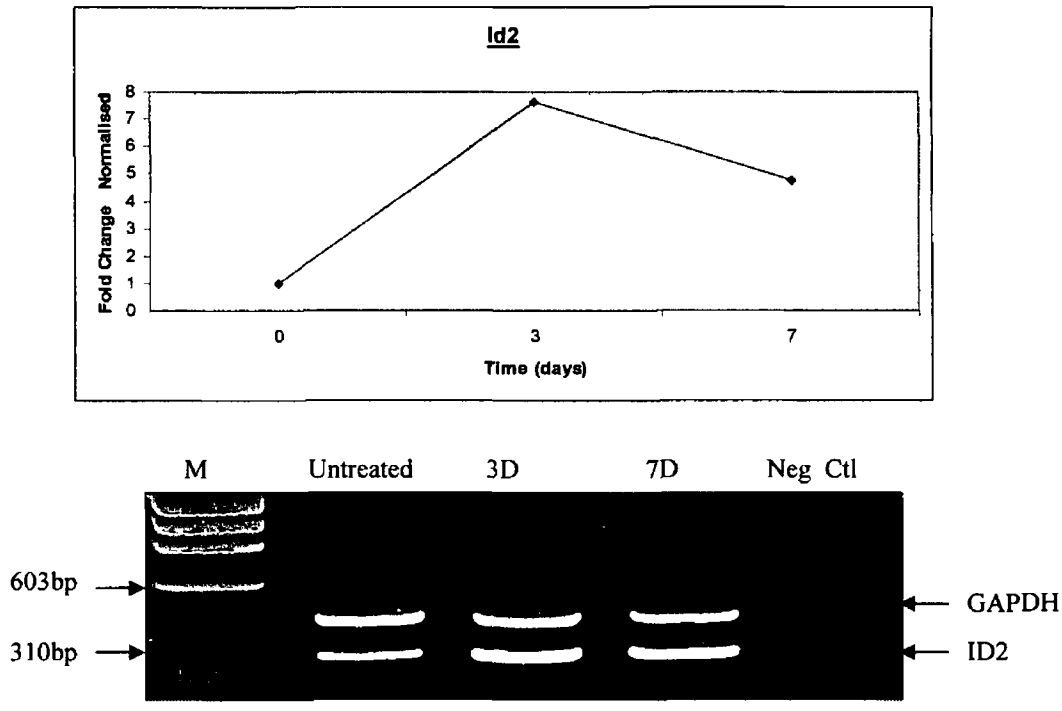


Figure 3 2 20 Top graph illustrates the fold change in Id2 mRNA following gene expression analysis RT-PCR analysis (bottom image) shows the actual level of Id2 at the various time points

Section 3.2.3.1.2 Id3

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and are now thought to be important m development, cell cycle control and tumour biology Microarray analysis revealed an increase in Id3 mRNA levels following exposure DLKP to 5,2 -FdU A 7 5- and 6 4-fold increase in Id3 mRNA levels was observed in day 3 and day 7 samples, respectively RT-PCR analysis confirmed this results and revealed a similar increase in Id3 transcript levels

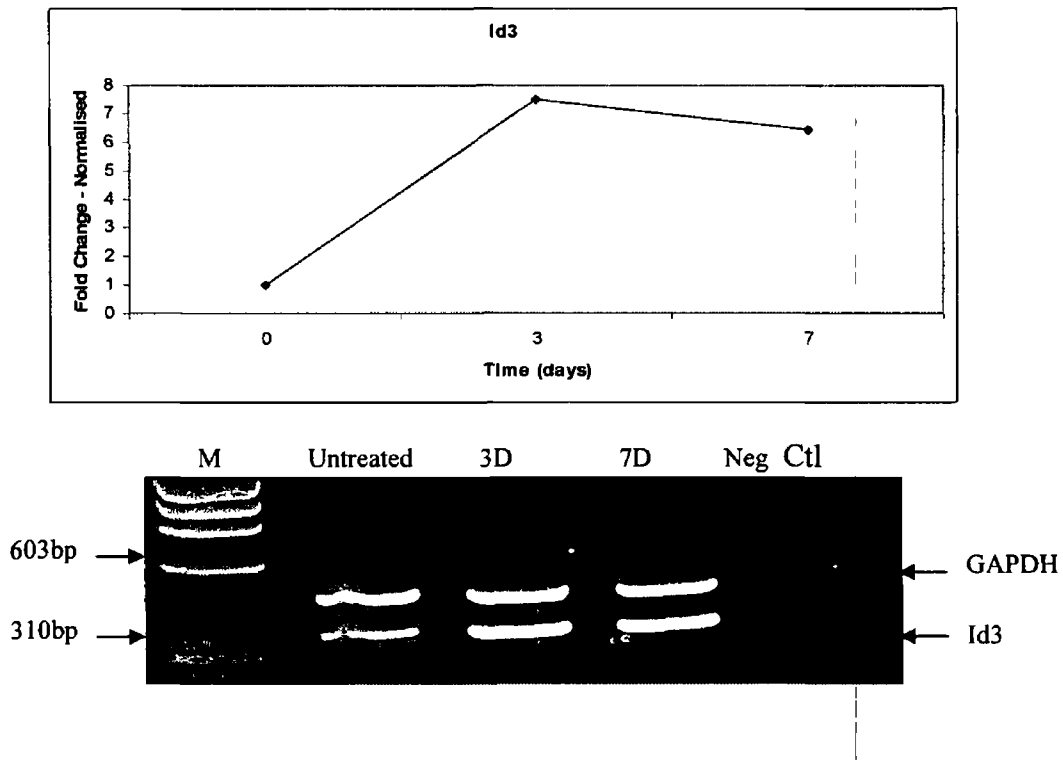
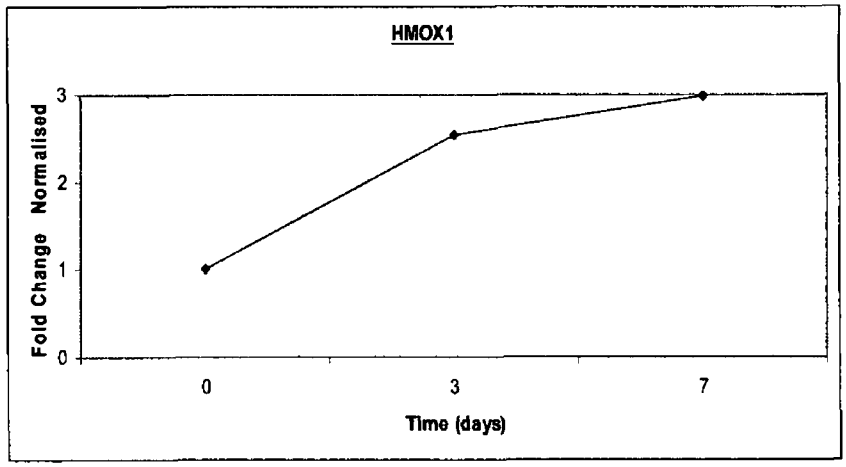


Figure 3 2 21 Top graph illustrates the fold change in Id3 mRNA following gene expression analysis RT-PCR analysis (bottom image) shows the actual level of Id3 at the various time points

Section 3.2 3 1 3 Heme Oxygenase1 (HMOX1)

HMOX1 is one of two isoforms of heme oxygenase that is involved in catabolising heme to biliverdin, carbon monoxide and free iron and is thought to be involved in iron homeostasis Following microarray analysis a 2 5-fold increase in HMOX1 mRNA expression levels was observed following three days of exposure of cells to drug A further increase in FHL2 expression was found in the day 7 sample where the genes mRNA levels had increased to nearly 3-fold RT-PCR analysis demonstrated a similar pattern of transcript levels for HMOX1



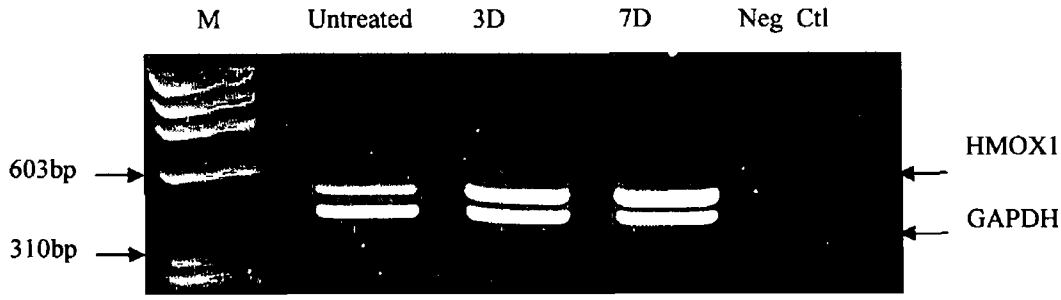
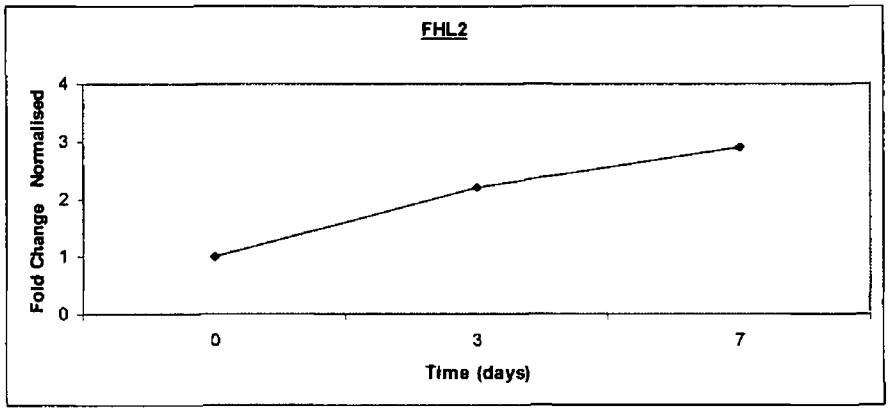


Figure 3 2 22 Top graph illustrates the fold change in HMOX1 mRNA following gene expression analysis RT-PCR analysis (bottom image) shows the actual level of HMOX1 at the various time points

Section 3.2 3 1 4 FHL2

FHL2 belongs to the family of LIM proteins, which are involved in protein-protein interaction and transcriptional regulation Recent evidence has suggests that FHL proteins may act as co-regulators involved in the modulation of tissue-specific gene expression by interacting with different transcription factors Such transcription factors include JUN and FOS and these associations have been shown to result in a powerful activation of AP-1-mediated transcription Following gene expression analysis, using GeneSpring analysis software, the FL1 gene was shown to increase in 5,2 -FdU-treated DLKP cells Microarray analysis revealed an increase in FHL2 transcript levels in drug-treated cells In the day 3 sample FHL2 mRNA levels were found to increased by 2 2-fold and levels were further increased to 2 9-fold in the day 7 sample Confirmation of this result was demonstrated following RT-PCR analysis with revealed a similar increase in FHL2 mRNA expression levels in the day 3 and day 7 samples



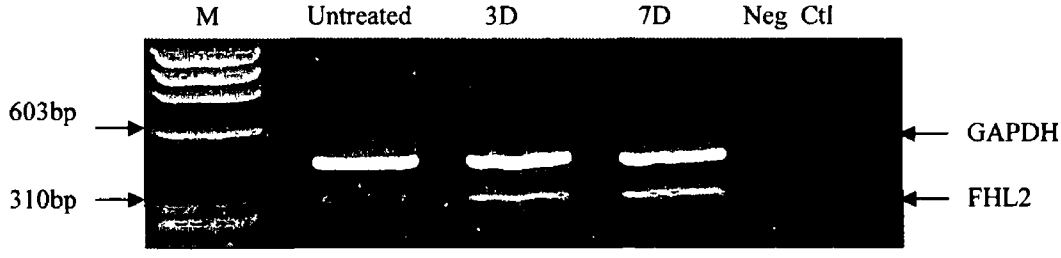
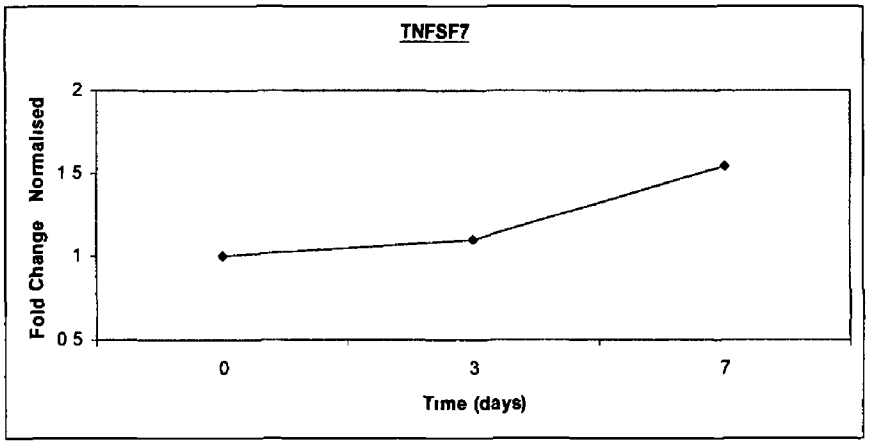


Figure 3 2 23 The top graph illustrates the fold change in FHL2 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of FHL2 mRNA following exposure of DLKP to 5,2 -FdU

Section 3 2 3 1 5 TNFSF7

TNFSF7 belongs to the tumour necrosis factor (TNF) ligand family and this cytokine has been reported to a play a role in the regulation of B-cell activation and IgG production Following microarray analysis the expression of TNFSF7 was demonstrated to increase to 1 5-fold in the day 7 sample, with a little change in mRNA expression levels at day 3 However, RT-PCR analysis reveal a signification increase in TNFSF7 mRNA transcription levels in both the day 3 and day 7 samples



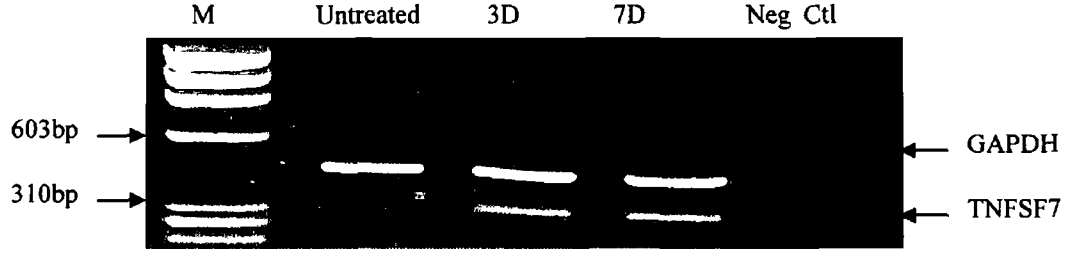


Figure 3 2 24 The top graph illustrates the fold change in TNFSF7 mRNA following gene expression analysis RT-RCR analysis (bottom image) shows the level of TNFSF7 mRNA following exposure of DLKP to 5,2 -FdU

Section 3.2.4 DLKP IdU DNA Microarray

Affymetrix U133 Plus 2 human expression DNA microarrays were probed with RNA from IdU-treated DLKP cells from a time course experiment Cells were harvested after 0 and 7 days exposure to the thymidine analogue (Section 2 7) and RNA isolated (Section 2 10) This experiment was set up in triplicate using biological replicates The RNA was quantified and shipped to Affymetrix where cRNA was prepared, hybridised to the GeneChips® and scanned using the Affymetrix Gene Chip scanner The data was received back from Affymetrix for gene expression analysis

As mentioned above, this experiment used the Affymetrix U133A Plus 2 whole genome GeneChips™ rather than the Affymetrix U133A GeneChips™, which had been used in the previous BrdU and 5,2 -FdU DNA microarrays It is therefore more difficult to compare the results from the BrdU and 5,2 -FdU microarrays with data generated from the IdU microarrays In order to get over this chip variation issue the IdU data sent was not put through the PLIER normalisation in GREX and an alternative normalisation method was applied

The Gene Chip data was normalised 'Per Chip' by using the 50^{th} percentile, using genes flagged as anything but 'Absent' in the calculation of the 50^{th} percentile The samples were then normalised 'Per Gene' to the mean of the day 0 samples The data was then imported into the GeneSpring analysis software where a new experiment was setup

A filter was then applied to the data to select genes that had a 'Present' or 'Marginal' flag in 3 out of the 6 samples The filter also contained a condition that selected for genes that crossed a 1 5 fold threshold, up or down in day 7

Section 3 2 5 Comparison of Up Regulated Genes between BrdU, 5,2`-FdU and IdU

The BrdU, and 5,2 -FdU microarrays experiments were performed on Affymetrix human U133A GeneChips™, whereas the IdU experiment was performed using the U133 Plus 2GeneChips™ In order to compare these arrays a different form of normalisation was performed on the IdU data set than was described for the BrdU Exp 3 and 5,2 -FdU microarray experiments

Since the chip types were different, they could not be combined in GREX for PLIER normalisation (as described in Section 2 15 3) and an alternative normalisation was used The GeneChips® were normalised to the 50th percentile, using genes flagged as anything but 'Absent' in the calculation of the of the 50th percentile The samples on the U133A GeneChips™ (BrdU and 5,2 -FdU microarrays) were then normalised to the mean of the day 0 samples Because the IdU microarrays were carried out on a different Gene Chip type, the samples on this Gene Chip were normalised to the mean day 0 on the U133 Plus 2 chip, and not to the day 0 of the U133A Gene Chip

Three pair-wise comparison experiments were then created, one for each of the three treatments and the appropriate day 0 sample Gene lists were then generated and called 'Present' or 'Marginal' in three of the nine samples, in each pair-wise experiment, and that went either 1 5-fold up or down with drug treatment The resulting gene lists from each drug treatment were then divided into up- and down-regulated differentially expressed genes

In the BrdU experiment a total of 1,093 genes were identified as being differentially expressed 812 of these genes were found to be up-regulated, and of 281 were to be down regulated

In the 5,2 -FdU DNA microarrays a total of 2,147 genes were identified as being differentially expressed Of this number 1,186 gene were found to be up-regulated, whereas 961 genes were found to be down-regulated

In the case of the IdU DNA microarray experiment a total of 722 genes were identified as being differentially expressed Of this number 471 genes were identified as being up-regulated, whereas 251 genes where found to be down-regulated In the IdU DNA microarrays only two time points were chosen for analysis, 0 and 7 days This may explain why a smaller number, 722 genes, were found to be differentially expressed in comparison to the BrdU and 5,2 -FdU microarrays where 1,093 and 2,147 differentially expressed genes were identified, respectively

From analysis of the differentiation studies preformed in the early part of this thesis (Section 3 1 and Section 4 2) it appeared that all the pyrimidine analogues investigated induced a similar pattern of differentiation To explore these earlier studies further, it was decided to investigate common genes that that may be potentially involved in the regulation of differentiation in the BrdU, 5,2 -FdU and IdU treatments In order to identify common differentially expressed genes in these three drug treatments, up/down-regulated gene lists from the three microarray experiments (Apendix 7 1, Appendix 7 3 and Appendix 7 4) were overlapped using Venn diagrams (Figure 3 2 25 and Figure 3 2 26)

In the case of the up-regulated gene list it was fond that a total of 179 genes were common to both the BrdU and 5,2 -FdU microarrays 29 genes were identified as common to only the 5,2`-FdU and IdU microarrays, and 132 genes common to only the BrdU and IdU microarrays A total of 93 up-regulated genes were found common between the BrdU, 5,2 -FdU and IdU microarrays

In the case of the down-regulated gene lists it was found that a total of 105 genes were common to both the 5,2 -FdU and BrdU microarrays 15 genes were common only to the 5,2 -FdU and IdU microarrays A total 24 genes were identified as being common to both the BrdU and IdU microarrays Only 9 genes were determined to be commonly down-regulated between the BrdU, 5,2 -FdU and IdU microarrays

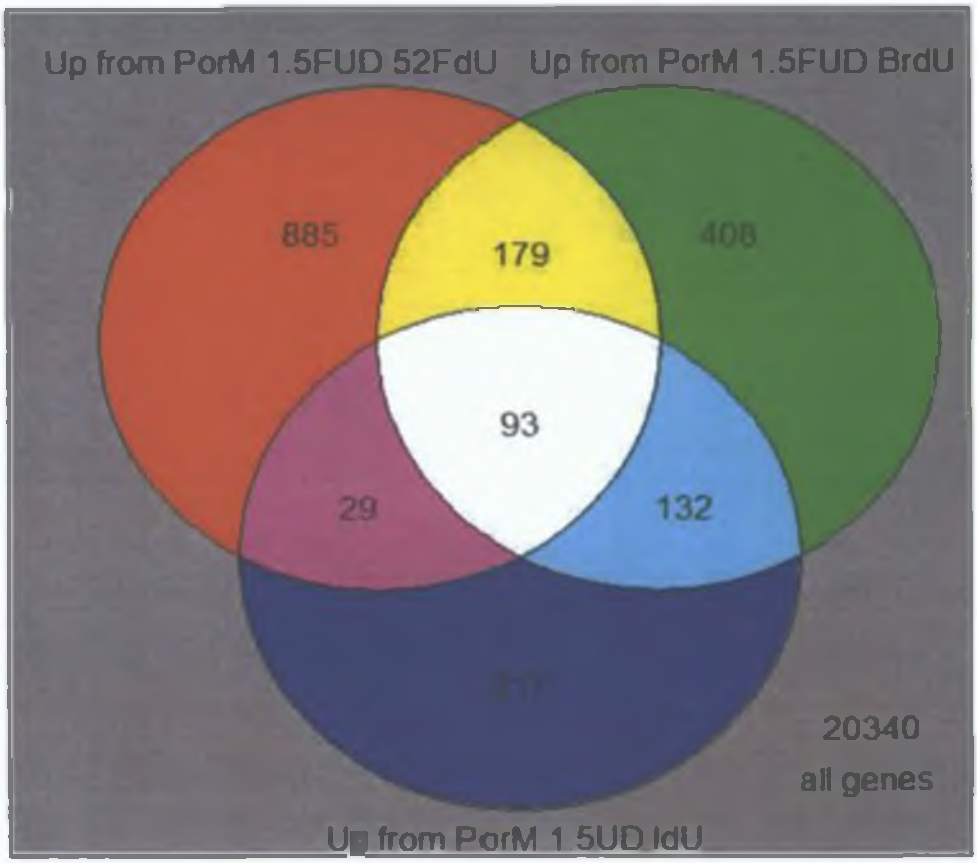


Figure 3.2.25 Venn Diagram of Genes Identified as being Up-regulated in the BrdU, 5,2'-FdU and IdU DNA Microarrays. The above Venn diagram illustrates the overlap of the genes determined to be up-regulated in all three microarrays experiments. The number in each overlapping section represents the number of up-regulated genes common to that section.

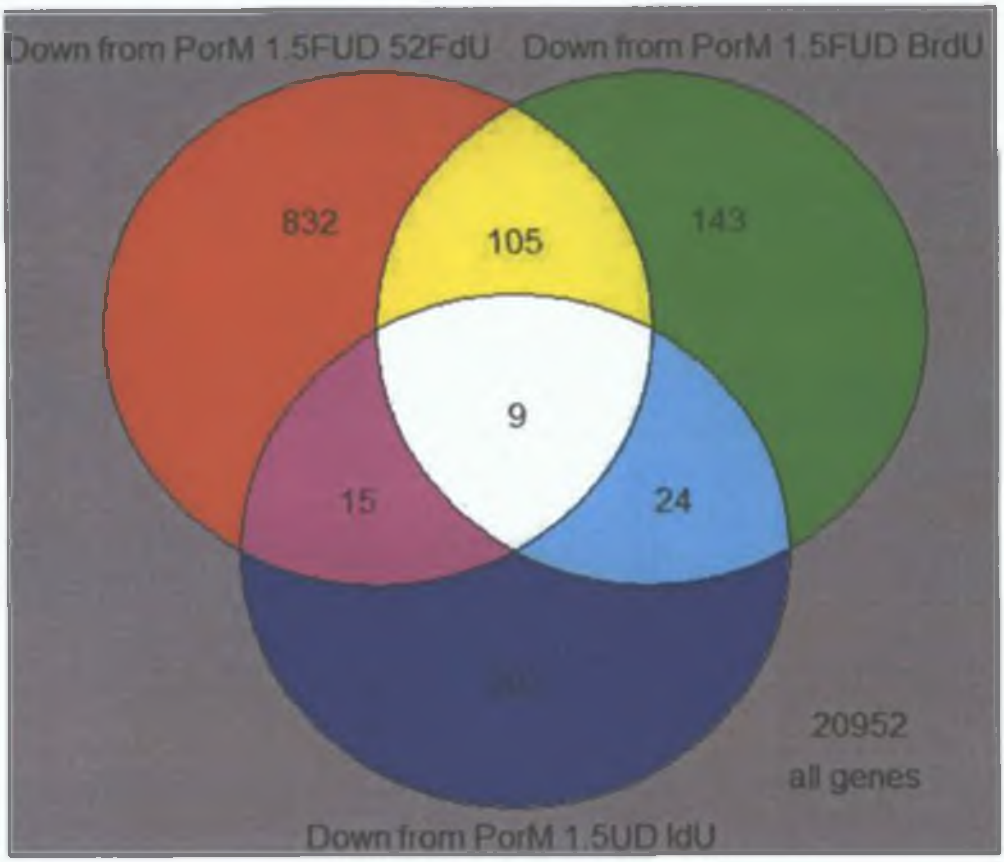


Figure 3.2.26 Venn Diagram of Genes Identified as being Down-regulated in the BrdU, 5,2`-FdU and IdU DNA Microarrays. The above Venn diagram illustrates the overlap of the genes determined to be down-regulated in all three microarrays experiments. The number in each overlapping section represents the number of down-regulated genes common to that section.

Further analysis of these results is described in the discussion.

Section 3.3 Transfections

Section 3 3 1 Overexpression of the transcription factor, c-myc

Several attempts were made to transfect DLKP and DLKP-SQ cell lines, both transiently (Section 2 14 4) and stably (Section 2 14 2), with an expression plasmid containing human c-*myc* cDNA insert

The plasmid appeared to readily transfect into both cell lines In the case of the stable transfections, several clones were isolated from the mixed population of transfected cells The clones were then assayed for up-regulation of c-*myc* mRNA and protein, however, no increase in either c-*myc* mRNA or protein levels were observed It was thought that during the selection process the clones lost the overexpression of c-*myc* and that the surviving cells were as a result of resistance to geneticin c-*myc* is widely known to induce apoptosis when up-regulated in many cell types, and this may also be a reason for the lack of overexpression seen in each round of stable clones generated

Several rounds of c-*myc* transient transfections were also carried out RNA and protein were harvested from the transfected cells at the following time points - 0, 24, 48 and 72 hours In these transfections an increase in c-*myc* mRNA and a marginal increase in c-Myc protein were noted However, the up-regulation of c-myc appeared to have a negative effect on all the other genes assayed for It was suspected that the transient up-regulation of c-*myc* was inducing apoptosis, hence, shutting off these genes

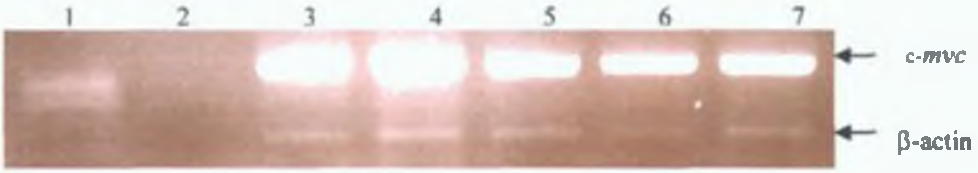
Gene Investigated	Transient	Stable
c-*myc*	↑mRNA ↑ Protein	- mRNA/Protein
YY1	↑ Protein	↑Protein
Mad1	↑ Protein	
eIF4E	↑ Protein	
Cytokeratin 8	↓mRNA	
β_1 integrin	- mRNA	

Table 3 3.1 Summary of Alterations in mRNA and Protein levels of genes investigated in c-*myc* Stable and Transient Transfections ↑= increase in expression, ↓= decrease in expression, - = no change in expression

Section 3.3.1.1 c-*myc* stable transfections

Section 3.3.1.1.1 RT-PCR analysis of c-*myc* Stable Transfections

Lane 1: Marker, **Lane 2:** DLKP, **Lane 3:** DLKP::c-*myc* Mixed Population, **Lane 4:** DLKP-SQ, **Lane 5:** DLKP-SQ::c-*myc* Mixed Population, **Lane 6:** DLKP-SQ::YY 1 Mixed Population, **Lane 7:** DLKP-SQ::c-*myc* Clone 1, **Lane 8:** DLKP-SQ::c-*myc* Clone 2, **Lane 9:** DLKP-SQ::c-*myc* Clone 5, **Lane 10:** DLKP-SQ::c-*myc* Clone 6, **Lane 11:** DLKP-SQ::c-*myc* Clone 7, **Lane 12:** DLKP-SQ::c-*myc* Clone 8.



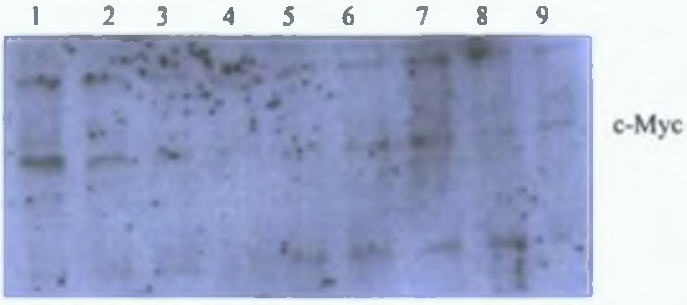
Lane 1: Marker, **Lane 2:** Blank, **Lane 3:** DLKP, **Lane 4:** DLKP::c-*myc* Mixed Population, **Lane 5:** DLKP-SQ, **Lane 6:** DLKP-SQ::c-*myc* Mixed Population, **Lane 7:** DLKP-SQ::YY1 Mixed Population

Lane 1: Marker, **Lane 2:** DLKP-SQ, **Lane 3:** DLKP-SQ::c-*myc* Clone 1, **Lane 4:** DLKP-SQ::c-*myc* Clone 2, **Lane 5:** DLKP-SQ::c-*myc* Clone 5, **Lane 6:** DLKP-SQ:: c-*myc* Clone 6, **Lane 7:** DLKP-SQ::c-*myc* Clone 7, **Lane 8:** DLKP-SQ::c-*myc* Clone 8

Figure 3.3.1 Expression of c-myc in DLKP and DLKP-SQ Stable Transfections. DLKP and DLKP-SQ cell lines were both stably transfected with a human c-myc expression plasmid and several stable clones were isolated from a mixed population of transfected cells. RT-PCR analysis revealed that there was no change in c-*myc* mRNA levels in any of the stable clones. 30μg of total protein was loaded per gel lane.

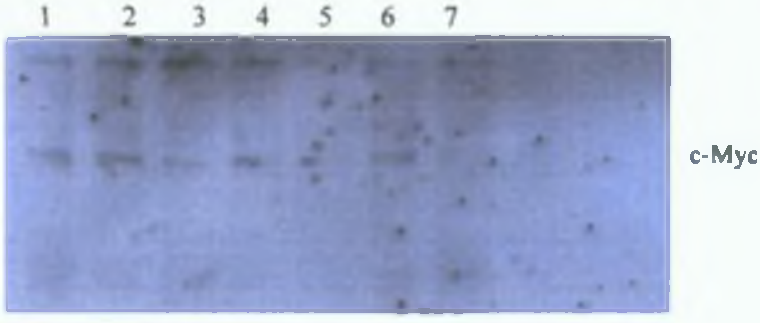
Section 3.3.1.1.2 Western Blot Analysis of c-*myc* Stably Transfected Clones

(A)



Lane 1: Positive Control, **Lane 2:** DLKP, **Lane 3:** DLKP::c-*myc* Mixed Population, **Lane 4:** DLKP::c-*myc* Clone 1, **Lane 5:** DLKP::c-*myc* Clone 2, **Lane 6:** DLKP::c-*myc* Clone 3, **Lane 7:** DLKP::c-*myc* Clone 4, **Lane 8:** DLKP::c-*myc* Clone 5, **Lane 9:** DLKP::c-*myc* Clone6.

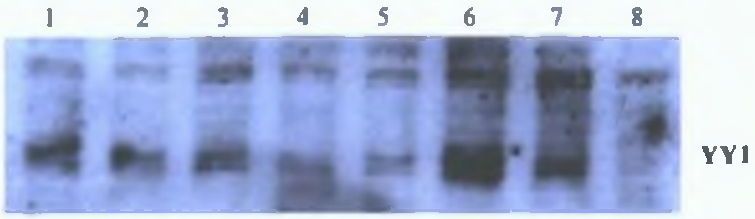
(B)



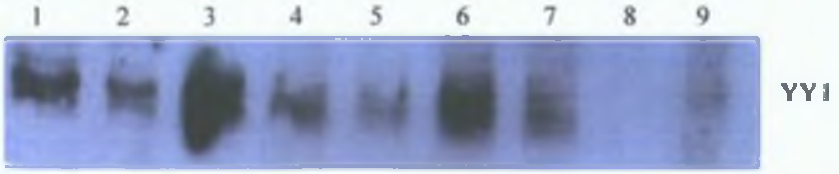
Lane 1: DLKP-SQ, **Lane 2:** DLKP-S::c-*myc* Mixed population, **Lane 3:** DLKP-SQ::YY1 Mixed Population, **Lane 4**: DLKP-SQ::c-*myc* Clone 4, **Lane 5:** DLKP-SQ::c-*myc* Clone 5, **Lane 6:** DLKP-SQ::c-*myc* Clone 7, **Lane 7:** DLKP-SQ::c-*myc* Clone 8.

Figure 3.3.2 Expression c-Myc in DLKP and DLKP-SQ Stable Transfections. c-Myc expression in DLKP and DLKP-SQ clones transfected with a full length human c-*myc* expression plasmid. Western blot analysis revealed that there was no up-regulation of c-Myc protein levels in any of the stable clones generated. 30μg of total protein was loaded per gel lane.

Section 3.3.1.1.3 Western Blot analysis of YY1 protein levels in DLKP::c*myc* and DLKP-SQ::c-*myc* stably transfected Clones.



Lane 1: DLKP, **Lane 2:** DLKP::c-*myc* Mixed Population, **Lane 3:** DLKP::c-*myc* Clone 1, **Lane 4 :**DLKP::c-*myc* Clone 2, **Lane 5:** DLKP::c-*myc* Clone 3, **Lane 6:** DLKP::c-*myc* Clone 4, **Lane 7:** DLKP::c-*myc* Clone 5, **Lane 8:**DLKP::c-myc Clone 6.



Lane 1: DLKP-SQ, **Lane 2:** DLKP-SQ::c-*myc* Mixed population, **Lane 3:** DLKP-SQ::YY1 Mixed Population, **Lane 4:** DLKP-SQ::c-*myc* Clone 4, **Lane 5:** DLKP-SQ::c-*myc* Clone 5, **Lane 6:** DLKP-SQ::c-*myc* Clone 7, **Lane 7:** DLKP-SQ::c-*myc* Clone 8, **Lane 8:** Blank, **Lane 9:** DLKP::c-*myc* Clone 8

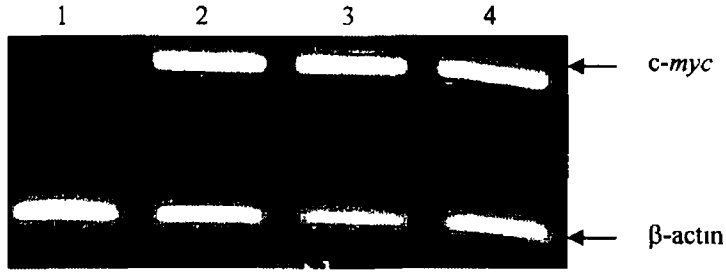
Figure 3.3.3 Expression of YY1 in DLKP and DLKP-SQ Stably Transfected with c-*myc*. YY1 expression in DLKP and DLKP-SQ cells stable transfected with a full length c-myc cDNA expression plasmid. An increase in YY1 protein levels was observed in some of the stable c-*myc* clones generated. 30μg of total protein was loaded per gel lane.

Section 3.3 1 2 c-myc Transient Transfections

Several attempts at transiently transfecting a full length human c-myc expression plasmid into the DLKP-SQ cell line were carried out Time points of 0, 24, 48 and 72 hours were selected for analysis In order to investigate the effect the up-regulation of c-myc had in the cell line, several different genes were assayed for and the results are listed in the following sections

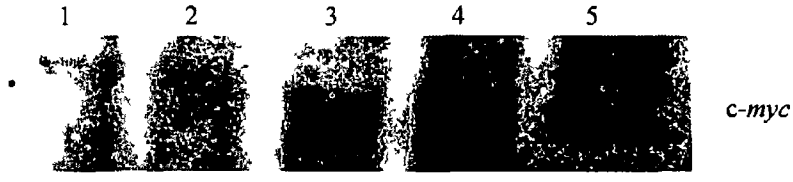
Section 3.3 1 2.1 RT-PCR analysis showing the increase in c-*myc* expression in DLKP-SQ cell line transiently transfected with the pCMV · c-*myc* plasmid

(A)



Lane 1 DLKP-SQ, **Lane 2** DLKP-SQ c-*myc* 24h, **Lane 3** DLKP-SQ c-*myc* 48h, **Lane 4** DLKP-SQ c-*myc* 72h

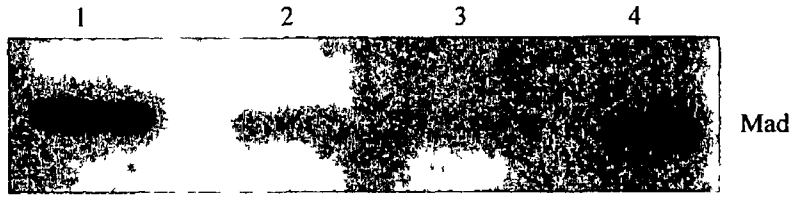
(B)



Lane 1 DLKP-SQ, **Lane 2** 24h, **Lane 3** 48h, **Lane 4** 72h, **Lane 5** Control Peptide

Figure 3 3 4 Expression of c-Myc in DLKP-SQ Transiently Transfected with c-*myc*. **(A)** PCR analysis for c-*myc* expression demonstrate an increase in c-*myc* mRNA after 24 hours of transfection This increase in c-*myc* was maintained over the course of the 72 hour transfection **(B)** Western blot analysis showed that the c-Myc protein level was increased after 24h of transfection and was demonstrated to increase further at 72h 30μg of total protein was loaded per gel lane

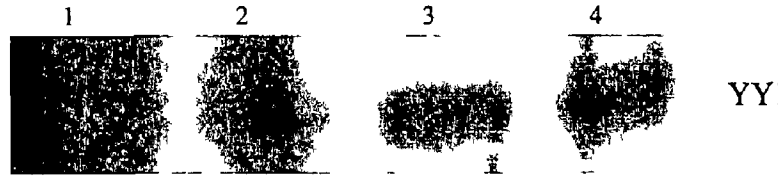
Section 3 3 1.2 2 Change in expression of Mad protein in DLKP-SQ transiently transfected with the pCMV.. c-*myc* plasmid.



Lane 1 DLKP-SQ, **Lane 2** 24h, **Lane 3** 48h, **Lane 4** 72h

Figure 3 3.5 Expression of Mad in DLKP-SQ Transiently Transfected with c-myc Western blot analysis for Mad expression in DLKP-SQ cells transiently tranfected with the pCMV c-*myc* plasmid showed that the up-regulation of c-Myc resulted in the reduction of Mad protein in the transfected cells It was observed from the analysed transfected cells that after 24h the level of Mad protein had been greatly reduced in comparison to the control untransfected DLKP-SQ cells However, after 72h the level of Mad protem was beginnig to return, but its expression was still very much reduced in comparison to control untransfected cells 30μg of total protein was loaded per gel lane

Section 3 3.1 2 3 Change in expression of YY1 protein in DLKP-SQ transiently transfected with the pCMV. c-*myc* plasmid



Lane 1 DLKP-SQ, **Lane 2** 24h, **Lane 3** 48h, **Lane 4** 72h

Figure 3 3 6 Expression of YY1 in DLKP-SQ Transiently Transfected with c-*myc* Western blot analysis for YY1 expression levels in DLKP-SQ cells transiently transfected with the pCMV c-*myc* plasmid showed that the YY1 protein slightly increased after 24h This increase was noted also after 48 and a greater increase in YY1 was seen following 72 hours of transfection 30μg of total protein was loaded per gel lane

Section 3 3 1 2 4 Change in expression of eIF4E RNA in DLKP-SQ cell line transiently transfected with the pCMV c-*myc* plasmid

Lane 1 DLKP-SQ, **Lane 2** 24h, **Lane 3** 48h, **Lane 4** 72h

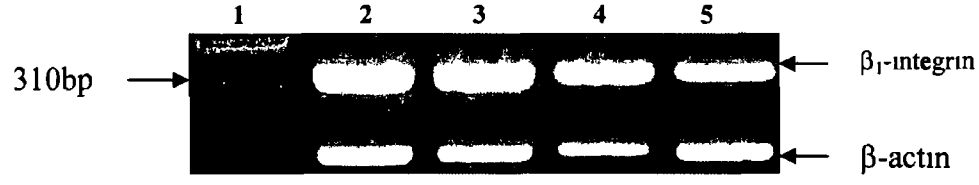
Figure 3 3 7 Expression of eIF4E in DLKP-SQ Transiently Transfected with c-*myc* Western blot analysis for eIF4E protein expression showed that there was an initial increase in the level of eIF4E protein in the transfected cells when compared to the untransfected DLKP-SQ control cells However, after 48h this increase in eIF4E expression had return to the levels observed in the control cells

Section 3 3 1 2 5 Change in expression of cytokeratin 8 RNA in DLKP-SQ transiently transfected with the pCMV c-*myc* plasmid

Lane 1 DLKP-SQ, **Lane 2** DLKP-SQ c-*myc* 24h, **Lane 3** DLKP-SQ c-*myc* 48h, **Lane 4** DLKP-SQ c-*myc* 72h

Figure 3 3 8 Expression of Cytokeratin 8 in DLKP-SQ Transiently Transfected with c-*myc* RT-PCR analysis of DLKP-SQ c-*myc* transiently transfected cells showed that upon the up-regulation of c-*myc*, the levels of CK8 where found to be decreased It was noted after 48h the eIF4E signal has more or less been turned off and that following 72h the CK8 levels started to increase once again

Section 3 3 1 2 6 Change in expression of β_1-integrin RNA in DLKP-SQ transiently transfected with the pCMV· c-*myc* plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ c-*myc* 24h, **Lane 4 DLKP-SQ** c-myc 48h, **Lane 5 DLKP-SQ** c-*myc* 72h

Figure 3 3.9 Expression of β_1-integrin in DLKP-SQ Transiently Transfected with c-*myc* RT-PCR analysis of DLKP-SQ c-*myc* transiently transfected cells showed that upon the up-regulation of c-*myc*, the levels of β_1-integrin where found to be decreased It was noted that after 48h and 72h the level of β_1-integrin had been reduced to nearly half that of the control untransfected cells

Section 3.3.2 Overexpression of Yin Yang 1, YY-1

A plasmid encoding the human transcription factor, YY1 was transfected into the DLKP-SQ cell line and proved reasonably efficient to transfect. This plasmid was transfected both stably and transiently into the DLKP-SQ cell line.

Several stable highly over-expression clones were generated in two separate transfections experiments. From the initial experiment three over-expression clones were isolated from a mixed population of transfected DLKP-SQ cells. And from the second experiment several more stable clones were isolated. Overexpression of YY1 in the three clones was shown to up-regulate c-Myc at the protein level (Figure 3.3.11), however, c-*myc* mRNA levels appeared unaffected by the overexpression of YY1. An increase in Mad1 (Figure 3.3.12), a member of the c-Myc/Max/Mad network, was observed.

An increase in the translation factors eIF4E (Figure 3.3.13) and eIF2α (Figure 3.3.19) was also noted in the three overexpressing clones. A significant increase in eIF4E protein levels was noted; however, due to problems with RT-PCR analysis of eIF4E, the effect of overexpression of YY1 on eIF4E RNA levels are not shown. Overexpression of YY1 also appeared to have an effect on the translation factor eIF2α. A slight increase in eIF2α was noted in the three stable clones generated (Figure 3.3.19). PCR analysis for eIF4e-binding protein-1 showed that there was no alteration in the expression of mRNA levels for this gene in any of the clones analysed.

The effect of overexpression of YY1 on cytokeratin expression was also investigated and it was found that there was no effect on the mRNA levels of cytokeratin 8 (Figure 3.3.14). However, some changes in the expression levels of both cytokeratins 18 (Figure 3.3.15) and cytokeratin 19 (Figure 3.3.16) were noted.

Unfortunately for some unknown reason, the YY1 clones lost all over-expression of YY1 during the freeze thaw back of the stable YY1 clones and due to time

constraints towards the end of this thesis it was not possible to generate more stable over-expression clones Therefore, it was decided to transiently transfect the YY1 plasmid and in order to investigate further the role which YY1 plays in the regulation of differentiation in our cell system

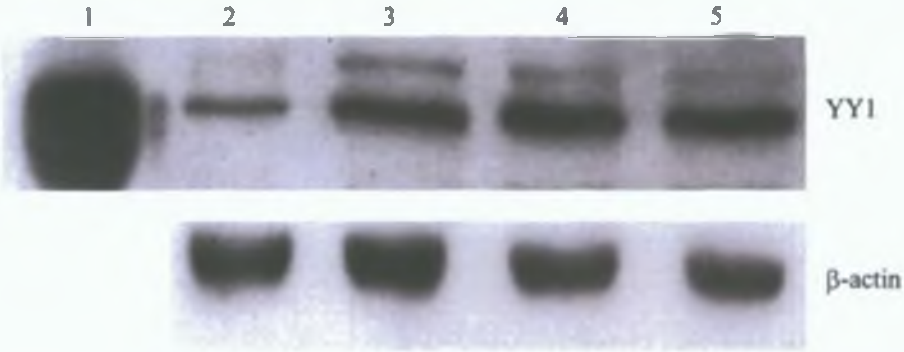
Gene Investigated	Transient	Stable
YY1	↑mRNA	↑Protein
c-*myc*	↓mRNA ↑Protein	-mRNA ↑Protein
Mad1	↑ Protein	↑Protein
eIF4E	↑ Protein	↑Protein
Cytokeratin 8	↓mRNA	-mRNA
Cytokeratin 18	↓mRNA at 24 h only	-mRNA
Cytokeratin 19	-mRNA	↑mRNA
b_1 integrin	↓mRNA	-mRNA ↓Protein
eIF4E-BP1	- mRNA	-mRNA
eIF2α	↓mRNA	↑Protein
Mnk2	-mRNA	
FHL1	↑mRNA	
FSTL1	-mRNA	
HMOX1	↑mRNA	
Id2	↑mRNA	
Id3	↑mRNA	

Table 3 3 2 Summary of the results obtained for the YY1 transient and stable transfection experiments ↑= increase in expression, ↓= decrease in expression, - = no change in expression

Section 3.3.2.1 Stable Over-expression YY1 Clones

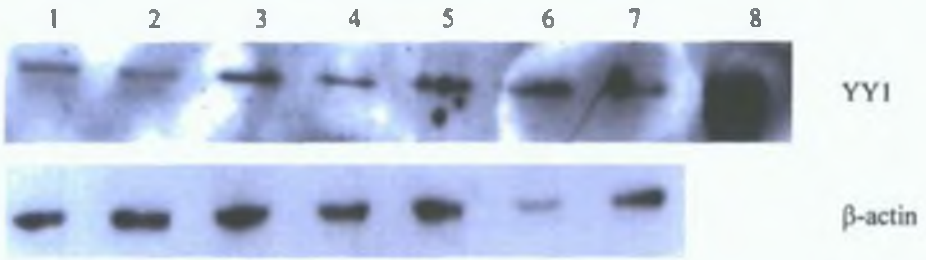
Section 3.2.2.1.1 Investigation of the increase of YY1 protein levels in DLKP::YY1 stably transfected cells.

(A)



Lane 1: YY1 Control Peptide, **Lane 2:** DLKP-SQ, **Lane 3:** DLKP-SQ::YY1 Clone 2, **Lane 3:** DLKP-SQ::YY1 Clone 4, **Lane 4:** DLKP-SQ::YY1 Clone 13.

(B)

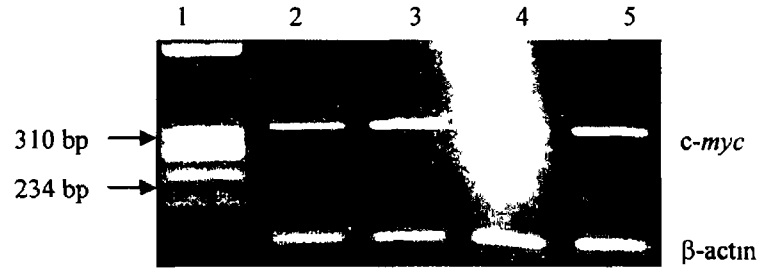


Lane 1: DLKP-SQ, **Lane 2:** Clone 6, **Lane 3:** Clone 4, **Lane 4:** Clone 13, **Lane 5:** Clone 14, **Lane 6:** Clone 7D, **Lane 7:** Clone8D, **Lane 8:** Positive Control.

Figure 3.2.10 Expression of YY1 in DLKP-SQ YY1 Stable Transfections. Western blot analysis was carried out on the DLKP-SQ::YY1 stable clones. **(A)** Level of YY1 protein overexpression in the initial round of YY1 stable transfections. **(B)** Level of YY1 protein overexpression in clones isolated from the second round of YY1 transfections. 30μg of total protein was loaded per gel lane.

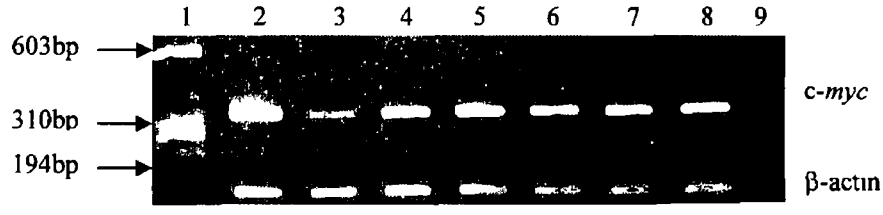
Section 3.2.2.1.2 Investigation of c-*myc* RNA and protein levels in DLKP YY1 stably transfected cells

(A)



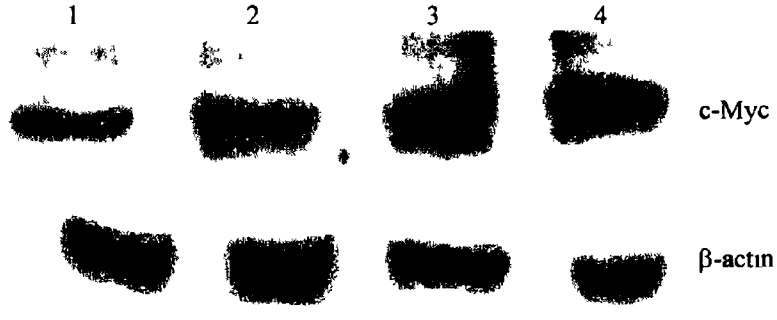
Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 2, **Lane 4** Clone 4, **Lane 5** Clone 13

(B)



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 6, **Lane 4** Clone 8, **Lane 5** Clone 13, **Lane 6** Clone 14, **Lane 7** Clone 7D, **Lane 8** Clone8D, **Lane 9** Neg Control

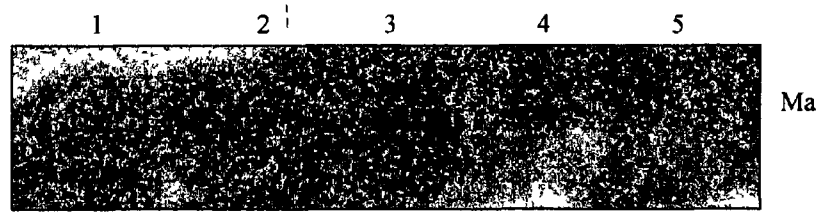
(C)



Lane 1 DLKP-SQ, **Lane 2** Clone 2, **Lane 3** Clone 4, **Lane 4** Clone 13

Figure 3 3.11 Expression of c-myc in Stable YY1 transfections **(A)** and **(B)** RT-PCR analysis for c-*myc* RNA in the stably transfected DLKP-SQ YY1 clones showed that there was no apparent change in expression in any of the isolated clones in the first and second round of transfections, respectfully **(C)** Western blot analysis was carried out on the DLKP-SQ YY1 stable clones revealed that all three clones had increased levels of c-Myc when compared to the untransfected DLKP-SQ cells 30μg of total protein was loaded per gel lane

Section 3 2 2 1 3 Investigation of Mad protein levels in DLKP- YY1 stably transfected cells

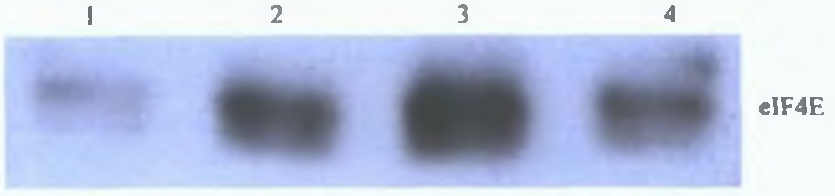


Lane 1 DLKP-SQ, **Lane 2** DLKP-SQ YY1 Clone 2, **Lane 3** DLKP-SQ YY1 Clone 4, **Lane 4** DLKP-SQ YY1 Clone 13, **Lane 5** A549

Figure 3 3 12 Mad Western expression in YY1 overexpressing DLKP-SQ Western blot analysis was carried out on YY1 stable clones revealed that all three clones had increased levels of Mad, with Clone 4 exhibiting the greatest increase

Section 3.2.2.1.4 Investigation of eIF4E protein levels in DLKP::YY1 stably transfected cells.

(A)



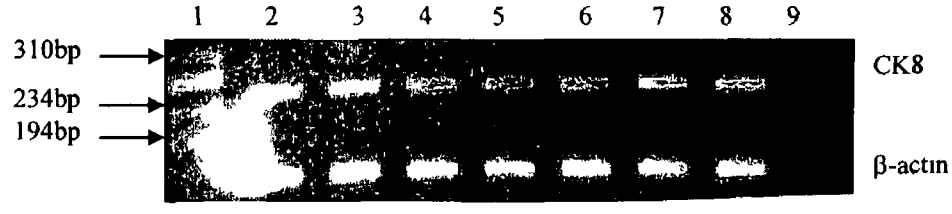
Lane 1: DLKP-SQ, **Lane 2:** DLKP-SQ::YY1 Clone 2, **Lane 3:** DLKP-SQ::YY1 Clone 4, **Lane 4:** DLKP-SQ::YY1 Clone 13.

(B)

Lane 1: DLKP-SQ, **Lane 2:** Clone 6, **Lane 3:** Clone 4, **Lane 4:** Clone 13, **Lane 5:** Clone 14, **Lane 6:** Clone 7D, **Lane 7:** Clone8D

Figure 3.3.13 eIF4E expression in YY1 Overexpressing DLKP-SQ. (A) Western blot analysis was carried out initial YY1 stable clones isolated showed that there was a significant increase in eIF4E. **(B)** The increase in eIF4E was also repeated in the second round of YY1 transfections and was confirmed by western blot analysis. 30µg of total protein was loaded per gel lane.

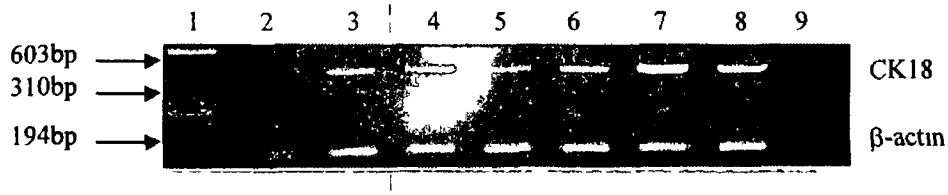
Section 3 2 2 1 5 Investigation of cytokeratin 8 expression levels in DLKP..YY1 stably transfected cells.



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 6, **Lane 4** Clone 8, **Lane 5** Clone 13, **Lane 6** Clone 14, **Lane 7** Clone 7D, **Lane 8** Clone8D, **Lane 9** Neg Control

Figure 3 3 14 Cytokeratin 8 mRNA Expression in YY1 Oversexpression DLKP-SQ **RT-PCR analysis of the stably transfected DLKP-SQ. YY1 clones for alterations in the expression of CK8 RNA revealed that there was no apparent change in the level of CK8 RNA in any of the stable clones isolated**

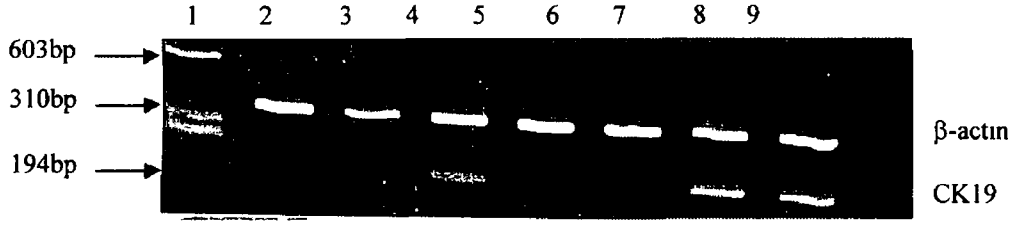
Section 3 2 2 1 1 6 Investigation of cytokeratin 18 expression levels in DLKP: YY1 stably transfected cells



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 6, **Lane 4** Clone 8, **Lane 5** Clone 13, **Lane 6** Clone 14, **Lane 7** Clone 7D, **Lane 8** Clone8D, **Lane 9** Neg Control

Figure 3.3 15 Cytokeratin 18 mRNA Expression in YY1 Overexpressing DLKP-SQ RT-PCR analysis of the stably transfected DLKP-SQ YY1 clones for alterations in the expression of CK18 RNA revealed that there was no apparent change in the level of CK18 RNA in any of the stable clones isolated

Section 3 2.2 1 1 7 Investigation of cytokeratin 19 expression levels in DLKP YY1 stably transfected cells

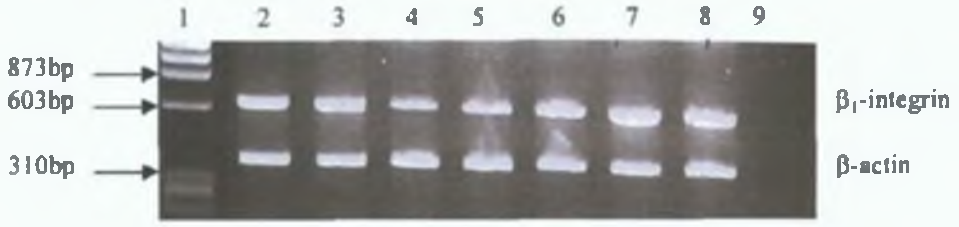


Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 6, **Lane 4** Clone 8, **Lane 5** Clone 13, **Lane 6** Clone 14, **Lane 7** Clone 7D, **Lane 8** Clone8D, **Lane 9** Neg Control

Figure 3.3.16 Cytokeratin 19 mRNA Expression in YY1 Overexpression DLKP-SQ RT-PCR analysis of the stably transfected DLKP-SQ YY1 clones for alterations in the expression of CK19 RNA revealed that Clones 4, 13, 14, 7D and 8D all showed an increase in CK19 RNA when compared to the DLKP-SQ untransfected cells

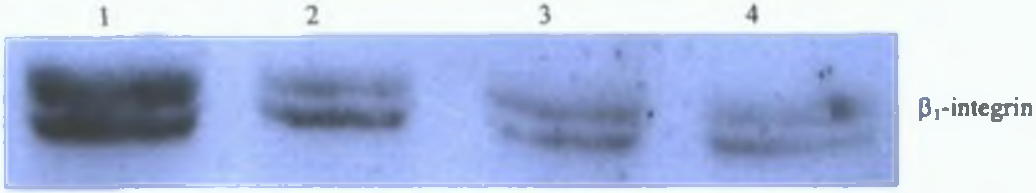
Section 3.2.2.1.1.8 Investigation of β_1-integrin expression levels in DLKP::YY1 stably transfected cells.

(A)



Lane 1: Marker, **Lane 2:** DLKP-SQ, **Lane 3:** Clone 6, **Lane 4:** Clone 8, **Lane 5:** Clone 13, **Lane 6:** Clone 14, **Lane 7:** Clone 7D, **Lane 8:** Clone8D, **Lane 9:** Neg. Control.

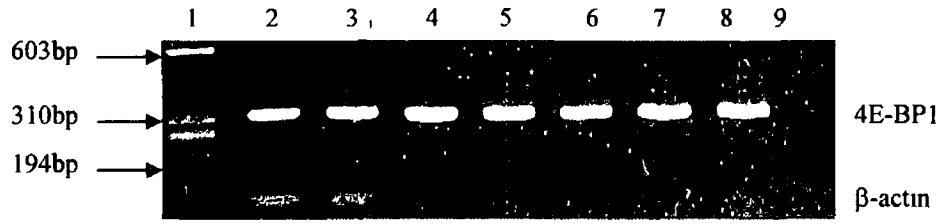
(B)



Lane 1: DLKP-SQ, **Lane 2:** Clone 2, **Lane 3:** Clone 3, **Lane 4:** Clone 13.

Figure 3.3.17 Expression of β_1-integrin in YY1 Overexpressing DLKP-SQ. **(A)** RT-PCR analysis of the YY1 stably transfections for β_1-integrin mRNA expression levels revealed that there was no apparent change. **(B)** Western blot analysis carried out on the three initial clones showed a reduction β_1-integrin protein levels when compared to untransfected cells. 30μg of total protein was loaded per gel lane.

Section 3 2 2 1.1 9 Investigation of eIF4E-binding protein 1 expression levels in DLKP..YY1 stably transfected cells



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** Clone 6, **Lane 4** Clone 8, **Lane 5** Clone 13, **Lane 6** Clone 14, **Lane 7** Clone 7D, **Lane 8** Clone8D, **Lane 9** Neg Control

Figure 3 3 18 Expression of eIF4E-binding protein-1 mRNA in YY1 Overexpressing DLKP-SQ RT-PCR analysis of the stably transfected YY1 clones revealed that there was no apparent change in the level of eIF4E-BP1 mRNA in any of the stable clones isolated

Section 3.2.2.1.10 Investigation of eIF-2α protein levels in DLKP::YY1 stably transfected cells.

Lane 1: DLKP-SQ, **Lane 2:** Clone 2, **Lane 3:** Clone 4, **Lane 4:** Clone 13

Figure 3.3.19 Expression of eIF2α in YY1 Overexpression DLKP-SQ. Western blot analysis was carried out on YY1 stable clones. A slight increase in eIF-2α protein levels was observed in clone 4. The other two clones exhibited similar eIF-2α as the DLKP-SQ control cells. 30μg of total protein was loaded per gel lane.

Section 3 2 2.2 Analysis RNA and protein changes in DLKP-SQ cells transiently transfected with the YY1 plasmid

As mentioned earlier, the stable YY1 expressing clones generated from the transfection of the DLKP and DLKP-SQ cell lines lost YY1 over-expression for unknown reasons during cell freezing/thaw back process Therefore, it was decided to further characterise the role that YY1 plays in our model system by transiently transfecting both cell lines with the YY1 plasmid

DLKP and DLKP-SQ cells were transiently transfected with two different YY1 plasmids The transfected cells were also analysed for alterations in expression levels of other proteins to elucidate what the effect the up-regulation of YY1 had

Due to historical miss-labelling of the YY1 plasmid stocks these transfections were performed using two different YY1 vectors, each containing a full length human YY1 cDNA sequence The initial transient transfections were performed using the plasmid described in Section 2 14, transfected into the DLKP-SQ cell line, while second set of experiments were performed using the His-tagged YY1 plasmid, transfected into DLKP The reasoning behind the use of two different YY1 plasmids was due to historical miss-labelling of plasmids stocks in the laboratory Both YY1 vectors used in this study contained a full length human YY1 cDNA sequence and only differed in the fact that one contained an added His-tag sequence

From analysis of these transfections we demonstrate that transient YY1 over-expression up-regulated c-Myc protein levels, which is in agreement with the stable over-expression of YY1 in our earlier work mRNA levels of c-*myc* were down-regulated in the transient transfections, whereas in the stable transfections the over-expression of YY1 was shown to have no effect of c-*myc* mRNA expression levels

We also demonstrate that the transient over-expression of YY1 also has a negative effect on the mRNA of the translation initiation factor eIF4α, but does

not affect the expression of other translation initiation factors such as eIF4E-binding protein-1 and Mnk2, a known kinase of eIF4E

mRNA levels of cytokeratin 8 and 18 were also investigated and it was demonstrated that a slight decrease in cytokeratin 8 mRNA expression in DLKP-SQ cells after 24 hours of transfection with YY1 The transient over-expression of YY1 did not appear to have an effect on the mRNA levels of cytokeratin 18 β_1-integrin levels were also investigated and mRNA levels of this gene were revealed to be significantly down-regulated after 24 and 48 hours of transfection However, expression levels for this gene and returned to near control levels after 72 hours of transfection

Section 3.2.2.2.1 Change in expression of YY1 mRNA in DLKP-SQ transiently transfected with the YY1 plasmid.

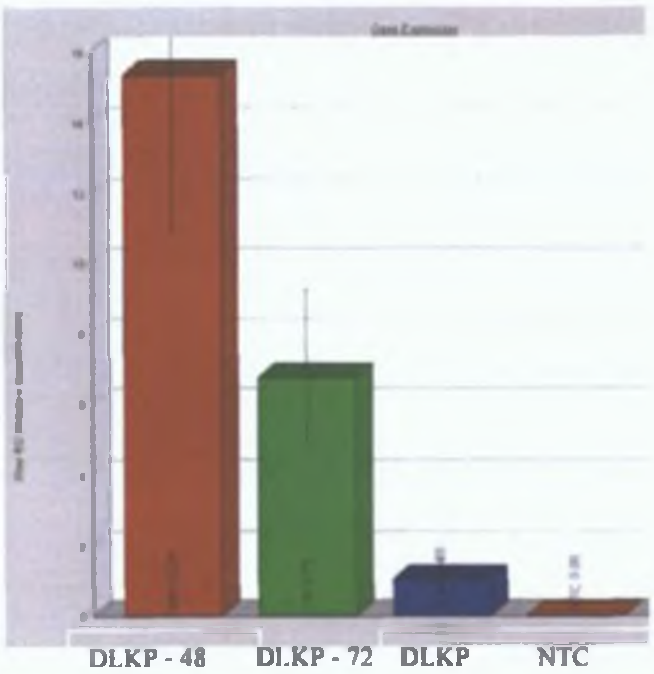
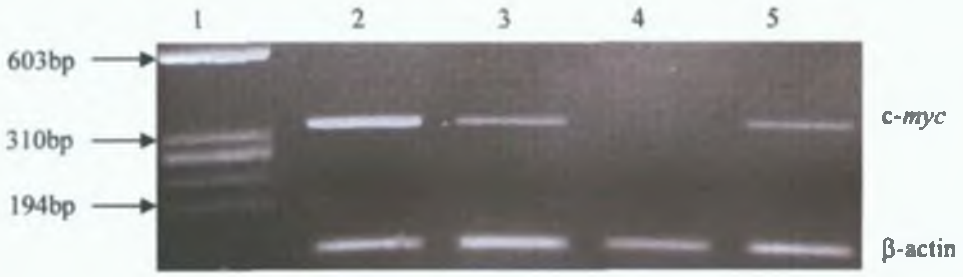


Figure 3.3.20 YY1 mRNA Fold change in DLKP cells transiently transfected with YY1

Taqman Real Time-PCR analysis for YY1 expression was preformed on YY1 transiently transfected DLKP cells. It was demonstrated that following transfection YY1 mRNA transcript levels were 15.35-fold increase after 48 hours and had decreased to 6.75-fold at 72 hours.

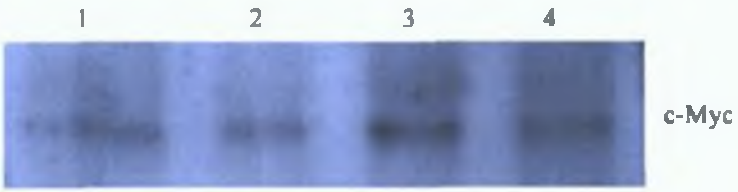
Section 3.2.2.2.2 Change in expression of c-*myc* RNA in DLKP-SQ transiently transfected with the YY1 plasmid.

(A)



Lane 1: Marker, **Lane 2:** DLKP-SQ, **Lane 3:** DLKP-SQ::YY1 24h, **Lane 4:** DLKP-SQ::YY1 48h, **Lane 5:** DLKP-SQ::YY1 72h.

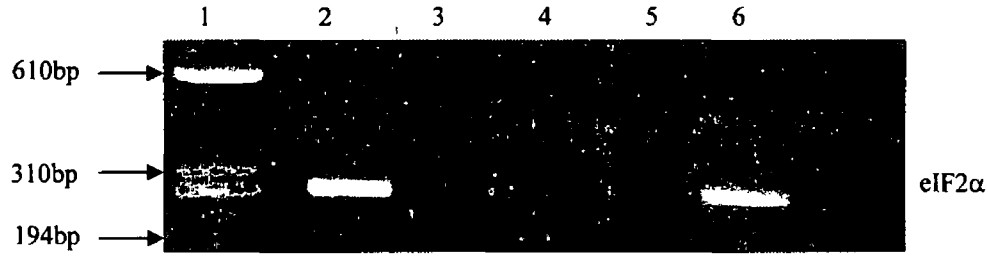
(B)



Lane 1: DLKP- SQ, **Lane 2:** DLKP-SQ::YY1 24h, **Lane 3:** DLKP-SQ::YY1 48h, **Lane 4:** DLKP-SQ::YY1 72h.

Figure 3.3.21 Expression of c-*myc* in YY1 Transient Transfected DLKP-SQ. **(A)** RT-PCR analysis for c-*myc* mRNA revealed that c-*myc* was down-regulated after 24h and following 48h c-*myc* message had been fully turned off. However, after 72h the level of c-*myc* RNA was found to be increasing once again, but not back to control levels. **(B)** Western blot analysis showed no increase in c-Myc protein after 24h, however, an increase was seen at 48 h and this was maintained at 72 h of transfection. 30µg of total protein was loaded per gel lane.

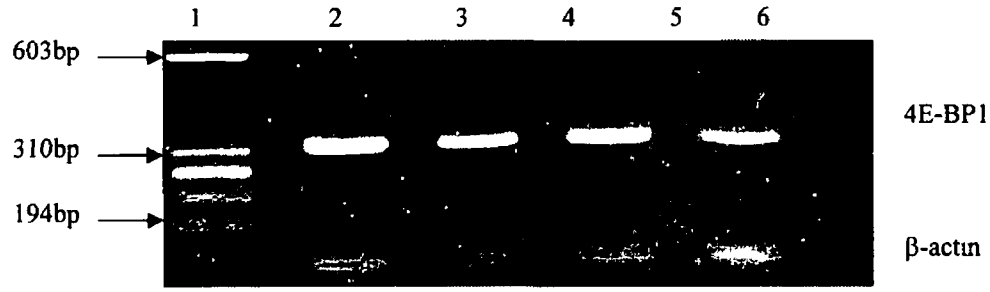
Section 3.2 2 2 3 Change in expression of eIF2α RNA in DLKP-SQ cells transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3 3 22 Expression of eIF2α in YY1 Transiently Transfected DLKP-SQ RT-PCR analysis for eIF2α showed that eIF2α was markedly down-regulated after 24h and 48h, when compared to the untransfected cells It was noted that after 72h the level of eIF2α had returned to that of the control untreated DLKP-SQ cells Control GAPDH mRNA levels did not amplify in this reaction, and due to time constraints this PCR was no repeated

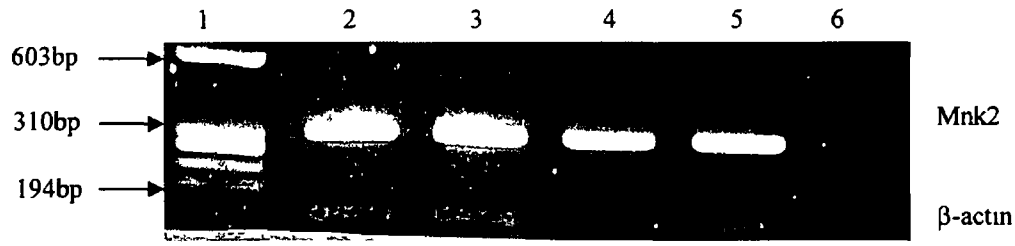
Section 3 2 2.2 4 Change in expression of eIF4E-binding protein 1 RNA in DLKP-SQ transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3.3.23 Expression of eIF4E-binding protein-1 in YY1 Transiently Transfected DLKP-SQ PCR analysis for 4E-BP1 mRNA showed no changed in expression levels following the overexpression of YY1

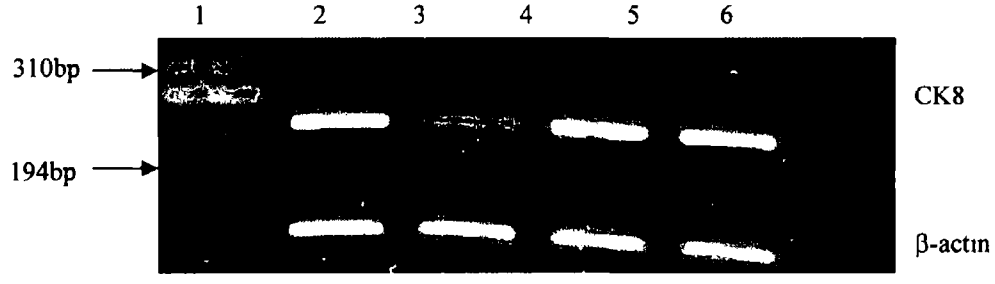
Section 3 2 2 2 5 Change in expression of Mnk2 RNA in DLKP-SQ transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3 3.24 Expression of Mnk2 in YY1 Transiently Transfected DLKP-SQ PCR analysis of Mnk2 levels in showed that there was no change in the expression of Mnk2 mRNA levels following the overexpression of YY1

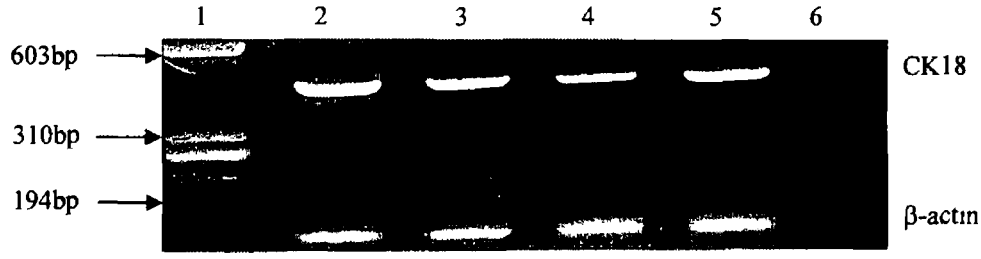
Section 3 2 2 2 6 Change in expression of cytokeratin 8 RNA in DLKP-SQ cells transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3.3 25 Expression of Cytokeratin 8 in YY1 Transiently Transfected DLKP-SQ RT-PCR analysis for CK8 RNA in YY1 transiently transfected cells revealed that the level of CK8 RNA was down-regulated after 24h However, after 48h the CK8 message had returned to that of the control untransfected cellstransfected cells

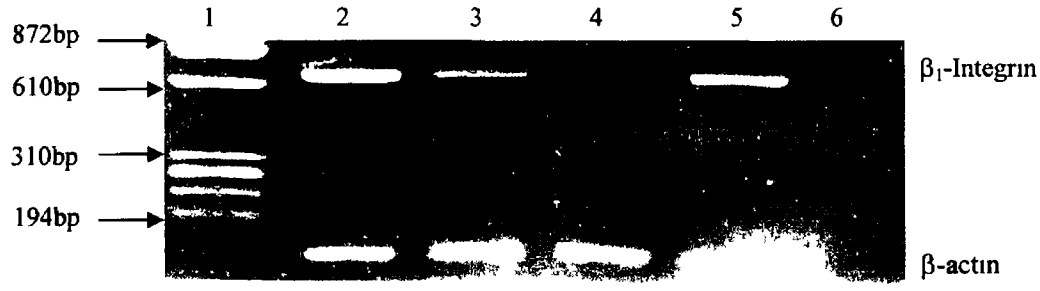
Section 3.2.2.2 7 Change in expression of cytokeratin 18 RNA in DLKP-SQ cells transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3 3.26 Expression of Cytokeratin 18 mRNA in YY1 Transiently Transfected DLKP-SQ. PCR analysis of CK18 mRNA showed that there was no significant change in expression following transient transfection with YY1

Section 3.2 2 2 8 Change in expression of β_1-integrin RNA in DLKP-SQ transiently transfected with the YY1 plasmid



Lane 1 Marker, **Lane 2** DLKP-SQ, **Lane 3** DLKP-SQ YY1 24h, **Lane 4** DLKP-SQ YY1 48h, **Lane 5** DLKP-SQ YY1 72h, **Lane 6** Neg Control

Figure 3 3 27 Expression of β_1-integrin mRNA in YY1 Transiently Transfected DLKP-SQ RT-PCR analysis for β_1-integrin mRNA revealed that the level of β_1-integrin RNA was significantly down-regulated after 24h In cells transfected for 48h the β_1-integrin message had nearly been fully switched off However, after 72h the β_1-integrin message had almost returned to that of the control untransfected cells

Section 3.2.2.3 Change in expression of Genes found regulated in BrdU Array in DLKP transiently transfected with the YY1 plasmid

Following the DNA microarray experiments which were performed towards the end of this thesis, it was decided to investigate if YY1 played a role in the regulation of some of the genes demonstrated to be differentially expressed in the microarray experiments

Following RT-PCR analysis we demonstrate that transient over-expression of YY1 did in fact have a positive effect on some of the genes identified in the DNA microarray experiments We show that the mRNA levels of FHL1, Id2, Id3 and HMOX1, to be increased While YY1 over-expression did not appear to have an effect on FSTL1 mRNA levels

Section 3.2.2.3.1 FHL1

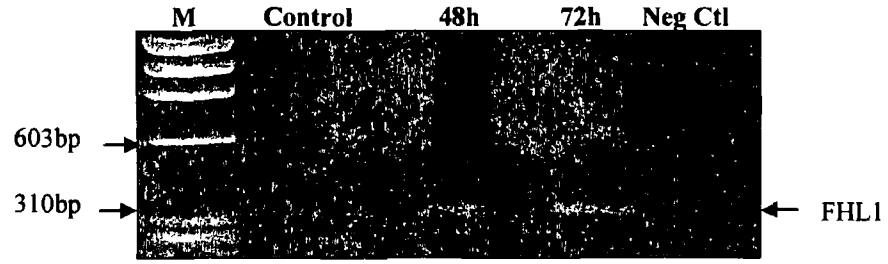


Figure 3 3 28 Expression of FHL1 mRNA in YY1 Transiently Transfected DLKP RT-PCR analysis for domonstreated that transcript levels for FHL1 were significantly up-regulated after 48 hours of transfectioin with YY1 This increase in FHL1 was maintained at 72 hours of transfection

Section 3 2 2 3 2 FSTL1

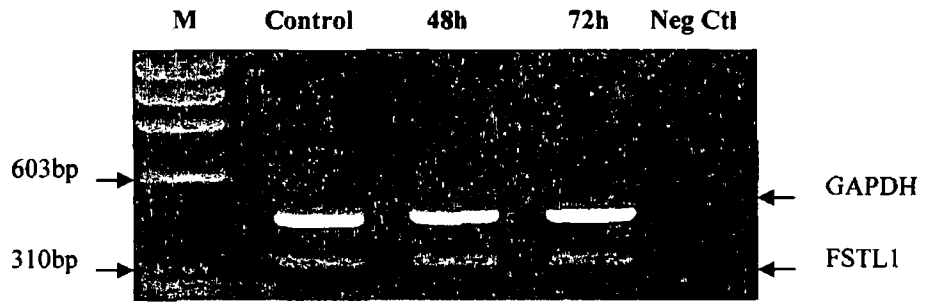


Figure 3 3 29 Expression of FSTL1 mRNA in YY1 Transiently Transfected DLKP RT-PCR analysis for revealed that the transient over-expression of YY1 had no effect on the mRNA levels of FSTL1

Section 3 2 2.3 3 HMOX1

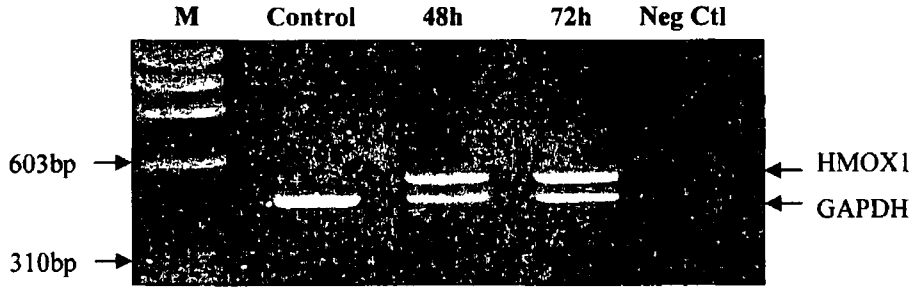


Figure 3 3 30 Expression of HMOX1 mRNA in YY1 Transiently Transfected DLKP RT-PCR analysis for domonstreated that transcript levels for HOMX1 were significantly up-regulated in DLKP cells after 48 hours of transfectioin with YY1 This increase in HMOX1 was maintained at 72 hours of transfection

Section 3 2 2 3.4 Id2

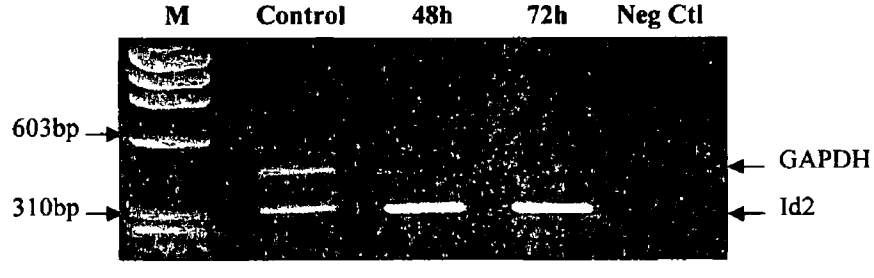


Figure 3 3 31 Expression of Id2 mRNA in YY1 Transiently Transfected DLKP. RT-PCR analysis for demonstarted a significant increase in Id2 transcript levels after 48 hours of transfection with YY1 This increase in Id2 was maintained at 72 hours of tranfection

Section 3 2.2.3 5 Id3

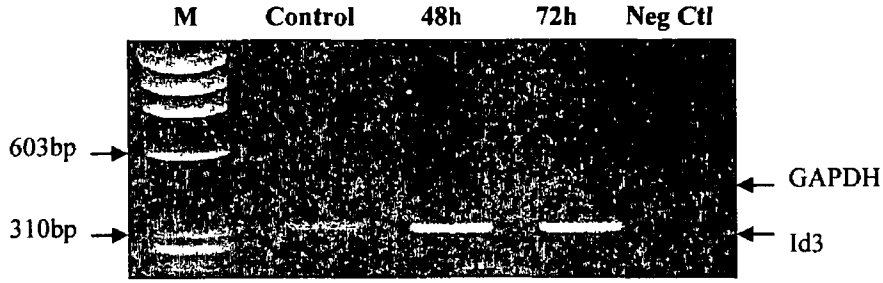


Figure 3.3.32 Expression of Id3 mRNA in YY1 Transiently Transfected DLKP RT-PCR analysis for revealed a large increase in Id3 mRNA expression leveles in transiently tranfected cells

Section 4.0 Discussion

4.1 General Introduction

The lung is a complex organ consisting of over 40 different cell types (Plopper, 1996), whose development has been divided into four chronological stages; I) the pseudoglandular stage, II) the canalicaular stage, III) the terminal sac stage and IV) the alveolar stage. While progress has been made in the developmental genetics in general, aided by huge developments in DNA microarray analysis and proteomic tools, the genetics of lung morphogenesis, the differentiation pathway and the genes involved in development of specific cell types are still largely unknown. The failure to identify a stem cell(s) of the lung (Emura, 1997) and the relative infancy of developmental genetics are part of the main problems in understanding the mechanisms regulating early development of the lung. We have been afforded the opportunity to study an *in vitro* model for early development using a very poorly differentiated lung cancer cell line, DLKP, which was isolated here at the NICB (Law, *et al.*, 1992).

DLKP is a poorly differentiated squamous carcinoma cell line which does not express keratin proteins or other epithelial markers such as epithelial-specific antigen or desmosomal proteins; despite being of epithelial origins (McBride, *et al.*, 1998). DLKP cells may represent a stem cell-like cell line of lung epithelial lineage which has the potential for both proliferation and differentiation. Also, the cells appear to be at a very early stage of differentiation, shown by the lack of expression of the simple epithelial keratins 8, 18 and 19, and can theoretically progress towards several different phenotypes.

Bromodeoxyuridine (BrdU) is a halogenated thymidine analogue that competes with thymidine for incorporation into DNA. BrdU has been found to modulate differentiation in a number of different cell types (Harding *et al.*, 1978). Specifically, BrdU has been reported in the literature to modulate differentiation in neuronal, muscle and haematopoietic lineages (Feyles *et al.*, 1991; Tapscott *et al.*, 1989; Yen *et al.*, 1987). It has been shown previously that this differentiation modulating agent is capable of inducing differentiation in our *in vitro* DLKP model system, and the lung adenocarcinoma cell line, A549, (McBride, *et al.*, 1999; Mcleady, PhD Thesis, 1997; O'Sullivan, PhD Thesis, 1999; Walsh, PhD

Thesis 1999, Doolan, PhD Thesis, 2001) Associated with this alteration in differentiation status of these two cell lines was an alteration in the expression levels of various proteins Protein expression of cytokeratins 8, 18 and 19, integrins α_2 and β_1 (Section 3 1), the eukaryotic initiation factors eIF4E (Section 1 8 and Section 3 1 14) and eIF2α (Doolan, PhD Thesis 2001) and the transcription factors Yin Yang 1 (Section 1 7 2) and c-myc (Section 1 7 1) were all up-regulated in DLKP and A549 cells when induced to differentiate by exposure to BrdU (Walsh, PhD Thesis, 1999) BrdU-induced differentiation of DLKP and A549 also resulted in the alteration of Ep-CAM protein expression (Section 3 1), along with global changes in the transcription profiles of the cells, which were investigated by DNA microarray experiments

In addition, immunocytochemical analysis of DLKP treated with BrdU for 7 days and then grown in the absence of BrdU for up to three months showed that these cells retained the pattern of keratin expression observed in BrdU-treated cells (Walsh, PhD, 1999) This result suggests that BrdU induces a pathway that causes irreversible differentiation of these cells

In this study, BrdU and other halogenated thymidine analogues, namely IdU, CdU, Bromouridme, Bromouracil, as well as 5-FU, 5,2 -FdU and 5,5-FdU, were investigated to determine if they had an effect of the differentiation status of both DLKP and A549 cell lines

4.2 Thymidine analogue-induced differentiation in epithelial cell lines

5-Bromo-2`-deoxyUridine (BrdU) has been reported to modulate differentiation in a number of different cell types, particularly its role in modulating differentiation in neuronal, muscle and haematopoietic lineages has been documented (Yen *et al.*, 1987; Sugimoto *et al.*, 1988; Valyi-Nagy *et al.*, 1993). We have demonstrated in our laboratory that BrdU treatment of DLKP and A549 cells induce these cells to differentiate. The mechanism(s) by which BrdU exerts its differentiation-modulating effects has not been elucidated. Several different theoretical models have been suggested to date (Section 1.4.1). It appears that the incorporation of BrdU into DNA is critical.

Results presented in this thesis demonstrate that following exposure of both DLKP and A549 cell lines to BrdU and the various other halogenated pyrimidine analogues a similar increase in the differentiation markers investigated was observed. Induction of cytokeratin expression was demonstrated in the DLKP system, with an up-regulation of cytokeratins observed in A549. However, not all the analogues induced/up-regulated the cytokeratins and integrins to the same level and a summary of these results are contain in Table 3.1 and Table 3.2.

In the DLKP and A549 systems, treatment with BrdU, IdU and CdU appeared to have a similar pattern of differentiation, with cytokeratins -8, -18 and -19 showing induction in expression. In DLKP cells increased expression of integrins, α_2 and β_1, were also demonstrated in our differentiation experiments.

Treatment of DLKP and A549 with 5,2`-FdU resulted in a dramatic alteration in the cell morphology, with the cells increasing in size to 3-4 times that of the control untreated cells. Induction of cytokeratin protein expression was noted in the DLKP system and an up-regulation found in A549 treated cells. Integrin and Ep-CAM protein expression was also noted in the two cell systems.

Bromouridine and Bromouracil did not seem to induce the differentiation markers to the same extent as the other halogenated thymidine analogues, though

some induction of cytokeratins was noted in treated DLKP cells Interestingly, toxicity assays for these two analogues revealed that treatment with high concentrations of these drugs, of up to 70μg/μl, had no effect on the growth of DLKP cells (results not shown) A small increase in expression of the cytokeratin proteins was seen in A549 treated cells with little change in α_2-and β_1- integrin observed (Section 3 2 21)

In order to ensure that the alteration of the differentiation status of both DLKP and A549 was not as a result of simple toxic exposure, both cell lines were treated with Adriamycin for 7 days Treatment with Adriamycin did not produce the same differentiating-modulating effects as was seen with BrdU and its analogues (Data not shown)

In summary, all the thymidine analogues investigated exhibited a similar induction in differentiation as was demonstrated previously with treatment of DLKP and A549 cells with BrdU (O'Sullivan, PhD 1999) The exact mechanisms(s) by which these analogues induced the expression of the set of differentiation markers investigated in this study remain unclear A surprising find was that all the analogues investigated demonstrated a similar overall pattern of cytokeratin and integrin protein expression 5,5 -FdU, 5,2 -FdU and 5-FU are known to have a different biological mode of action than BrdU, and are known to inhibit DNA synthesis However, treatment of DLKP and A549 with Adriamycin revealed no induction of cytokeratins or integrin proteins following 7 days exposure of cells to this drug Therefore, it was concluded that the induction of the selected differentiation markers investigated by BrdU and the other pyrimidine analogues in our cell system was not as a result of toxic shock in the cells In order to investigate these differentiation assays further DNA microarray analysis was preformed on a subset of treatments and the results of these studies will be discussed in Section 4 5

4.3 c-Myc Over-expression Studies

Epithelial cell proliferation and differentiation is a complex process The regulation of genes encoding structural proteins, such as cytokeratins, during epithelial cell growth and differentiation is relatively well known However, less is known about the roles of specific transcription factors (Lymboussakia, *et al*, 1996) Since the discovery of c-*myc*, the *myc* gene family and the proteins they encode have formed part of the research programme of many cancer research laboratories worldwide However, despite the numerous studies performed or currently under investigation, the role and mechanism of action of the c-*myc* proteins still remain enigmatic In fact, although recently large advances in understanding the role played by this oncogene and some of its binding partners have been achieved the data obtained appears extremely complex and in some cases contradictory Many authors believe that oncogenes simply promote cell growth or cell death, and that they block cellular differentiation Studies performed in this laboratory have shown results that contradict this view In our differentiation model system we have shown that up-regulation of the c-*myc* oncogene may be of importance in the lung cell differentiation process and it is possible that critical genes such as c-*myc*, may play different roles depending on cell type

Our research group has previously demonstrated that an increase in c-Myc expression is associated with the induction of differentiation, in both the DLKP and A549 cell lines, following treatment with BrdU (Walsh, PhD 1999) Western blot analysis revealed a significant increase in the levels of c-Myc protein during the differentiation of both epithelial cell lines Along with the increase in c-Myc, an increase in eIF4E was also observed eIF4E has been identified as one of the few known transcriptional targets of the c-myc proto-oncogene (Jones *et al*, 1996) Studies have shown that eIF-4E expression correlates with c-*myc* levels following growth induction and that overexpression of c-*myc* in rat embryo fibroblasts leads to an up-regulation of eIF-4E expression (Makhlouf, *et al*, 2001)

It may appear contradictory that both c-Myc and eIF4E are up-regulated during epithelial lung differentiation in the DLKP model since there have been extensive reports in the literature of the down-regulation of c-*myc* expression during differentiation (Yen and Forbes, 1990, Valyi-Nagy *et al*, 1993, Shimizu *et al*, 1994) correlated with growth arrest (Bennet *et al*, 1994) However, the roles of oncogenes such as c-*myc* and eIF4E in cellular differentiation are poorly understood

4 3 1 Stable Over-expression of c-*myc* in DLKP

In light of our earlier studies, which showed that there was a significant increase in c-Myc protein levels in both DLKP and A549 cells treated with BrdU (Walsh, PhD 1999), it was decided to transfect the DLKP and A549 cell lines with a full length human c-*myc* expression vector A549 is known to be a difficult cell line to transfect, following several attempts using different transfection techniques (Section 2 14 2 and Section 2 14 4) it was not possible to obtain over-expressing clones

DLKP and a clonal subpopulation of DLKP, DLKP-SQ, were both transfected (Section 2 14 2) with a full length human c-*myc* expression vector Several attempts were made to generate c-Myc over-expressing clones The plasmid appeared relatively easy to transfect into both the DLKP and DLKP-SQ cell lines Several stable clones were isolated from a mixed population, however, none of the clones isolated appeared to overexpress c-*myc* at the mRNA (Section 3 3 1 1 1) or protein level (Section 3 3 1 1 2) One possible reason for the lack of overexpression of c-*myc* may be due to the possible induction of an apoptosis pathway in cells over-expressing c-Myc and the clones isolated from the mixed population were cells that had become resistant to geneticin, but did not greatly over-express c-Myc

4 3 2 Transient Transfection of DLKP-SQ with c-*myc*

As a result of the inability to generate stable over-expressing c-Myc clones in both the DLKP and DLKP-SQ cell lines, we decided to attempt to investigate the role that c-*myc* plays in differentiation of lung cells by transiently transfecting (Section 2 14 4) the DLKP-SQ cell line The DLKP-SQ cell line was selected for the transient transfections simply because this subpopulation of DLKP is known to be easier to transfect, than DLKP

RT-PCR analysis of transiently transfected DLKP-SQ cells revealed that the mRNA level of c-*myc* was significantly increased after 24h, 48h and 72h time points when compared to the untransfected DLKP-SQ parental cells (Section 3 3 1 2 1) An increase in c-Myc protein levels after 48 hours of transfection and a further increase at 72 hours was demonstrated (Section 3 3 1 2 1) Investigation of mRNA and in some cases protein levels of potential c-*myc* target genes was then performed, the results are described in the following subsections

4 3 2 1 YY1 Expression in c-*myc* transient transfections

The YY1 transcription factor possess the unusual ability to both positively and negatively regulate the expression of a number of genes which are thought to be important in cellular differentiation (Riggs *et al*, 1991) (reviewed in Section 1 7 2) and we have previously (Walsh, Phd Thesis, 1999) have identified this transcription factor as an important factor in our differentiation assays The unusual properties exhibited by this transcription factor allow it to regulate the expression of different genes in opposing fashion, making it a pivotal factor in the regulation of developmental gene expression We have previously demonstrated in that YY1 is up-regulated in DLKP and A549 cells treated with BrdU (Walsh, PhD Thesis 1999) suggesting that this transcription factor may indeed be a critical factor in the induction of differentiation on our *in vitro* cell systems

Investigation of YY1 protein expression in the c-*myc* transient transfection experiments revealed an increase in this protein following the over-expression of

c-*myc* At 24 hours following transfection with the c-*myc* expression vector a increase in YY1 protein expression was demonstrated and this increase was maintained at 72 hours (Section 3 3 1 2 3) Due to problems with RT-PCR analysis for the YY1 gene, it was not possible to ascertain if the mRNA levels of YY1 were altered in these experiments These results demonstrate that c-Myc had positive effect on the expression levels of YY1 in cell system

4 3.2 2 eIF4E Expression in c-*myc* transient transfections

eIF4E is a 25 kDa phospho-protein responsible for Cap-binding specificity in eIF4F complexes during eukaryotic translation initiation events eIF4E is widely accepted as the limiting factor in translational initiation, particularly for mRNAs with complex 5' UTR's The core promoter region of the eIF4E gene contains a pair of E-box consensus sequences, CACGTG, which corresponds to the c-*myc* binding sequence The eIF-4E gene is one of the few known targets of the c-Myc protein Studies have shown that eIF-4E expression correlates with c-*myc* levels following growth induction and that over-expression of c-*myc* in rat embryo fibroblasts leads to an up-regulation of eIF-4E expression (Makhlouf, *et al*, 2001) which is in agreement with studies that we have performed in this laboratory and results presented in this thesis Examination of the transient over-expression of c-*myc* in DLKP-SQ revealed that eIF4E protein levels increased after 24 hours of transfection, but reverted back to levels of control cells following 48 hours (Section 3 3 1 2 4)

4.3 2 3 Mad expression in c-*myc* transient transfections

The Myc/Max/Mad family of transcription factors plays a fundamental role in the regulation of cell proliferation, oncogenic transformation, and cell differentiation However, it remains unclear whether different heterodimers, such as Myc/Max and Mad/Max, recognize the same or different target genes *in vivo* c-Myc and Mad1 belong to a superfamily of interacting proteins that regulate cell growth and differentiation Myc, Max Mad1, Mad2 (Mxi1), Mad3 Mad4 and Mnt are all members of this family In order for Myc to exert is cellular function it requires dimerization with another protein Blackwood and Eiseman (1991)

undertook a study to try and uncover a binding partner required for c-*myc* functionality and subsequently they identified a small, novel protein which they named Max Sequence analysis showed that Max was similar to *myc* in that is also contained a bHLH and leucine zipper motifs The structure of Max appeared to be extremely simple, comprising of only 160 amino acids, 60 of which constitute the DNA-bindmg/dimerization domain (Cole, 1991) Max has been found to be a very stable protein and appears to be constitutively expressed in resting as well as proliferating cells In proliferating cells, all newly synthesised c-*myc* is found associated with Max, suggesting that c-*myc* is rate-limiting in the formation of c-*myc*-Max dimers

Further work was carried out by Ager and Eiseman (1993) to investigate Myc's and Max's ability to interact with other proteins The fact that the highly stable Max protein is present at times when *myc* is not expressed, prompted Eiseman and Ager to examine further the possibility that Max might associate with other proteins This group isolated a Max-binding protein which in turn the named Mad (Ager and Eisenman, 1993)

The Mad protein is made up of a family of four closely related proteins Mad1, Mxi 1 (Mad2), Mad3 and Mad4 When Mad proteins are over-expressed in cells, they inhibit proliferation and apoptosis and suppress cell transformation not only by *myc*, but also by a variety of oncogene factors (Zhou and Hurlin, 2001) Transcriptional repression function of Mad proteins corresponds to its biological activity which is in direct opposition to that of *myc* proteins

The expression levels of Max were not investigated in the c-*myc* transfection experiments, nor how the DLKP-treated with BrdU affects the expression levels of Max or Mad proteins Results presented in this thesis demonstrate that c-Myc protein over-expression has negative effect on the expression of Mad1 protein (Section 3 3 1 2 2) The reason why overexpression of c-Myc decreased the expression levels of Mad1 proteins is not clear However, in terms of cell function it is not surprising that increased expression of c-Myc would have a negative effect on the expression levels of Mad proteins, given that these two

proteins have been demonstrated to have conflicting roles in cellular differentiation.

4.3.2.4 β1-Integrin Expression in c-*myc* transient transfections

Integrins form a large family of heterodimeric transmembrane glycoproteins that bind to components of the extracellular matrix. They are versatile adhesion receptors expressed by almost every cell type. In addition to mediating cell adhesion, integrins are known to function as signalling receptors, participating in a diverse array of cellular events including spreading, migration, proliferation, differentiation and apoptosis (Debhar, 1999). Integrins also appear to be important for normal differentiation in most cell types. Their role in differentiation of epidermal stem cells has been well established (Watt, 1998).

Cell adhesion promotes cellular proliferation through the regulation of gene expression, including the immediate early genes. However, the precise role of cell adhesion in the regulation of the c-Myc proto-oncogene is not clear, and the adhesion-dependent signalling pathway(s) regulating the expression of c-Myc has yet to be defined.

Our research group has previously demonstrated that treatment of DLKP and A549 with the differentiation-modulating agent, BrdU, resulted in increase expression of β_1-integrin protein (Meleady, and Clynes, 2000). An increase in c-Myc was also observed in these cells (Walsh, PhD, 1999). Interestingly, in the transient transfection of the DLKP-SQ cell line with c-*myc*, a slight decrease in the mRNA levels of β_1-integrin was observed. It has been previously been reported in the literature that constitutive expression of c-Myc in keratinocytes causes a reduction in β_1-integrin expression levels. Our study of the transient over-expression of the c-*myc* gene agrees with these studies (Section 3.3.1.2.6). However, this does not explain how the expression levels of β_1-integrin are increased in BrdU-treated DLKP and A549 cells in which c-Myc levels are increased. It is possible that the treatment of these cell lines with BrdU induces β_1-integrin proteins via some other pathway way which is independent of

regulation of c-Myc Another reason for this disparity between the BrdU-treatments and the c-*myc* transient over-expression studies potentially could be related to c-Myc phosphorylation In these experiments the phosphorylation status of c-Myc has not been investigated Future work is required to examine if the activity of c-Myc is a result of kinase-related effects in BrdU-treated cells

In summary, we have found that it was not possible to generate stably over-express c-Myc in the DLKP cell line, but we have demonstrated that the transient over-expression of c-Myc increased levels of YY1 and eIF4E protein expression levels were increased in these transient experiments Our results agree with published data where up-regulated c-Myc expression levels have been reported to increase the expression of eIF4E protein levels (Makhlouf, *et al* , 2001) Further work is required to investigate the exact role c-*myc* plays our model differentiation system The over-expression of c-*myc* may indeed be involved in the regulation of other important differentiation-related genes not investigated in these studies Genes that may warrant further investigation maybe those which we have identified as being differentially expressed and thought important in the regulation of differentiation by BrdU, 5,2 -FdU and IdU, following DNA microarray analysis (Section 3 2 and Section 4 5) In view of the fact that the microarray experiments performed in this thesis were conducted after this set of c-*myc* over-expression studies, and due to time constraints, it was not possible to investigate if c-*myc* was involved in their regulation of expression

4 4 Yin Yang 1 Over-expression Studies

The Yin Yang 1 (YY1) transcriptional regulator is thought to be of critical importance in the control of normal development (Riggs *et al*, 1991) (reviewed in Section 1 7 2) YY1 possesses the unusual property of regulating transcription in three ways In different cellular contexts, YY1 has been shown to activate, repress or initiate transcription of a number of cellular genes These opposing roles in the regulation of gene expression make YY1 a pivotal factor in the regulation of developmental gene expression YY1 is ubiquitously expressed transcription factor that is thought to play an important role in the regulation of many cellular and viral genes through the consensus *cis* recognition sequence (Yao, *et al*, 1998) It has been reported previously to up-regulate genes such as c-*myc* (Riggs *el al*, 1993, Lee *et al*, 1994) Previous investigation in our laboratory of the DLKP and A549 cell lines showed elevated expression of YY1 protein upon treatment with BrdU in both cell lines (Walsh, PhD 1999) BrdU has also been shown to up-regulate the levels of YY1 in embryonic myoblasts (Lee *et al*, 1992), further confirming work previously performed in this laboratory It appears that YY1 may play an important role in the control of normal differentiation and development, due to its unusual ability to differentially regulate expression of various genes

Despite the large number of genes found to be potentially regulated by YY1 and the increasingly large number of proteins that are claimed to interact with YY1, little is known concerning how YY1 itself is regulated YY1 has also been linked to cellular growth and differentiation One study by Lee *et al* (1992) demonstrated that the level of YY1 activity changes during myoblast differentiation It has been shown that YY1 levels increase rapidly in quiescent NIH3T3 cells in response to serum and insulin-like growth factor I treatment (Shi, *et al*, 1997) YY1 DNA binding activity has also been shown to be regulated during differentiation For example, YY1 DNA binding decreases during differentiation of human teratocarcinoma cells (Liu, *et al*, 1994)

In view of its pleiotropic activities, it is not surprising that association of YY1 with other proteins appears to be important in determining the activity of YY1

YY1 was first cloned because of it was found to bind to an E1A sensitive site in the Adenoassociated virus (AAV) P5 promoter YY1 has also been shown to associate with another transcriptional activator Sp1, YY1 and Sp1 together activate transcription in a synergistic manner (Lee, *et al*, 1993, Seto, *et al*, 1993) Also, studies using the P5 promoter in an *in vitro* system indicated that YY1 TFIIB and Pol II are sufficient to initiate transcription

YY1 has been demonstrated to increase the transcription from the P1 and P2 promoter of the c-*myc* gene (Riggs *et al*, 1993) In addition, it has been revealed that YY1 associates with the c-Myc protein itself (Shrivastava, *et al*, 1993) In co-transfections carried out by Shrivastava, *et al* (1993), over-expression of c-Myc inhibits both the transcriptional activation and repression abilities of YY1 YY1 is thought to compete with Max (Section 1 7 1 1), excluding it from association with c-Myc (Shrivastava *et* al, 1994) Since the 201-343 amino acid region of YY1 is required for association with c-Myc and is also required for association with Sp1 and E1A, c-Myc may inhibit YY1 activity by interfering with its ability to associate with other transcription proteins Studies have shown that c-Myc interferes with the ability of YY1 to contact the basal transcription factors TATA-binding protein (TBP) and TFIIB

c-Myc levels vary in response to many mitogens and growth signals and it may be that the varying levels of c-Myc modulate YY1 activity *in vivo* There has been some speculation that one function of the c-Myc oncogene is to modulate the expression of YY1-dependent developmental genes by virtue of is association with YY1 (Liu, *et al*, 1996) In co-transfection experiments, c-*myc* expression was able to reduce YY1 activating function from eight-fold in the absence of co-expressed c-*myc* to two-fold in its presence (Shrivastave, *et al*, 1993) In light of YY1s ability to activate c-*myc* gene transcription, association between these two proteins may form the basis of an auto-regulatory mechanism that controls the expression and activity of both proteins (Grignani, *et al*, 1990), preventing excessive loss of growth control during periods of elevated c-Myc expression

4.4.1 Role for YY1 in regulation of differentiation in DLKP

Previous work performed by our research group showed that the differentiation-modulating agent, BrdU, was capable of inducing differentiation in our *in vitro* DLKP cell system and the lung adenocarcinoma cell line, A549(McBride S., *et al.*, 1999; Meleady P., PhD Thesis 1997; O'Sullivan F., PhD Thesis 1999; Walsh D., PhD Thesis 1999; Dolan P., PhD Thesis 2001; Meleady and Clynes, 2001a; Meleady and Clynes, 2001b). It was also proposed by work performed by Walsh (PhD Thesis, 1999) that YY1 may play a critical role in the control and induction of differentiation in our model system. To investigate if YY1 is indeed a critical factor in this model differentiation process it was decided to transfect the cell line, DLKP, and a colonal subpopulation, DLKP-SQ, with an YY1 expression vector.

Stable highly over-expression YY1 clones were generated from the transfection of DLKP-SQ cells with a full length YY1 expression vector. Preliminary characterisation on the effect of the over-expression of the YY1 transcription factor was performed on these clones. Unfortunately, during the freezing and thaw-back, the cells lost the over-expression of YY1 gene.

Western blot analysis of the isolated over-expressing YY1 clones revealed a significant increase in expression of YY1 protein in comparison to the untransfected parental cell line (Section 3.2.2.1.1). As a result of difficulties experienced generating effective RT-PCR primers, it was not possible to investigate the YY1 mRNA levels in the isolated over-expression clones.

In order to characterise the DLKP-SQ YY1 clones, the expression levels of several genes were investigated at both the mRNA and protein level in an attempt to determine if the over-expression of YY1 plays an important role in our differentiation system. In studies performed by Walsh (PhD Thesis, 1999), it was demonstrated that BrdU-treatment of DLKP increased the expression of YY1 and c-Myc proteins; indicating that these two proteins maybe two important transcription factors in this differentiation model system. Also, YY1 has been reported in the literature to associate with c-Myc; therefore, we investigated the

expression levels of c-Myc in our YY1 to determine if YY1 played a role in the regulation of c-*myc* RT-PCR analysis for c-*myc* was performed on the clones (Section 3 2 2 1 2) revealing no change in expression of c-*myc* However, Western blot analysis demonstrated that there was a marked increase in c-Myc protein levels within the YY1 clones (Section 3 2 2 1 2) It appears that the up-regulation of YY1 had no effect on c-*myc* expression at the RNA level, but did cause an increase in c-Myc protein expression

In addition to c-Myc, Mad1 protein levels were investigated in the YY1 over-expressing clones Mad1 protein is reported to be expressed in resting and differentiating cells and is a known antagonist of c-Myc The expression of c-Myc and Mad proteins is tightly controlled and their relative concentrations are critical parameters in the regulation of cell growth Western blot analysis for Mad1 protein in the YY1 clones revealed that the level of Mad1 was increased, with Clone 4 exhibiting the greatest increase in expression (Section 3 2 2 1 3)

The effect of over-expression of YY1 on members of the family of eukaryotic translation factors (eIFs) were also investigated in this study It was found that over-expression of YY1 up-regulated the mRNA expression levels of eIF2α (Section 3 2 2 1 10) in all clones when compared to the untransfected parental cell line, DLKP-SQ mRNA levels of Mnk2, a known eIF4E kinase, were examined It was demonstrated that over-expression of YY1 in this cell model system had no effect of the mRNA levels of Mnk2

Western blot analysis for eIF4E in the YY1 clones was performed revealing that a substantial increase in the level of eIF4E protein (Section 3 2 2 1 4) All clones exhibited a marked increased in eIF4E protein, however, Clone 4 showed the greatest change Immunocytochemical analysis of the clones revealed that the over-expression of YY1 also increased the expression of Ornithine Decarboxylase (results not shown) which is known to be translationaly regulated by eIF4E over-expression Due to problems with RT-PCR primers it was not possible to investigate if the mRNA levels of eIF4E were altered in these clones An increase in the expression of eIF4E protein levels was also demonstrated in

BrdU-treated DLKP cells eIF4E has also been reported as a target for c-Myc and over-expression of c-Myc has previously been shown to up-regulate eIF4E protein levels (Makhlouf, *et al*, 2001), which was confirmed here Also, an increase in eIF4E protein expression was demonstrated in the treatment of A549 cell lines (Section 3 1 14) with BrdU and this further confirms the fact that YY1 maybe playing role in this differentiation system

Other members of the translationaly machinery were investigated, these included Mnk2, eIF4E-BP1 and eIF2α RT-PCR analysis of eIFF4E-binding protein-1 revealed that there was no change in mRNA expression in any of YY1 over-expressing clones RT-PCR analysis for eIF2α revealed an increase in mRNA levels in the clones Western blot analysis also demonstrated an increase in eIF2α protein levels

In our studies we demonstrate that the over-expression of YY1 in our system had an effect on some of the translation machinery of the cell In particular, a major increase in the expression eIF4E protein levels was demonstrated along with an increase in the expression of eIF2α mRNA and protein levels eIF2α is a subunit of eIF2 and is involved in the recruitment of the initiator tRNA (Met-tRNA) to the 40S Ribosomal subunit, in the initiation process of translation (Colhurst *et al* 1987) It has been suggested that eIF2α, along with eIF4E, may possibly be a limiting factor in initiation of protein synthesis Under conditions of elevated eIF4E expression, translation of complex or repressed mRNAs have been shown to be initiated more frequently, and can compete more efficiently for eIF2α, becoming translated at the same rate as 'normal' mRNAs The over-expression of these two important initiation factors may thus be playing important roles in the induction of cytokeratin protein expression in the YY1 over-expressing clones

We also examined by RT-PCR analysis β_1-integrin mRNA expression levels in the YY1 over-expressing clones and demonstrated that there was no change in the level of expression of β_1-integrin mRNA (Section 3 2 2 1 1 8) However, Western blot analysis revealed that upon up-regulation of YY1 protein, β_1-

integrin protein levels appeared to be down-regulated (Section 3 2 2 1 1 8) As mentioned earlier up-regulation of c-Myc protein has been shown to have an inhibitory effect on the expression levels of β_1-integrin In our *in vitro* system we have demonstrated that associated with the over-expression of YY1 protein, is an associated up-regulation in the expression levels of c-Myc protein This up-regulation in c-Myc protein may explain why a decrease β_1-integrin was observed in the YY1 over-expression experiments Also, as for c-Myc, YY1 over expression may have a different affect on the expression of certain genes, and the expression pattern of these genes may vary when compared to BrdU-treatment of cells

Immunocytochemical analysis performed by Derek Walsh (PhD Thesis, 1999) demonstrated that the over-expression of YY1 in DLKP induced cytokeratin expression in the cell line In order to investigate if YY1 over-expression altered the mRNA expression of these proteins, RT-PCR analysis was performed for cytokeratins 8, 18 and 19 This analysis revealed that the level of mRNA of cytokeratin 8 remained unaffected by the over-expression of YY1 (Section 3 2 2 1 5) However, the mRNA levels of both cytokeratin 18 and 19 (Section 3 2 2 1 1 6and Section 3 2 2 1 1 7) were both increased, indicating that YY1 maybe involved in the regulation of their expression For some unknown reason the stable YY1 clones generated in this study lost their YY1 over-expression during freeze/thaw back Several clone stock banks were prepared, however when these stocks were re-cultured, Western blot analysis revealed that the clones had lost all over-expression of YY1 protein Due to time constraints in this thesis, it was not possible to ascertain if the protein levels of these three cytokeratins were also altered and some further work needs to be performed to address this

Section 4.4 2 Transient Over-expression of YY1

Due to time constraints towards the end of thesis and problems with the YY1 plasmid, it was not possible to generate more stable over-expressing YY1 clones Therefore, it was decided to transiently transfect the DLKP and DLKP-SQ cell lines with a YY1 expression vector for up to 72 hours in order to further investigate the potential role that YY1 plays in our model differentiation system The initial transient transfections where performed with the YY1 expression vector described in Section 3 2 2 2 while the DLKP YY1 transient transfections were carried out using the vector described in Section 3 2 2 2 Both YY1 plasmids contain a full length human cDNA gene

From analysis of the transient over-expression of YY1 experiments it was found the YY1 mRNA levels were increased several fold following 72 hours of transfection (Section 3 2 2 2 1) Unfortunately, due to continual problems sourcing anti-YY1 antibody from Santz Biotec , it was not possible to examine the protein levels of YY1 in these experiments Transient over-expression of YY1 appeared to have a negative effect on the expression levels of c-*myc* mRNA, and analysis revealed that the c-*myc* signal was complete shutoff after 48 hours, but were found to increase again after 72 hours, but not back to control levels (Section 3 2 2 2 2) However, Western blot analysis demonstrated that c-Myc protein levels were increased upon the over-expression of the YY1 gene (Section 3 2 2 2 2) From analysis of the stable over-expression YY1 experiments in DLKP-SQ cells (Section 4 4 1), results demonstrated that the mRNA levels of c-*myc* were unchanged by the over-expression of YY1 protein, however c-Myc protein expression levels were increased in the YY1 clones

The YY1 transient over-expression studies performed confirmed that YY1 up-regulation in our cell system is capable of increasing c-Myc protein expression The disparity in the effect of YY1 over-expression on c-*myc* mRNA levels maybe explained by dosage levels of the YY1 gene in the transfected cells The over-expression of YY1 achieved in the transient experiments maybe much higher than the levels obtained in the stable clones, and the differences in the

levels of YY1 expression may have a bearing on the expression of c-*myc* mRNA in these experiments Further analysis is required to confirm this hypothesis

The effect of transient over-expression of YY1 on members of the translation initiation factors was also investigated These experiments confirmed results of the stable over-expression experiments where YY1 was demonstrated to have no effect on expression levels of eIF4E-binding protein-1 and Mnk2 (Section 3 2 2 2 4 and Section 3 2 2 2 5) In the stable YY1 clones it was found that the protein expression levels of eIF2α were increased, however, the mRNA levels for this gene were not investigated The results from the transient over-expression of YY1 revealed that the mRNA levels for eIF2α were greatly reduced after 24 hours of transfection, but after 72 hours returned to control levels Unfortunately, due to time constraints it was not possible to investigate the protein levels of eIF2α in these transient experiments

β1-integrin mRNA levels in the YY1 transient transfection experiments were also investigated The results of this analysis revealed a marked reduction in the expression of β1-integrin mRNA upon up-regulation of YY1, with virtually all β1-integrin mRNA signal shutoff following 48 hours of transfection with YY1 However, 72 hours after transfection the mRNA expression levels of β1-integrin had almost returned to that of the control, untransfected cells These results in part contradicted the RT-PCR analysis performed on the YY1 stably over-expression clones, which showed no apparent change in β1-integrin mRNA levels (Section 3 2 2 2 8) Western blot analysis demonstrated a marked reduction in β1-integrin protein levels In both the YY1 stable and transient over-expression studies we have demonstrated that YY1 is cable of up-regulating c-Myc protein levels in your system Therefore, it is plausible to assume that this up-regulation in c-Myc protein levels may potentially be regulating the levels of β1-integrin The regulation of β1-integrin by c-Myc has previously been reported

The effect of YY1 over-expression on the mRNA expression of cytokeratin 8 and 18 was examined It appeared that YY1 over-expression had no major impact

the mRNA levels of these two cytokeratins These experiments were preformed at the end of this thesis and due to time constraints it was no possible to investigate if transient YY1 over-expression induced cytokeratin protein expression, as was demonstrated by our research group in stable YY1 over-expression clones (Walsh, PhD Thesis, 1999)

In summary, we have demonstrated that the stable and transient over-expression of the YY1 transcription factor in our cell system is capable of inducing a similar pattern of genes as were found altered in BrdU-treated DLKP cells We demonstrate that both BrdU-treatment of DLKP and the over-expression of YY1 protein both increased the expression levels of c-Myc and eIF4E proteins These results suggest that these two genes may be playing a critical role in the regulation of the differentiation pathway induced in DLKP by BrdU

4.5 DNA Microarray Analysis

Temporal and spatial control of gene expression by transcription factors is the hallmark of development The program of lung development from the beginning is directed by the activity of key transcriptional factors DNA microarrays which permit assessment of gene expression patterns on a global level have become increasingly available Initial work in this study demonstrated that a range of thymidine analogues, including BrdU, IdU and 5,2 -FdU (Section 4 2), were capable of inducing differentiation-related proteins in our DLKP and A549 cell line model systems Analysis of DLKP cells treated with these three thymidine analogues illustrated that they all induced a similar pattern of differentiation-related genes (Section 4 2) All three analogues altered the morphology of DLKP upon treatment for 7 days In particular, the expression of cytokeratins 8, 18 and 19 proteins were induced following exposure of cells to the thymidine analogues (Section 3 1) To investigate the global changes that occurred in DLKP cells treated with BrdU, 5,2 -FdU and IdU, DNA microarray analysis was performed using Affymetrix U133A GeneChips® (Section 2 15) The aim of this analysis was to identify patterns of gene expression changes in all three thymidine analogue treatments and also to identify common gene alterations between the three DNA microarrays performed, thereby possibly identifying genes which maybe potentially regulatory in the pathway of differentiation induced by these three analogues

The first DNA microarrays to be performed were microarrays on BrdU-treated DLKP cells Three separate microarray experiments, labelled Exp 1, Exp 2 and Exp 3, were performed In the initial experiments, Expt 1, time points at 0, 1, 3, 7 and 14 days and in the Expt 2 experiment time points of 0, 1 and 7 days were chosen for analysis (Section 3 2 2 1) These initial microarray experiments were carried out using single samples from each time point Gene expression analysis on data sets was carried out as per Rushton *et al* , (2003) In Exp 1 over 9,100 genes were identified as being differentially expressed in the experiment, whereas in Exp 2 approximately 3,500 genes were identified A large difference in the numbers of genes identified as being differentially expressed between

these two experiments maybe due to the fact that 5 experiment time points were chosen in Exp 1 In Exp 2 only time points at 3 and 7 days were chosen

Following gene expression analysis on these data sets, a list of differentially expressed up- and down-regulated genes was created From this list, ten genes were selected and RT-PCR analysis was performed in order to verify the gene expression analysis results The genes were selected on the basis of being the most robust and the most reliably consistent in the two experiments The ten selected genes were then confirmed by RT-PCR analysis in order to validate the microarray data sets

Expt 3 was preformed in triplicate using biological replicates and time points of 0, 3 and 7 days were chosen (Section 3 2 2 3) Since this experiment was performed using biological triplicates, the promoter analysis (Section 3 2 2 5 and Section 4 6) for this thesis was focused on this experiment In this experiment following gene expression analysis a total of 1,093 genes were identified as being differentially expressed (Appendix 7 1) 812 of which were demonstrated as being up-regulated, whereas 281 genes were identified as being down-regulated at day 7

In the 5,2 -FdU microarray experiment, total of 2,240 genes were identified as being differentially expressed across the experiment (Appendix 7 3) At day 7, 1,186 of these genes were shown to be up-regulated and 961 genes down-regulated The total number of differentially expressed genes identified in the 5,2 -FdU array found to be almost twice that Epx 3 BrdU array In the case of the IdU microarray 722 genes were identified as being differentially expressed, 471 of which were found to be up-regulated at day 7, with a further 251 down-regulated

Exp 3 of the BrdU microarrays, the 5,2 -FdU and IdU microarrays were performed towards the end of this thesis and as a result time did not allow for

RT-PCR validation of wide set of genes identified as being differentially expressed However, the ten genes used to validate the initial BrdU arrays, Exp 1 and Exp 2, also emerged following analysis of the third BrdU microarray, Exp 3, and the 5,2 -FdU microarray These ten genes were used to validate the BrdU (Exp 3) and 5,2-FdU However, not all of these ten were found up-regulated in the IdU microarrays, therefore only a subset of these were used to validate this microarray

4 5 1 Comparison of the BrdU, 5,2 -FdU Microarray and IdU DNA Microarrays

Venn diagrams of differentially expressed genes were created in order to identify genes that that were commonly up-regulated at 7 days between the Exp 3 BrdU, 5,2 -FdU and IdU microarrays (Section 3 2 5) 179 up-regulated genes were shown to be common to BrdU and 5,2 -FdU only 132 up-regulated genes were found common to BrdU and IdU only, and a further 29 genes common only to IdU and 5,2 -FdU A total of 93 up-regulated genes (Appendix 7 5) were identified as being common to all three treatments

Similar to the up-regulated genes, the lists of differentially expressed down-regulated genes from the BrdU, 5,2 -FdU and IdU microarrays were overlapped using a Venn diagram in order to identify genes that are commonly down-regulated between the three treatments From the Venn diagram 105 down-regulated genes were found to be common to both the BrdU and 5,2 -FdU microarrays It was observed that 24 genes were commonly down-regulated between BrdU and IdU, with a further 15 genes common to 5,2 -FdU and IdU only A total of 9 genes were identified as being down-regulated between all three microarray experiments

It should also be noted that BrdU treatment of DLKP cells differentiate in response to treatment with BrdU Therefore one would expect some 'noise' from undifferentiated cells Also, the low percentage of cells induced to differentiate

in the BrdU differentiation assays agrees with the finding of relatively few genes found to overlap between the three microarray experiments.

4.5.2 Identification of biological themes with EASE

High density microarray experiments have enabled the discovery of global patterns of biological responses to experimental conditions. Huge amounts of effort have been placed on issues such as data normalisation and statistical testing of genes that are significantly clustered on the basis of expression profiles. The net result of these efforts is the creation of one or more often very long lists of differentially expressed genes. However, one area of microarray analysis that requires attention in trying to make biological sense of these lists is of identification of biological themes from the gene lists. Annotating genes from such lists can be extremely laborious using internet-based databases or manual literature searches. Even after performing such searches, it can be difficult to identify biological themes from the gene list in order to makes sense of the microarray data. Expression Analysis Systematic Explorer (EASE) is a publicly available software application (http://david.niaid.nih.gov/david) that can used for the determination of biological themes from lists of genes.

The main focus of the differentiation studies performed in this body of work was to identify genes that were common to all three thymidine analogues, BrdU, 5,2'-FdU and IdU, since all three induce similar patterns of differentiation. A total of 93 genes (Appendix 7.5) were identified as being up-regulated between the three microarray experiments. In order to identify biological themes within this set of 93 genes, they were entered into EASE.

Not surprisingly, due to the effect these thymidine analogues have on the cell, some of the categories that featured strongly in this analysis included cell death and apoptosis categories. However, other groups which featured heavily in the statistical scoring in the EASE analysis were the categories of morphogenesis, organogenesis and development (Table 4.1).

Common Name	Affymetrix Id
ID3	207826_s_at
LY6H	206773_at
PHLDA2	209803_s_at
GBX2	210560_at
CYP1B1	202436_s_at
ID1	208937_s_at
F2R	203989_x_at
TPM1	206116_s_at
GPC4	204983_s_at
CSRP2	207030_s_at
PAPSS2	203058_s_at
HMOX1	203665_at
MCAM	209087_x_at
EPB41L2	201719_s_at
KLF4	221841_s_at
TAGLN	205547_s_at
DPYSL2	200762_at
DKK3	214247_s_at
FGF2	204422_s_at
DTR	203821_at

Table 4.1 This table details the 20 genes categorised by EASE has being involved in 'Development'

A total of 20 (Table 4 1) of the 93 (Appendix 7 5) up-regulated genes common to all three microarrays fell into the development category, a subset of the more interesting genes are described in the following subsections

4 5 2 1 Id family

The Id family of four helix-loop-helix (HLH) transcription factors (Id1, Id2, Id3 and Id4) act as dominant negative regulators of basic HLH proteins The four Id proteins share a homologous HLH domain, but lack the basic DNA binding domain Id proteins act to sequester bHLH proteins by forming inactive dimers to prevent binding of bHLH proteins to E-box sites (Chaudhary *et al* , 2000)

Two genes from the Id family of transcription factors, Id1 and Id3, were found following EASE analysis, in the development category and were common to all three DNA microarray experiments **The expression of Id1 mRNA was found**

to be up-regulated in all three microarray experiments: 4.8-fold in BrdU, 3.6-fold in 5,2`-FdU and 1.6-fold in the IdU microarray. The mRNA expression levels of the Id3 family member was increased by 7.5-fold in the BrdU microarray, 7.9-fold and 1.6-fold in the 5,2`-FdU and IdU microarrays, respectively.

Two other Id family members, Id2 and Id4, were also found up-regulated in the DNA microarray experiments. However, EASE did not classify these two family members in to the 'Development' category. **The expression of Id2 mRNA was increased to 3.9-fold in both the BrdU and 5,2-`FdU microarray and 1.5 fold in the IdU microarray. The expression levels of Id4 mRNA were increased to 4.4-fold and down to 2.7-fold at day 3 and day 7, respectively, in the BrdU microarray. In the 5,2`-FdU microarray experiments the mRNA levels of Id4 were found to be 3.2-fold and 3.9-fold at day 3 and day 7 respectively. And in the IdU microarray the expression of Id4 mRNA was found to increase to 2.1-fold following 7 days of exposure to the analogue.**

The different Id members have been localised to different chromosomes and show marked differences in their pattern of expression and function (Fong *et al.*, 2003). Although the family members are similar in the HLH domain, the regions outside this sequence are distinct from each other. It is thought that this variance between the four family members may determine their tissue specific function, as well as the binding specificity for particular bHLH proteins. Since many bHLH proteins positively regulate sets of genes during cell fate determination and cell differentiation, Id proteins are thought to inhibit the ability of bHLH proteins from binding DNA and inhibit cell differentiation.

Although Id proteins traditionally have been viewed as negative regulators of differentiation, recent work has revealed much wider biological roles, and are now thought to be important in development, cell cycle control and tumour biology. In general, the Id proteins are expected to have overlapping function because of their ability to form non-functional dimers with differentiation-inducing bHLH proteins. Recent studies suggest that his may not be true, and some Id proteins may, in fact, be required to induce and maintain the

differentiation state of a particular cell (Lui, *et al*, 2000, Cooper and Newburger, 1998) It has been demonstrated that Id2 may act as inhibitor of proliferation and is required for the determination and maintenance of the differentiated state of alveolar epithelial cells (Liu *et al*, 2000) The constitutive expressions of Id2 and Id3 mRNA in Sertoli cells suggests that these proteins may have a significant role in maintaining Sertoli cell function (Chaudhary *et al*, 2000) Chaudhary *et al* (2000) have shown that post-mitotic and differentiated Sertoli cells express high levels of Id proteins This pattern of Id2 and Id3 expression agrees with the results presented in this study, where we show a signification increase of both genes in differentiating lung epithelial cells

In several epithelial cell types, the expression of this family of proteins has been positively correlated with proliferation (Barone *et al*, 1994) They are often up-regulated in proliferating and undifferentiated cells, and down-regulated upon induction of differentiation (Norton *et al*, 1998) Ectopic expression of Id proteins has also been shown to block differentiation functions in a number of cell types by sequestering cell-specific bHLH transcription factors (Melnikova and Christy 1996, Shoji *et al*, 1994) Experiments presented in our study show that mRNAs for the Id family members are up-regulated in DLKP cells induced to differentiate following treatment with halogenated pyrimidine analogues Therefore, we suggest that the Id family of transcription factors may potentially play an important role in the control of differentiation-related genes in our model cell system However, further work is required to elucidate the exact role this family of proteins plays in our differentiation system

4 5.2 2 Glypicans

Glypicans are a family of six glycosylphosphatidylinositol-anchored cell surface heparan sulfate proteoglycans implicated in the control of cellular growth and differentiation, which have been localized to rafts and caveolae (Fransson 2003) Glypicans are selective regulators of ligand-receptor binding and have been reported to control cellular growth and development In recent years it has become apparent that proteoglycans serve in several major developmental signaling-pathways It is now thought that several secreted and cell surface

molecules participate indirectly in growth-factor signaling, by influencing the interactions between growth factors and receptors The evidence is particularly strong for the pathways supported by FGFs, Wnts and BMPs, which all involved in a wide variety of developmental pathways (De Cat and David, 2001)

Proteoglycans are proteins, substituted with glycosaminoglycans These glycosaminoglycans bind to growth factors, extracellular matrix molecules, enzymes, protease inhibitors and many other proteins They are predominantly located on the cell surface Two major families of proteoglycans have been identified the syndecans and the glypicans Syndecans are transmembrane proteoglycans, whereas glypicans are attached to the cell surface Syndecans and glypicans show differential expression and have been found to be highly regulated during development The structures of the individual glypicans are extremely well conserved across the species It is not currently known if the glypican family members share common functions (De Cat and David, 2001)

Given the ability of glypicans to regulate the activity of cellular growth, this family of proteins has also been associated with some cancers Recent reports have showed that changes in their expression patterns maybe linked with tumour progression One such study established a connection between glypican 1 and pancreatic cancer, where the expression of this protein was significantly up-regulated (Kleef *et al* , 1998) **In our study we found that the expression of glypican-4 mRNA was increased by 1 6-fold at day 7 in the BrdU microarray, to 1.7-fold in both the 5,2`-FdU and IdU microarrays, respectively** The exact role that the glypicans play in our differentiation system remains unclear and further work is required to ascertain their exact role in the control of differentiation in the DLKP cell line

4 5 2 3 Kruppel-like factor 4

Following EASE analysis of the BrdU, 5,2 -Fdu and IdU microarray data sets, one interesting gene identified in the development category was KLF4 **Analysis of the microarray experiments revealed that the expression of KLF4 mRNA was increased 1 8-fold in BrdU-treated DLKP cells, 2 8-fold in 5,2`-FdU and 2 2-fold in IdU-treated DLKP cells following 7 days exposure**

Kruppel-like factors (KLFs) are DNA-binding transcriptional regulators that play a diverse role during differentiation and development The KLFs are zinc finger transcription factors, expressed in the epitheha of the skin, lungs and gastrointestinal tract as well as in many other organs Members of this family include erythroid (EKLF), lung (LKLF), basic (bKLF) and gut (GKLF, also known as KLF4)

KLF4 has been the most thoroughly investigated family member with respect to its role in cellular differentiation This gene has been shown to be important in the gastrointestinal tract and colon epithelium, where the gene is thought to regulate cell growth and differentiation Shie *et al* (2000) demonstrated that the expression of KLF4 mRNA is significantly decreased in neoplastic colonic tissues including adenoma and carcinoma and they have suggested that down-regulation of KLF4 may contribute to malignant transformation of the colon In this study, Shie *et al* (2000) showed that constitutive over-expression of KLF4 in human adenocarcinoma cells resulted in a decrease in [^{3}H]thymidine uptake, whereas inhibition of KLF4 led to an increase in DNA synthesis, suggesting that KLF4 may play an essential role in controlling growth arrest in the colon This trend in KLF4 expression was confirmed in another study by Sheilds *et al* (1996) who demonstrated that KLF4 mRNA levels were significantly decreased in proliferating NIH 3T3 cells The exact mechanism by which KLF4 exerts its effect in is not fully understood Shei *et al* (2000) reported that the expression of KLF4 is closely related to that of CD1 and CD1-associated kinase activity and that KLF4 in fact suppresses the CD1 promoter activity

Earlier work in this study examined the effect of the differentiation modulating agent, BrdU, and the various other halogenated thymidine analogues (Section 3 1 and Section 4 2) had on the differentiation status of both DLKP and A549 Markers of differentiation that were investigated in these assays included a group of intermediate filaments known as cytokeratins These proteins are known to be important in cellular differentiation and cytoskeleton organisation and have been subdivided into two main types The acidic type I is comprised of cytokeratins 9-20, whereas the basic type II include 1-8 (Section 1 5) One cytokeratin in particular, K19, has no known basic type II keratin partner, although its expression is often found in cells that express K8 (Quinlan *et al*, 1985) We have also demonstrated that the expression of K8 and K19 protein levels are induced our differentiation studies of both DLKP and A549 cells (Section 4 2)

Regulation of cytokeratin expression in the differentiating stratified squamous epithelium is governed by a complex interplay of both ubiquitous and tissue-specific transcription factors Little is known about the regulation of K19 expression Brembeck and Rustig (2000) reported that KLF4 and Sp1 modulate K19 promoter activity in a tissue-specific manner The role of Sp1 in the regulation of cytokeratins has been described previously The promoter of corneal-specific K3 gene is regulated by an overlapping Sp1/Ap2 site and endogenous levels of Sp1 and Ap2 define K3 gene transcription in differentiating corneal epithelial cells (Chen *et* al, 1996) The transcription of K5, K16, K17 and K18 has all been shown to be under the control of Sp1 (Ohtsuki *et al* 1993, Magnaldo *et al*, 1993, Gunther *et al*, 1995)

The KLF family of transcription factors had been phylogenetically linked to the Sp1 family Brembeck and Rustig (2000) in their study revealed that both Sp1 and KLF4 act positively on an overlapping Sp1/KLF4 site in the K19 promoter with preferential binding by KLF4 and postulated that the endogenous levels of Sp1 and KLF4 are important determinants in binding to this element In their study they showed that KLF4 contributes to the tissue-specific transcriptional regulation of K19 expression KLF4 appears to play an important role in cell-specific differentiation by activating K19 expression, which they demonstrated to be influenced by Sp1 in stratified squamous epithelial cells but is relatively

independent of Sp1 in pancreatic ductal epithelial cells Therefore, it is possible that KLF4 and Sp1 modulate K19 expression differently, in that Sp1 is important developmentally and KLF4 directs cell fate decisions during differentiation

Jenkins *et al* (1998) also reported that KLF4 increased the transcriptional activity of keratin 4 and Epstein-Barr virus ED-L2 promoters and suggested that KLF4 may function as a transcriptional activator in the oesophageal squamous epithelium to regulate cell differentiation It is also possible that KLF4 may function as either an activator or repressor of transcription and that this property is promoter or cell type specific (Yet *et al*, 1998) Is it possible that KLF4 is involved in the regulation of cytokeratin expression levels in our cell system? In order to answer this question the factors involved in the regulation of KLF4 itself require investigation

4 5 2 3 1 Regulation of KLF4

The transcriptional regulation of KLF4 itself is poorly understood and it was recently shown by Chen *et al*, (2000) that IFN-γ enhanced its expression IFN-γ, a pleiotropic cytokine with antiproliferative and immuno-modulating activities, has been shown to exert its functions by enhancing transcription of IFN-γ-responsive genes such as IRF-1, FcrR1 and Ly6-1A/E These genes, in general, share many common features, for example, their expression is mediated by tyrosine phosphorylation of latent pre-existing STAT1 and their promoters contain an IFN-γ-activation sequence that binds specifically to phosphorylated STAT1 (Chen *et al* 2002) A study performed by Chen *et al* (2002), established the molecular mechanism by which IFN-γ induced KLF4 expression in colon cancer cells This was accomplished by demonstrating that IFN-γ increased KLF4 and IFR-1 mRNA levels in a similar fashion Wong *et al* (2002) demonstrated that the induction of IRF-1 by IFN-γ was STAT1-dependent and that the expression of the STAT1 gene depended on IRF-1 Together, these data suggest that IFN-γ-induced KLF4 expression was mediated through STAT1 and this theory was further supported by findings showing that the effect of IFN-γ on KLF4 or IRF-1 gene expression was completely abolished in STAT1-deficient cells

4 5 2 3 2 STAT1 regulation of gene expression in response to IFN-γ

The transcription factor STAT-1 is activated by the tyrosine phosphorylation mediated by JAK family kinases during cellular responses to cytokine or growth factor signalling STAT-1 directly regulates the expression of key proteins in controlling the cellular processes of growth arrest of p21 (also known as CDKN1A) and cell death via expression of caspases (Wong *et al* , 2002) **In our study it was observed that p21 mRNA was up-regulated 4 9-fold in the BrdU microarray, 2.5-fold in 5,2`-FdU microarray and 2.2-fold in IdU-treated DLKP cells, after 7 days exposure** STAT-1 is also thought to be an essential element in a range of different transcription factor complexes The two most commonly identified are GAF and ISGF3, which are involved in the signal transduction pathways of type I and II INFs, respectively (Stark *et al* , 1998, Decker *et al* , 1997)

Genetic and biochemical analyses have revealed the importance of the protein tyrosine kinase Jak1 and Jak2 and the transcription factor STAT1 in IFN-γ-depended signalling Upon ligand binding, the receptor oligomerises and Jak1 and Jak2 are activated, leading to the phosphorylation of tyrosine 440 of the IFN-γ receptor subunit 1 (INFGR1) of the receptor, which provides a docking site for STAT1 STAT1 then is phosphorylated on tyrosine 701, leading to its dimerisation and translocation to the nucleus, where it binds to the gamma-activated sequence (GAS) elements of promoters to regulate expression of downstream genes (Rama *et al* , 2001) **In our study we found the that expression of Jak2 was increased by 1.7-fold in the DLKP IdU microarray experiment and increased by 1 8- and 2 2-fold at day 3 and day 7 in the 5,2-FdU microarray experiment, respectively** Therefore, Jak2 potentially may be involved in the phosphorylation of STAT1 in our model system

Wong *et al* (2002) proposed a role of IRF-1 in the regulation of STAT-1 expression They found that STAT-1 regulates the IRF-1 gene promoter and suggested that both gene products form a feedback loop that acts to regulate cellular responses to INFs, such as IFN-γ **In this study we found that STAT-1 mRNA was increased 1.8-fold in both the BrdU and IdU microarrays and**

1.6-fold in the 5,2`-FdU-treated DLKP cells These results indicate that STAT-1 may indeed be playing a role in the regulation of IFN-γ which in turn may potentially be controlling the expression levels of KLF4 in our differentiation model system The expression levels of IFN-γ are in the BrdU, 5,2 -FdU and IdU microarray experiments are unknown One proposed reason that they may not have been identified by microarrays analysis may be as result of less than optimal probe design by Affymetrix Affymetrix do not quality control each probe set and we know from other experiments that genes which we know are up-regulated at the mRNA levels are not detected by Affymetrix GeneChips However, from analysing the microarray data sets it would seem probably that IFN-γ is in fact up-regulated in treated DLKP cells The expression of other genes that are know targets of IFN-γ, such as IRF-1 and Interleukin 18 have been demonstrated as being increased several fold in the microarray experiments

It should be noted here that the Affymetrix GeneChip system has many advantages over other platforms currently available on the market However, this system does have some drawbacks Through out this study we have identified some failing in probe design, where the system has not identified genes that we know from previous studies to be altered following exposure of DLKP cells to BrdU Some of these genes include YY1, c-*myc* and α_2-integrin We have previously shown these genes in particular have increased mRNA and protein levels following treatment with drug

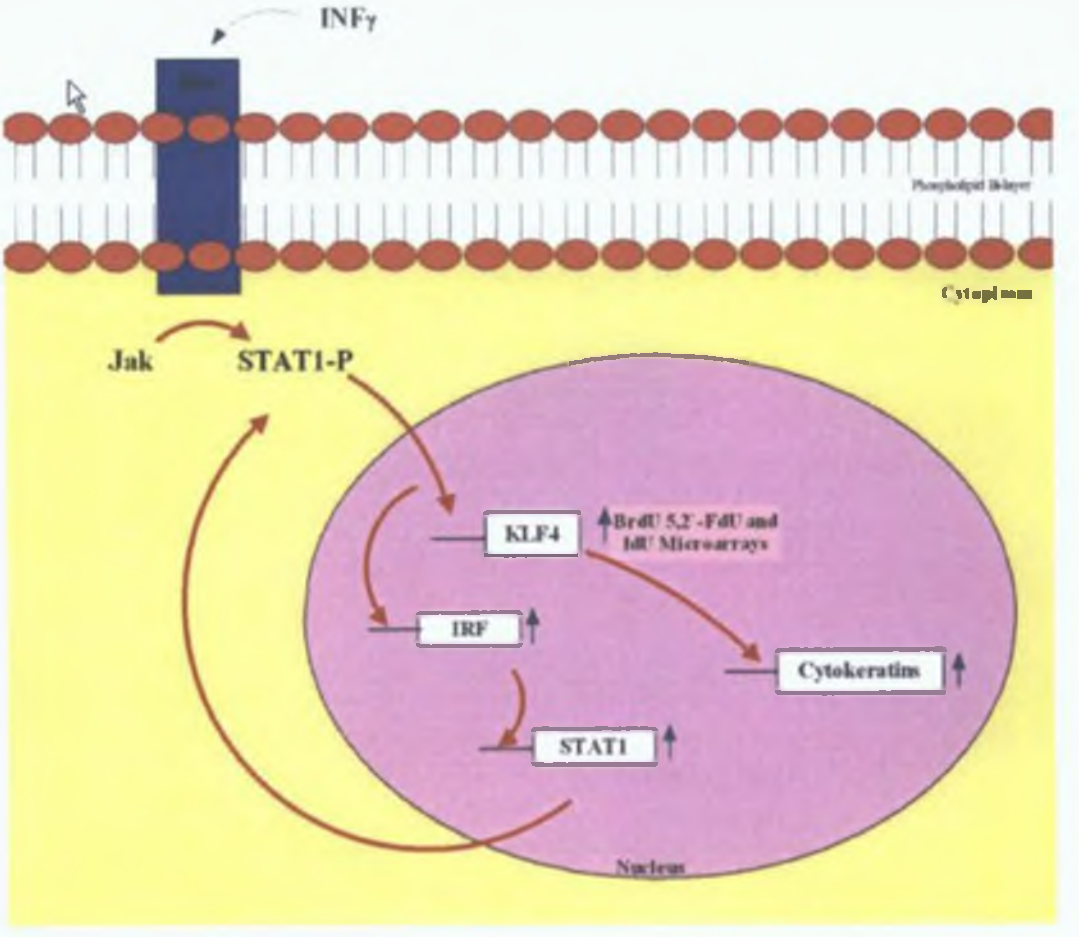


Figure 4.1 Schematic of the proposed regulation of cytokeratin expression in BrdU-, 5,2'-FdU- and IdU-treated DLKP Cells. STAT-1 is activated by phosphorylation mediated by the Jak kinases. Phosphorylated STAT-1 translocates to the nucleus where it is proposed to be involved in the regulation of KLF4 and IRF-1 gene expression. It is suggested that up-regulation of KLF4 increases the transcription of certain genes such as the cytokeratins. IRF-1 and STAT-1 form a feedback loop that acts to regulate the cellular responses to stimuli such as INF-γ.

4 5 2 3 3 Potential role for YY1 in regulation of IFN-γ

The nuclear factor of activated T cells (NFAT) originally described as an essential transcription factor for IL-2 gene expression in T cells, is thought to play a major role in the coordinating transcription of a number of cytokines Recent studies have demonstrated that the cytoplasmic NFAT components belong to a large family of regulatory transcription factors comprised of at least four members, NFAT 1-4, which are differentially expressed In T cells NFAT1 is expressed in both unstimulated and stimulated cells, whereas NFAT2 is expressed primarily in activated cells (Sweetser *et al* 1998)

Some studies have shown that NFAT1 (also know as NFATC2) may function in the regulation of the IFN-γ promoter Sweetser *et al* (1998) demonstrated that two strong NFAT binding sites were required for maximal expression of the IFN-γ reporter construct containing 538 bp of the IFN-γ promoter Also in this work, the group showed that YY1 did not mediate inhibition of basal IFN-γ expression Previous studies suggested that YY1 suppresses IFN-γ promoter function in Jurkat T cells by interacting at two regions within a silencer element located between the NFAT-binding sites (Ye *et al* , 1996) In contrast to this Yen *et al* (1996), Sweetser *et al* (1998) demonstrated that proteins binding to the NFAT and YY1 sites on the IFN-γ promoter may serve to initiate expression of IFN-γ in primary splenocytes Although YY1 has been shown to interact with many other transcriptional regulators, interaction with NFAT proteins has not yet been demonstrated

The mechanisms by which YY1 induces transcriptional activation of cellular genes such as c-*myc* and how proteins modulate the activating and repressive activities of YY1 remain yet unclear And this is the case of the effect of YY1 on the IFN-γ promoter, however, this transcription factor is thought to play a complex and context-dependent role in the regulation of IFN-γ expression

4 5.2.3 4 YY1 transient over-expression versus BrdU-treatment in DLKP cells

Following DNA microarray analysis performed on DLKP-treated cells with BrdU, 5,2 -FdU and IdU (Sections 3 2 and Section 4 5) it was decided to investigate if some of the differentially expressed genes identified in these microarrays experiments were also altered following the transient over-expression of YY1 RT-PCR analysis revealed that the transient over-expression of YY1 did in fact up-regulate the expression of Hmox1, Id2, Id3 and FHL1 mRNAs (Section 3 2) However, further RT-PCR analysis for FSTL1 mRNA expression demonstrated that YY1 over-expression had no effect on expression levels of FSTL1 (Section 3 2)

Promoter analysis on these four genes was also performed (Section 2 16) Interestingly, this analysis demonstrated that the promoter sequences of the Id2 and FHL1 genes both contained binding motifs for the Sp1 and KLF4 transcription factors Analysis on the BrdU, 5,2 -FdU and IdU microarrays demonstrated that KLF4 and the Id family of transcription factors may potentially play an important role in the induction of differentiation in our DLKP model system We have earlier discussed (Section 4 5 2 3 3) how YY1 has been shown to regulate the expression levels of IFN-γ in certain cell types and how in turn IFN-γ, as well as STAT-1, are involved in the regulation of KLF4 In previous studies, YY1 has been demonstrated to associate with the transcription factor Sp1 and the association of these two factors as been shown to activate the transcription of a number of cellular genes (Section 1 7 2) Therefore, it is plausible to hypothesise that YY1 is in fact a major controlling factor in the regulation of differentiation induced in our DLKP model system by BrdU, as previously suggested (Walsh D , PhD Thesis, 1999)

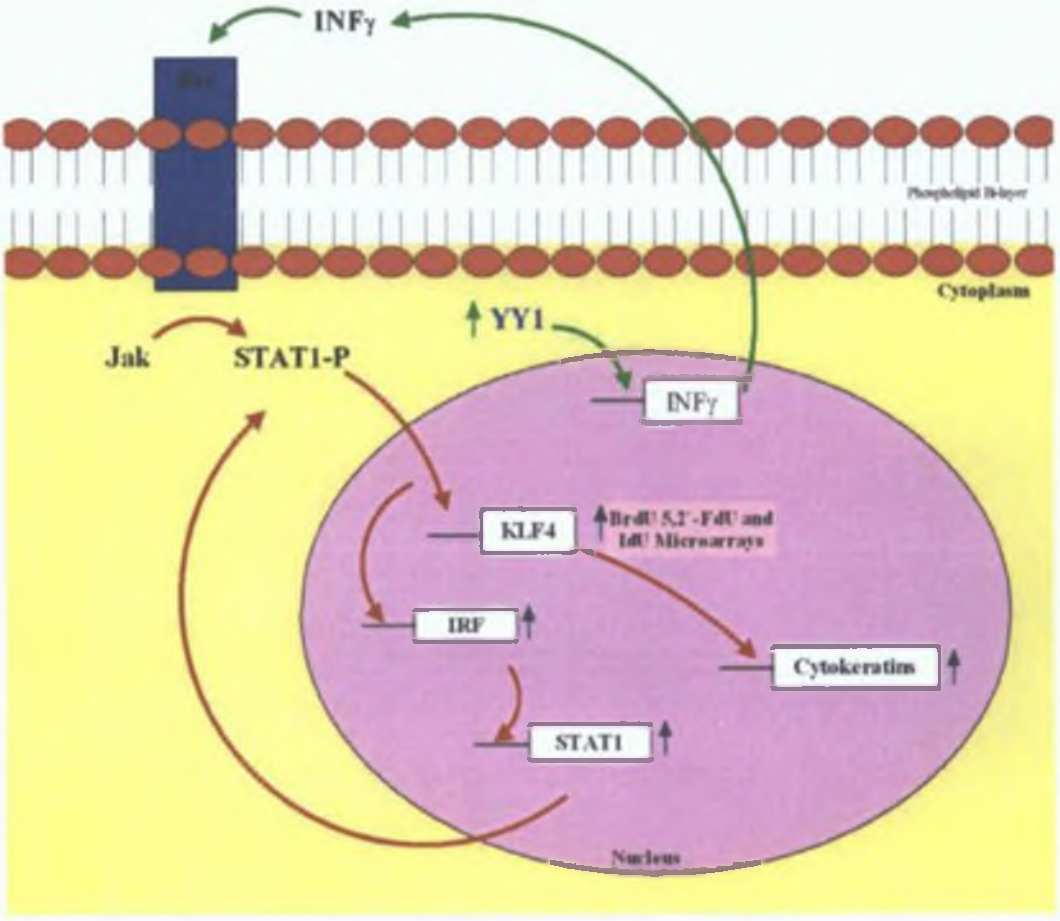


Figure 4.2 Hypothesised Involvement of YY1 in the Regulation of INFγ

It is hypothesised that YY1 may be involved in the regulation of expression of IFN-γ, and that IFN-γ then stimulates the phosphorylation of STAT-1, mediated by Jak, which results in the activation of transcription of KLF4.

4 5 2 3 5 Other genes identified in the development category included FGF-2 and LY6H

Fibroblast Growth Factor-2

Fibroblast growth factors (FGF) make up a large family of polypeptide growth factors that are found in a wide variety of organisms In vertebrates, 22 members have been identified and are thought to be highly conserved in both gene structure and amino acid sequence Most FGF genes are found distributed around the geneome (Ornitz and Itoh, 2001) The members of this family of proteins are differentially expressed in many tissue types, but the patterns and timing of expression differ Some FGFs are expressed exclusively during embryonic development (FGF 3, 4, 8, 15, 17, and 19), whereas others are expressed in embryonic and adult tissues (FGFs 1, 2, 5-7 16, 18 and 20-23)

In our differentiation system, DNA microarray analysis revealed that FGF-2 mRNA levels were increased in the BrdU, 5,2 -FdU and IdU microarrays The expression of FGF-2 mRNA was found to be increased to 1 6-fold in all three arrays The expression patterns of FGFs suggest that they have important roles in development They are often involved in the direct signalling across epithelial-mesenchymal boundaries (Hogan 1999), the expression of each family member is thought to be tightly controlled However, the exact mechanisms regulating FGFs activity *in vivo* are not yet fully understood The roles which FGFs play in development have also yet to be determined

LY6H

The ly6 family of molecules was first identified in mouse and are a class of glycosylphosphatidylinositol-anchored cell surface glycoproteins Highly restrictive patterns of expression of Ly6 genes in specific subpopulations of murine myeloid and lymphoid cells established Ly6 molecules as markers for T-cell differentiation and for hematopoietic stem cells

Murine Ly-6 molecules are a family of cell surface glycoproteins which have interesting patterns of tissue expression during haematopoiesis from multipotential stem cells to lineage committed precursor cells, and on specific leucocyte subpopulations in the peripheral lymphoid tissues These interesting patterns of tissue expression suggest an intimate association between the regulation of Ly-6 expression and the development and homeostasis of the immune system Ly-6 molecules are low molecular weight phosphatidyl inositol anchored glycoproteins with remarkable amino acid homology throughout a distinctive cysteine rich protein domain that is associated predominantly with O-linked carbohydrate These molecules are encoded by multiple tightly linked genes located on chromosome 15 which have conserved geneomic organization The *in vivo* functions of Ly-6 molecules are not known although *in vitro* studies suggest a role in cellular activation

Ly6H appeared to play a role in the differentiation pathway induced in DLKP suggestion that following the treatment with BrdU, 5,2 -FdU and IdU **It was observed that the mRNA expression levels of this gene was increased to 2 0- and 1 9-fold in the BrdU and 5,2`-FdU microarrays, and to 1 7-fold in the IdU experiment following 7 days exposure of DLKP to each of the analogues**

4.6 Investigation of potentially co-regulated genes in BrdU-treated cells

Time course microarray experiments reveal information about the temporal transcription profile of genes. Sets of genes with the same expression pattern can be grouped into clusters but the identification of molecular mechanisms responsible for co-expression requires further investigation. By using comparative promoter analysis it is possible to identify genes for which co-expression maybe a result of re-regulation. The aim of this analysis was to find promoter features that are potentially responsible for co-expression of different genes.

DLKP cells exposed to the differentiating-modulating agent, BrdU, undergo morphological change and several epithelia markers of lung cell differentiation are induced. In order to analyse global changes, the transcriptional profiles of BrdU-treated DLKP cells were examined using Affymetrix U133A DNA microarray GeneChips®. Following DNA microarray data was normalised and passed through various filters, as described in Section 3.2.2.3. Effective comparative promoter analysis requires tightly clustered gene expression profiles. To generate tight clusters the gene lists were subjected to ANOVA analysis (Section 3.2.2.5).

It is beyond the scope of this thesis to analyse all the clusters from the BrdU array for co-regulated set of differentially expressed genes. Therefore, it was decided to select one cluster and to demonstrate how it is possible to identify potentially co-regulated genes using the Genomatix Suite software (www.genomatix.de). In this worked example genes contained in Cluster 5 were chosen. The decision to choose Cluster 5 was made because genes contained in this cluster where found to be up-regulated following three days exposure to BrdU and remained at this level after 7 days.

A total of 115 genes were contained in Cluster 5 and were imported into the Genomatix BiblioSphere software package (Section 2.16) and were filtered using 'Biological Processes' Gene Ontology filter. After applying GO filter the genes were categorised as illustrated in figure 4.3 and ranked according o z-score. 15

genes (Table 4.2) were found to be classified into the 'Development' category and had a z-scoring of 1.68. These were chosen for further analysis. The hypothesis behind is experiment was that co-expressed genes that are involved in the same biological process may potentially be co-regulated at a promoter level. We could have classified the genes based on involvement in other cellular processes such as disease or molecular function alternatively, but since the overall aim of this thesis in the investigation of genes involved in cellular differentiation it was decided to select the 'Development' category.

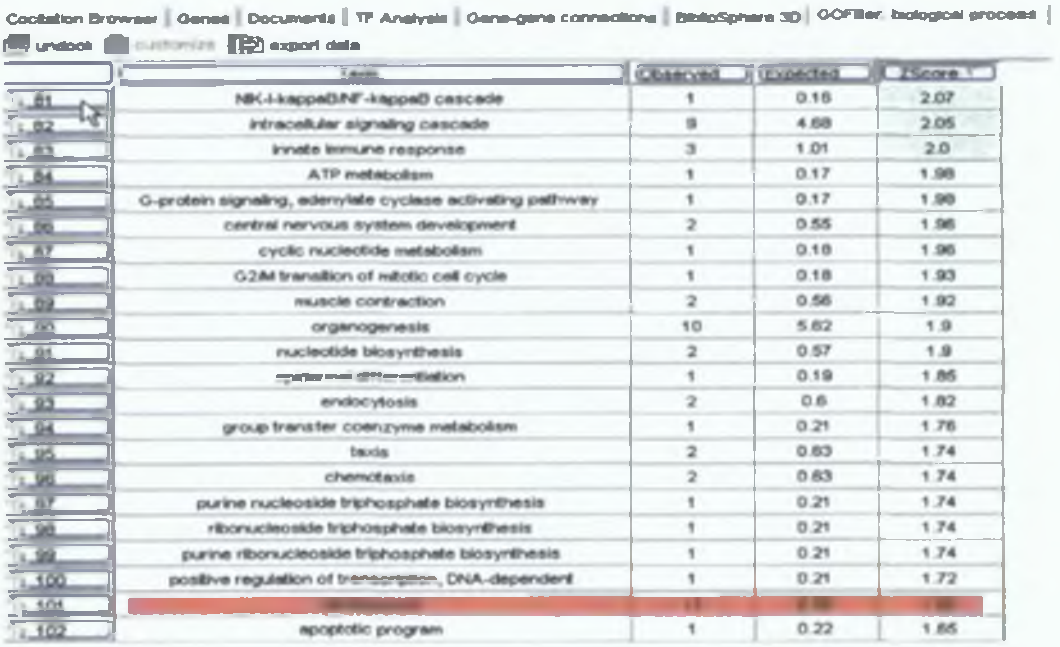


Figure 4.3 Biological Filter of genes contain in Cluster 5.

Common Name		LocusId	Affymetrix Id
EPAS1	endothelial PAS domain protein 1	2034	200878_at
SCML1	sex comb on midleg-like 1 (Drosophila)	6322	218793_s_at
MYH9	myosin, heavy polypeptide 9, non-muscle	4627	211926_s_at
PHLDA2	pleckstrin homology-like domain, family A, member 2	7262	209803_s_at
TPM2	tropomyosin 2 (beta)	7169	212654_at
TPM1	tropomyosin 1 (alpha)	7168	206117_at
SMTN	smoothelin	6525	209427_at
CXCR4	chemokine (C-X-C motif) receptor 4	7852	217028_at
COL1A1	collagen, type I, alpha 1	1277	202310_s_at
PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	9060	203060_s_at
ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	2114	201329_s_at
HMGA1	high mobility group AT-hook 1	3159	206074_s_at
RPS6KA6	ribosomal protein S6 kinase, 90kDa, polypeptide 6	27330	220738_s_at
ADORA2A	adenosine A2a receptor	135	205013_s_at
GPR64	G protein-coupled receptor 64	10149	206002_at

Table 4 2 15 genes contained in 'Development' Category

The promoters for each of these 15 genes were retrieved using Genomatix Gene2Promoter software packages and the promoter regions of the 15 genes were then searched for a common framework of transcription factor binding sites 500 bps upstream of the transcriptional start site, for each of the genes was analysed Following this analysis, several models were identified The model illustrated below (Figure 4 4) was chosen on the basis that it contained an E box transcription factor matrix Previous work in this laboratory has suggested that c-*myc* may play an important role in the control of the differentiation pathway of BrdU-treated DLKP cells (Walsh, PhD Thesis 1999) c-Myc is known to contain an E box binding motif (Section 1 7 1 1)

The model chosen (Figure 4 4) contains three transcription factor elements MAZF, ZBPF and Ebox, were found in 6 (40%) (Figure 4 4) of the 15 gene promoters from the 'Development' category

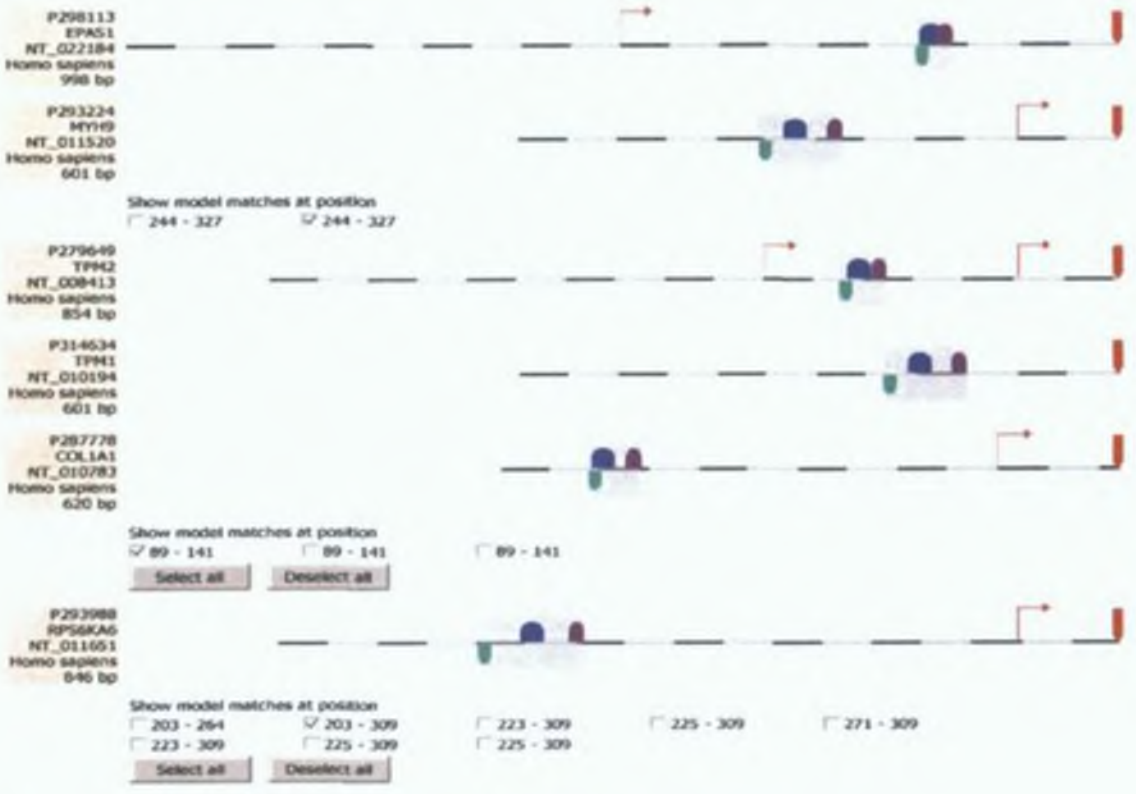


Figure 4.4 Binding sites for MAZF, ZBPF and Ebox in the upstream promoter regions of EPAS1, MYH9, TMP1, TMP2 COLA1 RPS6KA6.

4.6.1 Role for MAZF, ZPF and EBOX Regulation of other human promoter sequences

In order to investigate the specificity of the promoter model containing MAZF, ZBPF and E box this model was searched for in all known human promoter regions. This was performed by using Genomatix ModelInspector program. From this analysis it was found that the promoter model occurs in 94 human gene promoter sequences, two of which are involved in regulation of protein biosynthesis (eIFG1 and PRKAA1). Another set of genes whose promoters also contain this model transcription factor framework are these involved in the protein kinase cascade. Included in this list of genes were STK17B, ADRA2C, PRKAA1, F2R and RPS6KA2 (Figure 4.5).

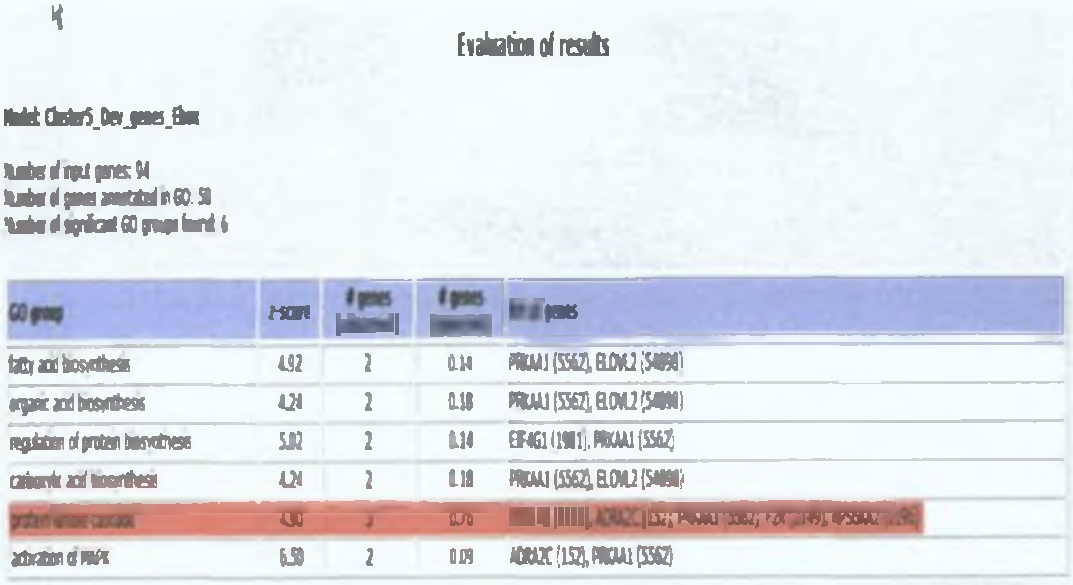


Figure 4.5 Evaluation of ModelInspector Matches

Interestingly, one gene, STK17A, whose closely related family member STK17B promoter region was found to contain the proposed transcription factor model motif; was also found in cluster 5. From DNA microarray analysis we found that STK17A mRNA expression levels were increased by 1.7- and 1.9-fold at day 3 and day 7, respectively, in the BrdU Exp. 3 microarray experiments. STK17B expression levels were also found increased in the DLKP BrdU Exp. 3 microarray experiment. At day 3 and day 7 the mRNA expression levels of this gene was found to be 1.7- and 1.8-fold increased, respectively. This may be further evidence that the transcription factor model system that we have identified in this example may indeed be biologically relevant.

Further experimental work is required to confirm the model transcription factor framework identified in this example. Also, in this example only one cluster from the BrdU Exp. 3 microarray data set was investigated and genes contained in this cluster were found up-regulated at day 3 and remained up-regulated at day 7. Genes whose expression levels increase and decrease across the experiment and genes that are down-regulated at day 3 and day 7 may be investigated for co-regulation. The work required to investigate further potentially co-regulated genes in the differentiation experiments, is beyond what is possible in the time allotted for this thesis and further work may identify other interesting models

systems that are responsible for the gene expression patterns observed in our *in vitro* differentiation model system

In light of this it is clear that the results generated in these microarray experiments could be extensively mined in the manner demonstrated to reveal countless groups of potentially co-regulated genes The breath of this analysis was beyond the scope of this thesis, therefore, it was decided to illustrate the powerful use of this type of analysis in one cluster of the DLKP BrdU microarray experiment The process just described is an iterative one and further work could potentially generate additional interesting transcription factor frameworks

4 7 Summary

Experiments performed in this study have demonstrated that the pyrimidine analogues investigated induce a very similar gene expression changes and differentiation pattern, in drug-treated DLKP and A549 cell lines These alterations in gene expression are reflected in a common set of transcriptional gene changes, as well as drug-specific changes We have identified a whole host of additional differentially expressed genes following BrdU exposure, as well as in 5,2 -FdU and IdU-treated DLKP cells by DNA microarray analysis And have validated some of these gene expression alternations by RT-PCR

It has also been shown in this thesis that YY1 is directly capable of regulating some, but not all, of the genes altered following BrdU exposure The results of these studies further the hypothesis put forward by Walsh (PhD Thesis, 1999), which suggested that YY1 potentially plays a key role in the regulation of differentiation in our *in vitro* model system

We have analysed all these results and discussed some possible models for the observed transcriptional alternations induced in our cell systems upon induction of differentiation and suggest some experiments to future work

Section 5.0 Conclusions and Future Work

5 1 Conclusions

❖ Pyrimdine Analogues Induce Differentiation in DLKP and A549 cell lines

This project began, as an investigation into the mechanism(s) by with the differentiating modulating agent, BrdU, is able to alter the differentiation status of epithelial lung cells A range of pyrmidine analogues, as well as BrdU, namely IdU, CdU, BromoUracil, BromoUridine, 5-FU, 5,2 -FdU and 5,5 -FdU, were also investigated to determine if similar differentiation affects were seen with these analogues as was seen with BrdU-treated DLKP and A549 cells

Our results demonstrate a common differentiation effect of all the pyrmindine analogues in both the DLKP and A549 cell lines The ability of these analogues to induce differentiation in both cell lines was assessed by examining their effect on protein expression levels of cytokeratin 8, 18 and 19, α2-mtegrin, β_1-intégrin and Ep-CAM This study has shown that these proteins are induced in DLKP cells and are increased A549 cells following exposure to these analogues

❖ cDNA Transfection of c-*myc* and YY1 into DLKP

In light of work performed in our laboratory which demonstrated that BrdU treatment of DLKP and A549 cells significantly increases in the expression of c-Myc and YY1 (Walsh, PhD Thesis, 1999) It has been suggest that these two transcription factors may potentially be important key regulators of differentiation in our cell system Therefore, it was decided to transfect full length human c-*myc* and YY1 cDNAs into the DLKP cell line in order to determine if their over-expression induced similar patters of gene expression as was demonstrated in BrdU-treatment of DLKP

Attempts to stably over-express the c-*myc* expression vector in our DLKP cell line proved unsuccessful Clones isolated from a mixed population of cells did

not exhibit any significant up-regulation in either c-*myc* mRNA or protein levels. Therefore, it was decided to transiently transfect the vector in order to determine if c-*myc* was a key regulator of differentiation. We demonstrated that the transient over-expression of c-*myc* had a positive effect on YY1 and eIF4E protein expression levels which is in agreement with results obtained from the treatment of DLKP cells with BrdU.

Over-expression of the transcription factor YY1, into DLKP cells also resulted in a significant increase in the expression levels of c-Myc and eIF4E proteins. Also associated with the transient increase in YY1 levels was a increase in genes which were identified as differentially expressed in the DNA microarrays performed in this study. We show that mRNA levels for FHL1, Id2, Id3 and HMOX1 are all increased in YY1 transiently transfected cells. This provides further evidence that YY1 is indeed an important factor in the regulation of differentiation and that YY1 transfection and BrdU treatment of the DLKP cell line may induce a similar pattern of differentiation in our cell system.

❖ **DNA Microarray Experiments**

DNA microarrays were also performed on DLKP cells treated with 5,2'-FdU, IdU and BrdU in order to examine global transcriptional changes which occur in DLKP cells following exposure to these differentiation-inducing drugs. 93 up-regulated differentially expressed genes were identified as common to all three microarray experiments. These genes were further investigated in order to determine their involvement in the process of differentiation in our cell system. These 93 genes were subjected to EASE analysis, which we used, helped to identify biological patterns in the gene list. Following this analysis we narrowed our focus on a subset of the 93 genes which are thought to be involved in cellular development. We propose that the Id family of transcription factors may be important regulators of differentiation in our cell models. This family of helix-loop-helix transcription factors has also been demonstrated as being a central regulator in the differentiation of other types of epithelial cells, besides the lung. These studies provide evidence that Id proteins may in fact be significant

controllers in our system and we suggest that these transcription factors may indeed have a wider role to play in cellular process than traditionally thought

Another finding from the microarray experiments performed in this thesis was the involvement of the transcription factor, KLF4 KLF4 has been shown to be important in the gastrointestinal tract where the gene is thought to regulate cell growth and differentiation This gene has also been associated with the regulation of cytokeratin expression in other cell systems and from the results of this thesis we hypothesis that this transcription factor could potentially be involved in the regulation of cytokeratin expression in BrdU-treated DLKP cells

From our microarray experiments we propose a role for KLF4 in the regulation of cytokeratin expression in our differentiation system We hypothesis that KLF4 mRNA levels are increase mediated by the phosphorylated STAT-1 and IFNγ and IRF-1 We also suggest a possible role for YY1 involvement in the regulation of IFNγ within the cell The involvement of YY1 in this model is further strengthen following promoter analysis of a subset of genes identified as differentially expressed in the DNA microarray experiments As mentioned earlier we have shown that YY1 is capable of inducing the expression of FHL1, Id2, Id3 and HMOX1 in DLKP cells following transient over-expression studies We demonstrate that the promoter sequences of Id2 and FHL1 genes both contain binding motifs for Sp1 and KLF4 transcription factors, which provides further evidence for important roles for both YY1 and KLF4 in our proposed model

Future Work.

The work described in this thesis has identified a number of key factors and the mechanism(s) that maybe critical in the control of differentiation of lung epithelial cells Areas that would be of interest for further study may include

1 **YY1 Transfections**

- DNA Arrays and Protein Arrays Perform DNA arrays on stable YY1 clones Investigate of YY1 over-expressing clones by DNA microarray analysis may provide further leads and help to identify gene targets for this unusual transcription factor Comparison of the results of YY1 and the microarray experiments preformed on pyrmidine-treated DLKP cells may identify common regulatory genes in our *in vitro* lung differentiation model Investigate on the altered protein profiles in YY1 transfected cells may also be of interest The use of new protein arrays, as provided Ciphergen SELI-TOF system, may generate interesting leads for further investigation

- DIGE 2D electrophoresis Analysis experiments on stable YY1 clones using DIGE analysis may also aid in the identification of proteins thought to be under either the activation or repression control of YY1

- Immunocytochemical and *in-situ* hybridisation studies using tissue sections from early lung and carcinoma samples may be used to identify the *in-vivo* relevance of factors, such as YY1, shown to be involved in differentiation in our *in-vitro* model

- In order to determine the percentage of transfect cells in the YY1 transient transfections co-transfection of the YY1 plasmid along with GFP or β-Gal should be preformed

2. c-Myc Transfections

- In order to investigate further the role which c-*myc* plays in our model differentiation system and to overcome the difficulties experienced in this study with stably transfecting the c-*myc* vector into the DLKP cell line, transfection with an inducible c-*myc* plasmid may offer an alternative approach
- Further work is required to confirm the hypothesis that overexpression of c-*myc* in our model system does in fact induce apoptosis RT-PCR and Western blot analysis for pro-apoptotic factors should be performed

3 Thymidine Analogues and DNA Microarrays

- Quantitative RT-PCR analysis confirm the microarray results using Real Time-PCR

- Transfection of KLF4 into the DLKP cell line From the results presented in this thesis it appears that KLF4 may be an important transcription factor in the regulation of cytokeratin expression in our cell system Therefore, it would be interesting to transfect a full length human cDNA for this gene into DLKP cells to investigate if it is possible to mimic the gene expression pattern with KLF4 over-expression as was demonstrated in pyrimidine-treated DLKP cells

- DNA microarray analysis generates vast amounts of data, analysis of some of which is only possible in the time provided for the completion of this thesis In this study only up-regulated genes where investigated Down-regulated genes may also play an important role in the regulation of cellular process and differentiation, therefore, warrant further investigation It is possible that the microarray data sets generated in this body of work could yet reveal more interesting

genes and pathways involved in the regulation early lung differentiation, if re-mined

- Further microarray analysis could reveal interesting targets for siRNA experiments It would be interesting to known-down key genes that are potentially regulating the process of differentiation in our cell system

- Promoter analysis in this study we demonstrated the possible use of promoter analysis software for the identification of potentially co-regulated genes in the BrdU microarray experiments As mentioned, this process is iterative, and is beyond the scope of this thesis to fully mine the data to its full potential Similar analysis of clusters is possible for the 5,2`-FdU and IdU microarray experiments In light of the model transcription factor framework identified in this thesis, it is clear that other framework models of more significant importance may yet be identified from the microarray data sets

- DIGE 2D electrophoresis and protein Arrays Comparison of control versus treated cells, using DIGE 2D electrophoresis and protein arrays may help to identify proteins that may be up- or down-regulated in our model system for lung cell differentiation Unknown proteins may then be tentatively identified using Mass Spec analysis

Section 6 0 **Bibliography**

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Section 7.0 Appendices

7 1 Appendix A – Differentially Expressed Genes Identified in BrdU Exp 3 DNA Microarray Experiment

List of differentially expressed genes identified from microarray analysis of BrdU Exp 3 DNA microarrays Genes listed are sorted by Affymetrix ID number

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
1405_i_at	6352	1	A	12.878	P,A	67.626	P	CCL5
200600_at	4478	1	P	1.354	P	1.630	P	MSN
200606_at	1832	1	P	1.275	P	1.626	P	DSP
200609_s_at	9948	1	P	1.529	P	1.480	P	WDR1
200611_s_at	9948	1	P	1.537	P	1.500	P	WDR1
200621_at	1465	1	P	1.570	P	1.708	P	CSRP1
200632_s_at	10397	1	P	1.329	P	1.531	P	NDRG1
200677_at	754	1	P	1.252	P	1.515	P	PTTG1IP
200678_x_at	2896	1	P	0.643	P	0.859	P	GRN
200696_s_at	2934	1	P	1.411	P	1.860	P	GSN
200697_at	3098	1	P	1.415	P	1.532	P	HK1
200715_x_at	23521	1	P	0.637	P	0.783	P	RPL13A
200742_s_at	1200	1	P	0.572	P	0.826	P	CLN2
200743_s_at	1200	1	P	0.559	P	0.784	P	CLN2
200760_s_at	10550	1	P	1.309	P	1.761	P	JWA
200761_s_at	10550	1	P	1.191	P	1.738	P	JWA
200762_at	1808	1	P	1.699	P	2.095	P	DPYSL2
200770_s_at	3915	1	P	1.234	P	1.717	P	LAMC1
200779_at	468	1	P	0.423	P	0.453	P	ATF4
200785_s_at	4035	1	P	1.290	P	1.521	P	LRP1
200787_s_at	8682	1	P	1.506	P	1.940	P	PEA15
200788_s_at	8682	1	P	1.486	P	1.880	P	PEA15
200790_at	4953	1	P	1.551	P	1.213	P	ODC1
200802_at	6301	1	P	0.652	P	0.659	P	SARS
200808_s_at	7791	1	P	1.880	P	1.962	P	ZYX
200838_at	1508	1	P	1.093	P	2.031	P	CTSB
200839_s_at	1508	1	P	1.062	P	1.769	P	CTSB
200841_s_at	2058	1	P	0.633	P	0.742	P	EPRS
200872_at	6281	1	P	1.473	P	2.429	P	S100A10
200878_at	29952	1	A	2.247	P,M,A	2.708	P	EPAS1
200887_s_at	6772	1	P	1.627	P	2.377	P	STAT1
200897_s_at	23022	1	P	1.398	P	1.836	P	KIAA0992
200904_at	3133	1	P	1.118	P	1.758	P	HLA-E
200905_x_at	3133	1	P	1.209	P	1.655	P	HLA-E
200907_s_at	23022	1	P,A	1.217	P	1.710	P	KIAA0992
200923_at	3959	1	A	1.150	M,A	2.423	P	LGALS3BP
200931_s_at	7414	1	P	1.474	P	1.745	P	VCL
200965_s_at	3983	1	P	0.544	P	0.800	P	ABLIM1
200983_x_at	966	1	P	1.362	P	1.767	P	CD59
200984_s_at	966	1	P	1.501	P	1.931	P	CD59
200985_s_at	966	1	P	1.581	P	2.096	P	CD59;
200986_at	710	1	P,A	1.259	P	1.908	P	SERPING1
200988_s_at	10197	1	P	1.665	P	1.565	P	PSME3
200989_at	3091	1	P	1.537	P	1.423	P	HIF1A
201010_s_at	10628	1	P	0.584	P	1.402	P	TXNIP
201015_s_at	3728	1	A	2.589	P,M	4.411	P	JUP
201037_at	5214	1	P	1.790	P	1.679	P	PFKP
201041_s_at	1843	1	P	1.355	P	1.848	P	DUSP1
201042_at	7052	1	P,A	13.556	P	22.821	P	TGM2
201058_s_at	10398	1	P	1.658	P	1.843	P	MYL9

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
201060_x_at	2040	1	P	1.433	P	1.743	P	STOM
201061_s_at	2040	1	P	1.314	P	1.669	P	STOM
201085_s_at	6651	1	P,A	1.374	P	1.570	P	SON
201110_s_at	7057	1	A	10.648	P	8.768	P,A	THBS1
201116_s_at	1363	1	P	1.085	P	1.537	P	CPE
201140_s_at	5878	1	P	1.487	P	1.703	P	RAB5C
201141_at	10457	1	P	1.287	P	1.578	P	GPNMB
201142_at	1965	1	P	1.386	P	1.509	P	EIF2S1
201144_s_at	1965	1	P	1.530	P	1.376	P	EIF2S1
201147_s_at	7078	1	A	2.093	A	3.727	P,M	TIMP3
201149_s_at	7078	1	P	1.905	P	2.995	P	TIMP3
201150_s_at	7078	1	A	1.292	P,A	2.131	P	TIMP3
201156_s_at	5878	1	P	1.355	P	1.592	P	RAB5C
201161_s_at	8531	1	P	1.241	P	1.555	P	CSDA
201162_at	3490	1	A	1.383	A	3.143	P	IGFBP7
201163_s_at	3490	1	P	1.634	P	3.422	P	IGFBP7
201170_s_at	8553	1	P	1.254	P	1.671	P	BHLHB2
201189_s_at	3710	1	P,A	1.362	P	1.681	P	ITPR3
201195_s_at	8140	1	P	0.680	P	0.553	P	SLC7A5
201206_s_at	6238	1	P	1.327	P	1.537	P	RRBP1
201214_s_at	5510	1	P	1.516	P	1.411	P	PPP1R7
201216_at	10961	1	P	0.593	P	0.717	P	ERP28
201232_s_at	5719	1	P	1.576	P	1.478	P	PSMD13
201233_at	5719	1	P	1.513	P	1.425	P	PSMD13
201263_at	6897	1	P	0.587	P	0.632	P	TARS
201266_at	7296	1	P	1.646	P	1.680	P	TXNRD1
201278_at		1	A	1.467	M,A	1.567	P,A	
201315_x_at	10581	1	P	0.870	P	1.524	P	IFITM2
201329_s_at	2114	1	A	1.617	P,A	1.713	P	ETS2
201348_at	2878	1	P	3.924	P	4.117	P	GPX3
201397_at	26227	1	P	0.436	P	0.417	P	PHGDH
201401_s_at	156	1	P	0.595	P,M,A	0.705	P,A	ADRBK1
201410_at		1	P	1.538	P	1.312	P	PLEKHB2
201416_at		1	P	1.303	P	1.740	P	SOX4
201417_at		1	P	1.198	P	1.571	P	SOX4
201422_at	10437	1	P	1.367	P	1.522	P	IFI30
201425_at	217	1	P	0.599	P	0.574	P	ALDH2
201427_s_at	6414	1	A	1.808	P,A	2.178	P,M	SEPP1
201460_at	9261	1	P	1.472	P	1.528	P	MAPKAPK2
201464_x_at	3725	1	A	2.573	P,M	3.470	P	JUN
201466_s_at	3725	1	A	2.201	P	2.898	P	JUN; AP1
201473_at	3726	1	P	1.369	P	1.899	P	JUNB
201475_x_at	4141	1	P	0.521	P	0.556	P	MARS
201482_at	5768	1	P	1.364	P	1.561	P	QSCN6
201489_at	10105	1	P	1.485	P	1.688	P	PPIF
201490_s_at	10105	1	P	1.493	P	1.827	P	PPIF
201498_at	7874	1	P	1.530	P	1.293	P	USP7
201502_s_at	4792	1	P	1.915	P	2.399	P	NFKBIA
201505_at	3912	1	P,A	1.903	P	2.354	P	LAMB1
201531_at	7538	1	P	1.184	P	1.773	P	ZFP36

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
201539_s_at	2273	1	P	1 454	P	2 417	P	FHL1
201540_at	2273	1	P	1 485	P	2 255	P	FHL1
201546_at	9320	1	P	1 577	P	1 540	P	TRIP12
201557_at	6844	1	P	0 617	P	0 790	P	VAMP2
201564_s_at	6624	1	P	1 496	P	1 609	P	FSCN1
201565_s_at	3398	1	P	3 928	P	1 901	P	ID2
201566_x_at	3398	1	P,M	5 879	P	2 464	P	ID2
201578_at	5420	1	M,A	1 188	M,A	1 558	P,M	PODXL
201596_x_at	3875	1	A	2 751	P,M	4 813	P	KRT18
201601_x_at	5805	1	P	0 833	P,M	1 587	P	IFITM1
201631_s_at	8870	1	P	2 594	P	3 867	P	IER3
201648_at		1	P	1 420	P	1 577	P	KIAA1579
201649_at	9246	1	P	1 161	P	1 818	P	UBE2L6
201655_s_at	3339	1	A	1 074	A	1 610	P	HSPG2
201666_at	7076	1	P	1 201	P	1 954	P	TIMP1
201673_s_at	2997	1	P	1 690	P	1 503	P	GYS1
201693_s_at	1958	1	P	1 009	P,A	1 554	P	EGR1
201695_s_at	4860	1	P	1 642	P	1 375	P	NP
201700_at	896	1	P	1 810	P	1 706	P	CCND3
201710_at	4605	1	P	1 687	P	1 163	P	MYBL2
201718_s_at	2037	1	P	1 446	P	1 651	P	EPB41L2
201719_s_at	2037	1	P	1 328	P	1 509	P	EPB41L
201739_at	6446	1	P	4 350	P	6 312	P	SGK
201744_s_at	4060	1	P	1 428	P	1 879	P	LUM
201762_s_at	5721	1	P	1 311	P	1 660	P	PSME2
201765_s_at	3073	1	P	0 592	P	0 840	P	HEXA
201798_s_at	26509	1	P,A	5 459	P	13 058	P	FER1L3
201810_s_at	9467	1	P	1 813	P	2 115	P	SH3BP5
201811_x_at	9467	1	P	2 037	P	2 081	P	SH3BP5
201841_s_at	3315	1	P	1 636	P	1 720	P	HSPB1
201860_s_at	5327	1	A	1 658	M,A	2 312	P,A	PLAT
201865_x_at	2908	1	P	1 479	P	1 561	P	NR3C1
201866_s_at	2908	1	P M	1 441	P	1 529	P	NR3C1
201877_s_at	5527	1	P	0 638	P	0 714	P	PPP2R5C
201891_s_at	567	1	P	1 282	P	1 598	P	B2M
201900_s_at	10327	1	P	0 521	P	0 748	P	AKR1A1
201915_at	11231	1	P	0 640	P	0 734	P	SEC63
201925_s_at	1604	1	P	0 611	P	0 771	P	DAF
201926_s_at	1604	1	P	0 627	P	0 722	P	DAF
201951_at	214	1	P	1 600	P	2 764	P	ALCAM
201952_at	214	1	P	1 483	P	2 448	P	ALCAM
201959_s_at	23077	1	P,A	1 408	P	1 698	P	MYCBP2
201960_s_at	23077	1	P	1 402	P	1 742	P	MYCBP2
201971_s_at	523	1	P	1 559	P	1 595	P	ATP6V1A
201972_at	523	1	P	1 458	P	1 614	P	ATP6V1A
201998_at	6480	1	P,M	1 529	P	1 496	P	SIAT1
202007_at	4811	1	P	1 340	P	2 095	P	NID
202008_s_at	4811	1	P	1 243	P	1 839	P	NID
202016_at	4232	1	P	1 862	P	1 731	P	MEST
202017_at	2052	1	P	1 519	P	1 532	P	EPHX1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
202023_at	1942	1	A	1 509	P,M,A	2 268	P	EFNA1
202052_s_at	26064	1	P	1 594	P	2 242	P	RAI14
202055_at		1	P,M A	1 533	P	1 546	P	KPNA1
202056_at		1	P	1 650	P	1 539	P	KPNA1
202058_s_at	3836	1	P	1 537	P	1 427	P	KPNA1
202059_s_at	3836	1	P	1 619	P	1 493	P	KPNA1
202069_s_at	3419	1	P	1 615	P	1 367	P	IDH3A
202071_at	6385	1	P	2 310	P	2 260	P	SDC4
202074_s_at	10133	1	P	1 143	P	1 560	P	OPTN
202080_s_at	22906	1	A	1 158	M,A	1 555	P,A	OIP106
202086_at	4599	1	A	2 340	P,M	6 578	P	MX1
202105_at	3476	1	P	0 645	P	0 722	P	IGBP1
202122_s_at	10226	1	P	1 468	P	1 635	P	M6PRBP1
202132_at	25937	1	M,A	1 536	P,M	1 656	P,M	TAZ
202133_at	25937	1	P	1 231	P	1 613	P	TAZ
202180_s_at	9961	1	P,A	1 104	P	1 649	P	MVP
202193_at	3985	1	P	1 711	P	1 601	P	LIMK2
202196_s_at	27122	1	A	3 642	P,A	4 850	P,M	DKK3
202241_at	10221	1	P	2 804	P	3 234	P	TRIB1
202258_s_at	10443	1	P	0 585	P	0 678	P	PFAAP5
202259_s_at	10443	1	P	0 629	P	0 787	P	PFAAP5
202272_s_at	23219	1	P	1 532	P	1 412	P	FBXO28
202284_s_at	1026	1	P	4 476	P	4 940	P	P21
202307_s_at	5696	1	P	1 881	P	2 580	P	TAP1
202310_s_at	1277	1	A	2 670	P,A	2 989	P,A	COL1A1
202328_s_at	5310	1	P	0 597	P	0 657	P	PKD1
202350_s_at	4147	1	P	1 668	P	1 884	P	MATN2
202361_at	9632	1	P	1 333	P	1 503	P	SEC24C
202370_s_at	865	1	P	1 601	P	1 336	P	CBFB
202389_s_at	3064	1	A	1 568	P	1 638	P	HD
202391_at	10409	1	P	3 198	P	4 368	P	BASP1
202402_s_at	833	1	P	0 560	P	0 602	P	CARS
202422_s_at	2182	1	P	1 501	P	1 625	P	ACSL4
202425_x_at	5530	1	P,M,A	1 237	P	1 524	P	PPP3CA
202430_s_at	5359	1	P	1 447	P	2 452	P	PLSCR1
202435_s_at	1545	1	A	1 335	P	1 670	P,A	CYP1B1
202436_s_at	1545	1	P,A	1 418	P	1 647	P	CYP1B1
202437_s_at	1545	1	A	1 486	P	2 040	P	CYP1B1
202446_s_at	5359	1	P	1 293	P	2 069	P	PLSCR1
202458_at	11098	1	A	3 120	P,M,A	6 194	P	SPUVE
202462_s_at	9879	1	P	1 543	P	1 268	P	DDX46
202468_s_at	8727	1	P	1 567	P	1 731	P	CTNNAL1
202481_at	9249	1	P	1 124	P	1 520	P	DHRS3
202524_s_at	9806	1	P	0 542	P	0 594	P	SPOCK2
202531_at	3659	1	P	1 909	P	2 375	P	IRF1
202575_at	1382	1	A	2 672	M,A	2 516	P,M,A	CRABP2
202598_at	6284	1	P	1 430	P	2 083	P	S100A13
202620_s_at	5352	1	P	1 826	P	2 047	P	PLOD2
202628_s_at	5054	1	A	17 716	A	55 277	P	SERPINE1
202630_at	10513	1	P	0 651	P	0 866	P	APPBP2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
202637_s_at	3383	1	P	1.367	P	2.120	P	ICAM1
202638_s_at	3383	1	P	1.482	P	2.282	P	ICAM1
202643_s_at	7128	1	A	1.678	P,A	2.349	P	TNFAIP3
202644_s_at	7128	1	P	1.788	P	2.315	P	TNFAIP3
202656_s_at	9792	1	P	1.594	P	1.556	P	SERTAD2
202660_at	23526	1	P	1.418	P	1.575	P	HA-1
202662_s_at	3709	1	P,M,A	1.343	P	1.679	P	ITPR2
202672_s_at	467	1	P	1.366	P	1.620	P	ATF3
202684_s_at	8731	1	P	1.883	P	1.362	P	RNMT
202686_s_at	558	1	M,A	1.259	P,M	1.829	P	AXL
202693_s_at	9263	1	P	1.691	P	1.690	P	STK17A
202695_s_at	9263	1	P,A	1.734	P	1.915	P	STK17A
202716_at	5770	1	P	1.583	P	1.633	P	PTPN1
202728_s_at	4052	1	P	0.577	P,A	0.844	P	LTBP1
202729_s_at	4052	1	P	0.652	P	0.914	P	LTBP1
202732_at	11142	1	P	1.085	P	1.566	P	PKIG
202743_at	8503	1	P	1.988	P	1.995	P	PIK3R3
202746_at	9452	1	P	2.315	P	3.499	P	ITM2A
202759_s_at	11217	1	A	1.472	P,A	1.842	P,A	PALM2
202760_s_at	11217	1	P,A	1.545	P,M,A	1.993	P,A	AKAP2
202761_s_at	23224	1	P	0.987	P	1.563	P	SYNE2
202766_s_at	2200	1	P	1.182	P	1.767	P	FBN1
202769_at		1	P	0.572	P	0.630	P	CCNG2
202794_at	3628	1	P	1.450	P	1.549	P	INPP1
202801_at	5566	1	P	1.590	P	1.454	P	PRKACA
202812_at	2548	1	P	0.515	P	0.759	P	GAA
202819_s_at	6924	1	P	1.762	P	1.460	P	TCEB3
202822_at	4026	1	P	1.414	P	1.735	P	LPP
202830_s_at	2542	1	P	0.497	A	0.630	P,A	SLC37A4
202846_s_at	5279	1	P	0.605	P	0.690	P	PIGC
202847_at	5106	1	P	0.382	P	0.381	P	PCK2
202854_at	3251	1	P	1.497	P	1.527	P	HPRT1
202870_s_at	991	1	P	1.511	P	1.396	P	CDC20
202883_s_at	5519	1	P	1.565	P	1.515	P	PPP2R1B
202887_s_at	54541	1	P	0.429	A	0.474	A	DDIT4
202934_at	3099	1	A	1.917	P,A	1.845	P,A	HK2
202948_at	3554	1	P,A	1.099	P,A	1.595	P,M,A	IL1R1
202949_s_at	2274	1	P	3.214	P	5.650	P	FHL2
202962_at	23303	1	M,A	1.689	P,A	1.867	P	KIF13B
202998_s_at	4017	1	P	1.214	P	2.216	P	LOXL2
203002_at	51421	1	P	1.636	P	1.783	P	AMOTL2
203023_at	51491	1	P	1.611	P	1.585	P	HSPC111
203051_at	22893	1	P	1.431	P	1.584	P	BAHD1
203058_s_at	9060	1	P	2.594	P	3.299	P	PAPSS2
203059_s_at	9060	1	P,M	1.694	P	2.184	P	PAPSS2
203060_s_at	9060	1	P	3.572	P	4.996	P	PAPSS2
203062_s_at	9656	1	P	1.511	P	1.563	P	MDC1
203065_s_at	857	1	P	1.411	P	1.629	P	CAV1
203066_at	51363	1	P	1.656	P	1.645	P	GALNAC4S-6ST
203072_at	4643	1	A	2.026	P	2.157	P	MYO1E

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203074_at	244	1	A	1 185	P,A	1 664	P	ANXA8
203102_s_at	4247	1	P	1 591	P	1 451	P	MGAT2
203119_at	79080	1	P	1 364	P	1 501	P	MGC2574
203140_at	604	1	P	1 299	P	2 011	P	BCL6
203148_s_at	9830	1	P	1 551	P	2 105	P	TRIM14
203153_at	3434	1	A	2 969	P,A	8 524	P	IFIT1
203158_s_at	2744	1	A	1 579	P	1 648	P	GLS
203178_at	2628	1	P	1 544	P	1 337	P	GATM
203184_at	2201	1	P	1 873	P	2 503	P	FBN2
203211_s_at	8898	1	P	1 550	P	1 336	P	MTMR2
203212_s_at	8898	1	A	1 565	P	1 536	P,A	MTMR2
203216_s_at	4646	1	P	1 547	P	1 323	P	MYO6
203242_s_at	10611	1	P,M	1 471	P	1 604	P	LIM
203243_s_at	10611	1	P	1 642	P	1 942	P	LIM
203252_at	10263	1	P	1 512	P	1 421	P	DOC-1R
203304_at	25805	1	P,M	2 356	P	2 293	P	BAMBI
203336_s_at	9270	1	P	1 559	P	1 518	P	ITGB1BP1
203359_s_at	26292	1	P	1 943	P	1 520	P	MYCBP
203360_s_at	26292	1	P	1 734	P	1 317	P	MYCBP
203381_s_at	348	1	P,M	1 376	P	2 272	P	APOE
203382_s_at	348	1	P,A	1 507	P	2 557	P	APOE
203386_at	9882	1	P,A	1 553	P	1 726	P	TBC1D4
203413_at	4753	1	P	1 253	P	1 852	P	NELL2
203423_at	5947	1	P	1 287	P	1 812	P	RBP1
203439_s_at	8614	1	P	0 674	P	0 609	P,A	STC2
203455_s_at	6303	1	P	1 713	P	2 535	P	SAT
203485_at	6252	1	P	1 575	P	1 516	P	RTN1
203504_s_at	19	1	P	1 913	P	2 457	P	ABCA1
203505_at	19	1	P	2 218	P	3 080	P	ABCA1
203526_s_at	324	1	P	1 347	P	1 584	P	APC
203558_at	9820	1	P	0 612	P M	0 914	P	CUL7
203563_at	60312	1	A	1 187	P,A	1 569	P,M,A	AFAP
203578_s_at	9057	1	P,M	1 746	P	1 392	P	SLC7A6
203585_at	7739	1	P	1 631	P	1 707	P	ZNF185
203592_s_at	10272	1	P M	1 896	P	1 801	P	FSTL3
203603_s_at	9839	1	P,A	1 574	A	1 463	P,A	ZFHX1B
203607_at	22876	1	P	1 591	P	1 529	P	INPP5F
203650_at	10544	1	P	1 335	P	1 510	P	PROCR
203657_s_at	8722	1	P	0 540	P	0 741	P	CTSF
203665_at	3162	1	P	2 443	P	2 435	P	HMOX1
203671_at	7172	1	P,A	1 647	P,M	1 565	P	TPMT
203674_at	9931	1	P	1 472	P	1 593	P	HELZ
203675_at	4925	1	P	1 180	P	1 626	P	NUCB2
203701_s_at	55621	1	P	1 548	P	1 424	P	FLJ20244
203710_at	3708	1	P	1 811	P	1 661	P	ITPR1
203725_at	1647	1	P	1 452	P	1 861	P	GADD45A
203728_at	578	1	P	1 507	P	1 486	P	BAK1
203736_s_at	8496	1	M,A	1 375	P	1 616	P	PPFIBP1
203743_s_at	6996	1	P	1 606	P	1 410	P	TDG
203767_s_at	412	1	P	2 006	P	1 691	P	STS

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203787_at	23635	1	P	1.717	P	2.234	P	SSBP2
203810_at	11080	1	P	1.593	P	1.458	P	DNAJB4
203811_s_at	11080	1	P,M,A	1.666	P	1.648	P	DNAJB4
203821_at	1839	1	A	3.276	P	2.477	P,M	DTR
203836_s_at	4217	1	A	1.462	P,A	1.899	P,A	MAP3K5
203843_at	6197	1	P	1.349	P	1.585	P	RPS6KA3
203851_at	3489	1	P,A	1.972	P	2.426	P	IGFBP6
203876_s_at	4320	1	P,A	0.573	P,M,A	0.695	P,A	MMP11;
203889_at	6447	1	M,A	1.463	P	2.005	P	SGNE1
203895_at		1	P	1.334	P	1.558	P	PLCB4
203910_at	9411	1	P	2.144	P	3.560	P	PARG1
203921_at	9435	1	P	0.439	P	0.546	P	CHST2
203927_at	4794	1	P,M	1.652	P	2.040	P	NFKBIE
203936_s_at	4318	1	P	1.434	P	1.608	P	MMP9
203946_s_at	384	1	P,A	2.360	P	2.222	P	ARG2
203951_at	1264	1	A	12.155	P	12.597	P	CNN1
203964_at	9111	1	P	1.874	P	1.995	P	NMI
203973_s_at	1052	1	P	1.188	P	1.604	P	CEBPD
203980_at	2167	1	P	1.538	P	1.611	P	FABP4
203981_s_at	5826	1	P	0.585	P	0.711	P	ABCD4
203986_at	8987	1	P	1.663	P	1.604	P	GENX-3414
203989_x_at	2149	1	A	1.891	P,A	2.739	P	F2R
204005_s_at	5074	1	P	1.501	P	1.615	P	PAWR
204024_at	734	1	P	1.539	P	1.159	P	C8orf1
204029_at	1952	1	P	0.616	P	0.643	P	CELSR2
204030_s_at	29970	1	P	1.948	P	2.721	P	SCHIP1
204035_at	7857	1	P	0.803	P	0.416	P	SCG2
204048_s_at	9749	1	P	1.321	P	1.539	P	C6orf56
204049_s_at	9749	1	P	1.515	P	1.693	P	C6orf56
204070_at	5920	1	P,A	1.431	P,A	2.723	P	RARRES3
204081_at	4900	1	P	2.118	P	1.856	P	NRGN
204082_at	5090	1	P	1.467	P	1.858	P	PBX3
204083_s_at	7169	1	P	1.545	P	1.656	P	TPM2
204094_s_at	9819	1	P	1.573	P	1.601	P	KIAA0669
204109_s_at	4800	1	P	1.530	P	1.251	P	NFYA
204135_at	11259	1	P	1.623	P	2.851	P	DOC1
204139_x_at	7593	1	P	0.602	A	0.762	P,A	ZNF42
204141_at	7280	1	P	2.284	P	2.008	P	TUBB
204159_at	1031	1	P	1.550	P	1.388	P	CDKN2C
204184_s_at	157	1	P	1.536	P	1.358	P	ADRBK2
204210_s_at	5130	1	P,A	1.534	P,A	1.414	P	PCYT1A
204218_at	25906	1	P	1.514	P	1.254	P	DKFZP564M082
204224_s_at	2643	1	P	1.961	P	1.709	P	GCH1
204238_s_at	10591	1	P	0.642	P	0.808	P	C6orf108
204260_at	1114	1	P	0.189	P	0.220	P	CHGB
204268_at	6273	1	A	2.756	P	4.154	P	S100A2
204279_at	5698	1	P	2.180	P	3.303	P	PSMB9
204284_at	5507	1	P	1.259	P,A	1.672	P	PPP1R3C
204313_s_at	1385	1	P	1.541	P	1.436	P	CREB1
204326_x_at	4500	1	P	2.032	P	2.455	P	MT1L

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
204332_s_at	175	1	P	0.600	P	0.718	P	AGA
204333_s_at	175	1	P	0.570	P	0.611	P	AGA
204347_at	205	1	P	1.642	P	1.952	P	AK3
204348_s_at	205	1	P	1.606	P	1.835	P	AK3
204358_s_at	23768	1	P	0.625	P,A	0.939	P	FLRT2
204359_at	23768	1	P	0.620	P	0.909	P	FLRT2
204360_s_at	4669	1	P	0.647	P	0.742	P	NAGLU
204368_at	6578	1	A	1.442	P,M,A	2.032	P	SLCO2A1
204396_s_at	2869	1	P	1.589	P	1.584	P	GRK5
204398_s_at	24139	1	P	0.643	P	0.825	P,M	EML2
204412_s_at	4744	1	P	1.572	P	1.257	P	NEFH
204416_x_at	341	1	A	1.584	P	1.572	P,M	APOC1
204420_at	8061	1	P	1.580	P	1.869	P	DIPA
204421_s_at	2247	1	P	1.488	P	1.563	P	FGF2
204422_s_at	2247	1	P	1.667	P	1.646	P	FGF2
204423_at	4289	1	P	1.696	P	1.558	P	MKLN1
204425_at	393	1	P	0.583	P,A	0.738	P	ARHGAP4
204455_at	667	1	A	1.901	P,A	3.900	P	BPAG1
204462_s_at	6567	1	P	1.414	P	1.766	P	SLC16A2
204465_s_at	9118	1	P	1.915	P	1.894	P	INA
204475_at	4312	1	P	3.658	P	6.287	P	MMP1
204485_s_at	10040	1	P	0.653	P	0.781	P	TOM1L1
204490_s_at	960	1	P,A	2.166	P,A	4.040	P,A	CD44
204493_at	637	1	P	1.569	P	1.427	P	BID
204514_at	1802	1	P	1.505	P	1.600	P	DPH2L2
204527_at	4644	1	P	1.519	P	1.513	P	MYO5A
204530_s_at	9760	1	P,A	1.481	A	1.702	P	TOX
204540_at	1917	1	P	1.556	P	1.229	P	EEF1A2
204567_s_at	9619	1	P	1.555	P	1.144	P	ABCG1
204616_at	7347	1	P	1.549	P	1.454	P	UCHL3
204627_s_at	3690	1	A	1.485	P,A	2.100	P	ITGB3
204632_at	8986	1	P	1.832	P	1.669	P	RPS6KA4
204646_at	1806	1	P	0.547	P	0.606	P	DPYD
204647_at	9454	1	P	1.685	P	2.199	P	HOMER3
204653_at		1	P,A	1.274	P	1.608	P	TFAP2A
204655_at	6352	1	A	2.186	P,A	7.657	P	CCL5
204665_at	80143	1	P	0.825	P	0.626	P	FLJ21168
204682_at	4053	1	P,A	1.357	P	2.179	P	LTBP2
204745_x_at	4495	1	P	1.463	P	1.882	P	MT1G
204748_at	5743	1	M,A	3.283	P	4.991	P	COX2
204749_at	4675	1	P	1.535	P	1.564	P	NAP1L3
204759_at	1102	1	P	1.684	P	1.274	P	CHC1L
204780_s_at	355	1	A	1.375	P,A	1.754	P	TNFRSF6
204788_s_at	5498	1	P	0.546	P	0.617	P	PPOX
204790_at	4092	1	P	1.807	P	1.313	P	SMAD7
204792_s_at	9742	1	P,A	0.594	P	0.819	P,A	KIAA0590
204806_x_at	3134	1	P	1.034	P	1.558	P	HLA-F
204811_s_at	9254	1	P	0.644	P	0.651	P	CACNA2D2
204859_s_at	317	1	P	1.512	P	1.599	P	APAF1
204865_at	761	1	A	1.506	P,A	1.757	P	CA3; CAIII

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
204875_s_at	2762	1	P	1 377	P	1 725	P	GMDS
204880_at	4255	1	P	0 655	P	0 764	P	MGMT
204897_at	5734	1	P	2 197	P	2 832	P	PTGER4
204908_s_at	602	1	P	1 734	P	2 267	P	BCL3
204928_s_at	8273	1	P	1 590	P	1 331	P	SLC10A3
204935_at	5771	1	P,A	1 546	P	1 237	P,A	PTPN2
204937_s_at	10782	1	P	1 525	P	1 349	P	ZNF274
204948_s_at	10468	1	A	1 305	P	1 701	P	FST
204955_at	8406	1	P	1 854	P	1 797	P	SRPX
204983_s_at	2239	1	P	1 249	P	1 621	P	GPC4
204985_s_at	79090	1	P	0 632	P	0 772	P	MGC2650
204991_s_at	4771	1	A	1 583	P,A	1 809	P,M	NF2
204995_at	8851	1	P	0 707	P	0 625	P,A	CDK5R1
204998_s_at	22809	1	P	0 532	P	0 660	P	ATF5
204999_s_at	22809	1	P	0 420	P	0 507	P	ATF5
205005_s_at	9397	1	P	1 593	P	1 426	P	NMT2
205006_s_at	9397	1	P	1 605	P	1 718	P	NMT2
205013_s_at	135	1	P,A	1 864	P	2 098	P	ADORA2A
205016_at	7039	1	A	2 225	P	2 039	P	TGFA
205034_at	9134	1	P	1 702	P	1 461	P	CCNE2
205047_s_at	440	1	P	0 279	P	0 271	P	ASNS
205081_at	1396	1	P	1 803	P	1 343	P	CRIP1
205088_at	10046	1	M	1 433	P	1 612	P	CXorf6
205097_at	1836	1	P	1 363	P	1 622	P	SLC26A2
205110_s_at	2258	1	P,A	1 482	P	1 643	P	FGF13
205111_s_at	51196	1	P	1 548	P	2 134	P	PLCE1
205112_at	51196	1	P,A	1 636	P	2 470	P	PLCE1
205115_s_at	9904	1	A	1 552	P	1 353	P,M	RBM19
205126_at	7444	1	P	1 504	P	1 542	P	VRK2
205172_x_at	1212	1	P	1 550	P	1 597	P	CLTB
205174_s_at	25797	1	P	2 687	P	3 129	P	QPCT
205192_at	9020	1	P,M	1 501	P	1 575	P	MAP3K14
205193_at	23764	1	P,M	1 594	P	1 360	P	MAFF
205196_s_at	1174	1	P,M	1 432	P,A	2 059	P,A	AP1S1
205205_at	5971	1	P	1 867	P	2 271	P	RELB
205214_at	9262	1	A	2 069	P,M,A	1 846	M,A	STK17B
205249_at	1959	1	P	1 041	P	1 649	P	EGR2
205280_at	2743	1	P	0 651	P	0 722	P	GLRB
205286_at	7022	1	A	2 102	P	3 094	P	TFAP2C
205288_at	8556	1	P	1 548	P	1 322	P,M	CDC14A
205350_at	1381	1	P	1 976	P	2 301	P	CRABP1
205352_at	5274	1	P,A	1 428	P	1 956	P	SERPINI1
205358_at	2891	1	P	0 622	P	0 774	P	GRIA2
205379_at	874	1	A	1 967	P,A	2 206	P	CBR3
205401_at	8540	1	P	1 522	P	1 327	P	AGPS
205407_at	8434	1	P	0 604	P	0 835	P	RECK
205423_at	162	1	P	1 614	P	1 524	P	AP1B1
205428_s_at	794	1	P,A	1 529	P	1 484	P	CALB2
205434_s_at	22848	1	P	1 576	P	1 496	P	AAK1
205443_at	6617	1	P	1 845	P	1 582	P	SNAPC1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
205479_s_at	5328	1	A	2 913	P	6 454	P	PLAU
205483_s_at	9636	1	P	1 597	P	2 631	P	G1P2
205493_s_at	10570	1	P	1 691	P	1 379	P	DPYSL4
205497_at	7728	1	P,A	0 649	M,A	0 781	M,A	ZNF175
205498_at	2690	1	P,M,A	1 635	P	1 803	P	GHR
205534_at	5099	1	A	1 711	P,A	2 180	P	PCDH7
205543_at	22824	1	P	1 395	P	1 560	P	APG-1
205547_s_at	6876	1	P	5 771	P	6 351	P	TAGLN
205569_at	27074	1	P	1 142	P	1 881	P	LAMP3
205606_at	4040	1	P	0 577	P	0 722	P	LRP6
205619_s_at	4222	1	A	1 342	P,A	2 228	P	MEOX1
205625_s_at	793	1	A	3 079	P	4 556	P	CALB1
205626_s_at	793	1	P,A	1 401	P	1 643	P	CALB1
205660_at	8638	1	M,A	1 247	P,A	2 008	P	OASL
205676_at	1594	1	P,A	1 479	P	1 575	P	CYP27B1
205715_at	683	1	M,A	1 517	P,M,A	1 399	M,A	BST1
205729_at	9180	1	P	1 035	P	1 544	P	OSMR
205777_at	1852	1	P	1 564	P	1 326	P	DUSP9
205807_s_at	7286	1	P	1 437	P	1 504	P	TUFT1
205828_at	4314	1	P	1 590	P	1 338	P	MMP3
205829_at	3292	1	P,A	1 885	P	1 625	P	HSD17B1
205830_at	1047	1	P	0 605	P	0 780	P	CLGN
205848_at	2620	1	P	0 656	P	0 514	P,M	GAS2
205876_at	3977	1	P,M	2 037	P	2 162	P	LIFR
205896_at	6583	1	P	1 458	P	1 508	P	SLC22A4
205899_at	8900	1	A	1 684	P,A	1 730	P,M	CCNA1
205924_at	5865	1	A	2 627	P	3 222	P	RAB3B
205925_s_at	5865	1	P	1 998	P	2 138	P	RAB3B
205937_at	10669	1	P	0 572	P	0 792	P	CGREF1
205973_at	9638	1	P,A	1 314	P	1 514	P	FEZ1
205986_at	9625	1	P,A	0 600	A	0 689	M,A	AATK
206002_at	10149	1	A	1 796	M,A	1 849	P A	GPR64
206023_at	10874	1	P	1 387	P	1 535	P	NMU
206036_s_at	5966	1	P	1 555	P	1 453	P	RELB
206067_s_at	7490	1	A	2 297	P,M,A	1 863	P,A	WT1
206068_s_at	33	1	M,A	1 636	P	1 448	P	ACADL
206074_s_at	3159	1	P	1 526	P	1 582	P	HMGA1
206100_at	1368	1	P,A	1 582	P	1 924	P	CPM
206103_at	5881	1	P	0 576	P	0 674	P	RAC3
206104_at	3670	1	P,A	1 864	P	1 713	P	ISL1
206116_s_at	7168	1	P	3 588	P	5 110	P	TPM1
206117_at	7168	1	A	3 077	P,A	4 184	P	TPM1
206128_at	152	1	P	0 621	P,A	0 816	P	ADRA2C
206137_at	9699	1	P,M	1 908	P	2 182	P	RIMS2
206142_at	7694	1	P,M	0 613	A	0 737	A	ZNF135
206157_at	5806	1	P	2 010	P	1 241	P	PTX3
206279_at	5616	1	P,A	1 471	P	1 573	P,M	PRKY
206295_at	3606	1	P,A	1 653	P	2 355	P	IL18
206343_s_at	3084	1	P	2 177	P	1 623	P	NRG1
206355_at	2774	1	P	0 623	P	0 645	P	GNAL

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
206401_s_at	4137	1	P	0 652	P,A	0 626	P,M,A	MAPT
206404_at	2254	1	P	0 695	P	0 652	P	FGF9
206424_at	1592	1	P	1 763	P	1 461	P	CYP26A1
206461_x_at	4496	1	A	1 974	P,A	2 623	P,A	MT1H
206463_s_at	10202	1	A	2 838	P,A	2 716	M,A	DHRS2
206504_at	1591	1	A	3 766	P,M	5 333	P,M,A	CYP24A1
206506_s_at	8464	1	P,A	0 640	P,A	0 811	M,A	SUPT3H
206508_at	970	1	P,A	1 738	P,A	3 310	P	TNFSF7
206580_s_at	30008	1	P	1 848	P	1 712	P	EFEMP2
206632_s_at	9582	1	P	1 920	P	2 098	P	APOBEC3B
206662_at	2745	1	P	1 832	P	2 075	P	GLRX
206675_s_at	6498	1	P,A	1 890	P	1 265	P,A	SKIL
206693_at	3574	1	P,M	1 108	P,A	1 915	P	IL7
206699_x_at	4861	1	P,A	1 553	P	1 429	P,M	NPAS1
206748_s_at	9043	1	P	1 599	P	1 443	P	SPAG9
206765_at	3759	1	P	0 629	P	0 611	P	KCNJ2
206773_at	4062	1	P,A	1 954	P	1 786	P	LY6H
206788_s_at	865	1	P	1 761	P	1 411	P	CBFB
206805_at	10371	1	P	1 173	P	1 859	P	SEMA3A
206825_at	5021	1	A	2 427	P	3 962	P	OXTR
206850_at	10633	1	P,A	1 882	P	1 420	P	RRP22
206907_at	8744	1	P	1 624	P	1 882	P	TNFSF9
207014_at	2555	1	P,M	1 312	P	1 718	P	GABRA2
207030_s_at	1466	1	P	2 743	P	2 341	P	CSRP2
207050_at	781	1	A	1 491	P,A	1 633	P,M,A	CACNA2D1
207147_at	1746	1	P	1 790	P	0 731	P,M	DLX2
207180_s_at	10553	1	P,M,A	1 511	P	1 248	P	HTATIP2
207196_s_at	10318	1	P	1 511	P	1 648	P	TNIP1
207219_at	65243	1	P	0 600	P	0 655	P	LOC65243
207281_x_at	51480	1	P	2 237	P	3 607	P	VCX2
207290_at	5362	1	P	0 385	P,A	0 403	A	PLXNA2
207302_at	6445	1	P	0 428	P	0 400	P	SGCG
207304_at	7596	1	P	2 175	P	1 509	P	ZNF45
207324_s_at	1823	1	P,M	0 597	A	0 528	A	DSC1
207332_s_at	7037	1	P	1 500	P	1 539	P	TFRC
207357_s_at	55568	1	P	1 161	P	1 527	P	GALNT10
207390_s_at	6525	1	P	1 523	P	1 530	P	SMTN
207415_at	22925	1	P,A	1 303	P M,A	1 716	P	PLA2R1
207535_s_at	4791	1	P	1 625	P	1 878	P	NFKB2
207563_s_at	8473	1	P	0 643	P	0 780	P	OGT
207574_s_at	4616	1	P	2 255	P	2 443	P	GADD45B
207643_s_at	7132	1	A	1 083	P,M	1 619	P	TNFRSF1A
207700_s_at	8202	1	P	1 456	P	1 560	P	NCOA3
207714_s_at	871	1	P	2 408	P	2 214	P	SERPINH1
207768_at	1961	1	A	3 446	P	3 661	P	EGR4
207813_s_at	2232	1	P	0 646	P	0 741	P	FDXR
207826_s_at	3399	1	P	7 473	P	4 465	P	ID3
207876_s_at	2318	1	P	1 365	P	1 739	P	FLNC
208003_s_at	10725	1	P	0 988	P	1 502	P	NFAT5
208018_s_at	3055	1	A	1 611	P,A	1 393	P,A	HCK

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
208055_s_at	26091	1	P	1.551	P	1.726	P	HERC4
208112_x_at	10938	1	P	1.504	P	1.679	P	EHD1
208116_s_at	4121	1	A	1.661	P,M,A	1.889	P	MAN1A1
208117_s_at	81887	1	P	1.630	P	1.524	P	FLJ12525
208178_x_at	7204	1	P,M,A	1.454	P	1.645	P	TRIO
208186_s_at	3991	1	P,M,A	1.847	P	1.823	P	LIPE
208309_s_at	10892	1	P	1.875	P	2.052	P	MALT1
208359_s_at	3761	1	P	0.497	P,A	0.569	P,A	KCNJ4
208370_s_at	1827	1	P	1.427	P	1.610	P	DSCR1
208433_s_at	7804	1	P	0.616	P	0.698	P	LRP8
208436_s_at	3665	1	P	1.157	P	1.636	P	IRF7
208456_s_at	22800	1	P	1.631	P	1.682	P	RRAS2
208576_s_at	8358	1	A	1.196	P,A	1.569	P,M,A	HIST1H3B
208581_x_at	4501	1	P	1.869	P	2.465	P	MT1X
208621_s_at	7430	1	P	1.501	P	1.603	P	VIL2
208622_s_at	7430	1	P	1.530	P	1.643	P	VIL2
208633_s_at	23499	1	P	1.191	P	1.635	P	MACF1
208637_x_at	87	1	P	1.511	P	1.704	P	ACTN1
208650_s_at	934	1	P,M,A	3.016	P	4.522	P	CD24
208651_x_at	934	1	P,A	2.132	P	2.999	P,A	CD24
208693_s_at	2617	1	P	0.558	P	0.562	P	GARS
208711_s_at	595	1	M,A	1.445	M,A	1.972	P	CCND1
208712_at	595	1	P,A	1.765	P	2.199	P	CCND1
208729_x_at	3106	1	P	1.113	P	1.955	P	HLA-B
208740_at	10284	1	P	1.352	P	1.635	P	SAP18
208779_x_at	780	1	P	1.342	P	1.583	P	DDR1
208782_at	11167	1	P	1.714	P	3.062	P	FSTL1
208789_at	22939	1	P	2.200	P	2.707	P	PTRF
208790_s_at	22939	1	P	1.590	P	2.630	P	PTRF
208791_at	1191	1	P	1.628	P	2.677	P	CLU
208792_s_at	1191	1	P	1.559	P	2.485	P	CLU
208813_at	2805	1	P	0.617	P	0.619	P	GOT1
208866_at	1452	1	P	1.301	P	1.633	P	CSNK1A1
208872_s_at	7905	1	P	1.521	P	1.377	P	DP1
208891_at	1848	1	P	0.518	P	0.753	P	DUSP6
208892_s_at	1848	1	P	0.522	P	0.720	P	DUSP6
208893_s_at	1848	1	P	0.391	P	0.656	P	DUSP6
208898_at	51382	1	P	2.051	P	1.758	P	ATP6V1D
208899_x_at	51382	1	P	2.028	P	1.817	P	ATP6V1D
208933_s_at	55127	1	P	1.257	P	1.724	P	FLJ10359
208934_s_at	3964	1	P	1.900	P	2.448	P	LGALS8;
208935_s_at	3964	1	P	1.185	P	1.857	P	LGALS8
208936_x_at	3964	1	P	1.626	P	1.911	P	LGALS8
208937_s_at	3397	1	P	4.800	P	2.532	P	ID1
208944_at	7048	1	P	1.585	P	2.663	P	TGFBR2
208949_s_at	3958	1	P	1.459	P	1.628	P	LGALS3
208950_s_at	501	1	P	0.631	P	0.722	P	ALDH7A1
208951_at	501	1	P	0.658	P	0.744	P	ALDH7A1
208989_s_at	22992	1	P,M	1.250	P	1.508	P	FBXL11
208991_at		1	P	1.704	P	2.387	P	STAT3

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
208992_s_at	6774	1	P,A	1 536	P	2 315	P	STAT3
208997_s_at	7351	1	P	1 438	P	1 589	P	UCP2
208998_at	7351	1	P	1 524	P	1 742	P	UCP2
209011_at	7204	1	P	1 414	P	1 536	P	TRIO
209015_s_at	10049	1	P	1 549	P	1 491	P	DNAJB6
209017_s_at	9361	1	P	0 646	P	0 691	P	PRSS15
209025_s_at	10492	1	P	1 379	P	1 517	P	SYNCRIP
209040_s_at	5696	1	M,A	2 224	P	3 927	P	PSMB8
209083_at	11151	1	A	2 468	P	2 216	P	CORO1A
209087_x_at	4162	1	P,A	2 137	P	1 898	P	MCAM
209098_s_at	182	1	P	0 588	P	0 621	P	JAG1
209099_x_at	182	1	P	0 597	P	0 626	P	JAG1
209102_s_at	26959	1	P	0 614	P	0 816	P	HBP1
209124_at	4615	1	P	1 413	P	1 580	P	MYD88
209131_s_at	8773	1	M,A	1 433	P,A	1 605	P,A	SNAP23
209140_x_at	3106	1	P	1 234	P	1 900	P	HLA-B
209155_s_at	22978	1	P	1 453	P	1 604	P	NT5C2
209166_s_at	4125	1	P	1 200	P	1 530	P	MAN2B1
209173_at	10551	1	P	0 598	A	0 549	A	AGR2
209188_x_at	1810	1	P	1 536	P	1 358	P	DR1
209189_at	2353	1	P	1 346	P	2 745	P	FOS
209191_at	84617	1	P	1 599	P	1 610	P	MGC4083
209193_at	5292	1	P	1 789	P	1 942	P	PIM1
209209_s_at	10979	1	P	1 811	P	1 712	P	PLEKHC1
209210_s_at	10979	1	P	1 619	P	1 599	P	PLEKHC1
209211_at	688	1	P	1 213	P	1 620	P	KLF5
209212_s_at	688	1	P	1 017	P	1 568	P	KLF5
209220_at	2719	1	P	0 550	P,A	0 718	P	GPC3
209260_at	2810	1	A	1 464	P,A	2 035	P,M	SFN
209267_s_at	64116	1	P,M	2 978	P	3 693	P	SLC39A8
209268_at	11311	1	P	1 446	P	1 540	P	VPS45A
209276_s_at	2745	1	P,M	1 660	P	1 565	P	GLRX
209277_at	7980	1	P	2 483	P	2 593	P	TFPI2
209278_s_at	7980	1	P	2 378	P	3 167	P	TFPI2
209288_s_at	10602	1	P	1 232	P	1 644	P	CDC42EP3
209291_at	3400	1	P	4 766	P	2 901	P	ID4
209292_at	3400	1	P	2 515	P	1 942	P	ID4
209293_x_at	3400	1	P	4 372	P	2 725	P	ID4
209304_x_at	4616	1	A	1 886	P	1 887	P,A	GADD45B
209305_s_at	4616	1	P,A	1 756	P	1 656	P	GADD45B
209340_at	6675	1	P	2 091	P	2 136	P	UAP1
209348_s_at	4094	1	P,A	1 513	P,A	1 958	P,M,A	MAF
209356_x_at	30008	1	P	2 008	P	1 941	P	EFEMP2
209366_x_at	1528	1	P	1 467	P	1 640	P	CYB5
209372_x_at	7280	1	P	1 619	P	1 625	P	TUBB
209383_at	1649	1	P	0 573	P,M	0 591	P,M,A	DDIT3
209407_s_at	10522	1	P	0 491	P,A	0 710	P	DEAF1
209420_s_at	6609	1	P,M	1 848	P	1 874	P	SMPD1
209427_at	6525	1	P,A	1 556	P	1 661	P	SMTN
209432_s_at	10488	1	P	1 598	P	1 600	P	CREB3

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
209453_at	6548	1	A	1.660	P	1.727	P	SLC9A1
209457_at	1847	1	P	2.416	P	3.400	P	DUSP5
209459_s_at	57416	1	A	4.488	P	5.435	P	ABAT
209478_at	201254	1	P	1.684	P	1.429	P	STRA13
209487_at	11030	1	A	2.105	P	2.085	P	RBPMS
209506_s_at	7025	1	P	0.641	P	0.777	P	NR2F1
209536_s_at	10916	1	P	1.810	P	2.176	P	MAGED2
209543_s_at	947	1	P	0.631	P	0.681	P	CD34
209560_s_at	8788	1	A	1.323	A	1.636	P	DLK1
209561_at	7059	1	P	1.170	P	1.595	P	THBS3
209574_s_at	753	1	P	1.409	P	1.547	P	C18orf1
209598_at	10687	1	P	1.568	P	1.861	P	PNMA2
209620_s_at	22	1	P	0.620	P	0.797	P	hABC7
209631_s_at		1	A	1.990	P,A	1.896	P,M,A	
209635_at	1174	1	P	1.474	P	2.123	P	AP1S1
209636_at	4791	1	P	1.876	P	2.559	P	NFKB2
209651_at	7041	1	P	1.739	P	1.698	P	TGFB1I1
209653_at	3840	1	P	1.709	P	1.728	P	KPNA4
209656_s_at	83604	1	P	1.613	P	1.497	P	TM4SF10
209666_s_at	1147	1	P	1.541	P	1.478	P	CHUK
209682_at	868	1	P	1.543	P	1.702	P	CBLB
209710_at	84724	1	P	2.005	P	0.954	P	GATA2
209715_at	23468	1	P	1.726	P	1.956	P	CBX5
209716_at	1435	1	P,M,A	1.173	P	1.713	P	CSF1
209758_s_at	8076	1	P	0.841	P	1.766	P	MFAP5
209759_s_at	1632	1	P	0.616	P	0.602	P	DC1
209771_x_at	934	1	P	2.774	P	4.169	P	CD24
209772_s_at	934	1	A	1.873	P,A	2.306	P	CD24
209773_s_at	6241	1	P	1.619	P	1.253	P	RRM2; R2
209788_s_at	51752	1	P	1.218	P	1.550	P	ARTS-1
209803_s_at	7262	1	P,M	5.997	P	6.779	P	PHLDA2
209818_s_at	22927	1	P	1.844	P	1.608	P	HABP4
209835_x_at	960	1	A	1.667	P,M	2.588	P	CD44
209846_s_at	11118	1	P,A	0.860	P,A	1.574	P	BTN3A2
209852_x_at	10197	1	P	1.647	P	1.621	P	PSME3
209853_s_at	10197	1	P	1.606	P	1.606	P	PSME3
209875_s_at	6696	1	A	1.714	P,A	2.386	P,A	SPP1
209894_at	3953	1	P,A	1.227	P	2.008	P,M	LEPR
209897_s_at	9353	1	P	1.021	P	1.533	P	SLIT2
209921_at	23657	1	P	0.513	P	0.703	P	SLC7A11
209960_at	3082	1	P	0.636	P	0.745	P	HGF
209967_s_at	1390	1	P	0.709	P	0.619	P	CREM
209969_s_at	6772	1	P	1.796	P	3.525	P	STAT1
210008_s_at	6183	1	P	1.762	P	1.545	P	MRPS12
210017_at	10892	1	P	1.764	P	1.728	P	MALT1
210018_x_at	10892	1	P	1.692	P	1.614	P	MALT1
210026_s_at	29775	1	P,A	1.518	P	1.440	P	CARD10
210050_at	7167	1	P,M	1.510	P	1.275	P	TPI1
210057_at	23049	1	P	1.206	P	1.702	P	SMG1
210074_at	1515	1	P	1.176	P	1.784	P	CTSL2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
210095_s_at	3486	1	P	1 570	P	1 793	P	IGFBP3
210100_s_at	20	1	P,M	0 658	A	0 871	P,A	ABCA2
210102_at	4013	1	P	1 014	P	1 527	P	LOH11CR2A,
210117_at	6674	1	P	1 783	P	1 224	P	SPAG1
210145_at	5321	1	M,A	1 545	P,A	1 693	P	PLA2G4A
210148_at	10114	1	P,A	1 980	P	1 492	P	HIPK3
210163_at	6373	1	A	1 927	A	2 936	P	CXCL11
210171_s_at	1390	1	P	0 598	P,M	0 577	P,A	CREM
210220_at	2535	1	P	0 587	P	0 703	P	FZD2
210221_at	1136	1	P,A	1 817	P	1 234	P,A	CHRNA3
210222_s_at	6252	1	P,A	1 365	P	1 503	P	RTN1
210233_at	3556	1	P,A	2 966	P	1 892	A	IL1RAP
210240_s_at	1032	1	P	1 578	P	1 086	P	CDKN2D
210298_x_at	2273	1	P	1 406	P	2 671	P	FHL1
210299_s_at	2273	1	P	1 698	P	3 269	P	FHL1
210336_x_at	7593	1	P	0 643	A	0 686	P,A	ZNF42
210358_x_at	84724	1	A	1 895	P,A	1 144	P,M,A	GATA2
210385_s_at	51752	1	P	1 205	P	1 669	P	ARTS-1
210396_s_at		1	P	1 666	P	1 321	P	
210410_s_at	4439	1	P	0 645	P,A	0 708	P	MSH5
210415_s_at	4957	1	P,M	1 612	P	1 356	P	ODF2
210457_x_at	3159	1	P	1 506	P	1 298	P	HMGA1
210463_x_at	55621	1	P	1 560	P	1 325	P	FLJ20244
210480_s_at		1	P,A	1 682	P	1 970	P	MYO6
210512_s_at	7422	1	P	0 617	P	0 543	P	VEGF
210514_x_at	3135	1	P	1 020	P	1 513	P	HLA-G
210538_s_at	330	1	P,A	10 016	P	9 540	P	BIRC3
210560_at	2637	1	P,M	3 388	P	2 070	P	GBX2
210570_x_at	5601	1	P	1 524	P	1 193	P	MAPK9
210592_s_at		1	P	1 817	P	2 819	P	SAT, SSAT
210605_s_at	4240	1	P	1 403	P	2 404	P	MFGE8
210612_s_at	8871	1	P,A	1 267	P,A	1 900	P	SYNJ2
210715_s_at	10653	1	P	1 934	P	3 224	P	SPINT2
210732_s_at	3964	1	P	1 928	P	2 242	P	LGALS8
210756_s_at		1	P	1 220	P	1 630	P	NOTCH2
210793_s_at	4928	1	P	1 813	P	1 575	P	NUP98
210797_s_at	8638	1	P	1 521	P	3 982	P	OASL
210829_s_at		1	P	1 629	P	2 203	P	SSBP2
210845_s_at	5329	1	P	2 671	P	3 584	P	PLAUR,
210869_s_at	4162	1	P,A	2 460	P	2 019	P	MCAM
210876_at	303	1	P,A	1 724	P	1 739	P	ANXA2P1
210926_at		1	P	1 447	P	1 531	P,M	FKSG30
210935_s_at		1	P	1 602	P	1 480	P	WDR1
210976_s_at	5213	1	P	0 655	P	0 848	P	PFKM
210986_s_at	7168	1	P	3 399	P	4 636	P	TPM1
210987_x_at	7168	1	P	3 168	P	4 265	P	TPM1
211003_x_at	7052	1	A	9 192	M A	13 843	P A	TGM2
211016_x_at	3308	1	P	1 516	P	1 391	P	HSPA4
211031_s_at	7461	1	P,A	1 320	P	1 654	P	CYLN2,
211043_s_at	1212	1	P	1 584	P	1 564	P	CLTB

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
211061_s_at	4247	1	P	1 858	P	1 770	P	MGAT2
211091_s_at	4771	1	P,A	1 508	P	1 496	P	NF2
211094_s_at	4763	1	P	0 997	P	1 583	P	NF1
211126_s_at	1466	1	P	2 302	P	2 088	P	CSRP2
211160_x_at	87	1	P,A	1 403	P,A	1 566	P	ACTN1
211162_x_at	6319	1	P	1 573	P	1 331	P	SCD
211340_s_at	4162	1	P,M,A	2 877	P	2 346	P	MCAM
211352_s_at	8202	1	A	1 204	P,A	1 526	P	CAGH16
211403_x_at	51481	1	P	1 991	P	2 939	P	VCX2
211456_x_at		1	P,A	1 744	P	2 486	P	
211527_x_at	7422	1	P	0 660	P	0 657	P,A	VEGFA
211528_x_at	3135	1	P	1 086	P	1 827	P	HLA-G
211529_x_at	3135	1	P	1 142	P	1 871	P	HLA-G
211530_x_at	3135	1	P	1 112	P	1 705	P	HLA-G
211538_s_at	3303	1	P	1 912	P	2 114	P	HSPA1A
211564_s_at	8572	1	P	1 343	P	1 633	P	PDLIM
211573_x_at	7052	1	A	1 795	P	2 031	P	TGM2
211600_at		1	P	0 938	P	0 534	P	PTPRO
211651_s_at	3912	1	A	1 608	P,A	2 073	P	LAMB1
211668_s_at	5328	1	P,M	2 528	P	5 235	P	PLAU, UPA
211671_s_at	2908	1	P	1 534	P	1 466	P	NR3C1
211725_s_at		1	P	1 700	P	1 575	P	
211792_s_at	1031	1	P	1 617	P	1 458	P	CDKN2C
211799_x_at	3107	1	P	0 962	P	1 664	P	HLA-C
211864_s_at	26509	1	A	3 119	P,M	6 485	P	FER1L3
211911_x_at	3106	1	P	1 121	P	2 071	P	HLA-B
211924_s_at	5329	1	P	2 665	P	3 589	P	PLAUR
211926_s_at	4627	1	P	1 555	P	1 717	P	MYH9
211928_at	1778	1	P	1 192	P	1 504	P	DNCH1
211950_at	23352	1	P	1 493	P	1 769	P	RBAF600
211962_s_at	677	1	P	1 239	P	1 834	P	ZFP36L1
211982_x_at	23214	1	P	1 607	P	1 516	P	XPO6
211986_at	195	1	P	1 915	P	2 401	P	MGC5395
211992_at	65125	1	P	1 282	P	1 535	P	PRKWNK1
211994_at		1	P	1 469	P	1 516	P	PRKWNK1
212010_s_at	55573	1	P	1 436	P	1 524	P	H41
212012_at		1	A	1 280	P,A	1 541	P,A	D2S448
212014_x_at	960	1	P,M	1 549	P,M,A	2 119	P	CD44
212022_s_at		1	P	1 619	P	1 656	P	MKI67
212023_s_at		1	P	1 549	P	1 521	P	MKI67
212032_s_at	53635	1	P	0 611	P	0 802	P	PTOV1
212061_at	23350	1	P	0 477	P	0 566	P	SR140
212063_at	960	1	A	1 971	P,A	3 607	P	CD44
212069_s_at	23121	1	P,M,A	1 440	P	1 544	P	KIAA0515
212076_at		1	P	1 130	P	1 559	P	MLL
212092_at	23089	1	P	2 584	P	3 170	P	PEG10
212094_at	23089	1	P	2 664	P	2 873	P	PEG10
212097_at	857	1	P	1 353	P	1 633	P	CAV1
212099_at		1	P	2 434	P	1 716	P	RHOB
212125_at	5905	1	P	1 450	P	1 624	P	RANGAP1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
212126_at		1	P	1.405	P	1.502	P	
212127_at	5905	1	P	1.589	P	1.903	P	RANGAP1
212135_s_at	493	1	P	1.354	P	1.919	P	ATP2B4
212136_at	493	1	P	1.290	P	1.860	P	ATP2B4
212143_s_at	3486	1	P	1.726	P	2.072	P	IGFBP3
212154_at	6383	1	P,M	1.757	P	1.761	P	SDC2
212158_at	6383	1	P,M	1.715	P	1.586	P	SDC2
212162_at	57498	1	P	1.333	P	1.513	P	KIDINS220
212171_x_at	7422	1	P	0.626	P	0.637	P	VEGF
212176_at	25957	1	P	0.623	P	0.685	P	C6orf111
212177_at		1	P	0.492	P	0.598	P	C6orf111
212179_at	25957	1	P	0.579	P	0.699	P	C6orf111
212185_x_at	4502	1	P	1.963	P	2.823	P	MT2A
212186_at	31	1	P	1.565	P	1.734	P	ACACA
212190_at	5270	1	P	1.880	P	1.499	P	SERPINE2
212192_at	115207	1	A	4.360	P,M	3.016	A	KCTD12
212196_at		1	P	1.385	P	1.513	P	IL6ST
212225_at	10209	1	P	0.558	A	0.821	P,A	SUI1
212249_at	5295	1	P,M,A	1.465	P	1.554	P	PIK3R1
212253_x_at	667	1	A	1.617	M,A	2.087	P,A	BPAG1
212254_s_at	667	1	P	1.535	P	2.146	P	BPAG1
212259_s_at	57326	1	P	0.521	P	0.701	P	PBXIP1
212268_at	1992	1	A	1.968	M,A	1.753	P,M,A	SERPINB1
212276_at	23175	1	P	1.346	P	1.536	P	LPIN1
212290_at		1	P	0.609	P	0.796	P	SLC7A1
212294_at	55970	1	P	1.322	P	1.740	P	GNG12
212295_s_at		1	P	0.634	P	0.686	P	SLC7A1
212307_s_at	8473	1	P	0.636	P	0.753	P	OGT
212312_at	598	1	P	1.588	P	1.623	P	BCL2L1
212325_at	22998	1	M,A	1.330	P,A	1.656	P,A	KIAA1102
212333_at	25940	1	P	1.598	P	1.443	P	DKFZP564F0522
212364_at	4430	1	P,A	1.678	P	1.866	P	MYO1B
212384_at	7919	1	P	0.655	P,A	0.774	P	BAT1
212387_at		1	P	0.648	P	0.771	P	TCF4
212412_at		1	P	1.489	P	1.652	P	LIM
212463_at		1	P	1.189	P	1.911	P	CD59
212470_at	9043	1	P	1.567	P	1.433	P	SPAG9
212473_s_at		1	P	1.365	P	1.964	P	
212501_at	1051	1	P	0.642	P	0.699	P	CEBPB
212511_at		1	P	1.594	P	1.461	P	PICALM
212527_at	27351	1	P	1.625	P	1.474	P	D15Wsu75e
212538_at	23348	1	P	1.225	P	1.563	P	DOCK9
212548_s_at	23045	1	P	1.504	P	1.674	P	KIAA0826
212565_at	23012	1	P,A	1.109	A	1.518	P,A	STK38L
212574_x_at	91304	1	P	0.688	P	0.628	P	R32184_3
212590_at	22800	1	P	1.672	P	1.611	P	RRAS2
212593_s_at	27250	1	P	0.643	P	0.740	P	PDCD4
212599_at	26053	1	P	1.378	P	1.623	P	AUTS2
212601_at	23140	1	P,M	1.362	P	1.655	P	ZZEF1
212624_s_at	1123	1	P	1.516	P	1.329	P	CHN1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
212636_at		1	P	1 277	P	1 581	P	QKI, QK3
212638_s_at	11059	1	P	1 627	P	1 605	P	WWP1
212641_at	3097	1	P	1 265	P	1 550	P	HIVEP2
212646_at	23180	1	A	4 120	P	4 448	P	RAFTLIN
212654_at	7169	1	P,A	1 842	P	2 106	P	TPM2
212662_at	5817	1	P	1 508	P	1 483	P	PVR
212665_at	25976	1	P	1 805	P	1 892	P	TIPARP
212693_at	23195	1	P	1 266	P	1 530	P	MDN1
212706_at	10156	1	P	1 617	P	1 485	P	RASA4
212708_at		1	P	0 565	P	0 686	P	CASC3
212714_at	113251	1	P	1 507	P	1 426	P	LOC113251
212722_s_at	23210	1	P	1 820	P	1 535	P	PTDSR
212723_at	23210	1	P	1 627	P	1 448	P	PTDSR
212748_at	57591	1	P	1 377	P	1 667	P	MKL1
212775_at	23363	1	P	1 634	P	1 636	P	KIAA0657
212776_s_at	23363	1	P	1 585	P	1 510	P	KIAA0657
212780_at	6654	1	P	1 525	P	1 345	P	SOS1
212786_at	23274	1	P,A	1 343	P	1 652	P	KIAA0350
212812_at		1	P	1 246	P	1 507	P	
212815_at	10973	1	P	1 684	P	1 789	P	HELIC1
212816_s_at	875	1	P	0 504	P	0 503	P	CBS
212828_at		1	P	1 378	P	1 755	P	SYNJ2
212829_at		1	P	1 501	P	1 689	P	
212845_at	23034	1	P	1 576	P	1 551	P	SAMD4
212848_s_at	84909	1	A	2 385	P	2 786	P	C9orf3
212859_x_at		1	P	1 746	P	2 152	P	MT2A
212884_x_at	348	1	P	1 242	P	1 771	P	APOE
212923_s_at		1	P	1 451	P	1 617	P	C6orf145
212944_at		1	P	1 201	P	2 249	P	MRPS6
212971_at	833	1	P	0 567	P	0 575	P	CARS
212980_at	23021	1	P	0 627	P,A	0 663	P,A	AHSA2
212990_at	8867	1	P	1 627	P	1 470	P	SYNJ1
213030_s_at	5362	1	P	0 474	P	0 501	P	PLXNA2
213069_at		1	M A	1 297	P,A	1 624	P	HEG
213096_at	9911	1	A	1 397	P,A	1 556	P,M	HUCEP11
213107_at	23043	1	P	1 449	P	1 907	P	TNIK
213115_at	115201	1	P	1 718	P	1 659	P	COL4A6
213118_at	23074	1	P	1 559	P	1 350	P	KIAA0701
213135_at		1	P	1 653	P	2 296	P	TIAM1
213164_at		1	P	1 183	P	2 589	P	MRPS6
213167_s_at		1	P,A	1 111	P	1 715	P	MRPS6
213191_at	148022	1	P,M,A	1 583	P	1 704	P	TRIF
213194_at	6091	1	P	1 514	P	1 827	P	ROBO1
213199_at	26005	1	P,M	1 333	P	1 579	P	DKFZP586P0123
213220_at	27250	1	P	0 657	P	0 652	P	LOC92482
213258_at		1	P	0 612	P	0 846	P,M	TFPI
213274_s_at	1508	1	P	1 152	P	2 165	P	CTSB
213275_x_at	1508	1	P	1 096	P	2 138	P	CTSB
213281_at	3725	1	P	2 050	P	3 041	P	JUN
213283_s_at	6297	1	P	0 603	P	0 768	P	SALL2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
213313_at	23637	1	P	0.647	P	0.758	P	GPR21
213338_at	25907	1	A	1.660	P	1.726	P	RIS1
213361_at	23424	1	P	1.263	P	1.558	P	TDRD7
213368_x_at	8541	1	P,A	1.530	P	1.350	P	PPFIA3
213373_s_at	841	1	P	0.728	P	0.651	P	CASP8
213411_at		1	P	0.536	M,A	0.617	P,A	ADAM22
213427_at	10799	1	P	1.669	P	1.374	P	RPP40
213438_at		1	P	0.609	P	1.038	P	
213449_at	10940	1	P	1.453	P	1.676	P,M,A	POP1
213469_at		1	P	1.283	P	1.604	P	FLJ12377
213506_at	2150	1	A	13.406	P,M,A	28.093	P	F2RL1
213558_at	27445	1	P	2.389	P	2.403	P	PCLO
213568_at	116039	1	P	1.810	P	1.926	P	OSR2
213572_s_at	1992	1	P,A	1.964	P	2.107	P	SERPINB1
213591_at		1	P	0.572	P	0.606	P	ALDH7A1
213618_at	116984	1	P	1.675	P	1.695	P	CENTD1
213622_at	1298	1	P	1.566	P	1.394	P	COL9A2
213629_x_at		1	P	1.950	P	1.916	P	MTIF
213650_at	23015	1	P	0.604	P	0.660	P	GOLGIN-67
213671_s_at	4141	1	P	0.478	P	0.526	P	MARS
213672_at	4141	1	P,A	0.828	P,A	0.636	P,A	MARS
213675_at		1	P,A	1.230	P	1.675	P	
213698_at		1	P	0.629	P	0.773	P	MGC14276
213712_at		1	M,A	1.294	P,A	1.894	P	CTNNAL1
213716_s_at	6398	1	M,A	3.568	P	3.135	P	SECTM1
213721_at	6657	1	P	0.528	P	0.543	P	SOX2
213722_at	6657	1	P,M	0.604	A	0.529	A	SOX2
213741_s_at	3836	1	P	1.609	P	1.536	P	KPNA1
213764_s_at		1	P	0.769	P	1.749	P	MFAP5
213765_at		1	P	0.941	P	1.879	P	MFAP5
213793_s_at	9456	1	P	1.893	P	2.020	P	HOMER1
213804_at		1	P	0.627	M,A	0.641	P,M,A	INPP5B
213832_at		1	P	1.592	P	1.948	P	
213859_x_at	8467	1	P	1.576	P	1.514	P	SMARCA5
213899_at	10988	1	P	1.548	P	1.359	P	METAP2
213906_at	4603	1	P	1.962	P	2.273	P	MYBL1
213924_at	65258	1	P	0.649	P	0.847	P	MPPE1
213926_s_at		1	P	1.591	P	1.480	P	HRB
213931_at	3398	1	M,A	8.131	P	2.690	P,A	ID2
213956_at	9857	1	P	1.274	P	1.537	P	CAP350
213984_at	23244	1	P	1.526	P	1.120	P	KIAA0648
213988_s_at	6303	1	P,A	1.661	P,M,A	2.970	P	SAT
213996_at	29799	1	P	0.356	P	0.487	P	YPEL1
213998_s_at	10521	1	P	0.513	P	0.735	P	DDX17
214022_s_at	5805	1	P	1.113	P	1.831	P	IFITM1
214023_x_at	7280	1	P	1.816	P	1.784	P	MGC8685
214030_at	131544	1	P	1.311	P	1.529	P	MINA
214053_at		1	P	0.521	P	0.641	P	
214071_at	65258	1	P	0.594	P	0.726	P	GNAL
214077_x_at	4213	1	P	0.646	P	0.722	P	MEIS4

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
214079_at		1	P,M,A	11 801	P	8 179	P	DHRS2
214091_s_at	2878	1	P	3 385	P	3 445	P	GPX3
214097_at	6227	1	P	1 463	P	1 702	P	RPS21
214155_s_at	113251	1	P	1 844	P	1 271	P	LOC113251
214175_x_at	8572	1	A	1 411	P,A	1 823	P,M	PDLIM4
214176_s_at	57326	1	P,A	0 629	P,A	0 934	P,A	PBXIP1
214196_s_at	1200	1	P	0 655	P	0 818	P	CLN2
214212_x_at	10979	1	P	1 405	P	1 526	P	PLEKHC1
214247_s_at	10530	1	M,A	2 019	P	3 501	P	DKK3
214368_at	10235	1	M,A	1 491	M,A	2 067	P,A	RASGRP2
214414_x_at	3039	1	A	2 589	P	2 073	P,M,A	HBA2
214437_s_at	6472	1	P	0 694	P	0 660	P	SHMT2
214505_s_at	2273	1	P	1 370	P	2 323	P	FHL1
214578_s_at	6093	1	P	1 552	P	1 466	P	ROCK1
214657_s_at		1	P	0 510	P,A	0 739	P,A	
214690_at	9014	1	P	0 649	P	0 834	P	TAF1B
214696_at	84981	1	P,A	1 945	P	1 641	P	MGC14376
214697_s_at	9991	1	P	1 733	P	1 499	P	ROD1
214722_at		1	P	1 168	P	1 758	P	LOC376745
214752_x_at	2316	1	P	1 315	P	1 524	P	FLNA
214764_at		1	P,A	0 594	P,A	0 784	P,A	KIAA0507
214784_x_at	23214	1	P	1 589	P	1 715	P	XPO6
214909_s_at	23564	1	P	1 500	P	1 267	P	DDAH2
214924_s_at	22906	1	P	1 243	P	1 712	P	OIP106
214930_at	26050	1	P	1 506	P	1 615	P	SLITRK5
214954_at	26032	1	P	2 460	P	2 485	P	KIAA0527
215014_at		1	A	3 565	P	5 117	P	
215016_x_at	667	1	P	1 415	P	1 993	P	BPAG1
215047_at	25893	1	P	1 553	P	1 332	P	DKFZp434C091
215136_s_at	11340	1	P	0 647	P	0 702	P	EXOSC8
215222_x_at	23499	1	P	1 272	P	1 528	P	MACF1
215313_x_at	3105	1	P	1 041	P	1 550	P	HLA-A
215446_s_at	114990	1	P,M A	1 575	P	1 510	P	LOX
215485_s_at	3383	1	P,A	1 287	P	1 814	P	ICAM1
215489_x_at	9454	1	P	1 539	P	2 194	P	HOMER3
215495_s_at	23034	1	P,A	2 971	P	2 475	P	SAMD4
215498_s_at	5606	1	P	1 415	P	1 566	P	MAP2K3
215499_at	5606	1	P	1 406	P	1 788	P	MAP2K3
215506_s_at	9077	1	P,A	1 211	P,M,A	1 670	P	ARHI, NOEY2
215629_s_at	79469	1	P	1 822	P	1 288	P	BCMSUNL
215643_at		1	P	0 491	P	0 541	P	SEMA3D
215684_s_at	84164	1	P	1 514	P	1 603	P	ASC1p100
215695_s_at	8908	1	P	1 396	P,M	1 571	P,A	GYG2
215706_x_at	7791	1	P	2 157	P	2 230	P	ZYX
215719_x_at	355	1	P	1 784	P,A	2 585	P	TNFRSF6
215780_s_at		1	P	0 702	P	0 633	P	
215783_s_at		1	P	1 356	P	1 774	P	ALPL
216041_x_at	2896	1	P	0 660	P	0 911	P	GRN
216060_s_at	23002	1	P A	1 451	P	1 591	P	DAAM1
216061_x_at	5155	1	P,A	1 583	P	1 763	P	PDGFB

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
216064_s_at	175	1	P	0.550	P	0.623	P	AGA
216230_x_at	6609	1	P,A	1.850	P	1.693	P	SMPD1
216231_s_at	567	1	P	1.334	P	1.683	P	B2M
216247_at	6224	1	P	0.578	P	0.724	P	RPS20
216252_x_at	355	1	A	1.664	P,A	1.814	P,A	TNFRSF6
216264_s_at	3913	1	P	0.523	P	0.737	P	LAMB2
216268_s_at	182	1	P	0.614	P	0.649	P	JAG1
216338_s_at	25844	1	P	0.648	P	0.790	P	KLIP1
216379_x_at	934	1	P	2.582	P	3.826	P	NaGLT1
216620_s_at	9639	1	P	1.237	P	1.552	P	ARHGEF10
216804_s_at		1	P	1.879	P	1.932	P	LIM
216870_x_at	8847	1	P	1.869	P	1.430	P	DLEU2
216975_x_at	4861	1	A	2.074	P,M	1.853	P,M,A	NPAS1
216983_s_at		1	P,A	0.656	P,A	0.705	P,A	ZNF224
217028_at	7852	1	A	2.416	P	2.644	P,A	CXCR4
217122_s_at	9906	1	P	0.593	P	0.750	P	MMP23B
217124_at	23288	1	P	1.566	P	1.029	P	KIAA1023
217165_x_at		1	A	1.862	P	1.965	P	MT1F
217168_s_at	9709	1	P	0.629	P	0.649	P	HERPUD1
217234_s_at	7430	1	P	1.425	P	1.547	P	VIL2
217270_s_at		1	P,A	0.648	P,A	0.701	M,A	
217289_s_at	2542	1	P	0.534	P	0.590	P	G6PC
217383_at		1	P,A	1.379	P,A	1.548	P	PGK1
217427_s_at	7290	1	P	0.651	P	0.858	P	HIRA
217436_x_at		1	P	1.076	P	1.678	P	HLA-J
217456_x_at	3133	1	P	1.218	P	1.624	P	HLA-E
217478_s_at	3108	1	P	1.367	P	1.611	P	HLA-DMA
217494_s_at	11191	1	A	1.501	P,A	1.255	P,A	PTENP1
217678_at		1	P	0.521	P	0.666	P	SLC7A11
217682_at		1	P	1.565	P	1.419	P	PRO0149
217728_at	6277	1	A	2.135	P	5.558	P	S100A6
217733_s_at	9168	1	P	1.387	P	1.675	P	TMSB10
217744_s_at	64065	1	P	1.460	P	1.738	P	PERP
217755_at	51155	1	P	1.683	P	1.535	P	HN1
217761_at	55256	1	P	1.741	P	1.408	P	SIPL
217792_at	27131	1	P	0.640	P	0.879	P	SNX5
217809_at	28969	1	P	1.530	P	1.731	P	BZW2
217835_x_at	55969	1	P	1.597	P	1.412	P	C20orf24
217841_s_at	51400	1	P	1.671	P	1.978	P	PME-1
217853_at	64759	1	A	1.601	P	3.073	P	TENS1
217867_x_at	25825	1	P	0.625	P	0.684	P	BACE2
217890_s_at	55742	1	A	2.583	P	2.377	P	PARVA
217892_s_at	51474	1	P	1.791	P	2.131	P	EPLIN
217897_at	53826	1	P,A	2.284	P	3.047	P	FXYD6
217904_s_at	23621	1	P	1.610	P	1.824	P	BACE1
217915_s_at	51187	1	P	0.653	P	0.659	P	C15orf15
217923_at	23578	1	P	1.517	P	1.418	P	PEF
217924_at	64771	1	P	1.512	P	1.592	P	C6orf106
217977_at	51734	1	P	1.540	P	1.326	P	SEPX1
217985_s_at	11177	1	P	1.943	P	1.717	P	BAZ1A

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
217986_s_at	11177	1	P	1.718	P	1.513	P	BAZ1A
217989_at	51170	1	P	0.633	P	0.705	P	DHRS8
217995_at	58472	1	A	1.242	A	1.761	P	SQRDL
217996_at	22822	1	P	2.021	P	2.062	P	PHLDA1
217997_at	22822	1	P	1.856	P	1.792	P	PHLDA1
218012_at	64061	1	P	1.544	P	1.413	P	SE20-4
218022_at	51231	1	P	0.624	P,A	0.760	P	VRK3
218029_at	79567	1	A	1.501	P,M,A	1.543	P,A	FLJ13725
218035_s_at	54502	1	A	1.961	P,A	3.047	P	FLJ20273
218060_s_at	79650	1	P,A	1.581	P	1.536	P	FLJ13154
218076_s_at	55114	1	P	1.404	P	1.517	P	ARHGAP17
218091_at	3267	1	P	1.426	P	1.539	P	HRB
218092_s_at	3267	1	P	1.642	P	1.496	P	HRB
218113_at	23670	1	A	1.791	P	1.937	P,A	TMEM2
218129_s_at	4801	1	P	1.526	P	1.462	P	NFYB
218145_at	57761	1	P	0.310	P	0.290	P	TRIB3
218181_s_at	54912	1	P	1.464	P	1.565	P	MAP4K4
218199_s_at	65083	1	P,A	1.833	P	1.962	P	NOL6
218217_at	59342	1	P	0.591	P	0.698	P	SCPEP1
218273_s_at	54704	1	P	1.236	P	1.661	P	PPM2C
218284_at	25856	1	P	1.203	P	1.640	P	DKFZP586N0721
218298_s_at	80017	1	P	0.551	P,A	0.769	P	C14orf159
218380_at	60368	1	P	2.194	P	2.337	P	NALP1
218400_at	4940	1	P	1.212	P	1.703	P	OAS3
218417_s_at	55652	1	P,M,A	1.661	P	1.811	P	FLJ20489
218526_s_at	29098	1	P	0.633	P	0.798	P	RANGNRF
218532_s_at	54463	1	A	1.570	P,M	1.456	P,A	FLJ20152
218543_s_at	64761	1	P	1.179	P	1.679	P	ZC3HDC1
218574_s_at	29995	1	P	1.330	P	1.778	P	LMCD1
218591_s_at	79954	1	P,A	1.522	P,A	1.324	P,A	FLJ14075
218611_at	51278	1	P	1.696	P	2.128	P	IER5
218625_at	51299	1	P	2.877	P	2.196	P	NRN1
218642_s_at	79145	1	P,M	2.139	P	2.043	P	CHCHD7
218691_s_at	8572	1	A	1.968	P,A	2.322	P,M,A	PDLIM4
218693_at	23555	1	A	1.344	P,A	2.011	P,A	NET-7
218706_s_at	65983	1	P	1.210	P	1.649	P	NS3TP2
218723_s_at	28984	1	P	0.558	P	0.677	P	RGC32
218736_s_at	54873	1	P	0.645	P	0.629	P,A	PALMD
218764_at	5583	1	A	1.927	P,A	1.891	M,A	PRKCH
218773_s_at	22921	1	P	0.657	P	0.828	P	MSRB
218793_s_at	6322	1	P	1.490	P	1.512	P	SCML1
218826_at	54733	1	P	1.614	P	1.679	P	SLC35F2
218848_at	79228	1	P	0.608	P	0.713	P	MGC2655
218849_s_at	10848	1	P	1.692	P	1.601	P	RAI
218880_at	2355	1	P	1.436	P	1.660	P	FOSL2
218885_s_at	79695	1	P	0.542	P	0.583	P	GALNT12
218915_at	51219	1	P	1.485	P	1.697	P	NF2
218951_s_at	55344	1	P	0.654	P	0.666	P	FLJ11323
218961_s_at	11284	1	P	0.645	P	0.765	P	PNKP
218974_at	55084	1	P	1.503	P	1.680	P	FLJ10159

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
218986_s_at	55601	1	M,A	1 277	P,M	2 224	P	FLJ20035
218997_at	64425	1	P	1 505	P	1 467	P	PAF53
219014_at	51316	1	P	1 583	P	2 032	P	PLAC8
219026_s_at	9462	1	P	1 571	P	1 747	P	RASAL2
219039_at	54910	1	P,A	0 632	M,A	0 722	P,M,A	SEMA4C
219073_s_at	114884	1	P,A	1 510	P	2 049	P	OSBPL10
219083_at	55164	1	A	1 597	P	1 594	P,A	FLJ10539
219094_at	29067	1	P,A	1 618	P	1 355	P	HSPC056
219119_at	51691	1	P	0 713	P	0 636	P	LSM8
219152_at	50512	1	P,M	0 454	A	0 550	P,M,A	PODLX2
219158_s_at	80155	1	P	1 492	P	1 576	P	TBDN100
219170_at	79187	1	P	0 578	P,A	0 658	P,A	FSD1
219174_at	80173	1	P	0 594	P	0 713	P	CCDC2
219188_s_at	28992	1	P	0 463	P	0 647	P	LRP16
219209_at	64135	1	A	1 765	P,A	4 172	P	MDA5
219211_at	11274	1	P	1 252	P	1 676	P	USP18
219250_s_at	23767	1	A	1 127	P,M	1 629	P,M	FLRT3
219258_at	54962	1	P	1 566	P	1 491	P	FLJ20516
219263_at	79589	1	P,A	1 921	P	2 275	P	RNF128
219270_at	79094	1	P	0 252	A	0 230	A	MGC4504
219306_at	56992	1	P	0 660	P	0 709	P	KNSL7
219321_at	64398	1	P	1 548	P	1 351	P	MPP5
219326_s_at	10678	1	P	0 591	P	0 568	P	B3GNT1
219352_at	55008	1	M,A	1 488	P	1 786	P	FLJ20637
219353_at	54835	1	P	0 639	P	0 748	P	NHLRC2
219361_s_at	64782	1	A	1 513	P,M	1 505	P,M,A	FLJ12484
219366_at	57099	1	P,A	1 610	P	1 472	P	AVEN
219410_at	55076	1	P	1 373	P	1 540	P	FLJ10134
219427_at	79633	1	P	1 387	P	1 585	P	FATJ
219477_s_at	55901	1	M,A	1 240	P,A	1 855	P,M	THSD1,
219493_at	79801	1	P	1 534	P	1 333	P	SHCBP1
219500_at	23529	1	P	1 286	P	1 525	P	CLC, BSF3
219522_at	24147	1	P,A	1 277	P	1 524	P	FJX1
219557_s_at	56675	1	P	1 600	P	1 677	P	NRIP3
219612_s_at	2266	1	A	1 585	P,M	2 257	P	FGG
219628_at	64393	1	P	1 604	P	1 524	P	WIG1
219634_at	50515	1	P	1 300	P	1 508	P	CHST11
219690_at	79713	1	P	1 684	P	1 539	P	FLJ22573
219692_at	79412	1	P	1 643	P	1 554	P	KREMEN2
219700_at	57125	1	P	0 682	P	0 617	P	PLXDC1
219705_at	79832	1	P	1 560	P	1 181	P	FLJ21924
219763_at	57706	1	P	1 340	P	1 506	P	KIAA1608
219825_at	56603	1	P,A	2 205	P	1 888	P	CYP26B1
219869_s_at	64116	1	P,A	1 882	P	1 861	P	SLC39A8
219895_at	55026	1	A	1 631	P,A	2 125	P	FLJ20716
219926_at	64208	1	P	0 654	P	0 733	P	POPDC3
219938_s_at	9050	1	P A	2 250	P	2 363	P	PSTPIP2
219944_at	79745	1	P,A	1 064	A	1 730	P	FLJ21069
219992_at	6866	1	P,M,A	1 436	P	1 770	P	TAC3
220033_at		1	P	0 634	P	0 700	P	

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
220038_at	23678	1	P	1 384	P	1 502	P	SGKL
220085_at	3070	1	P	1 222	P	1 627	P	HELLS
220092_s_at	84168	1	P,A	2 559	P	3 188	P	ANTXR1
220104_at	56829	1	M,A	1 346	P,M,A	1 672	P,A	ZC3HAV1
220134_x_at	55194	1	P	1 579	P	1 518	P	FLJ10647
220145_at	79884	1	P,M	0 694	P,A	0 656	P	FLJ21159
220217_x_at	64663	1	A	1 453	P,A	2 299	P	SPANXC
220321_s_at	79635	1	P	0 646	P	0 772	P	FLJ13646
220327_at	51159	1	P	0 646	P	0 681	P	FLJ38507
220358_at	55509	1	P,A	1 892	P	1 994	P	SNFT
220393_at	51557	1	P	0 629	P	0 901	P	GLULD1
220520_s_at	54830	1	M,A	1 540	P	1 336	P	FLJ20130
220534_at	79097	1	P,A	1 850	P	1 799	P	TRIM48
220551_at	57084	1	P	0 518	P	0 437	P	SLC17A6
220617_s_at	55205	1	P	0 628	P	0 745	P	ZNF532
220661_s_at	55657	1	P	0 623	P	0 789	P	FLJ20531
220668_s_at	1789	1	P	1 574	P	1 389	P	DNMT3B
220684_at	30009	1	P,A	1 518	P	1 257	P	TBX21
220703_at	55853	1	P,A	1 657	P	1 308	P,A	C10orf110
220738_s_at	27330	1	A	1 661	M,A	1 844	M,A	RPS6KA6
220794_at	64388	1	P	0 598	P,M	0 664	P,A	PRDC
220800_s_at	29766	1	P,M	1 521	P	1 345	P	TMOD3
220892_s_at	29968	1	P	0 367	P	0 334	P	PSAT1
220922_s_at	30014	1	A	1 103	A	1 739	P	SPANXA1
220954_s_at	29990	1	P	0 635	P	0 772	P	PILRB
220987_s_at	81788	1	P	1 810	P	1 953	P	SNARK
221009_s_at	51129	1	P,A	1 890	P	2 686	P	ANGPTL4
221011_s_at	81606	1	P	4 211	P	9 513	P	LBH
221039_s_at	50807	1	P	1 416	P	1 545	P	DDEF1
221059_s_at	4166	1	P	2 224	P	2 377	P	CHST6
221078_s_at	55704	1	P	1 525	P	1 393	P	FLJ10392
221123_x_at	55893	1	P	0 627	A	0 858	P,A	ZNF395
221195_at	51136	1	P	0 657	P,A	0 784	A	LOC51136
221213_s_at	54816	1	P,A	0 642	P,A	0 873	P,M,A	FLJ20086
221234_s_at	60468	1	A	1 650	P	1 366	P A	BACH2
221261_x_at	81557	1	P	0 627	P	0 613	P	MAGED4
221484_at	9334	1	P	1 320	P	1 522	P	B4GALT5
221489_s_at	81848	1	P	1 595	P	1 675	P	SPRY4
221510_s_at	2744	1	P	1 602	P	1 956	P	GLS
221539_at	1978	1	P	0 600	P	0 600	P	EIF4EBP1
221561_at	6646	1	P	1 525	P	1 319	P	SOAT1
221645_s_at	55769	1	P	0 640	P	0 838	P	ZNF83
221657_s_at	140459	1	P,A	1 585	P	1 455	P	ASB6
221664_s_at	50848	1	P	1 069	P	1 542	P	F11R
221676_s_at	23603	1	P	1 673	P	1 541	P	CORO1C
221710_x_at	55194	1	P	1 569	P	1 313	P	FLJ10647
221718_s_at	11214	1	P	1 582	P	2 189	P	AKAP13
221760_at		1	A	1 997	P	2 902	P	MAN1A1
221766_s_at	55603	1	P,M,A	1 621	P	1 962	P	C6orf37
221779_at	85377	1	P	1 529	P	1 594	P	MIRAB13

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
221810_at		1	P,A	1 857	P	1 769	P	
221831_at		1	A	1 504	M A	1 482	P A	LUZP1
221841_s_at		1	A	1 690	P,M	1 837	P	KLF4
221865_at	203197	1	P	0 713	P	0 637	P	C9orf91
221875_x_at	3134	1	P	1 219	P	2 057	P	HLA-F
221892_at		1	P	0 564	P,A	0 705	P	H6PD
221893_s_at	90956	1	P	1 511	P	1 333	P	ADCK2
221899_at	10443	1	P	0 624	P	0 792	P,A	PFAAP5
221911_at		1	P	0 647	P	1 097	P	ETV1
221951_at		1	P	0 537	P,A	0 652	P	LOC283232
221986_s_at	54800	1	P	0 643	P,A	0 627	P,M	DRE1
221998_s_at	51231	1	P	0 521	P	0 663	P	LOC51231
222024_s_at	11214	1	P	1 368	P	1 754	P	AKAP13
222028_at	7596	1	P	1 948	P	1 303	P	ZNF45
222043_at	1191	1	P	1 660	P	2 393	P	CLU
222074_at	7389	1	P,A	2 213	P	1 840	P,A	UROD
222108_at		1	P	1 990	P	2 402	P	
222125_s_at	54681	1	P	0 591	P	0 673	P	PH-4
222126_at	3668	1	M,A	1 506	P	1 446	P	HRBL
222154_s_at	26010	1	P	1 496	P	1 727	P	DNAPTP6
222204_s_at	54700	1	P	1 559	P	1 584	P	RRN3
222258_s_at	23677	1	P	1 613	P	1 953	P	SH3BP4
222305_at		1	P,A	1 747	P	1 573	P,M	HK2
222351_at	5519	1	A	1 765	P,A	1 680	P,A	PPP2R1B
266_s_at	934	1	P,A	3 273	P	4 303	P	CD24
32042_at	10495	1	P	1 298	P	1 572	P	COVA1
33322_i_at	2810	1	P	1 427	P	1 969	P	SFN
33323_r_at	2810	1	P	1 536	P	2 165	P	SFN
33767_at		1	P	1 548	P	1 327	P	NEFH
34697_at	4040	1	P	0 625	P	0 688	P	LRP6
36552_at	26005	1	P	1 481	P	1 630	P	DKFZP586P0123
38037_at	1839	1	P,A	2 201	P	1 886	P	DTR
39966_at	10675	1	P	0 568	P	0 564	P	CSPG5
40093_at	4059	1	P	1 153	P	2 270	P	LU,
41469_at		1	A	1 227	P,A	1 592	P	PI3
45297_at	115273	1	A	1 467	P	1 572	P	EHD2
48106_at	55652	1	P	1 452	P	1 534	P	FLJ20489
49077_at	51400	1	P	1 410	P	1 606	P	PME-1
54970_at	83637	1	P	1 721	P	1 814	P	DKFZp761I2123
55081_at	85377	1	P	1 507	P	1 423	P	MIRAB13
57540_at	64080	1	P	1 737	P	1 825	P	RBKS
59697_at		1	P	1 734	P	1 701	P	
61732_r_at	80173	1	P	0 602	P	0 734	P,A	CCDC2
64432_at	51275	1	P	0 626	P	0 712	P	FLJ39616
65630_at		1	P	0 641	P	0 760	P	LOC283232

Affymetrix Id	Day 0		Day 3		Day 7		Common Name
	Normalised	Flag	Normalised	Flag	Normalised	Flag	
AFFX-HUMISGF3A/M97935_3_at	1	P	1 805	P	2 543	P	STAT1
AFFX-HUMISGF3A/M97935_5_at	1	P	1 610	P	2 380	P	STAT1
AFFX-HUMISGF3A/M97935_MA_at	1	P,M	1 726	P	2 600	P	STAT1
AFFX-HUMISGF3A/M97935_MB_at	1	P	1 796	P	2 751	P	STAT1

7 2 Appendix B – Cluster 5 BrdU Exp. 3

List of genes contained in Cluster 5 of the BrdU Exp 3 DNA microarray experiment

Cluster 5 – BrdU Exp. 3			
Common Name	Affymetrix Id	Common Name	Affymetrix Id
SMARCD	204099_at	FLJ22028	219802_at
FAM11B	219253_at	LOC56901	214096_s_at
AUH	205052_at	C20orf31	218282_at
TSFM	212656_at	DECR2	219664_s_at
FASTK	210975_x_at	PIGO	209998_at
ILVBL; AHAS	202993_at	QARS	217846_at
ZNF580	220748_s_at	CGI-143	219345_at
WARP	218731_s_at	C6orf108	204238_s_at
FLJ20758	217895_at	MOSPD3	219070_s_at
TRIAD3	218426_s_at	G6PC3	221759_at
FLJ11822	215090_x_at	STAT1	209969_s_at
NAGA	202944_at	dJ222E13.1	217284_x_at
EBP; CPX	202735_at	MAPBPIP	218291_at
KIAA0974	213896_x_at	MYST1	221820_s_at
DLGAP1	210750_s_at	SGSH; HSS;	35626_at
PPGB	200661_at	INSIG1; C1-6	201627_s_at
ACP2	202767_at	PTPN18	203555_at
LOC55974	219125_s_at	ATP5D	213041_s_at
PIGQ; GPI1	204144_s_at	GGCX	214006_s_at
RAB12A	220500_s_at	ATF5; ATFX	204999_s_at
SLC25A11	207088_s_at	LLGL2; HGL	203713_s_at
MBTPS1	217543_s_at	DHPS	202802_at
FLJ10496	221934_s_at	LRP8	208433_s_at
FLJ35827	212969_x_at	FASTK	214114_x_at
RFXANK	202758_s_at	KIAA1164	37802_r_at
NAGA	202943_s_at	TAGLN	56256_at
INPP5A	203006_at	LEPREL2	204854_at
DKFZp762C186	91703_at	SLC25A1	210010_s_at
FLJ12681	46142_at	PCCA	203860_at
LOC51337	218500_at	C19orf27	221267_s_at
SUCLG2	214835_s_at	NFIB; NFIB2	211467_s_at
PMM1	203467_at	FAIM; FAIM1	220643_s_at
DHX9	212105_s_at	MAPT	206401_s_at
	AFFX-LysX-1_at	AD-017	218146_at
NDUFS7	211752_s_at	UROS	203031_s_at
C3F	202793_at	CPSF1	33132_at
TGFB1	203085_s_at	DEAF1; SPN	209407_s_at
DKFZP586B1621	218688_at	CORO1B	64486_at
H2AFX	212525_s_at	PLEKHJ1	218290_at
HTATIP	206689_x_at	CREM; ICER	210171_s_at
LDLR	202067_s_at	MSRB; CBS1	218773_s_at
PIR	207469_s_at		
MPG; AAG	203686_at		
C6orf108	39817_s_at		
ACYP2	206833_s_at		
VPS28	218679_s_at		
NASP	201970_s_at		
CPSF4	206688_s_at		
ACLY	210337_s_at		

7 3 Appendix C – Differentially Expressed Genes Identified in 5,2`-FdU DNA Microarray Experiment

List of differentially expressed genes identified from microarray analysis of 5,2 -FdU microarray experiment Genes listed are sorted by Affymetrix ID number

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
1053_at	5982	1	P	1.509	P	1.140	P	RFC2
1598_g_at	10609	1	P	0.751	P	0.644	P	GAS6; AXSF
177_at	5337	1	P	0.711	P	0.646	P	PLD1
200020_at	23435	1	P	1.520	P	1.569	P	TARDBP
200023_s_at	8665	1	P	0.873	P	0.609	P	eIF3-p47
200036_s_at	4736	1	P	0.747	P	0.612	P	RPL10A
200050_at	7705	1	P	1.556	P	1.569	P	ZNF146; OZF
200054_at	8882	1	P	1.937	P	1.743	P	ZNF259; ZPR1
200079_s_at	3735	1	P	1.519	P	1.500	P	KARS
200090_at	2339	1	P	1.593	P	1.518	P	FNTA
200597_at		1	P	1.209	P	1.509	P	EIF3S10
200609_s_at	9948	1	P	1.689	P	1.526	P	WDR1; NORI-1
200615_s_at	163	1	P	1.630	P	1.567	P	AP2B1
200635_s_at	5792	1	P	0.660	P	0.822	P	PTPRF
200646_s_at	4924	1	P	0.577	P	0.511	P	NUCB1
200661_at	5476	1	P	0.723	P	0.507	P	PPGB; GSL
200670_at	7494	1	P	0.630	P	0.585	P	XBP1; XBP2
200678_x_at	2896	1	P	0.631	P	0.778	P	GRN; PEPI
200685_at	9295	1	P	1.205	P	1.552	P	SFRS11
200697_at	3098	1	P	1.496	P	1.577	P	HK1; HK1
200715_x_at	23521	1	P	0.450	P	0.349	P	RPL13A
200719_at	6500	1	P	2.144	P	2.713	P	SKP1A
200730_s_at	7803	1	P	1.968	P	1.484	P	PTP4A1
200731_s_at	7803	1	P	1.632	P	1.389	P	PTP4A1
200732_s_at	7803	1	P	1.601	P	1.372	P	PTP4A1
200733_s_at	7803	1	P	1.698	P	1.365	P	PTP4A1; HH72
200742_s_at	1200	1	P	0.449	P	0.409	P	CLN2
200743_s_at	1200	1	P	0.482	P	0.415	P	CLN2; TPP1
200747_s_at	4926	1	P	0.579	P	0.607	P	NUMA1
200762_at	1808	1	P	1.026	P	1.698	P	DPYSL2
200779_at	468	1	P	0.591	P	0.602	P	ATF4; CREB2;
200786_at	5695	1	P	1.509	P	1.170	P	PSMB7; Z
200787_s_at	8682	1	P	1.621	P	1.952	P	PEA15; PED
200788_s_at	8682	1	P	1.744	P	1.999	P	PEA15; PED
200789_at	1891	1	P	0.625	P	0.594	P	ECH1; HPXEL
200790_at	4953	1	P	1.770	P	1.758	P	ODC1
200796_s_at	4170	1	P	0.623	P,A	0.581	P	MCL1; TM;
200802_at	6301	1	P	0.697	P	0.633	P	SARS; SERS;
200808_s_at	7791	1	P	1.756	P	1.469	P	ZYX
200810_s_at	1153	1	P	0.563	P	0.439	P	CIRBP; CIRP
200811_at	1153	1	P	0.545	P	0.402	P	CIRBP; CIRP
200813_s_at	5048	1	P	1.168	P	1.517	P	PAFAH1B1
200814_at	5720	1	P	0.644	P	0.582	P	PSME1; PA28A
200821_at	3920	1	P	1.176	P	1.511	P	LAMP2
200833_s_at	5908	1	P	1.522	P	1.555	P	RAP1B
200841_s_at	2058	1	P	0.592	P	0.468	P	EPRS
200868_s_at	55905	1	P	1.541	P	1.429	P	ZNF313
200872_at	6281	1	P	1.427	P	1.626	P	S100A10
200873_s_at	10694	1	P	1.571	P	1.395	P	CCT8; Cctq
200887_s_at	6772	1	P	1.329	P	2.005	P	STAT1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
200897_s_at	23022	1	P	1.562	P	1.976	P	KIAA0992
200898_s_at	10724	1	P	1.409	P	1.698	P	MGEA5
200899_s_at	10724	1	P	1.242	P	1.740	P	MGEA5; MEA5
200907_s_at	23022	1	P,A	1.473	P	1.615	P	KIAA0992
200908_s_at	6181	1	A	1.524	P,A	1.522	P,A	RPLP2; RPP2
200911_s_at	6867	1	P	1.594	P	1.585	P	TACC1; Ga55
200916_at	8407	1	P	1.618	P	1.697	P	TAGLN2
200917_s_at	6734	1	P	0.744	P	0.573	P	SRPR
200922_at	10945	1	P	0.735	P	0.608	P	KDELR1; ERD2
200962_at		1	P	1.256	P	1.803	P	RPL31
200976_s_at	8887	1	P	1.309	P	1.504	P	TAX1BP1;
200977_s_at	8887	1	P	1.371	P	1.534	P	TAX1BP1; T6BP
200979_at		1	P	1.492	P	1.592	P	
200988_s_at	10197	1	P	2.141	P	2.318	P	PSME3; Ki
200995_at	10527	1	P	1.360	P	1.559	P	IPO7
201006_at	7001	1	P,A	0.865	P,A	0.603	A	PRDX2; PRP
201013_s_at	10606	1	P	1.619	P	1.495	P	PAICS
201014_s_at	10606	1	P	1.768	P	1.524	P	PAICS; AIRC
201020_at	7533	1	P	1.508	P	1.392	P	YWHAH
201022_s_at	11034	1	P	1.433	P	1.528	P	DSTN; ADF
201024_x_at	9669	1	P	1.516	P	1.215	P	EIF5B; IF2
201027_s_at	9669	1	P	1.727	P	1.482	P	EIF5B; IF2
201041_s_at	1843	1	P	1.469	P	1.598	P	DUSP1
201042_at	7052	1	P,A	3.942	P	4.238	P	TGM2
201043_s_at	8125	1	P	0.718	P	0.603	P	ANP32A
201046_s_at	5886	1	P	1.535	P	1.313	P	RAD23A
201050_at	23646	1	P	0.686	P	0.644	P	PLD3; HU-K4
201058_s_at	10398	1	P	1.547	P	1.111	P	MYL9; LC20
201060_x_at	2040	1	P	1.364	P	1.672	P	STOM
201061_s_at	2040	1	P	1.207	P	1.636	P	STOM; BND7
201065_s_at	2969	1	P	0.678	P	0.625	P	GTF2I; DIWS;
201093_x_at	6389	1	P	0.859	P	0.609	P	SDHA; FP
201097_s_at	378	1	P	1.478	P	1.512	P	ARF4
201099_at		1	P	1.527	P	1.544	P	USP9X
201100_s_at	8239	1	P	1.623	P	1.602	P	USP9X; DFFRX
201102_s_at	5211	1	P	1.067	P	0.660	P	PFKL; PFK-B
201123_s_at	1984	1	P	0.692	P,A	0.447	A	EIF5A; EIF-5A
201139_s_at	6741	1	P	1.502	P	1.497	P	SSB
201142_at	1965	1	P	1.789	P	1.637	P	EIF2S1
201143_s_at	1965	1	P	1.616	P	1.666	P	EIF-2alpha
201144_s_at	1965	1	P	1.783	P	1.655	P	EIF2S1; EIF-2
201145_at	10456	1	P	0.724	P	0.638	P	HAX1
201153_s_at	4154	1	P	1.226	P	1.559	P	MBNL1; EXP
201167_x_at	396	1	P	0.978	P	0.585	P	ARHGDIA
201193_at	3417	1	P	0.659	P	0.516	P	IDH1; IDP; PICD
201195_s_at	8140	1	P	0.641	P	0.520	P	SLC7A5
201211_x_at	1654	1	P	0.812	P	0.561	P	DDX3X; DBX
201214_s_at	5510	1	P	1.570	P	1.358	P	PPP1R7
201216_at	10961	1	P	0.615	P	0.585	P	C12orf8
201219_at	1488	1	P	1.390	P	1.657	P	CTBP2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
201247_at	6721	1	P	0 538	P	0 489	P	SREBF2
201248_s_at	6721	1	P	0 629	P	0 482	P	SREBF2
201275_at	2224	1	P	0 680	P	0 502	P	FDPS, FPS
201282_at	4967	1	P	1 640	P	1 195	P	OGDH, E1k
201284_s_at	327	1	P	0 667	P	0 325	P	APEH
201295_s_at	26118	1	P	0 514	P,A	0 515	P,M,A	WSB1
201307_at	55752	1	P	1 465	P	1 598	P	FLJ10849
201323_at	10969	1	P	1 550	P	1 336	P	EBNA1BP2
201325_s_at	2012	1	P	1 668	P	1 592	P	EMP1
201326_at	908	1	P	1 566	P	1 627	P	CCT6A
201329_s_at	2114	1	A	1 529	P,A	1 558	P,A	ETS2
201331_s_at	6778	1	P	0 623	P	0 638	P	STAT6
201337_s_at	9341	1	P	0 854	P	0 590	P	VAMP3, CEB
201344_at	7322	1	P	1 417	P	1 916	P	UBE2D2
201367_s_at	678	1	P,M,A	0 798	P,A	0 568	A	ZFP36L2
201370_s_at	8452	1	P	1 380	P	1 640	P	CUL3
201397_at	26227	1	P	0 479	P	0 443	P	PHGDH
201401_s_at	156	1	P	0 689	P,M	0 466	P,A	ADRBK1
201416_at		1	P	1 421	P	2 110	P	SOX4, EVI16
201417_at		1	P	1 211	P	1 721	P	SOX4, EVI16
201421_s_at	79084	1	P	1 508	P	1 299	P	MEP50
201425_at	217	1	P	0 501	P	0 408	P	ALDH2
201432_at	847	1	P	0 593	P	0 484	P	CAT
201436_at	1977	1	P	1 650	P	1 585	P	EIF4E,
201446_s_at	7072	1	P	0 741	P	0 648	P	TIA1
201454_s_at	9520	1	P	0 738	P	0 639	P	NPEPPS
201460_at	9261	1	P	1 586	P	1 527	P	MAPKAPK2
201464_x_at	3725	1	A	3 589	P	4 645	P	JUN
201466_s_at	3725	1	A	3 794	P	5 536	P	JUN, AP1
201475_x_at	4141	1	P	0 624	P	0 508	P	MARS
201478_s_at	1736	1	P	1 628	P	1 574	P	DKC1
201479_at	1736	1	P	1 519	P	1 444	P	DKC1,
201489_at	10105	1	P	1 616	P	1 101	P	PPIF, CYP3
201498_at	7874	1	P	1 772	P	1 813	P	USP7
201501_s_at	2926	1	P	1 434	P	1 682	P	GRSF1
201502_s_at	4792	1	P	1 600	P	1 591	P	NFKBIA
201516_at	6723	1	P	1 677	P	1 249	P	SRM
201534_s_at	5412	1	P	1 253	P	1 500	P	UBL3
201535_at	5412	1	P	1 285	P	1 729	P	UBL3
201536_at	1845	1	P	1 693	P	1 561	P	DUSP3
201537_s_at	1845	1	P	2 311	P	1 843	P	DUSP3, VHR
201539_s_at	2273	1	P	1 160	P	1 637	P	FHL1, KYO-T
201540_at	2273	1	P	1 354	P	1 943	P	FHL1, KYO-T
201546_at	9320	1	P	1 506	P	1 436	P	TRIP12
201554_x_at	2992	1	P	1 569	P	1 422	P	GYG
201559_s_at	25932	1	P	0 656	P	0 707	P	CLIC4
201565_s_at	3398	1	P	4 103	P	3 504	P	ID2, ID2A
201566_x_at	3398	1	P,M	5 836	P	4 596	P	ID2, ID2A
201571_s_at	1635	1	P	1 547	P	1 451	P	DCTD
201572_x_at	1635	1	P	1 588	P	1 626	P	DCTD

Affymetrix Id		Day 0		Day 3		Day 7		Common Name
	LocusLink	Normalized	Flags	Normalized	Flags	Normalized	Flags	
201576_s_at	2720	1	P	0.664	P	0.515	P	GLB1
201579_at	2195	1	P	1.465	P	1.648	P	FAT; ME5;
201582_at	10483	1	P,M	1.718	P	1.377	P	SEC23B
201587_s_at	3654	1	P	1.505	P	1.527	P	IRAK1; pelle
201594_s_at	9989	1	P	1.477	P	1.541	P	PPP4R1
201595_s_at	55854	1	P	1.513	P	1.380	P	LEREPO4
201600_at	11331	1	P	0.905	P	0.653	P	REA; BAP
201607_at	11137	1	P	1.851	P	1.853	P	PWP1
201608_s_at	11137	1	P	1.581	P	1.388	P	PWP1;
201625_s_at	3638	1	P	0.609	P	0.421	P	INSIG1
201626_at	3638	1	P	0.657	P	0.468	P	INSIG1
201627_s_at	3638	1	P	0.708	P	0.468	P	INSIG1
201629_s_at	52	1	P	1.560	P	1.593	P	ACP1
201630_s_at	52	1	P	1.386	P	1.500	P	ACP1
201632_at	1967	1	P	1.671	P	1.516	P	EIF-2Balpha
201639_s_at	29894	1	P	0.913	P	0.617	P	CPSF1
201642_at	3460	1	P	0.768	P	0.629	P	IFNGR2; AF-1
201668_x_at	4082	1	P,A	0.541	A	0.798	P,A	MARCKS
201677_at	56941	1	P	1.469	P	1.622	P	ELF3
201688_s_at	7163	1	P	1.456	P	1.570	P	TPD52
201689_s_at	7163	1	P	1.609	P	1.669	P	TPD52
201690_s_at	7163	1	P	1.541	P	1.672	P	TPD52
201691_s_at	7163	1	P	1.551	P	2.199	P	TPD52; N8L
201704_at	955	1	P	0.917	P	0.601	A	ENTPD6
201708_s_at	8508	1	P	0.818	P	0.640	P	NIPSNAP1
201709_s_at	8508	1	P	0.773	P	0.632	P	NIPSNAP1
201710_at	4605	1	P	1.765	P	1.248	P	MYBL2; BMYB
201712_s_at	5903	1	P	1.432	P	1.596	P	RANBP2
201739_at	6446	1	P	3.163	P	4.549	P	SGK; SGK1
201744_s_at	4060	1	P	1.555	P	1.623	P	LUM; LDC
201790_s_at	1717	1	P	0.699	P	0.522	P	DHCR7
201791_s_at	1717	1	P	0.705	P	0.557	P	DHCR7; SLOS
201794_s_at	9887	1	P	1.696	P	1.500	P	EST1C
201796_s_at	7407	1	P	0.711	P	0.524	P,A	VARS2
201798_s_at	26509	1	P,A	3.222	P	5.656	P	FER1L3
201805_at	5571	1	P	0.702	P	0.545	P	PRKAG1
201814_at	9779	1	P	1.247	P	1.552	P	TBC1D5
201816_s_at	2631	1	P	0.879	P	0.532	P	GBAS
201823_s_at	9604	1	P	1.461	P	1.615	P	RNF14
201824_at	9604	1	P	1.714	P	1.907	P	RNF14
201830_s_at	10276	1	P	1.378	P	1.517	P	NET1
201865_x_at	2908	1	P	1.507	P	1.598	P	NR3C1
201872_s_at	6059	1	P	1.763	P	1.551	P	ABCE1
201873_s_at	6059	1	P	1.586	P	1.598	P	ABCE1
201890_at	6241	1	P	1.591	P	1.475	P	RRM2; R2
201896_s_at		1	P	0.644	P	0.794	P	
201900_s_at	10327	1	P	0.608	P	0.522	P	AKR1A1
201913_s_at	80347	1	P	0.894	P	0.642	P	COASY
201920_at	6574	1	P	1.742	P	1.362	P	SLC20A1;
201937_s_at	23549	1	P,M	1.511	P	1.203	P	DNPEP

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
201939_at	10769	1	P	1.986	P	1.431	P	PLK2; SNK
201948_at	29889	1	P	1.967	P	1.598	P	HUMAUANTIG
201951_at	214	1	P	1.484	P	2.712	P	ALCAM; MEMD
201952_at	214	1	P	1.467	P	2.398	P	ALCAM; MEMD
201959_s_at	23077	1	P,A	1.600	P	1.665	P,M	MYCBP2
201963_at	2180	1	P	1.532	P	1.505	P	ACSL1
201966_at	4720	1	P	1.008	P	0.643	P	NDUFS2
201970_s_at	4678	1	P	0.829	P	0.638	P	NASP
201985_at	9897	1	P	0.590	P	0.692	P	KIAA0196
202014_at	23645	1	P	2.393	P	3.054	P	PPP1R15A
202015_x_at	10988	1	P,A	1.231	P,M,A	1.535	P,A	METAP2; p67
202016_at	4232	1	P	0.704	P	0.651	P	MEST; PEG1
202017_at	2052	1	P	1.456	P	1.742	P	EPHX1; MEH
202030_at	10295	1	P	0.917	P	0.629	P	BCKDK
202052_s_at	26064	1	P	1.963	P	3.139	P	RAI14; RAI13
202056_at		1	P	1.421	P	1.540	P	KPNA1; RCH2
202059_s_at	3836	1	P	1.511	P	1.429	P	KPNA1; RCH2
202066_at	8500	1	P	1.351	P	1.511	P	PPFIA1; LIP1
202067_s_at	3949	1	P	0.764	P	0.527	P,A	LDLR
202068_s_at	3949	1	P	0.777	P	0.523	P	LDLR; FH; FHC
202070_s_at	3419	1	P	1.711	P	1.660	P	IDH3A
202071_at	6385	1	P	1.977	P	1.860	P	SDC4; SYND4
202073_at	10133	1	P	1.301	P	2.089	P	OPTN
202074_s_at	10133	1	P	1.481	P	2.040	P	OPTN; NRP
202076_at	329	1	P	1.569	P	1.711	P	BIRC2; API1;
202079_s_at	22906	1	P	0.740	P,A	0.638	P	OIP106
202083_s_at	6397	1	P	1.728	P	1.632	P	SEC14L1
202085_at	9414	1	P	1.400	P	1.758	P	TJP2; ZO2; X104
202105_at	3476	1	P	0.713	P	0.620	P	IGBP1; IBP1
202121_s_at	27243	1	P	0.713	P	0.580	P	BC-2
202129_s_at	8780	1	P,M	1.971	P	2.200	P	RIOK3
202130_at	8780	1	P	1.948	P	2.448	P	RIOK3
202131_s_at	8780	1	P	2.061	P	2.389	P	RIOK3; SUDD
202135_s_at	10120	1	P	0.868	P	0.623	P	ACTR1B
202141_s_at	10920	1	P	1.642	P	1.686	P	COPS8
202142_at	10920	1	P	1.513	P	1.541	P	COPS8; COP9
202146_at	3475	1	P	2.868	P	3.711	P	IFRD1
202147_s_at	3475	1	P	2.728	P	3.227	P	IFRD1
202148_s_at	5831	1	P	0.738	P	0.619	P	PYCR1; P5C
202149_at		1	P	1.464	P	1.676	P	dJ761I2.1
202172_at		1	P	1.301	P	1.561	P	ZNF161; DB1
202174_s_at	5108	1	P	1.329	P	1.641	P	PCM1; PTC4
202180_s_at	9961	1	P,A	0.642	P,M,A	0.529	A	MVP; LRP
202181_at	9766	1	P	1.374	P	1.556	P	KIAA0247
202188_at	9688	1	P	1.655	P	1.222	P	NUP93
202193_at	3985	1	P	1.565	P	1.496	P	LIMK2
202205_at	7408	1	P	0.720	P	0.593	P	VASP
202209_at	27258	1	P	2.117	P	2.262	P	LSM3; SMX4
202212_at	23481	1	P	1.679	P	1.406	P	PES1
202218_s_at	9415	1	P	0.522	P	0.431	P	FADS2; D6D

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
202240_at	5347	1	P	0 653	P	0 785	P	PLK1, STPK13
202241_at	10221	1	P	1 614	P	2 539	P	TRIB1, C8FW
202245_at	4047	1	P	0 672	P	0 563	P	LSS
202251_at	9129	1	P	1 459	P	1 552	P	PRPF3, PRP3
202253_s_at	1785	1	P	0 853	P	0 654	P,A	DNM2, DYNII
202265_at	648	1	P	1 416	P	1 846	P	BMI1, RNF51
202272_s_at	23219	1	P	1 593	P	1 611	P	FBXO28
202279_at	9556	1	P	1 535	P	1 434	P	C14orf2, MP68
202284_s_at	1026	1	P	2 609	P	2 528	P	CDKN1A, P21
202290_at	11333	1	P	0 657	P	0 499	P	PDAP1, PAP
202307_s_at	5696	1	P	1 286	P	1 510	P	TAP1, APT1
202308_at	6720	1	P	0 455	P	0 315	P,A	SREBF1
202313_at	5520	1	P	1 649	P	1 658	P	PPP2R2A
202328_s_at	5310	1	P	0 646	P	0 612	P	PKD1, PBP
202329_at	1445	1	P	1 511	P	1 359	P	CSK
202330_s_at	7374	1	P	1 611	P	1 552	P	UNG, DGU
202331_at	593	1	P	0 797	P	0 458	P	BCKDHA
202340_x_at	3164	1	P,A	1 679	P	1 078	P,A	NR4A1, HMR
202341_s_at	23321	1	M,A	1 456	P	1 906	P	TRIM2
202342_s_at	23321	1	P	1 288	P	1 602	P	TRIM2, RNF86
202344_at	3297	1	P	1 590	P	1 186	P	HSF1, HSTF1
202345_s_at	6181	1	P	1 822	P	1 544	P	FABP5, EFABP
202352_s_at	5718	1	P	1 520	P	1 263	P	PSMD12
202366_at	35	1	P	0 798	P	0 646	M,A	ACADS, SCAD
202375_at	9871	1	P	0 966	P	0 634	P	SEC24D
202384_s_at	6949	1	P	1 721	P	1 254	P	TCOF1, MFD1
202389_s_at	3064	1	A	1 506	P	1 045	M,A	HD, IT15
202391_at	10409	1	P	2 916	P	8 624	P	BASP1, CAP23
202393_s_at	7071	1	P	1 429	P	1 746	P	TIEG, EGRA,
202400_s_at	6722	1	P,A	1 589	P	1 747	P	SRF
202402_s_at	833	1	P	0 615	P	0 570	P	CARS, CYSRS
202407_s_at	26121	1	P	0 905	P	0 604	P	PRPF31
202413_s_at	7398	1	P	1 510	P	1 536	P	USP1
202425_x_at	5530	1	P,M,A	1 620	P	2 130	P	PPP3CA
202429_s_at	5530	1	P	1 404	P	2 009	P	PPP3CA
202444_s_at	10613	1	P	0 618	P	0 450	P	C10orf69,
202451_at	2965	1	P	1 526	P	1 840	P	GTF2H1
202457_s_at	5530	1	P	1 286	P	1 842	P	PPP3CA
202462_s_at	9879	1	P	2 138	P	1 961	P	DDX46
202464_s_at	5209	1	P	1 768	P	1 761	P	PFKFB3
202468_s_at	8727	1	P	1 911	P	2 094	P	CTNNAL1
202470_s_at	11052	1	P	1 225	P	1 633	P	CPSF6, CFIM
202472_at	4351	1	P	0 818	P	0 616	P,M,A	MPI, PMI, PMI1
202476_s_at	10844	1	P	0 809	P	0 636	P	TUBGCP2
202492_at	79065	1	P	0 761	P	0 635	P	FLJ22169
202498_s_at	6515	1	P	1 600	P	1 348	P	SLC2A3
202499_s_at	6515	1	P	1 885	P	1 634	P	SLC2A3
202500_at	3300	1	P	1 900	P	1 886	P	DNAJB2
202531_at	3659	1	P	1 552	P	1 624	P	IRF1
202532_s_at	1719	1	P	1 885	P	1 962	P	DHFR

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
202533_s_at	1719	1	P	1.511	P	1.345	P	DHFR
202534_x_at	1719	1	P	1.601	P	1.717	P	DHFR
202536_at	25978	1	P	1.565	P	1.704	P	DKFZP564O123
202541_at	9255	1	P	1.812	P	1.832	P	SCYE1
202542_s_at	9255	1	P	1.802	P	1.735	P	SCYE1; p43;
202580_x_at	2305	1	P	1.316	P	1.502	P	FOXM1; MPP2
202581_at	3304	1	P	0.772	P	0.586	P	HSPA1B
202583_s_at	10048	1	P	1.509	P	1.551	P	RANBP9
202594_at	23484	1	P	1.349	P	1.608	P	LEPROTL1
202599_s_at	8204	1	P	1.347	P	1.687	P	NRIP1; RIP140
202600_s_at	8204	1	P	1.138	P	1.589	P	NRIP1
202611_s_at	9282	1	P	1.351	P	1.557	P	CRSP2
202613_at	1503	1	P	2.057	P	1.811	P	CTPS
202620_s_at	5352	1	P	1.189	P	1.957	P	PLOD2
202644_s_at	7128	1	P	1.335	P	1.654	P	TNFAIP3; A2
202645_s_at	4221	1	P	0.811	P	0.610	P	MEN1
202656_s_at	9792	1	P	1.519	P	1.910	P	SERTAD2
202657_s_at	9792	1	P	1.564	P	1.828	P	TRIP-Br2
202671_s_at	8566	1	P	1.176	P	0.640	P	PDXK; PKH
202672_s_at	467	1	P	1.140	P	1.533	P	ATF3
202676_x_at	10922	1	P,A	0.720	P,A	0.554	M,A	FASTK
202678_at	2958	1	P	1.552	P	1.615	P	GTF2A2
202682_s_at	7375	1	P	0.837	P	0.659	P	USP4; UNP
202693_s_at	9263	1	P	1.509	P	1.481	P	STK17A
202695_s_at	9263	1	P,A	1.673	P	2.224	P	STK17A
202702_at	7726	1	P	1.780	P	1.577	P	TRIM26; AFP
202706_s_at	7372	1	P	1.518	P	1.352	P	UMPS; OPRT
202712_s_at	1159	1	P	0.702	P	0.589	P	CKMT1;
202725_at	5430	1	P	1.538	P	1.505	P	POLR2A
202727_s_at	3459	1	P	1.647	P	1.741	P	IFNGR1; CD119
202730_s_at	27250	1	P	0.606	P	0.544	P	PDCD4; H731
202731_at	27250	1	P	0.526	P	0.546	P	PDCD4; H731
202735_at	10682	1	P	0.763	P	0.573	P	EBP; CPX
202740_at	95	1	P	0.570	P	0.399	P	ACY1
202743_at	8503	1	P	1.835	P	2.000	P	PIK3R3
202746_at	9452	1	P	1.435	P	3.389	P	ITM2A; E25A
202758_s_at	8625	1	P	0.551	P	0.285	P	RFXANK; BLS
202759_s_at	11217	1	A	1.462	P	1.784	P	PALM2
202760_s_at	11217	1	P,A	1.402	P,A	2.073	P	AKAP2
202764_at	6786	1	P	1.548	P	1.636	P	STIM1
202767_at	53	1	P	0.673	P	0.437	P	ACP2
202769_at		1	P	0.435	P	0.537	P	CCNG2
202770_s_at	901	1	P	0.513	P	0.611	P	CCNG2
202777_at	8036	1	P	1.378	P	1.516	P	SHOC2
202779_s_at	27338	1	P	0.833	P	0.652	P	UBE2S
202785_at	4701	1	P	0.958	P	0.608	P,M	NDUFA7
202792_s_at	9701	1	P	0.696	P,M	0.618	M,A	KIAA0685
202793_at	10162	1	P	0.696	P	0.419	P,A	C3F
202794_at	3628	1	P	1.468	P	1.688	P	INPP1
202802_at	1725	1	P	0.719	P	0.447	P	DHPS

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
202812_at	2548	1	P	0.572	P	0.562	P	GAA; LYAG
202819_s_at	6924	1	P	1.585	P	1.418	P	TCEB3; SIII
202823_at	6921	1	P	1.554	P	1.425	P	TCEB1
202830_s_at	2542	1	P	0.527	M,A	0.299	A	SLC37A4
202838_at	2517	1	P	0.558	P	0.356	P	FUCA1
202847_at	5106	1	P	0.261	P	0.203	M,A	PCK2;
202852_s_at	79719	1	P	1.590	P	1.519	P	FLJ11506
202855_s_at	9123	1	P	0.636	P	0.483	P	SLC16A3
202856_s_at	9123	1	P	0.830	P	0.649	P	SLC16A3;
202867_s_at	54788	1	P	0.730	P	0.568	P	DNAJB12;
202868_s_at	10775	1	P	1.521	P	1.252	P	POP4; RPP29
202883_s_at	5519	1	P	1.529	P	1.174	P	PPP2R1B
202887_s_at	54541	1	P	0.401	M,A	0.358	A	DDIT4; Dig2;
202900_s_at	4927	1	P	1.743	P	1.625	P	NUP88
202903_at	23658	1	P	1.364	P	1.690	P	LSM5
202904_s_at	23658	1	P	1.557	P	1.493	P	LSM5;
202906_s_at	4683	1	P	1.615	P	1.409	P	NBS1
202912_at	133	1	P	1.545	P	1.325	P	ADM; AM
202930_s_at	8803	1	P	1.518	P	1.409	P	SUCLA2
202934_at	3099	1	A	2.805	P	2.985	P	HK2
202937_x_at	27341	1	P,M	1.612	P	1.138	P	CGI-96
202939_at	10269	1	P	1.385	P	1.502	P	ZMPSTE24
202943_s_at	4668	1	P	0.748	P	0.546	P	NAGA
202944_at	4668	1	P	0.783	P	0.618	P	NAGA
202945_at	2356	1	P	0.927	P	0.596	M,A	FPGS
202949_s_at	2274	1	P	2.215	P	2.887	P	FHL2; DRAL
202951_at	11329	1	P	1.480	P	1.643	P	STK38
202962_at	23303	1	M,A	1.326	P	1.671	P	KIF13B; GAKIN
202978_s_at	58487	1	P	1.524	P	1.627	P	ZF
202979_s_at	58487	1	P	1.470	P	1.580	P	ZF
202993_at	10994	1	P	0.774	P	0.597	P	ILVBL
202994_s_at	2192	1	P	1.026	P	1.606	P	E46L;
202998_s_at	4017	1	P	1.150	P	1.980	P	LOXL2; WS9-14
203002_at	51421	1	P	1.373	P	1.526	P	AMOTL2
203006_at	3632	1	P	0.810	P	0.644	P	INPP5A
203018_s_at	22892	1	P	1.411	P	1.545	P	SSX2IP
203019_x_at	22892	1	P	1.564	P	1.819	P	SSX2IP
203023_at	51491	1	P	1.726	P	1.295	P	HSPC111
203027_s_at	4597	1	P	0.634	P,M,A	0.518	A	MVD
203031_s_at	7390	1	P	0.809	P	0.642	P	UROS
203038_at	5796	1	M,A	1.512	P	1.783	P	PTPRK
203041_s_at	3920	1	P	1.197	P	1.678	P	LAMP2;
203042_at	3920	1	P	1.395	P	1.932	P	LAMP2
203053_at	10286	1	P	1.513	P	1.419	P	BCAS2; DAM1
203058_s_at	9060	1	P	1.635	P	2.323	P	PAPSS2
203060_s_at	9060	1	P	1.942	P	3.318	P	PAPSS2; SK2
203062_s_at	9656	1	P	1.290	P	1.504	P	MDC1
203068_at	9903	1	P	1.570	P	1.514	P	KIAA0469
203075_at	4087	1	P	1.645	P	1.674	P	SMAD2
203077_s_at	4087	1	P	1.337	P	1.516	P	SMAD2; JV18

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203078_at	8453	1	P	1.703	P	1.648	P	CUL2
203085_s_at	7040	1	P	0.839	P	0.644	P	TGFB1;
203095_at	4528	1	P	1.576	P	1.464	P	MTIF2
203113_s_at	1936	1	P	1.003	P	0.650	P	EEF1D;
203116_s_at	2235	1	P	0.792	P	0.649	P	FECH
203119_at	79080	1	P	1.850	P	1.648	P	MGC2574
203122_at	51112	1	P,A	0.860	P,A	0.571	A	TTC15; CGI-87
203125_x_at	4891	1	P,M,A	0.792	M,A	0.649	A	SLC11A2
203126_at	3613	1	P	0.861	P	0.649	P	IMPA2
203139_at	1612	1	P,A	1.428	P	1.541	P	DAPK1
203140_at	604	1	P	1.268	P	2.302	P	BCL6; BCL5
203150_at	10244	1	P	1.641	P	1.613	P	RAB9P40; p40
203154_s_at	10298	1	P	1.551	P	1.351	P	PAK4
203156_at	11215	1	P	1.646	P	1.670	P	AKAP11
203185_at	9770	1	P	1.276	P	1.605	P	RASSF2
203188_at	11041	1	P	0.634	P	0.511	P	B3GNT6
203192_at	10058	1	P	0.902	P	0.613	M,A	ABCB6
203197_s_at	54987	1	P	0.945	P	0.626	P	FLJ20580
203198_at	1025	1	P	0.814	P	0.577	P,A	CDK9; TAK
203200_s_at	4552	1	P	1.461	P	1.623	P	MTRR; MSR
203202_at	11103	1	P	2.083	P	2.042	P	HRB2
203203_s_at	11103	1	P	1.802	P	1.720	P	HRB2; RIP-1
203204_s_at	9682	1	P	0.620	P	0.632	P	JMJD2A
203205_at	9682	1	P	0.621	P	0.600	P	JMJD2A;
203211_s_at	8898	1	P	1.784	P	1.634	P	MTMR2;
203212_s_at	8898	1	A	1.899	P	2.085	P	MTMR2
203215_s_at	4646	1	P	1.375	P	1.638	P	MYO6
203216_s_at	4646	1	P	1.484	P	1.679	P	MYO6
203228_at	5050	1	P	0.865	P	0.607	P	PAFAH1B3
203234_at	7378	1	P	1.639	P	1.910	P	UPP1; UPASE
203239_s_at	4849	1	P	0.846	P	0.625	P	CNOT3
203252_at	10263	1	P	0.786	P	0.559	P	DOC-1R
203259_s_at	51020	1	P	1.573	P	1.631	P	C6orf74
203264_s_at	23229	1	P	0.675	P	0.597	P	ARHGEF9
203267_s_at	1819	1	P	0.914	P	0.644	P	DRG2
203272_s_at	11334	1	P	1.825	P	1.548	P	TUSC2
203292_s_at	55823	1	P	1.371	P	1.544	P	VPS11
203294_s_at	3998	1	P,A	0.867	P,A	0.656	P,A	LMAN1; MR60
203299_s_at	8905	1	P,A	1.481	P	1.805	P	AP1S2; DC22
203304_at	25805	1	P,M	2.048	P	3.419	P	BAMBI; NMA
203312_x_at	382	1	P	1.507	P	1.410	P	ARF6
203314_at	8225	1	P	1.522	P	1.037	P	PGPL
203315_at	8440	1	P,M,A	1.235	P	1.590	P	NCK2; GRB4
203320_at	10019	1	P	0.799	P	0.569	P	LNK
203336_s_at	9270	1	P	1.799	P	1.350	P	ITGB1BP1
203338_at	5529	1	P	1.373	P	1.506	P	PPP2R5E
203341_at	10153	1	P	1.554	P	1.415	P	CEBPZ
203344_s_at	5932	1	P	1.566	P	1.380	P	RBBP8
203359_s_at	26292	1	P	1.657	P	1.294	P	MYCBP
203360_s_at	26292	1	P	1.682	P	1.219	P	MYCBP

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203361_s_at	26292	1	A	1.714	P	1.692	P,M	MYCBP
203365_s_at	4324	1	P	0.728	P,A	0.607	A	MMP15
203367_at	11072	1	P	1.517	P	1.846	P	DUSP14
203368_at	78987	1	P	0.699	P	0.551	P	CRELD1
203381_s_at	348	1	P,M	0.827	P	1.712	P	APOE
203382_s_at	348	1	P,A	1.007	P,A	2.208	P	APOE
203386_at	9882	1	P,A	1.512	P	1.988	P	TBC1D4
203388_at	409	1	P	0.751	P	0.650	P	ARRB2
203392_s_at	1487	1	P	0.690	P	0.523	P	CTBP1
203394_s_at	3280	1	P	1.091	P	1.598	P	HES1
203395_s_at	3280	1	P	1.282	P	1.810	P	HES1
203403_s_at	6049	1	P	1.450	P	1.592	P	RNF6
203410_at	10947	1	P	1.715	P	1.592	P	AP3M2
203411_s_at	4000	1	P	0.584	P	0.631	P	LMNA
203413_at	4753	1	P	1.221	P	1.667	P	NELL2
203415_at	10016	1	P	0.762	P	0.605	P	PDCD6
203417_at	4237	1	P	0.573	P,A	0.607	P	MFAP2;
203422_at	5424	1	P	0.869	P	0.584	P	POLD1
203439_s_at	8614	1	P	0.531	M,A	0.541	P,A	STC2
203442_x_at	256364	1	P	0.829	P	0.569	P	FLJ35827
203446_s_at	4952	1	P	1.498	P	1.553	P	OCRL
203452_at	26229	1	P	0.695	P	0.526	P	B3GAT3
203456_at	11230	1	P	0.810	P	0.584	P	JM4
203466_at	4358	1	P	1.533	P	1.685	P	MPV17
203467_at	5372	1	P	0.791	P	0.625	P	PMM1
203468_at	8558	1	A	0.995	P,A	0.653	P,A	CDK10
203485_at	6252	1	P	1.231	P	1.843	P	RTN1; NSP
203490_at	2000	1	A	1.674	P,M	1.520	P,A	ELF4; MEF
203501_at	10404	1	P,A	1.500	P	1.426	P,M	PGCP
203502_at	669	1	P	1.617	P	1.991	P	BPGM
203510_at	4233	1	P	1.154	P	1.745	P	MET
203526_s_at	324	1	P	1.402	P	1.570	P	APC; GS;
203555_at	26469	1	P	0.814	A	0.620	A	PTPN18; BDP1
203562_at	9638	1	P	1.110	P	1.581	P	FEZ1
203573_s_at	5875	1	P	0.789	P	0.486	P	RABGGTA
203574_at	4783	1	P	1.122	P	1.561	P	NFIL3; E4BP4
203576_at	587	1	P	0.791	P	0.532	P	BCAT2; BCAM
203578_s_at	9057	1	P,M	1.525	P	1.464	P	SLC7A6
203588_s_at	7029	1	P	1.604	P	1.961	P	TFDP2
203589_s_at	7029	1	P,A	1.544	P	2.083	P	TFDP2; Dp-2
203599_s_at	11193	1	P	1.365	P	1.593	P	WBP4; FBP21
203603_s_at	9839	1	P,A	1.732	P,A	3.432	P	ZFHX1B; SIP1
203607_at	22876	1	P	1.469	P	1.519	P	INPP5F; SAC2;
203622_s_at	56902	1	P	1.662	P	1.434	P	LOC56902
203646_at	2230	1	P	1.424	P	1.591	P	FDX1; ADX
203648_at	9797	1	P	1.565	P	1.615	P	KIAA0218
203657_s_at	8722	1	P	0.558	P	0.639	P	CTSF; CATSF
203665_at	3162	1	P	2.534	P	2.982	P	HMOX1; HO-1
203669_s_at	8694	1	P	0.684	P	0.555	P	DGAT1
203671_at	7172	1	P,A	1.564	P	1.833	P	TPMT

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203674_at	9931	1	P	1.568	P	1.585	P	HELZ
203675_at	4925	1	P	1.450	P	1.884	P	NUCB2; NEFA
203679_at	11018	1	P	0.781	P	0.494	P	IL1RL1LG
203680_at	5577	1	P	1.352	P	1.671	P	PRKAR2B
203682_s_at	3712	1	P,M	0.868	P	0.651	A	IVD; ACAD2
203686_at	4350	1	P	0.849	P	0.634	P	MPG; AAG
203693_s_at	1871	1	P	1.539	P	1.455	P	E2F3; E2F-3
203696_s_at	5982	1	P	1.613	P	1.251	P	RFC2; A1
203710_at	3708	1	P	1.939	P	1.964	P	ITPR1
203711_s_at	26275	1	P	0.922	P	0.520	P	HIBCH
203712_at	9933	1	P	1.748	P	1.821	P	KIAA0020
203713_s_at	3993	1	P	0.758	P	0.524	P,A	LLGL2; HGL
203714_s_at	6905	1	P	1.475	P	1.510	P	TBCE; HRD
203720_s_at	2067	1	P	0.799	P	0.640	P	ERCC1; UV20
203722_at	8659	1	P	0.720	P	0.554	P	ALDH4A1
203737_s_at	23082	1	P	1.556	P	1.486	P	PPRC1
203738_at	55322	1	P	1.752	P	1.804	P	FLJ11193
203740_at	10200	1	P	1.684	P	1.464	P	MPHOSPH6
203743_s_at	6996	1	P	1.857	P	1.563	P	TDG
203746_s_at	3052	1	P	1.575	P	1.235	P	HCCS; CCHL
203767_s_at	412	1	P	1.514	P	1.148	P	STS
203771_s_at	644	1	P	1.548	P	1.198	P	BLVRA; BVRA
203778_at	4126	1	P	1.037	P,M	1.519	P	MANBA
203787_at	23635	1	P	1.678	P	1.752	P	SSBP2
203810_at	11080	1	P	2.306	P	2.987	P	DNAJB4
203811_s_at	11080	1	P,M,A	1.751	P	2.377	P	DNAJB4
203814_s_at	4835	1	P	0.836	P	0.584	P	NQO2;
203815_at	2952	1	P	0.606	P,M	0.527	P,A	GSTT1
203816_at	1716	1	P	2.034	P	2.302	P	DGUOK
203821_at	1839	1	A	2.686	P	2.629	P	DTR; DTS
203840_at	8548	1	P,A	1.508	P	1.463	P	BLZF1; JEM1
203851_at	3489	1	P,A	1.556	P	1.833	P	IGFBP6; IBP6
203853_s_at	9846	1	A	1.394	P,A	1.639	P,A	GAB2;
203856_at	7443	1	P	1.542	P	1.361	P	VRK1
203857_s_at	10954	1	P	0.768	P	0.644	P	PDIR
203860_at	5095	1	P	0.781	P	0.585	P,A	PCCA
203870_at	64854	1	P	1.623	P	1.712	P	USP46;
203876_s_at	4320	1	P,A	0.466	P,A	0.396	A	MMP11
203878_s_at	4320	1	P	0.575	P	0.432	P	MMP11
203880_at	10063	1	P	1.866	P	1.835	P	COX17
203882_at	10379	1	P	0.616	P	0.661	P	ISGF3G
203893_at	6880	1	P	1.654	P	1.778	P	TAF9
203910_at	9411	1	P	1.239	P	1.897	P	PARG1
203916_at	8509	1	P	0.696	P,A	0.548	A	NDST2
203917_at	1525	1	P	1.296	P	1.788	P	CXADR;
203919_at	6919	1	P	0.719	P	0.614	P	TCEA2; TFIIS
203921_at	9435	1	P	0.652	P	0.523	P	CHST2; C6ST
203926_x_at	513	1	P	0.720	P	0.450	P	ATP5D
203935_at	90	1	P	1.480	P	1.545	P	ACVR1
203944_x_at	11120	1	P	1.500	P	1.641	P	BTN2A1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
203946_s_at	384	1	P,A	1 760	P	1 353	P,A	ARG2
203960_s_at	51668	1	P	1 619	P	1 528	P	C1orf41
203967_at	990	1	P	1 778	P	1 624	P	CDC6,
203968_s_at	990	1	P	1 709	P	1 453	P	CDC6
203970_s_at	8504	1	P	1 409	P	1 593	P	PEX3
203976_s_at	10036	1	P	1 528	P	1 324	P	CHAF1A
203980_at	2167	1	P	1 999	P	2 462	P	FABP4
203981_s_at	5826	1	P	0 657	P	0 694	P	ABCD4
203986_at	8987	1	P	2 200	P	2 284	P	GENX-3414
203989_x_at	2149	1	A	1 755	P,A	3 086	P	F2R, TR
204019_s_at	26751	1	P	0 723	P	0 566	P	SH3YL1
204020_at	5813	1	P	1 323	P	1 515	P	PURA
204022_at	11060	1	P	0 822	P	0 619	P	WWP2
204023_at	5984	1	P	1 617	P	1 373	P	RFC4, A1,
204024_at	734	1	P	1 634	P	1 681	P	C8orf1, hT41
204030_s_at	29970	1	P	1 563	P	1 944	P	SCHIP1
204033_at	9319	1	P	1 658	P	1 526	P	TRIP13
204035_at	7857	1	P	1 612	P	1 267	P	SCG2
204051_s_at	6424	1	P	1 347	P	1 798	P	SFRP4
204054_at	5728	1	P,A	1 394	P	1 659	P,M	PTEN, BZS
204064_at	9984	1	P	1 600	P	1 545	P	THOC1, P84
204073_s_at	745	1	P	0 625	P	0 564	P	C11orf9,
204076_at	9583	1	P	0 834	P	0 553	P	LYSAL1
204077_x_at	9583	1	P	0 929	P	0 660	P,A	LYSAL1
204078_at	10609	1	P	0 816	P	0 579	P	SC65, NOL55
204081_at	4900	1	P	1 528	P	2 036	P	NRGN, RC3,
204083_s_at	7169	1	P	1 698	P	1 599	P	TPM2, DA1
204088_at	5025	1	P	0 605	P	0 463	P	P2RX4
204091_at	5147	1	P	0 675	P	0 520	P	PDE6D, PDED
204099_at	6604	1	P	0 788	P	0 625	P	SMARCD3
204106_at	7016	1	P	1 522	P	1 093	P	TESK1
204108_at	4800	1	P	1 673	P	1 428	P	NFYA,
204125_at	51103	1	P	1 611	P	1 447	P	NDUFAF1
204127_at	5983	1	P	1 511	P	1 661	P	RFC3
204128_s_at	5983	1	P	1 671	P	1 481	P	RFC3
204133_at	9136	1	P	1 536	P	1 169	P	RNU3IP2
204135_at	11259	1	P	1 238	P	1 740	P	DOC1, GIP90
204139_x_at	7593	1	P	0 661	A	0 466	P,A	ZNF42, MZF1
204141_at	7280	1	P	1 868	P	1 973	P	TUBB
204142_at	55556	1	P	0 877	P	0 647	P	HSRTSBETA
204144_s_at	9091	1	P	0 807	P,A	0 605	P,A	PIGQ, GPI1
204146_at	10635	1	P	1 601	P	1 809	P	PIR51
204149_s_at	2948	1	P	0 740	P	0 375	P	GSTM4
204182_s_at	23099	1	P,A	1 500	P	1 691	P	ZNF297B
204185_x_at	5481	1	P	1 525	P	1 543	P	PPID, CYPD
204186_s_at	5481	1	P	1 624	P	1 613	P	PPID
204208_at	8732	1	P	1 480	P	1 572	P	RNGTT, HCE
204217_s_at	6253	1	P	0 341	P	0 403	P	RTN2, NSP2
204218_at	25906	1	P	1 512	P	1 038	P	DKFZP564M082
204227_s_at	7084	1	P	0 861	P	0 652	P,M	TK2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
204235_s_at	51454	1	P	1.189	P	1.518	P	GULP1
204238_s_at	10591	1	P	0.712	P	0.451	P	C6orf108
204243_at	6018	1	P	1.390	P	1.579	P	RLF
204260_at	1114	1	P	0.419	P	0.311	P	CHGB; SCG1
204264_at	1376	1	P	0.840	P	0.571	P	CPT2; CPT1
204279_at	5698	1	P	1.323	P	1.765	P	PSMB9; LMP2
204285_s_at	5366	1	P,A	1.563	P,A	2.275	P	PMAIP1
204286_s_at	5366	1	A	2.536	P,A	3.391	P,A	PMAIP1; APR
204290_s_at	4329	1	P	0.704	P	0.551	P	ALDH6A1
204295_at	6834	1	P	0.810	P	0.551	P	SURF1
204313_s_at	1385	1	P	1.382	P	1.649	P	CREB1
204314_s_at	1385	1	P	1.489	P	1.642	P	CREB1
204317_at		1	P	1.429	P	1.889	P	GTSE1; B99
204320_at	1301	1	P	0.837	P	0.639	P	COL11A1
204330_s_at	6183	1	P	1.730	P	1.629	P	MRPS12
204331_s_at	6183	1	P	1.601	P	1.166	P	MRPS12
204333_s_at	175	1	P	0.613	P	0.696	P	AGA; AGU
204340_at	8269	1	P	0.631	P	0.611	P	CXorf12; ITBA1
204343_at	21	1	P	0.610	P	0.520	P	ABCA3; ABC3
204346_s_at	11186	1	P,M	1.376	P	1.849	P	RASSF1
204347_at	205	1	P	1.302	P	1.566	P	AK3
204352_at	7188	1	P	1.408	P	1.864	P	TRAF5; RNF84
204358_s_at	23768	1	P	0.694	P	0.524	P,A	FLRT2
204359_at	23768	1	P	0.632	P	0.458	P	FLRT2
204360_s_at	4669	1	P	0.508	P	0.399	P	NAGLU
204361_s_at	8935	1	P	1.433	P	1.661	P	SCAP2;
204362_at	8935	1	P	1.357	P	1.700	P	SCAP2
204371_s_at	8570	1	P	0.740	P	0.611	P	KHSRP
204394_at	8501	1	P	0.603	P	0.644	P,A	SLC43A1
204398_s_at	24139	1	P	0.689	P	0.547	P	EML2
204407_at	8458	1	P	1.791	P	1.785	P	TTF2; HuF2
204418_x_at	2946	1	P	0.923	P	0.556	P	GSTM2;
204420_at	8061	1	P	1.606	P	1.457	P	DIPA
204421_s_at	2247	1	P	1.809	P	1.850	P	FGF2
204422_s_at	2247	1	P	1.451	P	1.551	P	FGF2
204423_at	4289	1	P	1.855	P	1.961	P	MKLN1
204425_at	393	1	P	0.602	P,M	0.462	P,A	ARHGAP4
204435_at	9818	1	P	1.611	P	1.567	P	NUPL1
204441_s_at	23649	1	P	1.617	P	1.341	P	POLA2
204442_x_at	8425	1	P	0.723	P	0.654	P	LTBP4
204459_at	1478	1	P	1.614	P	1.194	P	CSTF2
204460_s_at	5810	1	P	1.566	P	1.402	P	RAD1; HRAD1
204465_s_at	9118	1	P	1.743	P	1.507	P	INA
204472_at	2669	1	P	1.182	P	1.735	P	GEM; KIR
204473_s_at	9640	1	P	1.564	P	1.757	P	KIAA0211
204475_at	4312	1	P	1.787	P	2.786	P	MMP1;
204477_at	5877	1	P	1.767	P	2.088	P	RABIF
204478_s_at	5877	1	P	1.726	P	1.590	P	RABIF
204481_at	7862	1	P,A	1.516	P	1.467	P,A	BRPF1; BR140
204490_s_at	960	1	P,A	1.341	M,A	2.521	P,A	CD44; IN

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
204492_at	9824	1	P	1 233	P	1 540	P	ARHGAP11A
204512_at	3096	1	P	1 563	P	1 528	P	HIVEP1, MBP-1
204514_at	1802	1	P	1 717	P	1 518	P	DPH2L2
204521_at	29902	1	P	0 845	P	0 642	P	HSU79274
204523_at	7699	1	P	1 479	P	1 602	P	ZNF140, pHZ-39
204530_s_at	9760	1	P,A	1 538	P,M,A	1 204	M,A	TOX
204531_s_at	672	1	P	1 640	P	1 746	P	BRCA1
204547_at	10966	1	P	0 652	P	0 557	P	RAB40B
204550_x_at	2944	1	P	0 880	P	0 520	P	GSTM1
204558_at	8438	1	P	1 986	P	1 587	P	RAD54L
204569_at	22858	1	P	0 765	P	0 622	P,A	ICK, MRK
204580_at	4321	1	P	2 706	P	3 028	P	MMP12, HME,
204589_at	9891	1	P	1 203	P	1 522	P	ARK5
204593_s_at	29787	1	P	1 542	P	1 381	P	FLJ20232
204603_at	9156	1	P	2 735	P	2 211	P	EXO1,
204605_at	10668	1	P	1 549	P	1 891	P	CGRRF1
204608_at	435	1	P	0 863	P	0 509	P,A	ASL
204616_at	7347	1	P	1 614	P	1 409	P	UCHL3
204622_x_at	4929	1	P	1 826	P	1 407	P	NR4A2,
204632_at	8986	1	P	1 720	P	1 535	P	RPS6KA4,
204633_s_at	9252	1	P,M	0 732	A	0 628	A	RPS6KA5
204639_at	100	1	P	0 925	P	0 654	P	ADA
204642_at	1901	1	P	1 690	P	1 541	P	EDG1
204646_at	1806	1	P	0 825	P	0 599	P	DPYD, DHP, DPD
204667_at	3169	1	P	1 348	P	1 552	P	FOXA1
204668_at		1	P	1 541	P	1 696	P	
204690_at	9482	1	P	1 463	P	1 647	P	STX8, CARB
204692_at	4034	1	P	0 832	P	0 538	P,A	LRCH4
204695_at	993	1	A	2 647	P	1 946	P	CDC25A
204696_s_at	993	1	A	1 623	P,M	1 196	M A	CDC25A
204700_x_at	27042	1	P	1 423	P	1 517	P	MGC29875
204702_s_at	9603	1	P	1 485	P	1 611	P	NFE2L3, NRF3
204703_at	8100	1	P	0 853	P	0 650	P	TTC10,
204712_at	11197	1	P	1 220	P	2 049	P	WIF1, WIF-1
204717_s_at	3177	1	P	0 697	P	0 533	P	SLC29A2
204727_at	11169	1	P,A	1 886	P	2 089	P	WDHD1
204728_s_at	11169	1	P	1 747	P	1 743	P	WDHD1, AND-1
204740_at	10256	1	A	1 449	M,A	1 605	P,M,A	CNKSR1, CNK1
204748_at	5743	1	M,A	1 950	P,A	2 226	P	COX2,
204749_at	4675	1	P	1 812	P	2 359	P	NAP1L3
204759_at	1102	1	P	2 688	P	3 253	P	CHC1L
204766_s_at	4521	1	P	1 821	P	1 512	P	NUDT1, MTH1
204772_s_at	7270	1	P	1 615	P	1 361	P	TTF1
204783_at	4291	1	P	1 841	P	1 900	P	MLF1
204784_s_at	4291	1	P	1 553	P	1 579	P	MLF1
204788_s_at	5498	1	P	0 597	P	0 522	P	PPOX
204790_at	4092	1	P	1 773	P	1 845	P	SMAD7
204791_at	7181	1	P	0 762	P	0 656	P	NR2C1
204793_at	9737	1	P	1 222	P	1 529	P	GASP

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
204805_s_at	8971	1	P	0.567	P	0.495	P	H1FX; H1X;
204807_at	10329	1	P	1.408	P	1.554	P	TMEM5
204808_s_at	10329	1	P	1.703	P	1.594	P	TMEM5;
204831_at		1	P	1.414	P	1.549	P	
204837_at	66036	1	P	1.407	P	1.533	P	MTMR9
204840_s_at	8411	1	P,A	1.536	P,A	1.919	P	EEA1
204849_at	10732	1	P	0.676	P	0.512	P	TCFL5;
204854_at	10536	1	P	0.730	P,A	0.517	A	LEPREL2
204857_at	8379	1	P	0.619	P	0.426	P	MAD1L1;
204858_s_at	1890	1	P	0.625	P	0.537	P	ECGF1; TP
204862_s_at	4832	1	P	0.859	P	0.552	P	NME3
204867_at	2644	1	P	0.735	P	0.589	P	GCHFR;
204868_at	3396	1	P	1.501	P	1.090	P	ICT1; DS-1
204880_at	4255	1	P	0.794	P	0.420	P	MGMT
204883_s_at	3364	1	P	1.992	P	1.587	P	HUS1
204886_at	10733	1	P	0.685	P	0.631	P	PLK4
204897_at	5734	1	P	1.775	P	2.393	P	PTGER4
204905_s_at	9521	1	P	1.584	P	1.469	P	EEF1E1; P18
204928_s_at	8273	1	P	1.667	P	1.339	P	SLC10A3
204936_at	5871	1	P,A	1.035	P	0.637	P,A	MAP4K2
204947_at	1869	1	P,M,A	1.612	P	1.430	P	E2F1; RBP3
204948_s_at	10468	1	A	1.166	P,A	2.265	P	FST
204955_at	8406	1	P	1.309	P	1.812	P	SRPX; ETX1
204957_at	5001	1	P	1.674	P	1.702	P	ORC5L; ORC5P
204967_at	357	1	P,A	1.177	P	1.620	P	APXL; HSAPXL
204977_at	1662	1	P	1.654	P	1.818	P	DDX10; HRH-J8
204979_s_at	6450	1	A	2.152	P	2.223	P	SH3BGR
204981_at	5002	1	P	0.598	P,A	0.503	A	SLC22A18
204983_s_at	2239	1	P	1.372	P	1.630	P	GPC4
204984_at	2239	1	P	1.350	P	1.743	P	GPC4
204985_s_at	79090	1	P	0.590	P	0.597	P	MGC2650
204990_s_at	3691	1	P	0.827	P,A	0.641	A	ITGB4
204991_s_at	4771	1	A	3.015	P	2.297	P	NF2; CAN
204998_s_at	22809	1	P	0.835	P	0.610	P	ATF5; ATFX
204999_s_at	22809	1	P	0.686	P	0.422	P	ATF5; ATFX
205003_at	9732	1	P,A	1.307	P	1.522	P	DOCK4
205006_s_at	9397	1	P	1.612	P	1.575	P	NMT2
205007_s_at	10518	1	P	0.806	P	0.609	P	CIB2
205034_at	9134	1	P	2.516	P	1.527	P	CCNE2; CYCE2
205047_s_at	440	1	P	0.377	P	0.278	P	ASNS; TS11
205052_at	549	1	P	0.638	P,M	0.428	P,M	AUH
205060_at	8505	1	P	1.275	P	1.549	P	PARG
205061_s_at	5393	1	P	1.541	P	1.328	P	EXOSC9; p5
205070_at	54556	1	P	1.400	P	1.667	P	ING3;
205076_s_at	10903	1	P	1.319	P	1.830	P	CRA
205081_at	1396	1	P	2.199	P	2.220	P	CRIP1
205085_at	4998	1	P	1.912	P	1.805	P	ORC1L;
205088_at	10046	1	M	1.179	P,A	1.702	P	CXorf6; CG1; F18
205090_s_at	51172	1	P	0.980	P	0.647	P	NAGPA

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
205092_x_at	22890	1	P,A	0 686	A	0 647	P,A	ZBTB1
205107_s_at	1945	1	P	0 680	P	0 594	P	EFNA4, EFL4
205115_s_at	9904	1	A	2 047	P	2 141	P	RBM19, NPO
205122_at	8577	1	P	1 599	P	1 672	P	TMEFF1
205123_s_at	8577	1	P	1 555	P	1 555	P	TMEFF1, H7365
205126_at	7444	1	P	1 795	P	1 769	P	VRK2
205134_s_at	26747	1	P	1 813	P	1 938	P	NUFIP1
205135_s_at	26747	1	P	1 782	P	1 759	P	NUFIP1
205136_s_at	26747	1	P,A	2 241	P	3 564	P	NUFIP1
205141_at	283	1	P	0 606	P,A	0 873	P	ANG, RNASE5
205153_s_at	958	1	P,M,A	0 926	P,M	0 643	P,M,A	TNFRSF5, p50
205158_at	6038	1	P	0 569	P	0 706	P	RNASE4
205174_s_at	25797	1	P	1 291	P	2 557	P	QPCT, QC, GCT
205192_at	9020	1	P,M	1 366	P	1 631	P	MAP3K14
205193_at	23764	1	P,M	1 544	P	1 242	P	MAFF, U-MAF
205205_at	5971	1	P	1 603	P	1 581	P	RELB, I-REL
205217_at	1678	1	P	1 828	P	1 924	P	TIMM8A, DDP
205218_at	10621	1	P	1 540	P	1 396	P	POLR3F
205219_s_at	2585	1	P	0 733	P	0 603	P	GALK2
205224_at	6835	1	A	1 955	P,A	1 740	P,M,A	SURF2
205249_at	1959	1	P	1 708	P	2 299	P	EGR2,
205264_at	10849	1	P	1 520	P	1 559	P	ASE-1
205268_s_at	119	1	P	1 643	P	2 027	P	ADD2, ADDB
205271_s_at	23552	1	A	1 684	P,A	1 732	P,A	CCRK
205279_s_at	2743	1	P	0 552	P	0 422	P	GLRB
205280_at	2743	1	P	0 502	P	0 393	P	GLRB
205282_at	7804	1	P	0 808	P	0 441	P	LRP8
205284_at	9816	1	A	1 630	P,M	1 845	P,M	KIAA0133
205286_at	7022	1	A	1 281	P,A	1 867	P,M,A	TFAP2C
205320_at	10297	1	P	0 600	P	0 669	P	APC2, APCL
205345_at	580	1	P	1 658	P	1 892	P	BARD1
205350_at	1381	1	P	1 279	P	1 882	P	CRABP1
205354_at	2593	1	P	0 635	P	0 454	P	GAMT
205358_at	2891	1	P	1 050	P	1 505	P	GRIA2, GLUR2
205361_s_at	5203	1	P	1 637	P	1 646	P	PFDN4
205405_at	9037	1	P	0 810	P	0 476	P,A	SEMA5A, semF
205406_s_at	53340	1	P	1 548	P	2 062	P	SPA17, SP17
205407_at	8434	1	P	0 458	P	0 398	P	RECK
205420_at	5191	1	P	1 017	P	0 592	P	PEX7, PTS2R
205427_at	6940	1	P	2 098	P	2 272	P	ZNF354A, EZNF
205429_s_at	51678	1	P	1 795	P	1 690	P	MPP6, VAM1
205441_at	79629	1	P	0 736	P	0 624	P	FLJ22709
205443_at	6617	1	P	1 486	P	1 592	P	SNAPC1, SNAP43
205447_s_at	7786	1	P	0 685	P,A	0 588	P,A	MAP3K12
205461_at	11021	1	P,M,A	0 772	M,A	0 530	A	RAB35, RAY
205479_s_at	5328	1	A	1 261	A	1 555	P	PLAU UPA
205493_s_at	10570	1	P	1 949	P	1 956	P	DPYSL4, CRMP3
205498_at	2690	1	P,M,A	1 253	P	1 560	P	GHR
205510_s_at	55056	1	P	0 547	P,M	0 535	P,A	GABPB2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
205519_at	79968	1	P	1 503	P	1 461	P	FLJ12973
205521_at	9941	1	P,M	1 565	P	1 276	P	ENDOGL1
205526_s_at	11104	1	P	1 378	P	1 621	P	KATNA1
205527_s_at	50628	1	P	1 657	P	1 464	P	GEMIN4
205534_at	5099	1	A	1 583	A	2 336	P	PCDH7
205547_s_at	6876	1	P	3 365	P	2 114	P	TAGLN
205561_at	79734	1	P	1 724	P	1 338	P,M,A	FLJ12242
205575_at	10882	1	P	0 578	P	0 520	P	C1QL1
205578_at	4920	1	A	1 139	M,A	1 606	P,A	ROR2
205581_s_at	4846	1	P,A	0 784	P,A	0 562	A	NOS3
205588_s_at	11116	1	P	1 610	P	1 653	P	FGFR1OP
205621_at	8846	1	P	1 543	P	1 490	P	ALKBH, ABH
205628_at	5558	1	P	2 090	P	2 006	P	PRIM2A, p58
205633_s_at	211	1	P	1 691	P	1 406	P	ALAS1
205634_x_at	79143	1	P	0 756	P	0 569	P	LENG4, BB1
205652_s_at	25809	1	P	0 630	P	0 527	P	TTLL1
205677_s_at	10301	1	P	1 712	P	2 001	P	DLEU1
205691_at	9143	1	P	0 546	P	0 360	P	SYNGR3
205742_at	7137	1	P	0 664	P,A	0 649	P,A	TNNI3, TNNC1
205748_s_at	55658	1	P	0 894	P	0 564	P	RNF126
205760_s_at	4968	1	P	1 634	P	1 298	P	OGG1
205763_s_at	8886	1	P	1 620	P	1 427	P	DDX18, MrDb
205770_at	2936	1	P	0 983	P	0 600	P	GSR
205771_s_at	9465	1	P	0 785	P	0 545	P	AKAP7
205774_at	2161	1	P	0 653	P	0 487	P,A	F12, HAF
205776_at	2330	1	P,M	0 595	P,A	0 653	A	FMO5
205781_at	9605	1	P	0 727	P	0 610	P,M	ATP-BL
205802_at	7220	1	P	1 339	P	1 709	P	TRPC1
205803_s_at	7220	1	P	1 409	P	1 790	P	TRPC1
205807_s_at	7286	1	P	1 500	P	1 541	P	TUFT1
205822_s_at	3157	1	P	0 768	P	0 611	P	HMGCS1
205828_at	4314	1	P	3 382	P	3 934	P	MMP3
205841_at	3717	1	M,A	1 789	P	2 204	P,A	JAK2
205842_s_at	3717	1	P,A	1 757	P	1 858	P	JAK2
205858_at	4804	1	A	3 290	M,A	6 523	P	NGFR
205876_at	3977	1	P,M	2 022	P	3 180	P	LIFR
205880_at	5587	1	P	1 364	P	1 582	P	PRKCM
205895_s_at	9221	1	P	1 579	P	1 399	P	NOLC1
205924_at	5865	1	A	2 226	P	1 677	P	RAB3B
205925_s_at	5865	1	P	1 583	P	1 413	P	RAB3B
205928_at	10224	1	P	1 614	P	1 876	P	ZNF443, ZK1
205932_s_at	4487	1	P,A	1 413	P	1 804	P	MSX1
205955_at	55310	1	P	0 800	P	0 596	P,A	TAF6L
205964_at	79088	1	P	1 561	P	1 670	P	ZNF426
205967_at	8364	1	P	0 567	P	0 654	P	HIST1H4C
205973_at	9638	1	P,A	1 656	P	1 389	P	FEZ1
205995_x_at	9657	1	A	1 563	M,A	1 728	P M A	IQCB1
206003_at	9662	1	P	1 701	P	1 860	P	KIAA0635
206016_at	28952	1	P	0 912	P	0 616	P	JM1
206036_s_at	5966	1	P	1 537	P	1 775	P	REL, C-Rel

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
206039_at	9363	1	P	0.557	P,M,A	0.639	P,A	RAB33A
206055_s_at	6627	1	P	1.776	P	1.444	P	SNRPA1
206067_s_at	7490	1	A	2.087	P,A	2.391	P	WT1; GUD;
206103_at	5881	1	P	0.423	P	0.217	A	RAC3
206104_at	3670	1	P,A	1.663	P	2.113	P	ISL1; Isl-1
206106_at	6300	1	P	0.856	P	0.586	P	MAPK12
206108_s_at	6431	1	P	0.631	P	0.545	P	SFRS6; B52
206110_at	8357	1	P	1.250	P	1.671	P	HIST1H3H
206116_s_at	7168	1	P	1.992	P	2.370	P	TPM1; CMH3
206128_at	152	1	P	0.759	P	0.602	P,M,A	ADRA2C
206140_at	9355	1	P	1.859	P	1.873	P	LHX2; LH2
206142_at	7694	1	P,M	0.654	A	0.638	P,A	ZNF135; ZNF61
206157_at	5806	1	P	2.827	P	2.316	P	PTX3; TSG-14
206172_at	3598	1	P	1.066	P	2.208	P	IL13RA2; IL-13R
206182_at	7693	1	P	1.395	P	1.507	P	ZNF134; pHZ-15
206204_at	2888	1	A	1.524	P,A	1.353	P	GRB14
206235_at	3981	1	P	1.573	P	1.669	P	LIG4
206261_at	8187	1	P	1.888	P	2.078	P	ZNF239; MOK2
206290_s_at	6000	1	P	1.427	P	1.586	P	RGS7
206299_at	27112	1	P,A	1.771	P	2.069	P	TMEM28; TED
206336_at	6372	1	P,A	1.060	P,A	1.565	M,A	CXCL6; GCP2
206343_s_at	3084	1	P	4.211	P	4.886	P	NRG1; GGF
206352_s_at	5192	1	A	1.639	P	1.401	P,M	PEX10; NALD
206377_at	2295	1	P	1.411	P	1.671	P	FOXF2; FKHL6
206397_x_at	2657	1	P	0.772	P	0.583	P,A	GDF1
206401_s_at	4137	1	P	0.646	P,A	0.408	P,A	MAPT; TAU
206424_at	1592	1	P	1.631	P	1.340	P	CYP26A1
206440_at	8825	1	A	1.832	P	1.330	P,A	LIN7A; VELI1
206448_at	22891	1	P	1.376	P	1.980	P	ZNF365
206452_x_at	5524	1	P	1.526	P	1.334	P	PPP2R4
206489_s_at	9229	1	P	1.073	P	0.644	P	DLGAP1
206507_at	9753	1	P	2.250	P	3.405	P	ZNF305
206508_at	970	1	P,A	1.099	P	1.545	P	TNFSF7;
206550_s_at	9631	1	P	1.515	P	1.516	P	NUP155
206552_s_at	6863	1	M,A	1.164	P,A	1.521	P,A	TAC1
206578_at	1482	1	P	1.692	P	1.600	P	NKX2-5
206613_s_at	9015	1	P	2.270	P	2.403	P	TAF1A
206615_s_at	53616	1	P,A	0.858	P,A	0.625	A	ADAM22
206632_s_at	9582	1	P	1.306	P	1.749	P	APOBEC3B
206653_at		1	P	1.609	P	1.160	P	POLR3G
206662_at	2745	1	P	1.696	P	2.747	P	GLRX; GRX
206667_s_at	9522	1	P	0.622	P	0.722	P	SCAMP1
206675_s_at	6498	1	P,A	2.301	P	2.118	P,M	SKIL; SNO
206688_s_at	10898	1	P	0.811	P	0.609	P	CPSF4
206689_x_at	10524	1	P	0.788	P	0.574	P	HTATIP
206693_at	3574	1	P,M	1.603	P	3.207	P	IL7; IL-7
206699_x_at	4861	1	P,A	1.581	P	1.253	P,A	NPAS1;
206721_at	57821	1	P	1.503	P,M	1.703	P	LOC57821
206770_s_at	23443	1	P	1.354	P	1.521	P	SLC35A3
206772_at	5746	1	P,A	1.246	P	1.653	P	PTHR2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
206773_at	4062	1	P,A	1 322	P,M	1 661	P	LY6H, NMLY6
206782_s_at	3338	1	P	0 639	P,M	0 537	P,A	DNAJC4
206809_s_at		1	P	1 596	P	1 860	P	HNRPA3
206826_at	5375	1	P	1 330	P	1 517	P	PMP2, FABP8
206833_s_at	98	1	P	0 839	P	0 650	P	ACYP2
206846_s_at	10013	1	P	0 677	P	0 499	P	HDAC6, HD6,
206848_at	3204	1	P	1 379	P	1 589	P	HOXA7, ANTP
206891_at	89	1	P	0 677	P,A	0 565	P,A	ACTN3
206906_at	7087	1	P	0 652	P	0 652	P	ICAM5
207006_s_at	29903	1	P	0 661	P,A	0 473	P,A	HSU79303
207030_s_at	1466	1	P	2 372	P	2 355	P	CSRP2
207039_at	1029	1	P	1 272	P	1 541	P	CDKN2A
207088_s_at	8402	1	P	0 815	P	0 644	P	SLC25A11
207145_at	2660	1	P,A	2 027	P	1 671	P	GDF8, MSTN
207147_at	1746	1	P	2 217	P	2 191	P	DLX2, TES1
207153_s_at	11146	1	P	1 859	P	1 471	P	GLMN, GVM
207164_s_at	10472	1	P	1 750	P	1 605	P	ZNF238, RP58
207199_at	7015	1	P,A	1 539	P	1 396	P	TERT, TP2, TRT
207302_at	6445	1	P	0 681	P	0 572	P	SGCG, A4, MAM
207332_s_at	7037	1	P	1 556	P	1 342	P	TFRC, CD71
207391_s_at	8394	1	P	1 352	P	1 503	P	PIP5K1A
207415_at	22925	1	P,A	1 455	P,A	1 509	P,A	PLA2R1
207437_at	4857	1	P	1 281	P	1 760	P	NOVA1,
207469_s_at	8544	1	P	0 558	P	0 236	P	PIR
207574_s_at	4616	1	P	2 624	P	2 869	P	GADD45B
207633_s_at	4593	1	P,M,A	1 527	P	1 126	P,M	MUSK
207688_s_at	3626	1	P	1 517	P	1 390	P	INHBC, IHBC
207713_s_at	10616	1	P	0 769	P	0 626	P	C20orf18, XAP4
207714_s_at	871	1	P	1 797	P	1 770	P	SERPINH1
207722_s_at	55643	1	P	0 720	P	0 519	P,A	BTBD2
207753_at	57343	1	P	1 459	P	1 751	P	ZNF304
207768_at	1961	1	A	1 995	P	2 562	P,A	EGR4
207781_s_at	7552	1	P	1 425	P	2 020	P	ZNF6, ZNF4
207813_s_at	2232	1	P	0 457	P	0 220	P	FDXR, ADXR
207826_s_at	3399	1	P	7 454	P	6 419	P	ID3, HEIR-1
207831_x_at	1725	1	P	0 856	P	0 595	P	DHPS
207855_s_at	23155	1	P	1 613	P	1 347	P	MCLC
207876_s_at	2318	1	P	1 512	P	1 685	P	FLNC, ABPA
207891_s_at	11219	1	P	1 559	P	1 001	P	TREX2
207978_s_at	8013	1	P	2 087	P	1 290	P	NR4A3, CHN
208003_s_at	10725	1	P	1 505	P	1 738	P	NFAT5
208018_s_at	3055	1	A	1 611	P,A	1 861	P,A	HCK
208021_s_at	5981	1	P	1 856	P	2 057	P	RFC1
208025_s_at	8091	1	P	1 003	P	0 640	P	HMGA2
208055_s_at	26091	1	P	1 618	P	1 558	P	HERC4
208078_s_at	6935	1	P	1 776	P	1 290	P,M	TCF8, BZP
208114_s_at	81875	1	P	2 005	P	1 741	P	FLJ12671
208117_s_at	81887	1	P	1 605	P	1 411	P	FLJ12525
208119_s_at	81931	1	P	1 399	P	1 520	P	ZNF505

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
208180_s_at	8365	1	P	1.717	P	2.090	P	HIST1H4H
208264_s_at	8669	1	P	1.670	P	1.558	P	eIF3-alpha
208270_s_at	6051	1	P	0.887	P	0.653	P	RNPEP
208273_at	57116	1	P,A	1.401	A	1.958	P,M	LOC57116
208290_s_at	1983	1	P	2.199	P	2.124	P	EIF5; EIF-5A
208309_s_at	10892	1	P	1.807	P	1.742	P	MALT1
208336_s_at	9524	1	P	0.631	P	0.558	P	GPSN2
208361_s_at	661	1	P,M	1.682	P	1.656	P	POLR3D
208368_s_at	675	1	P,A	1.803	P	1.779	P	BRCA2; FAD
208370_s_at	1827	1	P	1.388	P	1.633	P	DSCR1; CSP1
208433_s_at	7804	1	P	0.593	P	0.308	P	LRP8; APOER2
208447_s_at	5631	1	P	1.536	P	1.299	P	PRPS1
208478_s_at	581	1	P	0.536	P	0.361	P	BAX
208611_s_at	6709	1	P	1.415	P	1.506	P	SPTAN1
208634_s_at	23499	1	P	1.468	P	1.590	P	MACF1
208649_s_at	7415	1	P	0.932	P	0.610	P	VCP; p97
208650_s_at	934	1	P,M,A	2.429	P	5.150	P	CD24
208651_x_at	934	1	P,A	1.757	P	3.434	P	CD24; CD24A
208676_s_at		1	P	1.796	P	1.422	P	PA2G4
208677_s_at	682	1	P	0.668	P	0.630	P	BSG
208693_s_at	2617	1	P	0.692	P	0.650	P	GARS; CMT2D
208699_x_at	7086	1	P	0.900	P	0.642	P	TKT
208705_s_at	1983	1	P	1.678	P	1.593	P	EIF5; EIF-5A
208706_s_at	1983	1	P	1.899	P	1.949	P	EIF5; EIF-5A
208708_x_at	1983	1	P	2.023	P	1.994	P	EIF5; EIF-5A
208711_s_at	595	1	M,A	1.707	P	1.648	P,A	CCND1
208712_at	595	1	P,A	2.242	P	2.582	P	CCND1
208735_s_at	10106	1	P	0.717	P	0.567	P	CTDSP2
208740_at	10284	1	P	2.951	P	3.168	P	SAP18
208741_at	10284	1	P	2.042	P	2.295	P	SAP18
208742_s_at	10284	1	P	1.994	P	1.968	P	SAP18
208745_at	10632	1	P	1.410	P	1.608	P	ATP5L
208759_at	23385	1	P	0.902	P	0.648	P	NCSTN
208782_at	11167	1	P	1.079	P	1.681	P	FSTL1
208789_at	22939	1	P	1.593	P	1.765	P	PTRF
208791_at	1191	1	P	1.180	P	1.510	P	CLU
208792_s_at	1191	1	P	1.160	P	1.534	P	CLU
208798_x_at	23015	1	P	1.550	P	1.814	P	GOLGIN-67
208802_at	6731	1	P	1.536	P	1.432	P	SRP72
208803_s_at	6731	1	P	1.649	P	1.513	P	SRP72
208813_at	2805	1	P	0.610	P	0.571	P	GOT1
208828_at	54107	1	P	1.513	P	1.274	P	POLE3;
208832_at	25814	1	P	1.755	P	1.839	P	E46L
208843_s_at	26003	1	P	1.529	P	1.551	P	GORASP2; p59
208848_at	128	1	P	1.403	P	1.551	P	ADH5
208851_s_at	7070	1	P	1.525	P	1.936	P	THY1; CD90
208865_at	1452	1	P	1.489	P	1.827	P	CSNK1A1
208866_at	1452	1	P	1.716	P	2.209	P	CSNK1A1
208867_s_at	1452	1	P	1.383	P	1.573	P	CSNK1A1
208871_at	1822	1	A	1.722	P	1.654	P,M,A	DRP1A

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
208874_x_at	5524	1	P	1 511	P	1 357	P	PPP2R4
208879_x_at	24148	1	P	0 761	P	0 521	P	C20orf14
208883_at	51366	1	P	1 089	P	1 523	P	DD5
208884_s_at	51366	1	P	1 273	P	1 520	P	DD5, EDD
208886_at	3005	1	P	0 367	P	0 371	P	H1F0, H10
208891_at	1848	1	P	0 653	P	0 596	P	DUSP6
208892_s_at	1848	1	P	0 662	P	0 624	P	DUSP6
208893_s_at	1848	1	P	0 512	P	0 437	P	DUSP6
208896_at	8886	1	P	1 711	P	1 600	P	DDX18, MrDb
208898_at	51382	1	P	2 456	P	2 748	P	ATP6V1D
208899_x_at	51382	1	P	2 242	P	2 568	P	ATP6V1D
208911_s_at	5162	1	P	0 929	P	0 629	P	PDHB, PHE1B
208916_at	6510	1	P	0 730	P	0 632	P	SLC1A5, R16
208917_x_at	65220	1	P	0 872	P	0 546	P	FLJ13052
208918_s_at	65220	1	P	0 993	P	0 590	P	FLJ13052
208919_s_at	65220	1	P	0 805	P	0 583	P	FLJ13052
208920_at	6717	1	P	1 682	P	1 908	P	SRI
208922_s_at	10482	1	P	1 407	P	1 628	P	NXF1, TAP
208930_s_at	3609	1	P	0 638	P	0 442	P	ILF3
208931_s_at	3609	1	P	0 922	P	0 471	P	ILF3, MMP4
208932_at	5531	1	P	0 932	P	0 657	P	PPP4C, PPX
208937_s_at	3397	1	P	4 295	P	3 094	P	ID1
208939_at	22929	1	P	1 184	P	1 526	P	SEPHS1
208940_at	22929	1	P	1 024	P	1 526	P	SEPHS1
208944_at	7048	1	P	1 236	P	1 516	P	TGFBR2
208955_at	1854	1	P	1 625	P	1 955	P	DUT, dUTPase
208968_s_at	57019	1	P	1 514	P	1 178	P	LOC57019
208969_at	4704	1	P	1 618	P	1 597	P	NDUFA9
208975_s_at	3837	1	P	1 541	P	1 443	P	KPNB1
208985_s_at	8669	1	P	1 579	P	1 499	P	eIF3-alpha
209003_at	8402	1	P	0 841	P	0 632	P	SLC25A11
209010_s_at	7204	1	A	4 240	A	12 140	P,M	TRIO
209014_at	9500	1	P	0 717	P	0 569	P	MAGED1
209015_s_at	10049	1	P	1 754	P	2 181	P	DNAJB6, MRJ
209017_s_at	9361	1	P	0 608	P	0 403	P	PRSS15, LON
209019_s_at	65018	1	P	1 306	P	1 740	P	PINK1, BRPK,
209025_s_at	10492	1	P	1 512	P	1 265	P	SYNCRIP
209040_s_at	5696	1	M,A	1 168	M,A	1 668	P	PSMB8
209052_s_at	7468	1	P	0 903	P	0 655	P,A	WHSC1
209068_at	9987	1	P	1 293	P	1 642	P	HNRPDL
209076_s_at	56270	1	P	1 556	P	1 549	P	LOC56270
209085_x_at	5981	1	P	1 637	P	1 654	P	RFC1
209087_x_at	4162	1	P,A	1 438	P	1 657	P	MCAM
209090_s_at		1	P	1 447	P	1 778	P	
209102_s_at	26959	1	P	0 490	P	0 683	P	HBP1
209106_at	8648	1	P	1 228	P	1 561	P	NCOA1
209113_s_at	10362	1	P	0 632	P	0 533	P	HMG20B
209117_at	23558	1	P	0 703	P	0 613	P	WBP2
209139_s_at	8575	1	P	1 530	P	1 284	P	PRKRA
209152_s_at	6929	1	P	1 745	P	1 668	P	TCF3

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
209156_s_at	1292	1	P	0 590	P	0 557	P	COL6A2
209161_at	9128	1	P	2 141	P	1 732	P	PRPF4
209162_s_at	9128	1	P	1 836	P	1 528	P	PRPF4, PRP4
209165_at	26574	1	P	1 540	P	1 472	P	AATF, DED
209172_s_at	1063	1	P	1 195	P	1 780	P	CENPF, CENF
209173_at	10551	1	P	2 030	P	1 954	P	AGR2, AG2
209175_at	11196	1	P	1 557	P	1 543	P	SEC23IP
209180_at	5876	1	P	1 707	P	1 786	P	RABGGTB
209181_s_at	5876	1	P	1 544	P	1 503	P	RABGGTB
209187_at	1810	1	P	1 661	P	1 910	P	DR1
209188_x_at	1810	1	P	1 471	P	1 615	P	DR1, NC2
209192_x_at	10524	1	P	0 711	P	0 543	P	HTATIP
209196_at	9277	1	P	1 794	P	1 554	P	C6orf11, BING4
209205_s_at	8543	1	P	1 581	P	2 534	P	LMO4
209211_at	688	1	P	1 455	P	1 708	P	KLF5
209213_at	873	1	P	0 733	P	0 619	P	CBR1
209221_s_at	9885	1	P	1 457	P	1 682	P	OSBPL2
209233_at	10436	1	P	1 666	P	1 304	P	C2F
209234_at	23095	1	P	0 817	P	0 595	P	KIF1B
209238_at	6809	1	P	1 706	P	2 020	P	STX3A
209242_at	5178	1	P	1 510	P	1 405	P	PEG3
209243_s_at	23619	1	P	1 721	P	1 459	P	PEG3
209244_s_at	10749	1	A	2 048	P	1 504	A	KIF1C
209263_x_at	7106	1	P	0 747	P	0 580	P	TM4SF7
209264_s_at	7106	1	P	0 625	P	0 514	P	TM4SF7
209267_s_at	64116	1	P,M	1 907	P	2 684	P	SLC39A8
209268_at	11311	1	P	1 660	P	1 336	P	VPS45A
209273_s_at	81689	1	P	1 737	P	1 349	P	HBLD2
209276_s_at	2745	1	P,M	1 321	P	2 187	P	GLRX
209277_at	7980	1	P	1 915	P	3 052	P	TFPI2
209278_s_at	7980	1	P	1 532	P	2 279	P	TFPI2
209286_at		1	A	1 954	P,A	2 430	P,M	CDC42EP3
209288_s_at	10602	1	P	1 401	P	2 037	P	CDC42EP3
209291_at	3400	1	P	4 160	P	5 427	P	ID4
209292_at	3400	1	P	3 185	P	3 789	P	ID4
209293_x_at	3400	1	P	2 886	P	2 748	P	ID4
209294_x_at	8795	1	P	0 700	P,A	0 604	P,A	TNFRSF10B
209304_x_at	4616	1	A	2 200	P	2 556	P,M,A	GADD45B
209305_s_at	4616	1	P,A	1 778	P	1 867	P	GADD45B
209306_s_at	23075	1	P	1 453	P	1 527	P	SWAP70
209307_at	23075	1	P	1 481	P	1 687	P	SWAP70
209330_s_at	3184	1	P	0 753	P	0 639	P	HNRPD, P37
209340_at	6675	1	P	2 249	P	2 366	P	UAP1, AgX
209344_at	7171	1	P	1 515	P	1 317	P	TPM4
209348_s_at	4094	1	P,A	1 701	P,A	2 435	P,A	MAF
209349_at	10111	1	P	1 598	P	1 667	P	RAD50
209366_x_at	1528	1	P	1 580	P	1 386	P	CYB5
209367_at	6813	1	P	0 702	P	0 551	A	STXBP2
209379_s_at	54462	1	P	1 427	P	1 538	P	KIAA1128
209383_at	1649	1	P	0 951	P	1 605	P	DDIT3, CHOP

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
209384_at	11212	1	P	1 531	P	1 505	P	PROSC
209407_s_at	10522	1	P	0 600	P	0 340	A	DEAF1
209417_s_at	3430	1	P	0 482	P	0 493	P	IFI35, IFP35
209428_s_at	7542	1	P	0 695	P	0 358	P,A	ZFPL1
209432_s_at	10488	1	P	1 518	P	1 560	P	CREB3
209440_at	5631	1	P	1 575	P	1 533	P	PRPS1, PRSI
209457_at	1847	1	P	1 427	P	1 695	P	DUSP5, HVH3
209459_s_at	57416	1	A	2 323	M,A	1 992	P,A	ABAT, GABAT
209461_x_at	57418	1	P,A	1 101	P	0 624	A	WDR18
209468_at	4041	1	P	0 686	P	0 638	P,A	LRP5, HBM
209478_at	201254	1	P	2 051	P	1 580	P	STRA13
209490_s_at	9374	1	P	0 933	P	0 619	P	PPT2
209504_s_at	58473	1	P	0 610	P,M,A	0 559	A	PLEKHB1
209516_at	10322	1	P,M	1 584	P	1 381	P	SMYD5
209519_at	4686	1	P,A	1 506	P	1 654	P	NCBP1
209522_s_at	1384	1	P	0 813	P	0 566	P	CRAT, CAT1
209523_at	6873	1	P	1 527	P	1 480	P	TAF2, TAF2B
209527_at	23404	1	P	1 581	P	1 421	P	EXOSC2
209529_at	8612	1	P	0 647	P	0 597	P	PPAP2C
209531_at	2954	1	P	0 632	P	0 425	M,A	GSTZ1
209532_at	9373	1	P,A	1 690	P,M	1 539	P	PLAA
209533_s_at	9373	1	P	1 772	P	1 581	P	PLAA
209544_at	8767	1	P,A	1 257	P	1 653	P	RIPK2
209549_s_at	1716	1	P	1 484	P	1 553	P	DGUOK
209556_at	23154	1	P	1 543	P	1 459	P	NCDN
209561_at	7059	1	P	0 977	P	1 661	P	THBS3, TSP3
209567_at	23212	1	P	1 690	P	1 675	P	RRS1
209568_s_at	23179	1	P	1 528	P	1 803	P	RGL1
209587_at	5307	1	P,A	1 877	P	1 549	P	PITX1
209592_s_at	10238	1	P	0 650	P	0 474	P	HAN11
209593_s_at	27348	1	P	1 516	P	1 405	P	TOR1B
209598_at	10687	1	P	2 262	P	4 527	P	PNMA2
209607_x_at	6818	1	P	1 454	P	1 529	P	SULT1A3
209611_s_at	6509	1	P,A	0 725	P,A	0 660	A	SLC1A4
209620_s_at	22	1	P	0 806	P	0 621	P	hABC7
209631_s_at		1	A	1 145	A	2 884	P	
209651_at	7041	1	P	1 443	P	1 746	P	TGFB1I1
209653_at	3840	1	P	1 989	P	1 706	P	KPNA4
209656_s_at	83604	1	P	1 573	P	2 707	P	TM4SF10
209658_at	8881	1	P	1 625	P	1 494	P	CDC16, APC6
209666_s_at	1147	1	P	2 045	P	1 912	P	CHUK, IKK1
209674_at	1407	1	P	1 379	P	1 626	P	CRY1, PHLL1
209694_at	5805	1	P	1 528	P	0 971	P	PTS, PTPS
209707_at	10026	1	P	1 562	P	1 851	P	PIGK, GPI8
209708_at	26002	1	P	1 371	P	1 766	P	MOXD1
209710_at	84724	1	P	2 409	P	2 266	P	GATA2
209725_at	27340	1	P	1 526	P	1 377	P	DRIM
209750_at	9975	1	P,A	1 426	P	1 506	P	NR1D2
209759_s_at	1632	1	P	0 607	P	0 464	P	DCI
209771_x_at	934	1	P	2 313	P	4 957	P	CD24

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
209772_s_at	934	1	A	1.698	P,A	2.682	P,M	CD24; CD24A
209773_s_at	6241	1	P	1.596	P	1.266	P	RRM2; R2
209777_s_at	6573	1	A	1.629	P,A	1.407	P,M,A	SLC19A1
209781_s_at	10656	1	P,M,A	1.808	P	2.380	P	KHDRBS3
209782_s_at	1628	1	P	0.557	P	0.633	P	DBP; DABP
209803_s_at	7262	1	P,M	1.975	P	2.322	P	PHLDA2
209805_at	5395	1	M,A	1.573	P	1.480	P,M	PMS2
209818_s_at	22927	1	P	1.722	P	2.078	P	HABP4
209822_s_at	7436	1	P	0.631	P	0.771	P	VLDLR
209831_x_at	1777	1	P	0.643	P	0.577	P	DNASE2
209834_at	9469	1	P,A	2.077	P	2.175	P	CHST3
209835_x_at	960	1	A	1.292	P	1.919	P	CD44; IN
209836_x_at	79008	1	P	2.284	P	2.104	P	MGC5178
209838_at		1	P	1.820	P	1.925	P	TRIP15
209840_s_at	54674	1	P	1.563	P	1.584	P	LRRN3
209841_s_at	54674	1	P	1.613	P	1.610	P	LRRN3
209845_at	23608	1	P	1.649	P	1.731	P	MKRN1; RNF61
209852_x_at	10197	1	P	1.772	P	1.512	P	PSME3
209865_at	23443	1	P	1.371	P	1.661	P	SLC35A3
209875_s_at	6696	1	A	1.126	P,M,A	2.060	P	SPP1
209892_at	2526	1	P	1.479	P	1.623	P	FUT4
209917_s_at	11257	1	P	0.782	P	0.565	P,M,A	TP53AP1
209921_at	23657	1	P	0.594	P	0.555	P	SLC7A11
209927_s_at	26097	1	P	1.547	P	1.346	P	DKFZP547E1010
209934_s_at	27032	1	P	1.468	P	1.618	P	ATP2C1
209935_at	27032	1	P	1.320	P	1.668	P	ATP2C1
209943_at	26235	1	P	1.516	P	1.522	P	FBXL4; FBL4
209944_at	57862	1	P	1.431	P	1.512	P	ZNF410; APA1
209945_s_at	2932	1	P	1.524	P	1.358	P	GSK3B
209959_at	8013	1	P,M	3.086	P	2.098	P	NR4A3
209961_s_at	3082	1	P	1.011	P	0.630	P	HGF; SF; HPTA
209963_s_at	2057	1	P	0.814	P	0.643	P	EPOR
209969_s_at	6772	1	P	0.692	P	0.445	A	STAT1;
209998_at	84720	1	P	0.745	P	0.511	P	PIGO
210006_at	25864	1	P	0.761	P	0.638	P	DKFZP564O243
210007_s_at	2820	1	P	1.624	P	1.722	P	GPD2; GDH2
210008_s_at	6183	1	P	2.028	P	1.545	P	MRPS12
210009_s_at	9570	1	P	1.679	P	1.354	P	GOSR2
210010_s_at	6576	1	P	0.813	P	0.645	P	SLC25A1
210017_at	10892	1	P	1.486	P	1.529	P	MALT1
210018_x_at	10892	1	P	1.654	P	1.635	P	MALT1
210028_s_at	23595	1	P	1.590	P	1.225	P	ORC3L
210033_s_at	9576	1	P,A	1.390	P,M	2.134	P	SPAG6
210070_s_at	1375	1	P	1.203	P	1.672	P	CPT1B
210073_at	6489	1	P,A	1.910	P,M	1.135	P,A	SIAT8A
210093_s_at	4116	1	P	1.584	P	1.259	P	MAGOH
210095_s_at	3486	1	P	1.781	P	1.937	P	IGFBP3
210105_s_at	2534	1	P	1.064	P	1.518	P	FYN; SLK
210112_at	3257	1	P	1.754	P	2.189	P	HPS1; MGC5277
210115_at	116832	1	P	1.592	P	1.522	P	RPL39L

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
210117_at	6674	1	P	1.977	P	2.118	P	SPAG1
210130_s_at	7108	1	P	0.348	P	0.229	P	TM7SF2; ANG1
210138_at	8601	1	P	1.544	P	0.982	P	RGS20
210150_s_at	3911	1	P	0.626	P	0.539	P	LAMA5;
210151_s_at	8444	1	A	1.814	P	1.872	P	DYRK3
210171_s_at	1390	1	P	0.735	P	0.513	P,A	CREM
210175_at	6936	1	P	1.626	P	1.294	P	C2orf3;
210180_s_at	6434	1	P	0.771	P,M	0.649	P	SFRS10
210205_at	8705	1	P	0.589	P,A	0.557	P	B3GALT4;
210215_at	7036	1	P	0.919	P	0.637	P	TFR2
210216_x_at	5810	1	P	1.537	P	1.419	P	RAD1; HRAD1
210220_at	2535	1	P	0.606	P	0.621	P	FZD2
210221_at	1136	1	P,A	1.546	P,M	1.882	P	CHRNA3
210233_at	3556	1	P,A	2.028	P,A	3.241	P	IL1RAP
210236_at	8500	1	P	1.685	P	1.947	P	PPFIA1; LIP1
210241_s_at	11257	1	P	0.700	P,A	0.623	P	TP53AP1
210243_s_at	8703	1	P	0.737	P	0.503	P	B4GALT3
210253_at	10553	1	P	0.522	P,M	0.401	P	HTATIP2
210255_at	5890	1	P,A	1.502	P	1.665	P	RAD51L1
210284_s_at	23118	1	P	1.143	P	1.553	P	MAP3K7IP2
210285_x_at	9589	1	P	0.784	P	0.609	P	WTAP
210298_x_at	2273	1	P	1.139	P	1.714	P	FHL1
210299_s_at	2273	1	P	1.600	P	2.131	P	FHL1; KYO-T
210306_at	26013	1	P,A	0.632	P,A	0.858	P,A	L3MBTL
210312_s_at		1	P	0.579	P	0.611	P	LOC90410
210320_s_at	11056	1	P	1.495	P	1.671	P	DDX52; ROK1
210336_x_at	7593	1	P	0.662	P,A	0.502	P,A	ZNF42; MZF1
210337_s_at	47	1	P	0.796	P	0.578	P	ACLY
210346_s_at		1	P	1.401	P	1.908	P	CLK1
210358_x_at	84724	1	A	2.211	P	1.935	P,M	GATA2
210394_x_at	6759	1	P	0.712	P	0.644	P	SSX4
210396_s_at		1	P	1.734	P	1.485	P	
210405_x_at	8795	1	P	0.632	P,A	0.536	P,A	TNFRSF10B
210415_s_at	4957	1	P,M	2.124	P	1.788	P	ODF2
210416_s_at	11200	1	P	1.443	P	1.502	P	CHEK2
210425_x_at	23015	1	P	1.407	P	1.627	P	GOLGIN-67
210465_s_at	6619	1	P	1.591	P	1.692	P	SNAPC3
210480_s_at		1	P,A	1.650	P	1.856	P	MYO6
210513_s_at	7422	1	P	0.732	P	0.613	P	VEGF
210534_s_at	27077	1	P	0.883	P	0.653	P	EPPB9
210538_s_at	330	1	P,A	5.671	P	6.522	P	BIRC3; AIP1
210560_at	2637	1	P,M	3.801	P	3.943	P	GBX2
210567_s_at	6502	1	P	1.038	P	0.620	P	SKP2; FBL1
210580_x_at	6818	1	P	1.460	P	1.563	P	SULT1A3
210605_s_at	4240	1	P	1.592	P	2.192	P	MFGE8; BA46
210612_s_at	8871	1	P,A	1.301	P	1.788	P,A	SYNJ2; INPP5II
210622_x_at	8558	1	P	0.831	P	0.576	P,A	CDK10
210643_at	8600	1	A	1.536	A	2.189	P	TNFSF11
210653_s_at	594	1	P	0.735	P	0.446	P	BCKDHB; E1B
210667_s_at	8209	1	P	0.704	P	0.521	P	C21orf33; ES1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
210697_at	113835	1	A	1.536	P	1.733	P	ZNF257
210715_s_at	10653	1	P	0.609	P	0.577	P	SPINT2
210720_s_at	63941	1	P	0.754	P	0.629	P	APBA2BP
210732_s_at	3964	1	P	0.879	P	0.488	P	LGALS8
210750_s_at	9229	1	P	0.773	P,A	0.586	A	DLGAP1
210755_at	3082	1	P	1.676	P	2.079	P	HGF; SF; HPTA
210776_x_at		1	P	1.554	P	1.571	P	TCF3
210802_s_at	27292	1	P	0.910	P	0.477	P	HSA9761
210809_s_at	10631	1	P	1.581	P	1.667	P	POSTN;
210815_s_at	10203	1	P	0.766	P	0.659	P	CALCRL
210869_s_at	4162	1	P,A	1.420	P,M	1.589	P	MCAM
210871_x_at	22892	1	P	1.411	P	1.705	P	SSX2IP
210876_at	303	1	P,A	1.519	P,A	1.649	P	ANXA2P1
210892_s_at	2969	1	P	0.504	P	0.383	P	GTF2I
210912_x_at	2948	1	P	0.895	P	0.583	P	GSTM4
210975_x_at	10922	1	P	0.769	P,M	0.594	P,A	FASTK
210986_s_at	7168	1	P	2.091	P	2.377	P	TPM1
210987_x_at	7168	1	P	1.917	P	2.251	P	TPM1
211009_s_at	10778	1	P	1.374	P	1.639	P	ZNF271
211015_s_at	3308	1	P	1.529	P	1.425	P	HSPA4
211017_s_at	4771	1	P,M	1.893	P	1.562	P	NF2
211019_s_at	4047	1	P	0.548	P	0.416	P,A	LSS; OSC
211023_at	5162	1	P	0.814	P	0.591	P	PDHB; PHE1B
211027_s_at	3551	1	P	0.671	M,A	0.471	A	IKBKB
211049_at	51407	1	P	0.763	P,A	0.532	P,A	TLX2
211052_s_at	6904	1	P	0.651	P	0.492	P	TBCD
211088_s_at		1	P	0.600	P	0.474	P	PLK4
211091_s_at	4771	1	P,A	2.657	P	2.214	P	NF2
211092_s_at	4771	1	A	2.125	P,M,A	1.779	P,M	NF2
211126_s_at	1466	1	P	1.874	P	1.829	P	CSRP2
211200_s_at	84288	1	A	3.178	P	4.783	P	FGR
211212_s_at	5001	1	P	1.528	P	1.502	P	ORC5L
211219_s_at	9355	1	A	1.586	P	1.532	P,M	LHX2
211256_x_at	11120	1	P	1.556	P	1.710	P	BTN2A1
211273_s_at	6899	1	P	0.673	P	0.476	P	TBX1
211284_s_at	2896	1	P	0.607	P	0.738	P	GRN; PEPI
211299_s_at	2319	1	P	0.701	P	0.521	P	FLOT2; ESA
211340_s_at	4162	1	P,M,A	1.500	P	2.044	P	MCAM
211425_x_at	6757	1	P	0.699	P	0.623	P	SSXT/SSX4
211458_s_at	23766	1	P	1.227	P	1.530	P	GABARAPL3
211467_s_at	4781	1	P	0.785	P	0.599	P	NFIB
211471_s_at	9609	1	P	1.552	P	1.370	P	RAB36
211474_s_at	5269	1	P	0.723	P	0.651	P	SERPINB6
211475_s_at	573	1	P	0.508	P	0.357	P	BAG1
211527_x_at	7422	1	P	0.649	P,M	0.501	P,M,A	VEGF; VEGFA
211540_s_at	5925	1	A	1.537	P,A	2.079	P	RB1; OSRC
211552_s_at	8659	1	P,A	0.691	P,M,A	0.578	M,A	ALDH4A1
211558_s_at	1725	1	P	0.820	P	0.558	P	DHPS
211559_s_at	901	1	P	0.640	P	0.625	P	CCNG2
211575_s_at	10109	1	P	1.542	P	1.490	P	ARPC2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
211576_s_at	6573	1	P	1.633	P	1.292	P	SLC19A1
211595_s_at	64963	1	P	1.832	P	1.626	P	MRPS11
211615_s_at	10128	1	P	1.825	P	2.026	P	LRPPRC
211622_s_at	377	1	P	0.763	P	0.573	P	ARF3
211658_at	7001	1	P	0.894	P	0.573	P	PRDX2
211666_x_at	6122	1	P	0.705	P	0.607	P	RPL3
211671_s_at	2908	1	P	1.535	P	1.628	P	NR3C1
211672_s_at	10093	1	P	0.674	P	0.526	P	ARPC4
211675_s_at	29969	1	P	1.183	P	1.527	P	HIC
211676_s_at	3459	1	P	1.724	P	1.740	P	IFNGR1; CD119
211700_s_at	7216	1	P	1.254	P	1.931	P	TRO
211701_s_at	7216	1	P	1.239	P	1.822	P	TRO
211707_s_at	9657	1	P	1.795	P	1.888	P	IQCB1
211721_s_at	90233	1	P	1.526	P	1.407	P	ZNF551
211724_x_at	54468	1	P	1.533	P	1.339	P	FLJ20323
211725_s_at		1	P	1.703	P	1.677	P	
211752_s_at	4727	1	P	0.697	P	0.485	P	NDUFS7
211800_s_at	7375	1	P	0.821	P	0.621	P	USP4; UNP
211810_s_at	2581	1	P	0.653	P	0.560	P	GALC
211814_s_at	9134	1	P	2.067	P	1.190	P	CCNE2; CYCE2
211833_s_at	581	1	P	0.585	P	0.415	P	BAX
211852_s_at	8455	1	P	0.686	P	0.656	P,A	ATRN
211855_s_at	9016	1	P	1.527	P	1.671	P	SLC25A14
211864_s_at	26509	1	A	1.785	P,A	2.653	P,M,A	FER1L3
211928_at	1778	1	P	1.465	P	1.510	P	DNCH1; p22
211929_at		1	P	1.393	P	1.548	P	hnRNPA3
211932_at		1	P	1.676	P	1.807	P	hnRNPA3
211933_s_at		1	P	1.563	P	1.745	P	hnRNPA3
211937_at	1975	1	P	0.624	P	0.547	P	EIF4B; EIF-4B
211947_s_at	23215	1	P,A	1.536	P	1.304	P	XTP2
211948_x_at	23215	1	P	1.688	P	1.553	P	XTP2
211951_at	9221	1	P	1.710	P	1.755	P	NOLC1
211952_at	3843	1	P	1.382	P	1.559	P	KPNB3
211953_s_at	3843	1	P	1.484	P	1.561	P	KPNB3
211973_at		1	P	0.749	P	0.658	P	
211976_at		1	P	0.780	P	0.633	P	
211982_x_at	23214	1	P	1.548	P	1.551	P	XPO6
211986_at	195	1	P	1.600	P	2.087	P	MGC5395
211988_at	6605	1	P	1.504	P	1.573	P	SMARCE1
211990_at	3113	1	P,M	1.035	P	2.069	P	HLA-DPA1
212010_s_at	55573	1	P	1.697	P	1.801	P	H41
212012_at		1	A	0.978	M,A	1.638	P,A	D2S448
212014_x_at	960	1	P,M	1.150	P,A	1.635	P	CD44; IN
212016_s_at	5725	1	P	0.958	P	0.629	P	PTBP1
212032_s_at	53635	1	P	0.556	P	0.385	P	PTOV1
212037_at	5411	1	P	1.695	P	1.712	P	PNN
212038_s_at	7416	1	P	1.566	P	1.387	P	VDAC1
212046_x_at	5595	1	P	0.817	P	0.566	P,A	MAPK3; ERK1
212052_s_at	23061	1	P	0.786	P	0.659	P	KIAA0676
212058_at	23350	1	P	0.732	P	0.624	P	SR140

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
212061_at	23350	1	P	0.611	P	0.728	P	SR140
212063_at	960	1	A	1.154	A	2.336	P	CD44
212064_x_at	4150	1	P	1.525	P	1.268	P	MAZ
212068_s_at	23121	1	A	1.304	P	1.523	P	KIAA0515
212069_s_at	23121	1	P,M,A	1.739	P	1.525	P	KIAA0515
212085_at	293	1	P	0.602	P	0.411	P	SLC25A6
212086_x_at	4000	1	P	0.616	P	0.680	P	LMNA; FPL
212092_at	23089	1	P	1.623	P	2.188	P	PEG10
212094_at	23089	1	P	1.510	P	2.244	P	PEG10
212097_at	857	1	P	1.505	P	1.269	P	CAV1
212099_at		1	P	1.794	P	1.471	P	RHOB
212105_s_at	1660	1	P	0.683	P	0.470	P	DHX9
212129_at	81614	1	P	1.617	P	1.561	P	NIPA2
212143_s_at	3486	1	P	1.870	P	1.935	P	IGFBP3; IBP3
212147_at	23381	1	A	2.328	P	2.305	P,M	EST1B
212154_at	6383	1	P,M	1.446	P	1.731	P	SDC2
212157_at	6383	1	P	1.581	P	1.986	P	SDC2
212158_at	6383	1	P,M	1.448	P	1.961	P	SDC2
212163_at	57498	1	P	1.331	P	1.522	P	KIDINS220
212165_at	92703	1	P	0.981	P	0.640	P	C1orf37
212171_x_at	7422	1	P	0.752	P	0.653	P	VEGF
212184_s_at	23118	1	P	1.548	P	1.974	P	MAP3K7IP2
212190_at	5270	1	P	1.767	P	1.903	P	SERPINE2
212194_s_at	9777	1	P	0.827	P	0.601	P	KIAA0255
212211_at	26057	1	P	1.542	P	1.582	P	ANKRD17
212214_at	4976	1	A	1.802	P	2.280	P	OPA1
212215_at	9581	1	P	1.337	P	1.890	P	KIAA0436
212217_at		1	P	1.186	P	1.630	P	KIAA0436
212218_s_at	2194	1	P	0.816	P	0.607	P	FBXO9; FBX9
212222_at	23198	1	P	1.310	P	1.658	P	PSME4
212225_at	10209	1	P	0.620	P,M,A	0.468	A	SUI1
212234_at	23393	1	P	1.245	P	1.597	P	ASXL1
212249_at	5295	1	P,M,A	1.597	P	1.400	P,A	PIK3R1
212254_s_at	667	1	P	1.433	P	1.606	P	BPAG1
212259_s_at	57326	1	P	0.402	P,A	0.518	P	PBXIP1
212274_at	23175	1	A	1.711	P,A	1.836	P,A	LPIN1
212276_at	23175	1	P	1.703	P	2.027	P	LPIN1
212294_at	55970	1	P	1.441	P	1.880	P	GNG12
212299_at	91754	1	P	1.256	P	1.588	P	NEK9
212307_s_at	8473	1	P	0.652	P	0.761	P	OGT
212310_at	23124	1	P	1.373	P	1.831	P	FLJ39207
212312_at	598	1	P	1.720	P	1.476	P	BCL2L1
212336_at	2036	1	P	1.964	P	2.035	P	EPB41L1
212339_at	2036	1	A	1.471	P,A	1.942	P	EPB41L1
212350_at	23216	1	P	1.511	P	1.461	P	TBC1D1
212361_s_at		1	P	0.803	P	0.554	P	ATP2A2
212364_at	4430	1	P,A	1.546	P	2.223	P	MYO1B; myr1
212365_at	4430	1	P	1.284	P	1.504	P	MYO1B; myr1
212366_at	23036	1	P	1.499	P	1.863	P	ZNF292
212395_s_at	23065	1	P	1.417	P	1.502	P	KIAA0090

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
212403_at	89910	1	P	0.818	P	0.611	P	UBE3B
212418_at	1997	1	P	1.423	P	1.678	P	ELF1
212421_at	23313	1	P	1.390	P	1.579	P	C22orf9
212422_at	22984	1	P	1.512	P	1.387	P	PDCD11
212434_at	80273	1	P	1.603	P	1.308	P	GRPEL1
212436_at		1	P	1.517	P	1.363	P	TRIM33
212459_x_at	8801	1	P	0.927	P	0.582	P	SUCLG2
212461_at	51582	1	P	1.683	P	1.681	P	OAZIN
212465_at	84193	1	P	1.574	P	1.796	P	FLJ23027
212473_s_at		1	P	1.549	P	1.571	P	
212483_at	25836	1	P	1.399	P	1.711	P	IDN3; CDLS
212492_s_at	23030	1	P	0.539	P	0.626	P	JMJD2B
212499_s_at		1	P	1.399	P	1.522	P	C14orf32
212501_at	1051	1	P	0.545	P	0.492	P	CEBPB
212511_at		1	P	1.386	P	1.544	P	PICALM
212514_x_at	1654	1	P	0.757	P	0.568	P	DDX3X
212525_s_at	3014	1	P	0.806	P	0.584	P	H2AFX
212542_s_at	55023	1	P	1.454	P	1.621	P	PHIP
212559_at	5575	1	P,A	0.655	P,A	0.590	P,A	PRKAR1B
212565_at	23012	1	P,A	1.596	P	1.861	P	STK38L; NDR2
212574_x_at	91304	1	P	0.419	P	0.303	P	R32184_3
212593_s_at	27250	1	P	0.582	P	0.647	P	PDCD4
212594_at	27250	1	P	0.593	P	0.742	P	PDCD4
212599_at	26053	1	P	1.728	P	2.244	P	AUTS2
212603_at	10240	1	P	1.610	P	1.245	P	MRPS31
212611_at	23220	1	P	1.154	P	1.530	P	MPEG1
212619_at	23306	1	P	1.520	P	1.799	P	KIAA0286
212621_at	23306	1	P	1.204	P	1.514	P	KIAA0286
212622_at	23027	1	P	1.506	P	1.517	P	KIAA0033
212623_at	23027	1	P	1.586	P	1.485	P	KIAA0033
212624_s_at	1123	1	P	2.008	P	2.265	P	CHN1
212632_at		1	P	1.568	P	1.842	P	STX7
212635_at		1	P	1.546	P	1.701	P	TNPO1
212636_at		1	P	1.518	P	1.895	P	QKI; QK3
212638_s_at	11059	1	P	1.509	P	1.340	P	WWP1
212641_at	3097	1	P	1.935	P	2.381	P	HIVEP2
212642_s_at	3097	1	P	1.778	P	1.980	P	HIVEP2
212654_at	7169	1	P,A	1.857	P	1.458	P	TPM2
212656_at	10102	1	P	0.796	P	0.635	P	TSFM
212662_at	5817	1	P	1.632	P	1.196	P	PVR
212665_at	25976	1	P	1.861	P	2.235	P	TIPARP
212687_at		1	P	1.554	P	1.710	P	LIMS1
212689_s_at	55818	1	P	1.557	P	1.603	P	JMJD1A
212708_at		1	P	0.567	P	0.444	P	CASC3
212709_at	23279	1	P	1.644	P	1.405	P	NUP160
212710_at	157922	1	P	1.630	P	1.966	P	CAMSAP1
212714_at	113251	1	P	1.577	P	1.678	P	LOC113251
212721_at	140890	1	P	1.472	P	1.575	P	SFRS12
212724_at	390	1	P	1.665	P	2.057	P	ARHE
212739_s_at	4833	1	P	0.861	P	0.619	P	NME4

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
212751_at	7334	1	P	1.321	P	1.519	P	UBE2N
212765_at	23271	1	P	1.853	P	1.972	P	KIAA1078
212766_s_at	81875	1	P	2.169	P	1.782	P	FLJ12671
212767_at	92170	1	P	0.843	P	0.532	P	Spm
212774_at	10472	1	P	1.705	P	1.834	P	ZNF238
212780_at	6654	1	P	1.534	P	1.433	P	SOS1
212803_at	4665	1	P	1.545	P	1.349	P	STAT6
212815_at	10973	1	P	1.847	P	1.838	P	HELICI
212816_s_at	875	1	P	0.521	P	0.390	P	CBS
212822_at	57493	1	A	1.501	P,A	1.542	M,A	HEG
212826_s_at	293	1	P	0.630	P	0.450	P	SLC25A6
212828_at		1	P	1.444	P	1.763	P	SYNJ2; INPP5B
212829_at		1	P	1.661	P	1.814	P	
212830_at	1955	1	P	0.744	P	0.599	P	EGFL5
212833_at	91137	1	P	1.508	P	1.587	P	LOC91137
212835_at	23172	1	P	1.524	P	1.400	P	KIAA0157
212837_at	23172	1	P	1.859	P	1.805	P	KIAA0157
212838_at	23268	1	P	1.626	P	1.937	P	DNMBP
212839_s_at		1	P	2.098	P	1.924	P	SSA2
212845_at	23034	1	P	1.822	P	1.638	P	SAMD4
212847_at		1	P	1.093	P	1.511	P	NEXN
212866_at		1	P,M	0.915	P	0.595	A	LOC203069
212877_at	3831	1	P	2.041	P	2.167	P	KNS2
212884_x_at	348	1	P	0.929	P	1.560	P	APOE
212886_at	26112	1	M,A	1.435	P,A	1.536	P	DKFZP434C171
212893_at	26009	1	P	1.641	P	1.704	P	ZZZ3
212928_at	23270	1	P	1.521	P	1.262	P	RPS5P1
212934_at		1	P	1.328	P	1.520	P	LOC137886
212955_s_at	5438	1	P	0.954	P	0.628	P	POLR2I
212957_s_at		1	P	1.331	P	1.813	P	LOC92249
212961_x_at	3423	1	P	1.642	P	1.500	P	LOC91966
212962_at	85360	1	P,A	1.627	P	1.474	P,A	7h3; FLJ13511
212969_x_at	256364	1	P	0.756	P	0.561	P	FLJ35827
212971_at	833	1	P	0.590	P	0.562	P	CARS
212977_at	57007	1	M,A	1.989	P	2.528	P	CMKOR1
212980_at	23021	1	P	1.344	P	1.781	P	AHSA2
212990_at	8867	1	P	1.808	P	1.700	P	SYNJ1; INPP5G
213012_at	4734	1	P	1.837	P	2.103	P	NEDD4
213015_at		1	P	1.046	P,M,A	1.700	P	BBX
213016_at		1	P	1.262	P	2.513	P	BBX
213021_at		1	P	1.637	P	1.636	P	GOSR1
213025_at	55623	1	P	1.567	P	1.448	P	FLJ20274
213032_at		1	P	0.774	P	0.623	P	NFIB
213033_s_at		1	P,M	0.840	P	0.566	P,A	NFIB
213035_at	23243	1	P	1.283	P	1.913	P	ANKRD28
213038_at	127544	1	P	1.607	P	1.411	P	FLJ90005
213041_s_at	513	1	P	0.642	P	0.338	P	ATP5D
213044_at	6093	1	P	1.666	P	2.125	P	ROCK1
213064_at	79882	1	P	1.448	P	1.601	P	FLJ11806
213069_at		1	M,A	1.948	P	2.214	P	HEG

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
213072_at	157542	1	P	1.892	P	2.322	P	MGC13010
213081_at	9278	1	P	0.768	P	0.622	P	ZNF297
213088_s_at	23234	1	P	1.547	P	1.408	P	KIAA0974
213092_x_at	23234	1	P	2.274	P	2.153	P	KIAA0974
213093_at		1	P	0.680	P	0.566	P	PRKCA
213096_at	9911	1	A	1.340	P,A	1.506	P,M,A	HUCEP11
213097_s_at	27000	1	P	1.663	P	1.536	P	ZRF1
213115_at	115201	1	P	1.607	P	1.563	P	COL4A6
213118_at	23074	1	P	1.500	P	1.683	P	KIAA0701
213124_at	25888	1	P	1.537	P	1.385	P	ZNF473
213126_at	112950	1	P	0.742	P	0.645	P	MED8
213128_s_at	7337	1	P	1.507	P	1.451	P	UBE3A
213130_at	25888	1	P	1.367	P	1.553	P	ZNF473
213135_at		1	P	1.511	P	1.399	P	TIAM1
213169_at		1	P	0.725	P	0.426	P,A	SEMA5A
213170_at	27234	1	P	0.897	P	0.629	P	GPX7
213176_s_at	8425	1	P	0.449	A	0.372	A	LTBP4
213190_at	91949	1	P	0.937	P	0.633	P	COG7
213191_at	148022	1	P,M,A	1.368	P	1.503	P	TRIF
213199_at	26005	1	P,M	1.527	P	1.554	P	DKFZP586P0123
213206_at	9570	1	P	1.620	P	1.704	P	GOSR2
213207_s_at	9570	1	P,A	1.775	P	1.669	P	GOSR2
213211_s_at	10629	1	P	0.680	P,A	0.548	P,A	TAF6L
213216_at	23252	1	P	1.905	P	1.733	P	KIAA0459
213223_at	6158	1	P	0.576	P,M	0.519	P,M,A	RPL28
213224_s_at	27250	1	P,A	1.346	P	1.638	P	LOC92482
213225_at	5495	1	P	1.540	P	2.187	P	PPM1B
213238_at	57205	1	P	1.342	P	1.752	P	ATP10D
213246_at	26175	1	P	1.635	P	1.614	P	C14orf109
213248_at		1	M,A	1.227	P,A	1.503	P,A	
213251_at		1	P	1.419	P	1.525	P	
213256_at		1	A	1.636	P,M	1.449	P	MGC48332
213259_s_at	23098	1	P,M,A	1.416	P	1.517	P	SARM1
213263_s_at	7786	1	P	0.593	P	0.695	P	MAP3K12
213269_at	57209	1	P	1.228	P,A	1.618	P	LOC57209
213279_at		1	P	0.596	P	0.533	P	DHRS1
213281_at	3725	1	P	2.935	P	3.450	P	JUN
213288_at		1	P	1.496	P	1.743	P	LOC129642
213298_at	4782	1	P	0.765	P,A	0.602	P,A	NFIC
213306_at	8777	1	P	0.654	P	0.528	P	MPDZ
213310_at		1	P	1.573	P	1.664	P	EIF2C2
213315_x_at	3423	1	P	1.744	P	1.541	P	LOC91966
213316_at		1	P	1.745	P	2.185	P	
213322_at	221443	1	P	0.686	P	0.577	P	MGC19570
213325_at	25945	1	P,A	1.183	P,A	1.525	P	PVRL3
213334_x_at	11219	1	P	1.500	P	1.024	P	TREX2
213338_at	25907	1	A	1.759	P	1.264	P,A	RIS1
213342_at	10413	1	P	1.454	P	1.500	P	YAP1
213350_at	6205	1	P	1.644	P	1.540	P	RPS11
213365_at	57638	1	P	1.537	P	1.756	P	KIAA1504

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
213389_at		1	P,A	1.506	P	1.703	P	PNKP
213391_at		1	P	1.345	P	1.635	P	LOC286148
213397_x_at	6038	1	P	0.617	P	0.860	P	RNASE4
213402_at		1	P	1.689	P	1.276	P	LOC126208
213410_at	26098	1	P	1.645	P	1.523	P	EDRF1
213411_at		1	P	0.536	A	0.602	A	ADAM22
213413_at	11037	1	P,A	1.362	P	1.581	P	SIBLF
213419_at	323	1	P	0.927	P	0.653	P,A	hFE65L
213427_at	10799	1	P	2.554	P	2.132	P	RPP40
213452_at	7738	1	P	1.644	P	2.052	P	ZNF184
213469_at		1	P	1.545	P	1.921	P	FLJ12377
213471_at	261734	1	P	1.560	P	1.620	P	NPHP4
213474_at	154881	1	P	2.093	P	2.131	P	KCTD7
213479_at	4885	1	P	1.568	P	1.722	P	NPTX2; NP2
213496_at	9890	1	P	1.706	P	2.126	P	PRG1
213504_at	10980	1	P	1.517	P	1.264	P	COPS6
213505_s_at	10147	1	P	1.585	P	1.605	P	SFRS14
213523_at	898	1	P	2.189	P	1.416	P	CCNE1
213540_at	7923	1	P	0.525	P,M,A	0.380	A	HSD17B8
213549_at		1	P	1.687	P	1.591	P	SLC18A2
213558_at	27445	1	P	1.275	P	1.509	P	PCLO
213581_at	5134	1	P	1.557	P	1.152	P	PDCD2
213595_s_at	9876	1	P,A	1.452	P	1.786	P	CDC42BPA
213599_at	11339	1	P	1.530	P	1.561	P	OIP5
213604_at		1	P	1.401	P	1.554	P	TCEB3
213606_s_at	396	1	P	0.988	P	0.529	P	ARHGDIA
213607_x_at	65220	1	P	0.932	P	0.553	P,A	FLJ13052
213618_at	116984	1	P	1.800	P	2.212	P	CENTD1
213634_s_at	55687	1	P	1.620	P	1.176	P	FLJ10140
213644_at	201134	1	P	1.679	P	1.888	P	MGC33887
213647_at	1763	1	P	1.661	P	1.624	P	DNA2L
213653_at		1	P,A	1.365	P	1.574	P	METTL3
213671_s_at	4141	1	P	0.581	P	0.505	P	MARS
213689_x_at	6125	1	P	0.749	P	0.641	P	RPL5
213696_s_at	112950	1	P	0.895	P	0.638	P	MED8
213704_at	5876	1	P	1.760	P	1.893	P	RABGGTB
213716_s_at	6398	1	M,A	1.629	P	2.457	P	SECTM1
213722_at	6657	1	P,M	0.650	A	0.564	P,A	SOX2
213737_x_at		1	P	1.423	P	1.689	P	
213742_at	9295	1	P	1.636	P	0.931	P	SFRS11
213757_at	1984	1	P	1.551	P	1.372	P	EIF5A
213761_at	56890	1	P	1.700	P	1.566	P	MDM1
213780_at		1	A	2.122	P,M,A	2.556	P,A	THH
213786_at	8887	1	P	1.543	P	1.904	P	TAX1BP1
213787_s_at	10682	1	P	0.694	P	0.555	P	EBP
213793_s_at	9456	1	P	1.898	P	1.983	P	HOMER1
213795_s_at	5786	1	P	0.811	P	0.584	P	PTPRA
213804_at		1	P	0.721	P,A	0.601	P,A	INPP5B
213811_x_at	6929	1	P	1.592	P	1.605	P	TCF3
213812_s_at	10645	1	P	0.726	P	0.645	P,M	CAMKK2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
213822_s_at	89910	1	P	1 757	P	1 669	P	UBE3B
213838_at	51406	1	P	1 559	P	1 641	P	NOL7
213846_at	1350	1	P	1 508	P	1 376	P	COX7C
213861_s_at	25895	1	P	1 663	P	1 685	P	DKFZP586D0919
213869_x_at	7070	1	P	1 348	P	1 801	P	THY1
213878_at	79912	1	P	0 730	P	0 568	P	RECQL
213882_at	83941	1	P,A	1 648	P,A	1 744	P,A	BBP
213887_s_at	5434	1	P	0 914	P	0 643	P	POLR2E
213889_at	9487	1	P,M,A	1 259	P	1 608	P,M	PIGL
213892_s_at	353	1	P	0 961	P	0 637	P	APRT
213896_x_at	23234	1	P	0 760	P,A	0 564	A	KIAA0974
213899_at	10988	1	P	1 778	P	1 785	P	METAP2
213906_at	4603	1	P	1 971	P	2 328	P	MYBL1
213931_at	3398	1	M,A	7 620	P	4 792	P	ID2
213939_s_at		1	P,A	1 538	P	1 552	P	RIPX
213951_s_at	29893	1	P	1 757	P	1 241	P	HUMGT198A
213986_s_at	91304	1	P	0 415	P	0 291	P,A	C19orf6
213989_x_at	54093	1	A	2 067	P	2 216	P	C21orf18
213996_at	29799	1	P	0 449	P	0 369	P	YPEL1, FKSG3
214005_at	2677	1	P	0 895	P	0 568	P	GGCX
214006_s_at	2677	1	P	0 756	P	0 520	P	GGCX
214012_at	51752	1	P	0 600	P	0 586	P	ARTS-1
214023_x_at	7280	1	P	1 522	P	2 003	P	MGC8685
214030_at	131544	1	P	1 458	P	1 609	P	MINA
214052_x_at	23215	1	P	1 745	P	1 528	P	XTP2
214055_x_at	23215	1	P	1 803	P	1 539	P	XTP2
214083_at	5527	1	A	1 769	P,A	2 598	P	PPP2R5C
214096_s_at	6472	1	P	0 755	P	0 526	P	LOC56901
214097_at	6227	1	P	2 074	P	2 319	P	RPS21
214107_x_at	9520	1	P	0 738	P	0 595	P	NPEPPS
214112_s_at	3423	1	P	1 563	P	1 394	P	LOC91966
214113_s_at	9939	1	P	1 565	P	1 529	P	RBM8A
214114_x_at	10922	1	P	0 821	P	0 660	P	FASTK
214126_at		1	A	1 409	P	1 505	P,A	MCART1
214151_s_at	9488	1	P	1 326	P	1 641	P	CPR8
214152_at	9488	1	P	1 186	P	1 601	P	CPR8
214155_s_at	113251	1	P	1 532	P	1 404	P	LOC113251
214157_at	2778	1	P	2 270	P	2 470	P	GNAS
214177_s_at	57326	1	P	0 515	P	0 748	P	PBX1P1
214182_at	382	1	P	1 517	P	1 510	P	ARF6
214193_s_at	27042	1	P	1 562	P	1 729	P	MGC29875
214196_s_at	1200	1	P	0 613	P	0 572	P	CLN2
214205_x_at	10539	1	P	1 565	P	1 661	P	TXNL2, PICOT
214210_at	10478	1	P	1 506	P	1 382	P	SLC25A17
214240_at	2586	1	P	1 747	P	1 651	P	GAL
214247_s_at	10530	1	M,A	1 262	P,A	2 240	P	DKK3
214251_s_at	4926	1	P	0 526	A	0 379	A	NUMA1
214252_s_at	1203	1	P	0 745	P	0 646	P	CLN5
214258_x_at	10524	1	P	0 720	P	0 549	P	HTATIP
214260_at	10920	1	P,A	1 712	P	1 757	P	COPS8

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
214266_s_at	9260	1	P	0.995	P	0.592	P,A	PDLIM7
214290_s_at	8337	1	P	0.651	P	0.739	P	HIST2H2AA
214293_at	55752	1	P	2.149	P	2.454	P	FLJ10849
214306_at	4976	1	P	1.422	P	1.581	P	OPA1
214310_s_at	7542	1	P	0.803	P	0.516	P,M	ZFPL1
214313_s_at	9669	1	P,M	1.993	P	1.565	P	IF2
214336_s_at	1314	1	P	0.581	P	0.523	P	PEX19
214383_x_at	116138	1	P	0.738	P	0.562	P	KLHDC3
214409_at	10737	1	P,A	1.564	P	1.481	P	RFPL3S
214427_at	4839	1	P	1.670	P	1.497	P	NOL1; p120
214434_at	9893	1	P	1.454	P	1.763	P	HSPA12A
214437_s_at	6472	1	P	0.654	P	0.441	P	SHMT2; GLYA
214442_s_at	9063	1	P	1.504	P	1.634	P	PIAS2; miz
214500_at	9555	1	P	1.610	P	1.673	P	H2AFY; H2A.y
214505_s_at	2273	1	P	1.179	P	1.627	P	FHL1
214507_s_at	23404	1	P	1.731	P	1.451	P	EXOSC2; p7
214578_s_at	6093	1	P	1.962	P	2.297	P	ROCK1
214581_x_at	27242	1	A	2.256	P	2.572	P	TNFRSF21
214594_x_at	5205	1	P	1.361	P	1.582	P	ATP8B1
214639_s_at	3198	1	P	1.975	P	2.030	P	HOXA1
214649_s_at	8898	1	P	1.768	P	1.610	P	MTMR2
214657_s_at		1	P	0.624	P	0.658	P,A	
214662_at	23160	1	P	1.615	P	1.666	P	KIAA0007
214683_s_at	1195	1	P	1.269	P	1.748	P	CLK1
214690_at	9014	1	P	1.501	P	1.549	P	TAF1B
214691_x_at	54629	1	P	0.649	P	0.682	P,A	KIAA1164
214696_at	84981	1	P,A	1.757	P	1.684	P	MGC14376
214716_at	55589	1	P,A	1.361	P,A	1.693	P,A	BMP2K
214722_at		1	P	1.285	P	1.820	P	LOC376745
214727_at		1	P	1.690	P	1.702	P	BRCA2; FAD
214742_at	22994	1	P,M	0.790	P,A	0.567	A	AZI1; AZ1
214778_at	1954	1	P	0.413	P,A	0.378	P,A	EGFL4; MEGF8
214784_x_at	23214	1	P	1.555	P	1.487	P	XPO6
214794_at		1	P	1.629	P	1.492	P	PA2G4
214816_x_at	91442	1	P	1.723	P	1.505	P	MGC32020
214828_s_at	91695	1	P	1.778	P	1.323	P	dJ222E13.2
214835_s_at	8801	1	P	0.765	P	0.594	P	SUCLG2
214878_at	7587	1	P,A	1.563	P,A	1.470	P	ZNF37A
214909_s_at	23564	1	P	1.487	P	1.548	P	DDAH2
214924_s_at	22906	1	P	1.576	P	1.699	P	OIP106
214934_at	11071	1	P	0.618	P	0.604	P,M	ATP9B
214960_at	8539	1	M,A	1.508	P	1.309	P,A	API5;
214983_at	64595	1	P	1.735	P	1.751	P	TTTY15
214992_s_at	1777	1	P	0.396	P	0.393	P	DNASE2
215001_s_at	2752	1	P	1.544	P	1.835	P	GLUL; GLNS
215009_s_at		1	P	1.715	P	1.862	P	SEC31L1
215014_at		1	A	2.582	P,A	2.067	P,A	
215016_x_at	667	1	P	1.359	P	1.543	P	BPAG1; BP240
215047_at	25893	1	P	1.532	P	1.524	P	DKFZp434C091
215073_s_at		1	P	1.171	P	1.517	P	NR2F2

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
215079_at		1	P	1 351	P	1 574	P	
215090_x_at	9520	1	P	0 773	P	0 596	P	FLJ11822
215099_s_at	6257	1	P	0 509	P,A	0 386	M,A	RXRB
215111_s_at	8848	1	P	1 344	P	1 642	P	TGFB1I4
215116_s_at	1759	1	P	0 644	P	0 529	P	DNM1
215143_at		1	P	1 271	P	1 504	P	FLJ36166
215146_s_at	23331	1	P	0 672	P	0 658	P	KIAA1043
215158_s_at	9191	1	P	1 502	P	1 265	P	DEDD, DEFT
215159_s_at	65220	1	P	0 870	P	0 561	P,A	FLJ13052
215165_x_at	7372	1	P	1 571	P	1 279	P	UMPS, OPRT
215167_at	9282	1	A	1 504	P,A	1 396	P	CRSP2
215170_s_at	22995	1	P,A	1 249	P,M,A	1 510	P	KIAA0912
215171_s_at	10440	1	P	1 560	P	1 339	P	TIMM17A
215204_at		1	A	1 324	P,M,A	1 575	P,A	
215220_s_at		1	P	0 713	P	0 588	P	TPR
215242_at		1	A	1 579	P	1 712	P,A	PIGC
215324_at	223117	1	P	0 962	P	0 649	P	SEMA3D
215359_x_at	7595	1	P	1 424	P	1 714	P	ZNF44
215380_s_at	79017	1	P	1 549	P	1 479	P	MGC3077
215424_s_at	22938	1	P	1 645	P	1 485	P	SKIIP
215425_at	10950	1	P	1 612	P	1 852	P	BTG3, ANA
215440_s_at	56271	1	P	0 601	P	0 747	P	BEXL1
215446_s_at	114990	1	P,M,A	1 166	P,A	1 554	P	LOX
215493_x_at	11120	1	P	1 625	P	1 776	P	BTN2A1
215495_s_at	23034	1	P,A	2 971	P	2 223	P	SAMD4
215532_x_at	57615	1	P	1 641	P	1 867	P	ZNF492
215548_s_at	23256	1	P	1 624	P	1 393	P	SCFD1
215629_s_at	79469	1	P	0 950	P	0 564	P	BCMSUNL
215643_at		1	P	0 676	P	0 503	P	SEMA3D
215684_s_at	84164	1	P	1 802	P	1 708	P	ASC1p100
215695_s_at	8908	1	P	1 692	P	2 522	P	GYG2
215706_x_at	7791	1	P	1 978	P	1 729	P	ZYX
215719_x_at	355	1	P	1 317	P,A	1 961	P,A	TNFRSF6
215722_s_at	6627	1	P	1 995	P	1 759	P	SNRPA1
215723_s_at	5337	1	P	0 723	P	0 568	P	PLD1
215728_s_at	11332	1	P	0 804	P	0 540	P	BACH
215735_s_at	7249	1	P	0 881	P	0 612	P	TSC2
215743_at	10557	1	P	1 402	P	1 748	P	RPP38
215747_s_at	1104	1	P	0 898	P	0 660	P	CHC1, RCC1
215760_s_at	22904	1	P,M	0 784	P,A	0 544	P,A	KIAA0963
215765_at	10489	1	P	1 704	P	1 931	P	MUF1
215772_x_at	8801	1	P	0 968	P	0 567	P	SUCLG2
215780_s_at		1	P	0 797	P	0 658	P	
215812_s_at		1	P	1 576	P	1 364	P	SLC6A10
215842_s_at	23250	1	P	0 793	P	0 520	P	ATP11A
215891_s_at	2760	1	P	0 649	P M A	0 625	M,A	GM2A, SAP-3
215945_s_at	23321	1	P	1 304	P	1 634	P	TRIM2 RNF86
216041_x_at	2896	1	P	0 633	P	0 778	P	GRN, PEPI
216044_x_at	6125	1	P	0 692	P	0 608	P	RPL5
216064_s_at	175	1	P	0 635	P	0 568	P	AGA

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
216080_s_at	3995	1	P	0.716	P	0.570	M,A	FADS3
216228_s_at	11169	1	P	1.783	P	1.705	P	WDHD1
216246_at	6224	1	P	1.512	P	1.300	P	RPS20
216248_s_at	4929	1	P	1.955	P	1.338	P	NR4A2; NOT
216250_s_at	9404	1	P	1.477	P	1.986	P	LPXN
216253_s_at	29780	1	P	0.893	P	0.645	P	PARVB
216282_x_at	5432	1	P	1.579	P	1.305	P	POLR2C
216299_s_at	7517	1	P,A	2.575	P	2.219	P	XRCC3
216305_s_at	6936	1	P	1.979	P	2.205	P	MRPL19
216338_s_at	25844	1	P	0.617	P	0.537	P	KLIP1
216379_x_at	934	1	P	2.247	P	4.929	P	NaGLT1
216411_s_at	196951	1	P	0.673	P,A	0.539	P,A	FLJ32800
216537_s_at	27036	1	P,A	1.631	P,A	1.743	P	SIGLEC7; p75
216556_x_at		1	P	0.622	P	0.673	P	
216559_x_at		1	P	0.710	P	0.570	P	
216685_s_at		1	P,A	1.085	P	0.619	P,A	MTAP
216705_s_at		1	P,M,A	0.778	P,M	0.509	A	ada
216733_s_at	2628	1	P	0.602	P	0.727	P	GATM; AGAT
216746_at		1	A	1.363	M,A	1.515	P,A	
216855_s_at	3192	1	P	0.676	P	0.610	P,A	HNRPU
216860_s_at	10220	1	P	0.661	P,A	0.481	A	GDF11
216899_s_at	8935	1	P	1.360	P	1.617	P	SCAP2
216941_s_at	9014	1	P	1.544	P	1.411	P	TAF1B
216969_s_at	3835	1	P	0.694	P	0.529	P	KNSL4
216971_s_at	5339	1	P,A	0.571	A	0.486	M,A	PLEC1
216975_x_at	4861	1	A	1.927	P	1.496	M,A	NPAS1
216977_x_at	6627	1	P	1.935	P	1.670	P	SNRPA1
217007_s_at	8751	1	P	0.599	P	0.595	P	ADAM15
217010_s_at	995	1	P	0.590	P	0.619	P,A	CDC25C
217028_at	7852	1	A	1.586	P,A	3.898	P	CXCR4
217127_at	1491	1	P	0.608	P	0.590	P	CTH
217150_s_at	4771	1	P,A	1.900	P	1.784	P	NF2
217168_s_at	9709	1	P	0.562	P	0.467	P	HERPUD1;
217173_s_at	3949	1	P	0.730	P,A	0.595	A	LDLR; FH; FHC
217185_s_at		1	P	2.112	P	1.671	P	ZNF259P
217188_s_at	11161	1	P	0.704	P	0.507	P	C14orf1
217250_s_at	26038	1	P,A	1.525	P	1.394	P	CHD5
217284_x_at	253190	1	P	0.745	P,M,A	0.515	A	dJ222E13.1
217289_s_at	2542	1	P	0.584	P	0.428	M,A	G6PC
217299_s_at	4683	1	P	1.578	P	1.280	P	NBS1
217309_s_at	10311	1	P,M	0.695	P,A	0.643	P,A	DSCR3
217317_s_at	60438	1	P	1.539	P	1.877	P	MN7
217364_x_at		1	P	1.527	P	1.392	P	
217370_x_at		1	P	0.724	P	0.548	P	
217416_x_at		1	P,A	1.350	P	1.502	P	VAPA
217437_s_at	6867	1	P,A	1.638	P	1.501	P	TACC1
217492_s_at	11191	1	P	1.555	P	1.407	P	PTENP1
217494_s_at	11191	1	A	1.647	P	1.291	P,M,A	PTENP1
217543_s_at	8720	1	P	0.801	P	0.637	P	MBTPS1
217554_at		1	P	1.659	P	1.629	P	

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
217585_at		1	P	1.893	P	2.274	P	NEBL
217591_at		1	P	1.648	P	1.462	P	SKIL
217604_at		1	P,A	1.533	P	1.314	P	
217618_x_at		1	P	1.555	P	1.525	P	HUS1
217631_at	23560	1	P,A	1.498	P	1.714	P	GTPBP4
217678_at		1	P	0.599	P	0.602	P	SLC7A11
217682_at		1	P	1.474	P	1.773	P	PRO0149
217722_s_at	51335	1	P	1.491	P	1.554	P	NEUGRIN
217738_at	10135	1	P	1.524	P	1.838	P	PBEF1
217752_s_at	55748	1	P	1.723	P	1.313	P	CNDP2
217766_s_at	23585	1	P	1.244	P	1.579	P	SMP1
217784_at	10652	1	P	1.607	P	1.343	P	YKT6
217786_at	10419	1	P	1.570	P	1.320	P	SKB1
217787_s_at	2590	1	P	0.866	P	0.632	P	GALNT2
217789_at	58533	1	P,A	1.380	P	1.619	P	SNX6
217790_s_at	6747	1	P,A	1.345	P,A	1.524	P	SSR3; TRAPG
217806_s_at	26073	1	P	1.743	P	1.434	P	POLDIP2
217807_s_at	29997	1	P	0.726	P	0.556	P	GLTSCR2
217808_s_at	79109	1	P	1.567	P	1.269	P	MAPKAP1
217809_at	28969	1	P	1.843	P	1.913	P	BZW2
217820_s_at	55740	1	P	1.708	P	1.932	P	ENAH; mena
217829_s_at	10713	1	P	1.555	P	1.549	P	USP39
217835_x_at	55969	1	P	1.628	P	1.503	P	C20orf24
217842_at	51631	1	P	1.654	P	1.806	P	LUC7L2
217844_at	58190	1	P	0.733	P	0.574	P	CTDSP1
217846_at	5859	1	P	0.771	P	0.552	P	QARS
217858_s_at	51566	1	P	1.399	P	1.866	P	ARMCX3
217859_s_at	55334	1	P	1.455	P	1.605	P	SLC39A9
217872_at	55011	1	P	0.690	P	0.514	P	FLJ20643
217890_s_at	55742	1	A	1.689	P,A	1.605	P,A	PARVA
217892_s_at	51474	1	P	1.506	P	1.577	P	EPLIN; SREBP3
217895_at	55037	1	P	0.743	P	0.544	P	FLJ20758
217896_s_at	80011	1	A	1.551	P	1.621	P	NIP30
217897_at	53826	1	P,A	1.533	P,M	2.496	P	FXYD6
217901_at		1	P,A	1.161	P	1.823	P	DSG2
217905_at	79892	1	P	1.460	P	1.565	P	FLJ13081
217907_at	29074	1	P	1.624	P	1.402	P	MRPL18
217919_s_at	28977	1	P	1.948	P	1.713	P	MRPL42
217923_at	23578	1	P	1.514	P	1.197	P	PEF; PEFLIN
217930_s_at	54472	1	P	0.774	P	0.573	M,A	TOLLIP
217932_at	51081	1	P	1.523	P	1.209	P	MRPS7
217936_at		1	P	1.319	P	1.794	P	ARHGAP5
217941_s_at	55914	1	P	1.427	P	1.633	P	ERBB2IP
217942_at	60488	1	P	1.634	P	1.605	P	MRPS35
217966_s_at	116496	1	P	0.727	P	0.606	P	C1orf24; NIBAN
217967_s_at	116496	1	P	0.652	P	0.619	P	C1orf24; NIBAN
217980_s_at	54948	1	P	0.849	P	0.610	P	MRPL16
217985_s_at	11177	1	P	1.614	P	1.279	P	BAZ1A
217986_s_at	11177	1	P	1.563	P	1.344	P	BAZ1A
217987_at	54529	1	P	1.895	P	1.720	P	NS3TP1

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
217988_at	57820	1	P	1.421	P	1.786	P	CCNB1IP1
217992_s_at	79180	1	P	1.505	P	1.453	P	EFHD2
217994_x_at	54973	1	P	0.710	P	0.527	P	FLJ20542
217996_at	22822	1	P	1.680	P	1.598	P	PHLDA1
217997_at	22822	1	P	2.140	P	2.698	P	PHLDA1
218012_at	64061	1	P	1.892	P	2.115	P	SE20-4
218021_at	10901	1	P	0.866	P	0.592	P,M	DHRS4
218022_at	51231	1	P	0.724	P,M,A	0.647	P,M,A	VRK3
218024_at	51660	1	P	1.651	P	1.078	P	BRP44l.
218029_at	79567	1	A	1.656	P	1.665	P	FLJ13725
218050_at	51569	1	P	1.366	P	1.552	P	Ufm1; BM-002
218060_s_at	79650	1	P,A	1.814	P	1.548	P	FLJ13154
218061_at	4201	1	P	1.573	P	1.342	P	MEA; HYS
218066_at	10723	1	P	1.391	P	1.645	P	SLC12A7
218070_s_at	29926	1	P	1.025	P	0.622	P	GMPPA
218071_s_at	23609	1	P	1.554	P	1.523	P	MKRN2
218091_at	3267	1	P	1.469	P	1.843	P	HRB
218092_s_at	3267	1	P	1.708	P	1.800	P	HRB
218099_at	55852	1	P	1.416	P	1.579	P	HT008
218100_s_at	55081	1	P	1.767	P	1.785	P	ESRRBL1
218104_at	54881	1	P	1.255	P	1.559	P	TEX10
218105_s_at	51073	1	P	0.842	P	0.580	P	MRPL4
218106_s_at	55173	1	P	1.551	P	1.634	P	MRPS10
218107_at	80232	1	P	1.569	P	1.714	P	WDR26
218108_at	55148	1	P	1.565	P	1.423	P	C14orf130
218117_at	9978	1	P	1.560	P	1.482	P	RBX1; ROC1
218124_at	54884	1	P	0.639	P	0.512	P	FLJ20296
218138_at	8195	1	P	1.382	P	1.550	P	MKKS
218139_s_at	55745	1	P	1.482	P	1.757	P	FLJ10813
218145_at	57761	1	P	0.262	P	0.251	P	TRIB3; NIPK
218146_at	55830	1	P	0.682	P	0.448	P	AD-017
218147_s_at	55830	1	P	0.554	P	0.326	P	AD-017
218156_s_at	55720	1	P	1.643	P	1.307	P	FLJ10534
218163_at	28985	1	P	1.731	P	1.638	P	MCTS1; MCT-1
218181_s_at	54912	1	P	1.420	P	1.770	P	MAP4K4
218187_s_at	65265	1	P	1.746	P	1.817	P	FLJ20989
218199_s_at	65083	1	P,A	1.915	P	1.766	P	NOL6; NRAP
218205_s_at	2872	1	P	0.870	P	0.615	P	MNK2
218214_at	60673	1	P	1.514	P	1.478	P	FLJ11773
218244_at	55035	1	P	1.867	P	1.760	P	NOL8; Nop132
218248_at	63901	1	P	1.510	P	1.685	P	FLJ22794
218261_at	10053	1	A	1.737	P	1.907	P	AP1M2
218262_at	64777	1	P	0.800	P	0.551	P	FLJ22318
218269_at	29102	1	P	1.773	P	1.844	P	RNASE3L
218272_at	55020	1	P,A	0.860	P,A	0.590	A	FLJ20699
218275_at	1468	1	P	0.852	P	0.514	P,M,A	SLC25A10; DIC
218282_at	55741	1	P	0.776	P	0.561	P	C20orf31
218290_at	55111	1	P	0.775	P	0.574	P	PLEKHJ1
218291_at	28956	1	P	0.751	P	0.520	P	MAPBPIP
218299_at	53838	1	A	1.803	P	1.628	P	C11orf24

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
218303_x_at	51315	1	P	1.326	P	1.548	P	LOC51315
218304_s_at	114885	1	P	1.587	P	1.589	P	OSBPL11
218307_at	55316	1	P	0.767	P	0.451	P	FLJ11164
218309_at	55450	1	P	1.236	P	1.859	P	CaMKIINalpha
218310_at	27342	1	P	1.327	P	1.566	P	RABGEF1
218332_at	55859	1	P	1.447	P	2.236	P	BEX1
218333_at	51009	1	P	1.519	P	1.240	P	F-LANa
218335_x_at	79155	1	P,M,A	1.512	P	1.510	P	TNIP2
218336_at	5202	1	P	1.746	P	1.605	P	PFDN2; PFD2
218341_at	79717	1	P	1.535	P	1.466	P	FLJ11838
218348_s_at	29066	1	P	1.407	P	1.561	P	ZC3HDC7
218355_at	24137	1	P	1.290	P	1.517	P	KIF4A;
218356_at	29960	1	P	1.912	P	1.603	P	FTSJ2; FJH1
218358_at	79174	1	P	0.788	P	0.463	P	MGC11256
218364_at	9209	1	P	1.583	P	1.956	P	LRRFIP2
218367_x_at	27005	1	P	0.798	P	0.535	P	USP21
218374_s_at	57102	1	P	1.685	P	1.754	P	C12orf4
218380_at	60368	1	P	1.776	P	2.641	P	NALP1
218383_at	54930	1	P	0.704	P	0.527	P	C14orf94
218385_at	55168	1	P	1.556	P	1.319	P	MRPS18A
218388_at	25796	1	P	0.770	P	0.639	P	PGLS; 6PGL
218394_at	79641	1	P	0.635	P	0.464	P	FLJ22386
218408_at	26519	1	P	1.671	P	1.489	P	TIMM10;
218414_s_at	54820	1	P	0.598	P	0.698	P,A	NDE1;
218417_s_at	55652	1	P,M,A	2.012	P	1.914	P	FLJ20489
218424_s_at	55240	1	P	1.722	P	1.318	P	TSAP6
218426_s_at	54476	1	P	0.737	P	0.541	M,A	TRIAD3
218434_s_at	65985	1	P	0.605	P	0.577	P	AACS
218438_s_at	80306	1	P	0.852	P	0.591	P	EG1
218442_at	7268	1	P	1.800	P	1.705	P	TTC4
218456_at	65981	1	P	1.487	P	1.682	P	C1QDC1
218465_at	55161	1	P	1.599	P	1.797	P	FLJ10525
218466_at	79735	1	P	0.713	P	0.542	P	TBC1D17
218488_at	8891	1	P	1.510	P	1.331	P	EIF2Bgamma
218490_s_at	55900	1	P	1.117	P	1.570	P	ZNF302
218493_at	79622	1	P	1.545	P	1.137	P	C16orf33
218496_at	246243	1	P	1.563	P	1.461	P	RNASEH1
218497_s_at	246243	1	P	1.579	P	1.223	P	RNASEH1
218499_at	51765	1	P	1.290	P	1.564	P	MST4; MASK
218500_at	51337	1	P	0.718	P	0.529	P,M	LOC51337
218507_at	29923	1	P	1.447	P	1.521	P	HIG2
218508_at	55802	1	P,A	1.520	P	1.281	P	HSA275986
218513_at	55319	1	P	1.584	P	1.579	P	FLJ11184
218517_at	79960	1	P	1.442	P	1.593	P	PHF17;
218518_at	51306	1	P	1.117	P	1.579	P	C5orf5; N61
218523_at	64077	1	P	0.606	A	0.417	A	LHPP
218535_s_at	55781	1	P	1.637	P	1.683	P	RIOK2
218536_at		1	P	0.895	P	0.654	P	MRS2L
218545_at	55297	1	P	1.480	P	1.781	P	FLJ11088; p56
218547_at	79947	1	P	1.580	P	1.366	P	DHDDS

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
218561_s_at	57128	1	P	1.821	P	1.477	P	C6orf149
218564_at	55159	1	P	1.714	P	1.825	P	FLJ10520
218566_s_at	26973	1	P	1.794	P	1.825	P	CHORDC1
218574_s_at	29995	1	P	1.989	P	1.766	P	LMCD1
218575_at	64682	1	P	1.672	P	1.502	P	ANAPC1
218576_s_at	11266	1	P	1.662	P	1.763	P	DUSP12; YVH1
218579_s_at	60625	1	P,M,A	1.738	P	1.392	P	DHX35
218585_s_at	51514	1	P	1.974	P	2.025	P	RAMP; L2DTL
218588_s_at	10827	1	P	1.236	P	1.591	P	C5orf3; 133K02
218590_at	56652	1	P	1.757	P	1.689	P	PEO1; TWINL
218591_s_at	79954	1	P,A	1.556	P,A	1.538	P,A	FLJ14075
218608_at	23400	1	P	0.768	P,A	0.488	P,A	HSA9947
218625_at	51299	1	P	1.937	P	2.374	P	NRN1
218640_s_at	79666	1	P	1.514	P	1.939	P	PLEKHF2
218642_s_at	79145	1	P,M	2.367	P	2.145	P	CHCHD7
218647_s_at	79693	1	P	1.771	P	1.577	P	FLJ23476
218664_at	51102	1	P	0.843	P	0.619	P	CGI-63; NRBF1
218670_at	80324	1	P	1.812	P	1.590	P	PUS1; MLASA
218672_at	79005	1	P,A	1.655	P	1.623	P	SCNM1
218675_at	57100	1	P,A	1.305	P	1.712	P	SLC22A17
218679_s_at	51160	1	P	0.772	P	0.526	P	VPS28
218681_s_at	23753	1	P	0.949	P	0.617	P	SDF2L1
218688_at	26007	1	P	0.712	P	0.438	P,A	DKFZP586B1621
218689_at	2188	1	A	1.654	P,A	1.803	P	FANCF; FAF
218692_at	55638	1	P	1.338	P	1.573	P	FLJ20366
218701_at	51110	1	P	1.298	P	1.548	P	LACTB2; CGI-83
218708_at	29107	1	P	1.755	P	1.500	P	NXT1; P15
218710_at	55622	1	P	1.656	P	1.628	P	FLJ20272
218712_at	54955	1	P	1.597	P	1.447	P	FLJ20508
218714_at	78994	1	P	0.848	P	0.559	P	MGC3121
218715_at	55813	1	P	1.621	P	1.443	P	HCA66
218719_s_at	64785	1	A	1.640	P	1.328	P	FLJ13912
218721_s_at	54953	1	P	1.661	P	1.865	P	FLJ20505
218731_s_at	64856	1	P	0.615	P,A	0.378	A	WARP
218733_at	55167	1	P	1.573	P	1.672	P	FLJ10546
218736_s_at	54873	1	P	1.563	P	1.437	P	PALMD
218740_s_at	80279	1	P	0.839	P	0.635	P	CDK5RAP3
218750_at	79101	1	P	1.754	P	1.834	P	MGC5306
218751_s_at	55294	1	P	1.541	P	1.416	P	FBXW7; AGO
218754_at	79707	1	P	1.565	P	1.487	P	FLJ23323
218757_s_at	65109	1	P	1.456	P	1.539	P	UPF3B
218760_at	51004	1	P	1.701	P	1.388	P	COQ6
218767_at	57109	1	P,M,A	1.537	P	1.392	P	XPMC2H
218768_at	57122	1	P	1.680	P	1.703	P	NUP107
218772_x_at	55151	1	P	1.523	P	1.808	P	C9orf87
218773_s_at	22921	1	P	0.746	P	0.514	P	MSRB
218774_at	28960	1	P	1.524	P	1.088	P	DCPS
218777_at	80346	1	P	0.681	P	0.636	P	PP432
218783_at	25896	1	P	1.709	P	2.043	P	DKFZP434B168
218813_s_at	56904	1	P	0.747	P	0.546	P	SH3GLB2

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218820_at	56967	1	P	1 258	P,M	1 785	P	C14orf132
218825_at	51162	1	P	0 578	A	0 448	A	EGFL7, ZNEU1
218834_s_at	54972	1	P	0 635	P	0 577	P	HSPA5BP1
218836_at	79897	1	P	1 633	P	1 323	P	RPP21
218838_s_at	64427	1	P	0 949	P	0 653	P	FLJ12788
218847_at	10644	1	P	1 759	P	1 615	P	IMP-2
218851_s_at	55339	1	P,A	2 367	P	3 437	P	WDR33
218853_s_at	56180	1	P	1 435	P	1 655	P	MOSPD1
218871_x_at	55454	1	P	1 504	P	1 581	P	GALNACT-2
218880_at	2355	1	P	2 146	P	1 984	P	FOSL2
218881_s_at	79579	1	A	1 561	P	1 534	M,A	FOSL2
218882_s_at	10885	1	P	1 697	P	1 651	P	WDR3
218884_s_at	60558	1	P	1 701	P	1 852	P	FLJ13220
218885_s_at	79695	1	P	0 501	P	0 335	P	GALNT12
218886_at	55003	1	P	2 000	P	1 604	P	PAK1IP1
218889_at	64318	1	P	1 513	P	1 356	P	C10orf117
218901_at	57088	1	P,A	1 478	P,A	1 596	P,M,A	PLSCR4
218904_s_at	55071	1	P	1 605	P	1 531	P	FLJ10110
218915_at	51219	1	P	2 219	P	2 288	P	NF2, CAN
218918_at	57134	1	P	0 568	P,A	0 674	P,A	MANIC1, HMIC
218921_at	59307	1	P	0 682	P	0 539	P	SIGIRR
218923_at	1486	1	P	1 300	P	1 766	P	SPATA1
218924_s_at	1486	1	P	1 225	P	1 665	P	CTBS
218929_at	55602	1	P	1 411	P	1 703	P	CARF
218932_at	54680	1	P	2 273	P	2 580	P	FLJ20729
218938_at	79176	1	P,M	0 742	A	0 639	M,A	MGC11279
218941_at	26190	1	P	1 564	P	1 423	P	FBXW2, FBW2
218948_at	55278	1	P,A	1 646	P	1 628	P,M	QRSL1, GatA
218949_s_at	55278	1	P	1 658	P	1 474	P	QRSL1
218951_s_at	55344	1	P	0 641	P	0 707	P	FLJ11323
218953_s_at	78991	1	P	0 759	P	0 388	P	MGC3265
218959_at	3226	1	P	1 272	P	1 609	P	HOXC10
218961_s_at	11284	1	P	0 613	P	0 397	P	PNKP
218972_at	55761	1	P	1 527	P	1 823	P	TTC17
218977_s_at	54952	1	P	0 900	P	0 624	P	SECP43
218981_at	57001	1	P	1 635	P	1 620	P	ACN9, DC11
218983_at	51279	1	P	0 303	A	0 252	P,A	C1RL, C1RL1
218995_s_at	1906	1	A	2 095	P,M	1 952	P,M	EDN1, ET1
218997_at	64425	1	P	1 915	P	1 583	P	PAF53
219000_s_at	79075	1	P	2 121	P	2 086	P	MGC5528
219004_s_at	54069	1	P	1 643	P	1 610	P	C21orf45
219007_at	79700	1	P	1 549	P	1 579	P	NUP43
219008_at	60526	1	P	1 701	P	1 366	P	FLJ21820
219010_at	55765	1	P	1 398	P	1 623	P	FLJ10901
219014_at	51316	1	P	1 861	P	2 168	P	PLAC8, C15
219022_at	64897	1	P	1 576	P	1 373	P	FLJ12448
219026_s_at	9462	1	P	1 696	P	1 785	P	RASAL2
219031_s_at	51388	1	P	1 711	P	1 518	P	CGI-37
219032_x_at	23596	1	P	0 952	P	0 490	P	OPN3
219037_at	51018	1	P	1 598	P	1 501	P	CGI-115

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		Normalized	Flags	Normalized	Flags	Normalized	Flags	
219038_at	79710	1	P	1 248	P	1 550	P	ZCWCC2
219049_at	55790	1	P M	1 639	P	1 430	P	ChGn
219066_at	60490	1	P,M	1 553	P	1 464	P	MDS018
219069_at	54851	1	P	1 548	P	1 652	P	FGIF, FLJ20189
219070_s_at	64598	1	P	0 782	P	0 572	P	MOSPD3
219073_s_at	114884	1	P,A	1 149	P	1 608	P	OSBPL10
219081_at	54882	1	P	1 326	P	1 508	P	ANKHD1, MASK
219083_at	55164	1	A	1 777	P	1 791	P,M	FLJ10539
219088_s_at	79177	1	A	1 545	P,M	1 168	P,M	ZNF576
219094_at	29067	1	P,A	1 820	P	1 903	P	HSPC056
219102_at	57333	1	P	0 634	P	0 637	P	RCN3, RLP49
219105_x_at	23594	1	P	1 631	P	1 546	P	ORC6L
219109_at	79582	1	P	1 345	P	1 524	P	PF20, WDR29
219125_s_at	55974	1	P	0 813	P	0 645	P	LOC55974
219126_at	55274	1	P	0 604	P	0 521	P	PHF10
219130_at	54482	1	P	1 297	P	1 603	P	FLJ10287
219138_at	9045	1	A	1 544	P,A	1 169	P,A	RPL14
219142_at	65997	1	P	1 280	P	1 628	P	RASL11B
219147_s_at	54981	1	P	0 654	P	0 567	P	NRK1
219149_x_at	51163	1	P,M	1 658	P	1 387	P	DBR1
219152_at	50512	1	P,M	0 706	P,A	0 413	A	PODLX2
219158_s_at	80155	1	P	1 909	P	1 727	P	TBDN100
219163_at	54811	1	P	1 509	P	1 421	P	ZNF562
219175_s_at	54946	1	P	0 726	P	0 622	P	SLC41A3
219177_at	55299	1	P	1 639	P	1 464	P	BRIX, FLJ11100
219178_at	79691	1	P	1 737	P	1 665	P	QTRTD1
219188_s_at	28992	1	P	0 425	P	0 265	P,A	LRP16
219202_at	79651	1	P	0 771	P	0 589	P	RHBDL6
219211_at	11274	1	P	1 391	P	1 748	P	USP18
219214_s_at	30833	1	P,A	0 764	P,A	0 503	A	RBAK
219240_s_at	80007	1	P	1 597	P	1 681	P	FLJ13490
219244_s_at	26589	1	P	1 635	P	1 433	P	MRPL46
219248_at	80745	1	P	1 514	P	1 607	P	THUMPD2
219250_s_at	23767	1	A	1 361	P,A	1 502	P,A	FLRT3
219253_at	79134	1	P	0 813	P	0 657	P	FAM11B
219254_at	79701	1	P	1 022	P A	0 623	P,A	FLJ22222
219258_at	54962	1	P	2 314	P	2 346	P	FLJ20516
219263_at	79589	1	P,A	1 851	P	2 232	P	RNF128
219266_at	59348	1	P,A	1 563	P,M	2 048	P	ZNF350
219270_at	79094	1	P	0 274	M,A	0 242	A	MGC4504
219274_at	23554	1	P,A	1 391	P,M,A	1 809	P	TM4SF12
219275_at	9141	1	P	1 799	P	1 663	P	PDCD5
219283_at	29071	1	P	0 573	P	0 510	P	C1GALT2
219288_at	57415	1	P	2 172	P	1 595	P	HT021
219292_at	55145	1	P	1 496	P	1 758	P	THAP1
219293_s_at	29789	1	P	1 509	P	1 539	P	PTD004
219299_at	55039	1	P	1 553	P	1 587	P	FLJ20772
219303_at	79596	1	P	1 746	P	1 810	P	C13orf7
219312_s_at	65986	1	P	1 102	P	1 534	P	ZBTB10

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219313_at	54762	1	M,A	1.180	P,A	1.545	P,A	DKFZp434C0328
219321_at	64398	1	P	1.555	P	1.481	P	MPP5
219324_at	79159	1	P	1.720	P	1.198	P	MGC3731
219343_at	55664	1	P	1.488	P	1.577	P	CDC37L1
219345_at	51027	1	P	0.816	P	0.616	P	CGI-143
219346_at	79414	1	P	0.974	P	0.659	P	LRFN3
219347_at	55270	1	P	1.769	P	1.545	P	NUDT15
219361_s_at	64782	1	A	2.215	P	2.006	P	FLJ12484
219362_at	79688	1	P,A	1.521	P	1.328	P	MAK10
219363_s_at	51001	1	P	1.750	P	1.585	P	CGI-12
219366_at	57099	1	P,A	1.679	P	1.319	P	AVEN; PDCD12
219371_s_at	10365	1	P	1.910	P	1.396	P	KLF2; LKLF
219373_at	54344	1	P	0.739	P	0.617	P	DPM3
219376_at	79692	1	P	1.568	P	1.654	P	ZNF322A
219377_at	64762	1	P	1.532	P	1.656	P	C18orf11
219384_s_at	23536	1	P	1.826	P	1.699	P	HADAT1
219387_at	55580	1	P,M	1.673	P	1.611	P	LOC55580
219401_at	64132	1	P	0.610	P	0.610	A	XYLT2; XT2
219410_at	55076	1	P	1.261	P	1.731	P	FLJ10134
219416_at	51435	1	P	1.336	P	1.501	P	SCARA3
219427_at	79633	1	P	1.611	P	1.496	P	FATJ; FLJ23056
219447_s_at	51006	1	P	0.892	P	0.641	P	SLC35C2
219459_at	55703	1	P,M	1.578	P	1.451	P	POLR3B
219460_s_at	55654	1	P,A	1.523	P	1.434	P	FLJ20507
219469_at	79659	1	P	1.509	P	1.579	P	FLJ11756
219470_x_at	54619	1	P	1.524	P	1.226	P	CCNJ
219472_at	79172	1	P	1.550	P	1.376	P	MGC11266
219473_at	54834	1	P	1.505	P	1.574	P	GDAP2
219477_s_at	55901	1	M,A	1.852	P	2.185	P	THSD1
219479_at	79070	1	P	0.925	P	0.582	P	KDELC1
219484_at	29915	1	P	1.599	P	1.739	P	HCFC2
219490_s_at	64858	1	P	1.691	P	1.406	P	DCLRE1B
219493_at	79801	1	P	1.554	P	1.334	P	SHCBP1
219495_s_at	7733	1	P	1.516	P	1.471	P	ZNF180
219499_at	55176	1	P	1.882	P	2.024	P	SEC61A2
219523_s_at	55714	1	P	0.837	P	0.547	P	FLJ10474
219540_at		1	P	1.416	P	1.688	P	ZNF267
219555_s_at	55839	1	P	1.550	P	1.234	P	BM039
219557_s_at	56675	1	P	1.475	P	1.662	P	NRIP3
219560_at	79680	1	P	1.529	P	1.124	P	FLJ21125
219562_at	25837	1	P	0.325	A	0.249	A	RAB26; V46133
219567_s_at	64789	1	P	1.427	P	1.785	P	FLJ21144
219575_s_at	64146	1	P	0.836	P	0.617	P	PDF
219577_s_at	10347	1	P	0.666	P	0.652	P,M,A	ABCA7
219582_at	79627	1	P	1.940	P	1.812	P	OGFRL1
219595_at	7574	1	A	1.976	P	2.431	P	ZNF26
219612_s_at	2266	1	A	2.124	P	2.150	P	FGG
219626_at	79649	1	P	1.310	P	1.505	P	FLJ12649
219628_at	64393	1	P	1.870	P	1.949	P	WIG1
219631_at	29967	1	P	1.876	P	2.302	P	ST7

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219646_at	54849	1	P	1.738	P	1.465	P	FLJ20186
219648_at	55686	1	P	1.520	P	1.436	P	FLJ10116
219650_at	54821	1	P	1.483	P	1.642	P	FLJ20105
219664_s_at	26063	1	P	0.826	P	0.649	P	DECR2; PIXR
219690_at	79713	1	P	1.608	P	1.349	P	FLJ22573
219703_at	55329	1	P	1.687	P	1.701	P	MNS1
219709_x_at	65990	1	P	0.827	P	0.643	P	MGC2494
219717_at	54876	1	P	1.289	P	1.514	P	FLJ20280
219736_at	55521	1	P,A	1.380	P	1.943	P	TRIM36
219742_at	80758	1	P	0.830	P	0.545	P	MGC10772
219760_at	64130	1	P	1.763	P	1.140	P	LIN7B
219774_at	54520	1	P	1.589	P	1.421	P	FLJ10996
219800_s_at	79896	1	P	1.335	P,A	1.566	P	TIINSL1
219802_at	79912	1	P	0.818	P	0.620	P	FLJ22028
219805_at	63932	1	P	1.380	P	1.535	P	FLJ22965
219825_at	56603	1	P,A	2.387	P	2.119	P	CYP26B1
219834_at	79800	1	P	0.551	A	0.792	P,A	ALS2CR8
219861_at	55192	1	P	0.956	P	0.613	P,A	FLJ10634
219884_at	26468	1	P	1.658	P	1.510	P	LHX6; LHX6.1
219895_at	55026	1	A	1.092	A	2.294	P,M	FLJ20716
219917_at	80001	1	P,A	1.553	P	1.117	P,A	FLJ23024
219933_at	51022	1	P	1.549	P	1.524	P	GLRX2; GRX2
219938_s_at	9050	1	P,A	1.408	P,A	1.613	P	PSTPIP2
219944_at	79745	1	P,A	1.905	P,A	2.992	P	FLJ21069
219945_at	29118	1	P	0.386	P,A	0.423	P	DDX25; GRTH
219966_x_at	54971	1	P	1.494	P	1.606	P	BANP
219984_s_at	57110	1	P	1.126	P,A	1.531	P,M,A	HRASLS
219987_at	79584	1	P,A	1.556	P	1.622	P	FLJ12684
219990_at	79733	1	P	1.652	P	1.538	P	FLJ23311
219997_s_at	64708	1	P	1.557	P	1.404	P	COPS7B
219998_at	29094	1	P	1.673	P	1.228	P	HSPC159
220011_at	79000	1	P	1.607	P	1.413	P	MGC2603
220014_at	51334	1	P	1.625	P	1.662	P	LOC51334
220044_x_at	51747	1	P	1.653	P	1.469	P	LUC7A; CROP
220051_at	10942	1	P	0.724	P	0.627	P,M	PRSS21
220092_s_at	84168	1	P,A	1.462	P,M,A	1.653	P	ANTXR1
220140_s_at	29916	1	P	1.501	P	1.378	P	SNX11
220143_x_at	55692	1	P	1.939	P	2.100	P	LUC7L2
220172_at	80067	1	P	1.716	P	1.613	P	FLJ13096
220179_at	64180	1	P	0.518	P	0.739	P	DPEP3
220183_s_at	11162	1	P	0.749	P,A	0.655	P,M	NUDT6
220199_s_at	64853	1	P	1.826	P	1.849	P	FLJ12806
220205_at	7179	1	P	0.615	P	0.725	P	TPTE; PTEN2
220253_s_at	29967	1	P	1.508	P	1.902	P	ST7; FLJ12929
220254_at	29967	1	P	1.500	P	1.736	P	ST7; FLJ12929
220262_s_at	65989	1	P	0.713	P,M	0.626	P,M	EGFL9
220358_at	55509	1	P,A	1.819	P	1.783	P,M	SNFT; JUNDM1
220368_s_at	55671	1	P	1.559	P	1.479	P	KIAA2010
220397_at	56890	1	A	2.070	P	2.824	P	MDM1
220432_s_at	51302	1	P	1.161	P	1.641	P	CYP39A1

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
220452_x_at		1	M,A	1 479	P	1 771	P,M	
220466_at	80071	1	P	1 746	P	1 796	P	FLJ13215
220484_at	55283	1	P,A	1 156	P	1 562	P	MCOLN3
220488_s_at	54828	1	P	0 619	P,M	0 488	A	BCAS3
220500_s_at	11159	1	P	0 825	P	0 657	P	RABL2A
220520_s_at	54830	1	M,A	1 483	P	1 974	P	FLJ20130
220545_s_at	60385	1	P	0 606	P	0 583	P,A	TSKS, TSKS1
220565_at	2826	1	P,M	0 644	P,M,A	0 675	P	GPR2, CCR10
220610_s_at	9209	1	P	1 745	P	1 892	P	LRRFIP2
220633_s_at	50809	1	P	0 770	P	0 649	P,A	HP1-BP74
220643_s_at	55179	1	P	0 823	P	0 643	P	FAIM, FAIM1
220647_s_at	51287	1	P	1 569	P	1 348	P	E2IG2
220651_s_at	55388	1	P	2 028	P	1 748	P	MCM10
220688_s_at	51154	1	P	1 707	P	1 373	P	C1orf33
220707_s_at	80020	1	P,A	0 832	P,M,A	0 598	A	FLJ23322
220748_s_at	51157	1	P	0 722	P	0 517	P	ZNF580
220771_at	51152	1	P	1 227	P	1 815	P	LOC51152
220839_at	29081	1	P,A	1 960	P,A	1 583	P,A	HSPC133
220840_s_at	55732	1	P	1 832	P	1 842	P	FLJ10706
220841_s_at	54806	1	P	1 574	P	1 304	P	AHI1
220892_s_at	29968	1	P	0 354	P	0 300	P	PSAT1
220934_s_at	79064	1	P	0 638	P,M,A	0 537	A	MGC3196
220936_s_at	55766	1	P,A	1 212	P,A	1 529	P	H2AFJ
220937_s_at	27090	1	P	0 987	P	0 563	P	SIAT7D
220987_s_at	81788	1	P	1 556	P	1 560	P	SNARK
220992_s_at	81627	1	P	1 691	P	2 013	P	C1orf25
221011_s_at	81606	1	P	1 150	P	2 247	P	LBH
221014_s_at	83452	1	P	1 566	P	1 511	P	RAB33B
221021_s_at	56259	1	P	1 528	P	1 589	P	CTNNBL1
221031_s_at	81575	1	P,M	1 265	P	1 677	P	DKFZP434F0318
221045_s_at	8863	1	P M	1 889	P	1 933	P	PER3
221059_s_at	4166	1	P	1 682	P	1 503	P	CHST6, MCDC1
221104_s_at	55335	1	P	1 249	P	1 551	P	NIPSNAP3B
221156_x_at	9236	1	P	1 485	P	1 609	P	CPR8
221190_s_at	29919	1	P	1 562	P	1 437	P	C18orf8,
221193_s_at	54819	1	P	1 635	P	1 812	P	ZCCHC10
221196_x_at	79184	1	P,A	1 524	P	1 270	P	C6 1A
221206_at		1	P	1 436	P	1 596	P	
221213_s_at	54816	1	P,A	0 703	P,A	0 638	P,A	FLJ20086
221219_s_at	54758	1	P	1 711	P	1 381	P	DKFZp434G0522
221255_s_at	83460	1	P	1 513	P	1 303	P	MGC2963
221260_s_at	81566	1	P	1 519	P	1 652	P	C12orf22
221267_s_at	81926	1	P	0 774	P	0 574	P	C19orf27
221270_s_at	81890	1	P,A	0 894	P,A	0 552	A	QTRT1, TGT
221434_s_at	81892	1	P	1 867	P	1 867	P	C14orf156
221435_x_at	81888	1	P	0 819	P	0 585	P	HT036
221437_s_at	64960	1	P	1 857	P	1 754	P	MRPS15
221448_s_at	56154	1	P,M	1 247	P	1 711	P	TEX15
221489_s_at	81848	1	P	1 551	P	1 553	P	SPRY4
221503_s_at	3839	1	P	1 556	P	1 493	P	KPNA3

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
221510_s_at	2744	1	P	1.833	P	2.008	P	GLS; GLS1
221511_x_at	57499	1	P	1.214	P	1.638	P	CPR8; KIAA1254
221515_s_at	51451	1	P	1.549	P	1.368	P	LCMT1; CGI-68
221523_s_at	58528	1	P	0.978	P	1.602	P	RAGD
221524_s_at	58528	1	P	0.922	P	1.580	P	RRAGD
221528_s_at	63916	1	P	1.669	P	1.380	P	ELMO2
221535_at	55341	1	P	1.848	P	1.862	P	FLJ11301
221536_s_at	55341	1	P	1.943	P	1.910	P	FLJ11301
221537_at	84202	1	P	1.634	P	1.402	P	DKFZp564A176
221550_at	1355	1	P	1.526	P	1.124	P	COX15
221551_x_at	27090	1	P,A	0.801	P	0.609	P,A	SIAT7D
221559_s_at	79003	1	P	1.507	P	1.509	P	MIS12
221562_s_at	23410	1	P	0.706	P	0.488	P,M,A	SIRT3; SIR2L3
221577_x_at	9518	1	P,M	0.441	A	0.273	A	GDF15
221582_at	92815	1	P	0.581	P,M	0.469	P,A	HIST3H2A
221589_s_at	4329	1	P	0.749	P	0.629	P	C14orf45
221598_s_at	9442	1	A	1.789	P	1.696	P,M	CRSP8
221628_s_at	84656	1	P	0.582	P,M,A	0.572	P,A	N-PAC
221633_at	29781	1	P,A	1.538	P	1.300	P	384D8-2
221648_s_at		1	P	1.527	P	1.493	P	PNAS-4
221652_s_at	55726	1	P	1.843	P	1.922	P	FLJ10637
221664_s_at	50848	1	P	0.529	A	0.489	A	F11R;
221676_s_at	23603	1	P	1.642	P	1.619	P	CORO1C
221677_s_at	29980	1	P	1.563	P	1.598	P	DONSON
221693_s_at	55168	1	P	1.529	P	1.274	P	MRPS18A
221695_s_at	10746	1	P	1.487	P	1.798	P	MAP3K2
221704_s_at	79720	1	P	1.591	P	1.544	P	FLJ12750
221713_s_at	79929	1	P,A	1.756	P	1.680	P	FLJ12748
221718_s_at	11214	1	P	1.591	P	1.552	P	AKAP13
221727_at	10923	1	P	1.517	P	1.795	P	PC4
221741_s_at	54915	1	P	1.575	P	1.651	P	dJ963E22.1
221754_s_at	57175	1	P	0.790	P	0.553	P	CORO1B
221755_at	254102	1	P	0.647	P,M	0.469	P,A	DKFZp762C186
221759_at	92579	1	P	0.734	P	0.487	P,A	G6PC3
221760_at		1	A	1.742	P	2.686	P	MAN1A1
221768_at		1	P	1.601	P	1.638	P	SFPQ
221780_s_at	55661	1	P	1.501	P	1.237	P	DDX27
221788_at	5238	1	P	1.134	P	1.547	P	PGM3
221800_s_at	80233	1	P	0.863	P	0.605	P	FLJ22175
221810_at		1	P,A	1.513	P	1.488	P	
221820_s_at	84148	1	P	0.814	P	0.638	P	MYST1
221823_at	90355	1	P	1.703	P	2.045	P	LOC90355
221832_s_at		1	P	1.392	P	1.535	P	LUZP1
221837_at	84861	1	P	0.739	P,A	0.454	M,A	FLJ14360
221841_s_at		1	A	1.647	A	1.684	P	KLF4
221864_at	93129	1	P	0.567	P	0.605	P	MGC13024
221873_at	7702	1	P	1.410	P	1.507	P	ZNF143
221892_at		1	P	0.599	P	0.532	P	H6PD
221919_at	3178	1	P	1.530	P	1.499	P	HNRPA1

Affymetrix Id	Locus Link	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
221934_s_at	55152	1	P	0.738	P	0.533	P	FLJ10496
221951_at		1	P	0.566	P,A	0.534	P,A	LOC283232
221953_s_at	10893	1	M,A	0.824	P,A	0.529	A	MMP24
221970_s_at	25926	1	P	1.656	P	1.514	P	DKFZP586L0724
221973_at		1	P	0.816	P,A	1.506	P	LOC150759
221987_s_at	55720	1	P	1.784	P	1.202	P	FLJ10534
221998_s_at	51231	1	P	0.620	P	0.553	P	LOC51231
222006_at		1	P	1.623	P	1.382	P	FGFR3
222010_at	39	1	P	1.890	P	2.057	P	ACAT2
222011_s_at	6950	1	P	1.702	P	1.775	P	ACAT2
222040_at	3178	1	P	1.590	P	1.306	P	HNRPA1
222043_at	1191	1	P	1.116	P	1.552	P	CLU
222045_s_at	63935	1	P	0.951	P	0.657	P,M	C20orf67
222074_at	7389	1	P,A	2.217	P	2.461	P	UROD
222088_s_at	144195	1	P	1.554	P	1.342	P	SLC2A14
222103_at	466	1	P	1.429	P	1.640	P	ATF1
222108_at		1	P	1.393	P	1.626	P	
222111_at		1	P	0.643	P	0.677	P	KIAA1164
222125_s_at	54681	1	P	0.579	P	0.580	P	PH-4; FLJ20262
222130_s_at	29960	1	P	1.878	P	1.537	P	FTSJ2; FJH1
222149_x_at		1	A	1.346	P,A	1.519	P,M	DKFZp434P162
222154_s_at	26010	1	P	1.348	P	1.658	P	DNAPTP6
222156_x_at	57499	1	P	1.251	P	1.601	P	CPR8
222162_s_at	9510	1	A	1.512	P,M	1.463	P	ADAMTS1
222204_s_at	54700	1	P	1.857	P	1.703	P	RRN3
222231_s_at	55379	1	P	1.611	P	1.356	P	PRO1855
222233_s_at	64421	1	P	1.526	P	1.558	P	DCLRE1C
222234_s_at	79007	1	P,A	0.707	P,M	0.631	P,A	MGC3101
222235_s_at		1	P	1.906	P	1.879	P	dJ19N1.1
222240_s_at	51477	1	P	0.664	P	0.518	P	ISYNA1
222249_at		1	P,A	1.632	P,A	1.595	P	KIAA1651
222250_s_at	25896	1	P	2.134	P	2.027	P	DKFZP434B168
222258_s_at	23677	1	P	1.589	P	2.227	P	SH3BP4
222263_at	79939	1	P,M	0.957	P	0.658	P,A	SLC35E1
222266_at		1	P	1.618	P	1.621	P	C19orf2
222269_at		1	A	1.916	P,A	1.682	P,A	UNQ8193
222274_at	150244	1	A	1.544	P,A	1.537	P	FLJ31568
222305_at		1	P,A	3.630	P	3.539	P	HK2
222312_s_at	5350	1	P,A	1.429	P	1.518	P	PLN
222360_at	51611	1	P	1.587	P	1.785	P	CGI-30
222382_x_at	23165	1	P,A	1.699	P,A	1.537	P	NUP205
266_s_at	934	1	P,A	2.416	P	5.457	P	CD24; CD24A
31845_at	2000	1	P	1.572	P	1.333	P	ELF4
32088_at	8548	1	P,M,A	1.340	P	1.632	P	BLZF1
32094_at	9469	1	P	1.807	P	1.822	P	CHST3
33132_at	29894	1	P	0.801	P	0.627	P	CPSF1
33307_at	27341	1	P	1.551	P	1.124	P	CGI-96
33778_at	25771	1	P	0.726	P	0.564	P	C22orf4
34408_at	6253	1	P	0.403	P	0.463	P	RTN2
34868_at	23381	1	P	1.907	P	1.878	P	EST1B

Affymetrix Id	LocusLink	Day 0		Day 3		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	Normalized	Flags	
35156_at		1	P	0 830	P	0 578	P	LOC203069
35179_at	26229	1	P	0 855	P	0 625	P	B3GAT3
35436_at	2801	1	P	0 875	P	0 648	P	GOLGA2
35626_at	6448	1	P	0 707	P	0 459	P	SGSH, HSS
36545_s_at	9814	1	P	0 838	P	0 604	P	KIAA0542
36552_at	26005	1	P	1 574	P	1 743	P	DKFZP586P0123
36553_at	8623	1	P	0 931	P	0 580	P	ASMTL
36554_at	8623	1	P	0 861	P	0 533	P	ASMTL
36564_at	127544	1	P	1 692	P	1 466	P	IBRDC3
36711_at	23764	1	P	1 550	P	1 338	P	MAFF
37028_at	23645	1	P	1 894	P	2 523	P	PPP1R15A
37170_at	55589	1	P	1 487	P	1 745	P	BMP2K
37408_at	9902	1	P	0 680	P	0 639	P	MRC2
37796_at	4034	1	P	0 793	P	0 505	P	LRRN1
37802_r_at	54629	1	P,M,A	0 741	P	0 532	P,A	KIAA1164
37892_at	1301	1	P	0 842	P	0 614	P	COL11A1
37950_at	5550	1	P	0 884	P	0 650	P	PREP
37966_at	29780	1	P	0 881	P	0 641	P	PARVB
38037_at	1839	1	P,A	1 804	P	2 034	P	DTR
39729_at	7001	1	P	0 904	P	0 582	P	PRDX2
39817_s_at	10591	1	P	0 803	P	0 582	P	C6orf108
40489_at	1822	1	P	1 821	P	1 574	P	DRPLA, B37
40665_at	2328	1	P,M,A	0 629	A	0 858	P,A	FMO3
41660_at	9620	1	P	0 605	P	0 510	P	CELSR1
44654_at	92579	1	P	0 874	P	0 586	P	G6PC3
45633_at	64785	1	P	1 961	P	1 606	P	FLJ13912
46142_at	64788	1	P,A	0 654	P,M,A	0 443	A	FLJ12681
46167_at	7268	1	P	1 768	P	1 589	P	TTC4
48106_at	55652	1	P	1 755	P	1 523	P	FLJ20489
48808_at	1719	1	P	1 685	P	1 707	P	DHFR
50965_at	25837	1	P	0 441	P	0 383	P	RAB26
51176_at	9442	1	P	1 558	P	1 483	P	CRSP8
52164_at	53838	1	P	1 659	P	1 484	P	C11orf24
52940_at	59307	1	P	0 703	P	0 553	P	SIGIRR
56256_at	51092	1	P	0 708	P	0 482	P	TAGLN
57539_at	84619	1	P	0 887	P	0 650	P	FLJ20406
58308_at	55223	1	P	1 445	P	1 655	P	FLJ10759
59625_at	8996	1	P	0 824	P	0 659	M,A	LOC283849
60794_f_at		1	P	1 512	P	1 575	P	
60815_at	5439	1	P,A	1 427	P	1 837	P	POLR2J
61732_r_at	80173	1	P	0 692	P,A	0 659	P,A	CCDC2
61734_at	57333	1	P	0 560	P	0 615	P	RCN3
63825_at		1	P	1 736	P	1 654	P	ABHD2
64371_at	10147	1	P,A	1 369	P	1 576	P	SFRS14
64486_at	57175	1	P	0 771	P	0 571	P	CORO1B
65517_at	10053	1	P	1 728	P	1 970	P	AP1M2
65588_at		1	P	1 609	P	1 540	P	
65630_at		1	P	0 633	P	0 452	P	LOC283232
91703_at	254102	1	P	0 737	P	0 550	P	DKFZp762C186

Affymetrix Id	Day 0		Day 3		Day 7		Common Name
	Normalised	Flag	Normalised	Flag	Normalised	Flag	
AFFX-DapX-3_at	1	P	0 687	P	0 566	P	
AFFX-DapX-5_at	1	P	0 559	P	0 490	P	
AFFX-DapX-M_at	1	P	0 662	P	0 542	P	
AFFX-HUMISGF3A/M97935_3_at	1	P	1 348	P	1 949	P	STAT1
AFFX-HUMISGF3A/M97935_MB_at	1	P	1 285	P	1 760	P	STAT1
AFFX-LysX-3_at	1	P	0 737	P	0 550	P	
AFFX-LysX-5_at	1	P	0 707	P	0 576	P	
AFFX-LysX-M_at	1	P	0 696	P	0 550	P	
AFFX-M27830_5_at	1	P,A	0 955	P,A	1 841	P,M,A	
AFFX-PheX-3_at	1	P	0 756	P	0 598	P	
AFFX-PheX-5_at	1	P	0 629	P	0 510	P	
AFFX-PheX-M_at	1	P	0 675	P	0 568	P	
AFFX-r2-Bs-dap-3_at	1	P	0 746	P	0 632	P	
AFFX-r2-Bs-dap-5_at	1	P	0 611	P	0 488	P	
AFFX-r2-Bs-dap-M_at	1	P	0 658	P	0 529	P	
AFFX-r2-Bs-lys-3_at	1	P	0 696	P	0 589	P	
AFFX-r2-Bs-lys-5_at	1	P	0 693	P	0 493	P	
AFFX-r2-Bs-lys-M_at	1	P	0 691	P	0 557	P	
AFFX-r2-Bs-phe-3_at	1	P	0 674	P	0 540	P	
AFFX-r2-Bs-phe-5_at	1	P	0 536	P	0 457	P	
AFFX-r2-Bs-phe-M_at	1	P	0 687	P	0 518	P	
AFFX-r2-Bs-thr-3_s_at	1	P	0 682	P	0 598	P	
AFFX-r2-Bs-thr-5_s_at	1	P	0 599	P	0 522	P	
AFFX-r2-Bs-thr-M_s_at	1	P	0 621	P	0 472	P	
AFFX-ThrX-3_at	1	P	0 677	P	0 543	P	
AFFX-ThrX-5_at	1	P	0 549	P	0 424	P	
AFFX-ThrX-M_at	1	P	0 631	P	0 474	P	

7 4 Appendix E – Differentially Expressed Genes Identified in IdU DNA Microarray Experiment

List of differentially expressed genes identified from microarray analysis of IdU microarray experiment Genes listed are sorted by Affymetrix ID number

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
207331_at	1063	1	P	0.645	P	CENPF
1405_i_at	6352	1	P,A	2.996	P	CCL5
200696_s_at	2934	1	P	1.552	P	GSN
200697_at	3098	1	P	1.622	P	HK1
200762_at	1808	1	P	1.536	P	DPYSL2
200768_s_at	4144	1	P	0.544	P	MAT2A
200838_at	1508	1	P	1.543	P	CTSB
200872_at	6281	1	P	1.588	P	S100A10
200878_at	29952	1	P,A	1.539	P	EPAS1
200887_s_at	6772	1	P	1.857	P	STAT1
200983_x_at	966	1	P	1.602	P	CD59
200985_s_at	966	1	P	1.505	P	CD59
201015_s_at	3728	1	P,M,A	1.715	P	JUP
201042_at	7052	1	P,A	1.989	P	TGM2
201058_s_at	10398	1	P	1.528	P	MYL9
201060_x_at	2040	1	P	1.541	P	STOM
201110_s_at	7057	1	P,A	3.583	P	THBS1
201122_x_at	1984	1	P	1.584	P	EIF5A
201123_s_at	1984	1	P	1.689	P	EIF5A
201141_at	10457	1	P	1.920	P	GPNMB
201149_s_at	7078	1	P,A	2.564	P	TIMP3
201150_s_at	7078	1	P,A	1.750	P,A	TIMP3
201162_at	3490	1	P,A	2.617	P	IGFBP7
201163_s_at	3490	1	P	2.360	P	IGFBP7
201169_s_at	8553	1	P,A	1.888	P	BHLHB2
201185_at	5654	1	P,A	2.074	P	PRSS11
201205_at	6238	1	P,A	1.504	P	RRBP1
201266_at	7296	1	P	1.610	P	TXNRD1
201295_s_at	26118	1	P	0.647	P	WSB1
201330_at	5917	1	P	0.499	P	RARS
201341_at	8507	1	P,A	1.746	P	ENC1
201348_at	2878	1	P	1.669	P	GPX3
201427_s_at	6414	1	P,A	1.795	P,A	SEPP1
201464_x_at	3725	1	P,A	1.863	P	JUN
201466_s_at	3725	1	P,A	2.065	P	JUN; AP1
201473_at	3726	1	P	1.871	P	JUNB
201502_s_at	4792	1	P	1.555	P	NFKBIA
201505_at	3912	1	P	1.933	P	LAMB1
201506_at	7045	1	A	2.585	P,M	TGFBI
201578_at	5420	1	P,M,A	1.529	P,M,A	PODXL
201596_x_at	3875	1	P,A	2.271	P	KRT18
201631_s_at	8870	1	P	1.775	P	IER3
201666_at	7076	1	P	1.638	P	TIMP1
201693_s_at	1958	1	P	1.813	P	EGR1
201719_s_at	2037	1	P	1.558	P	EPB41L2
201733_at		1	P	0.657	P	CLCN3
201739_at	6446	1	P	2.196	P	SGK; SGK1
201793_x_at	9887	1	M,A	1.732	P,A	C1orf16
201798_s_at	26509	1	P,A	2.298	P	FER1L3
201843_s_at	2202	1	P,A	1.682	P,A	EFEMP1

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
201927_s_at	8502	1	P	1.541	P	PKP4
201945_at	5045	1	A	1.679	P,M	FURIN
202008_s_at	4811	1	P	1.533	P	NID
202017_at	2052	1	P	1.622	P	EPHX1
202086_at	4599	1	P,A	1.925	P	MX1
202132_at	25937	1	P	1.550	P	TAZ
202159_at	2193	1	P	1.565	P	FARSLA
202284_s_at	1026	1	P	2.209	P	P21
202307_s_at	5696	1	P	1.669	P	TAP1
202328_s_at	5310	1	P	0.619	P,M	PKD1; PBP
202424_at	5605	1	P	1.619	P	MAP2K2
202436_s_at	1545	1	P,A	1.542	P	CYP1B1
202446_s_at	5359	1	P	1.827	P	PLSCR1
202458_at	11098	1	P,A	2.022	P	SPUVE
202472_at	4351	1	P	0.620	P	MPI
202524_s_at	9806	1	P	0.402	P	SPOCK2
202575_at	1382	1	M,A	1.965	P	CRABP2
202598_at	6284	1	P	1.560	P	S100A13
202662_s_at	3709	1	A	1.654	P	ITPR2
202665_s_at	7456	1	P,A	1.649	P	WASPIP
202672_s_at	467	1	P,A	2.023	P	ATF3
202686_s_at	558	1	P,A	2.035	P	AXL; UFO
202687_s_at	8743	1	P,A	2.233	P	TNFSF10
202688_at	8743	1	P,A	1.673	P,A	TNFSF10
202700_s_at	9725	1	P,A	0.587	P,A	KIAA0792
202719_s_at	26136	1	P,A	1.846	P	TES
202720_at	26136	1	P	1.696	P	TES
202747_s_at	9452	1	P,A	3.918	P,A	ITM2A
202760_s_at	11217	1	P,M,A	1.590	P,A	AKAP2
202765_s_at	2200	1	P,A	1.780	P	FBN1
202766_s_at	2200	1	P	1.545	P	FBN1
202920_at		1	P	0.525	P,A	ANK2
202949_s_at	2274	1	P	1.899	P	FHL2
202996_at	57804	1	P	1.613	P	POLD4
203058_s_at	9060	1	P	1.641	P	PAPSS2
203065_s_at	857	1	P	1.943	P	CAV1
203066_at	51363	1	P	1.604	P	GALNAC4S-6ST
203072_at	4643	1	P,A	1.876	P,A	MYO1E
203091_at	8880	1	P	1.514	P	FUBP1; FBP
203109_at	9040	1	P	1.524	P	UBE2M
203117_s_at	9924	1	P	0.586	P	USP52
203140_at	604	1	P,A	2.202	P	BCL6
203153_at	3434	1	P,A	2.185	P	IFIT1
203172_at	9513	1	P	1.508	P	FXR2
203184_at	2201	1	P	2.158	P	FBN2
203227_s_at	6302	1	P	0.622	P	SAS
203229_s_at	1196	1	P	0.612	P,M,A	CLK2
203243_s_at	10611	1	P	1.514	P	LIM; ENH
203304_at	25805	1	P,A	1.722	P	BAMBI
203368_at	78987	1	P	1.554	P	CRELD1

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
203394_s_at	3280	1	P	1 619	P	HES1
203423_at	5947	1	P	1 703	P	RBP1
203446_s_at	4952	1	P	0 604	P	OCRL
203452_at	26229	1	P	1 524	P	B3GAT3
203455_s_at	6303	1	P	1 832	P	SAT
203469_s_at	8558	1	P,A	1 610	P,A	CDK10
203501_at	10404	1	P	0 520	P	PGCP
203504_s_at	19	1	P	1 556	P	ABCA1
203646_at	2230	1	P	1 686	P	FDX1
203665_at	3162	1	P	1 622	P	HMOX1
203722_at	8659	1	P	1 528	P	ALDH4A1
203725_at	1647	1	P	1 921	P	GADD45A
203821_at	1839	1	P,M	1 612	P	DTR
203837_at	4217	1	P	1 566	P	MAP3K5
203882_at	10379	1	P	1 612	P	ISGF3G
203910_at	9411	1	P,A	3 529	P	PARG1
203926_x_at	513	1	P	1 522	P	ATP5D
203929_s_at	4137	1	P,A	0 602	P,A	MAPT
203952_at	22926	1	P,A	0 569	P,M,A	ATF6
203980_at	2167	1	P	1 515	P	FABP4
203989_x_at	2149	1	P,A	1 843	P,A	F2R
203999_at		1	P,A	1 923	P	SYT1
204030_s_at	29970	1	P	1 636	P	SCHIP1
204035_at	7857	1	P	0 338	P	SCG2
204036_at	1902	1	P	0 432	P	EDG2
204037_at	1902	1	P	0 618	P	EDG2
204038_s_at	1902	1	P	0 580	P	EDG2
204135_at	11259	1	P	1 624	P	DOC1, GIP90
204260_at	1114	1	P	0 537	P	CHGB, SCG1
204268_at	6273	1	P,A	2 684	P	S100A2,
204279_at	5698	1	P	1 597	P	PSMB9
204326_x_at	4500	1	P	1 771	P	MT1L
204346_s_at	11186	1	P,A	1 671	P	RASSF1
204359_at	23768	1	P	1 593	P	FLRT2
204421_s_at	2247	1	P	1 610	P	FGF2
204422_s_at	2247	1	P	1 556	P	FGF2
204439_at	10964	1	P	0 481	P	C1orf29
204452_s_at	8321	1	P	0 654	P,M,A	FZD1
204455_at	667	1	P,A	2 264	P	BPAG1
204475_at	4312	1	P	2 186	P	MMP1
204490_s_at	960	1	P,A	1 850	P,A	CD44
204529_s_at	9760	1	P,A	1 583	P,M	TOX
204564_at	10336	1	P,A	1 513	P	RNF3
204577_s_at	23059	1	P	0 602	P,A	KIAA0643
204589_at	9891	1	P	1 729	P	ARK5
204604_at	5218	1	P,A	1 904	P	PFTK1
204612_at	5569	1	P	0 542	P,A	PKIA
204627_s_at	3690	1	P,A	2 471	P,A	ITGB3
204653_at		1	P,A	1 730	P	TFAP2A
204655_at	6352	1	P,A	2 353	P	CCL5

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
204665_at	80143	1	P,A	1 527	P,A	FLJ21168
204682_at	4053	1	P,A	1 573	P	LTBP2
204697_s_at	1113	1	P,A	1 540	P	CHGA, CGA
204718_at	2051	1	P,A	0 613	P,A	EPHB6, HEP
204748_at	5743	1	P,A	2 701	P	COX2
204762_s_at	2775	1	P,M,A	0 512	M,A	GNAO1
204779_s_at	3217	1	P	1 647	P	HOXB7
204840_s_at	8411	1	P,M	1 614	P	EEA1
204841_s_at	8411	1	P,A	1 529	P	EEA1
204859_s_at	317	1	P	1 558	P	APAF1
204864_s_at	3572	1	P	1 687	P	IL6ST
204865_at	761	1	P,A	2 305	P	CA3, CAIII
204897_at	5734	1	P	1 895	P	PTGER4
204947_at	1869	1	P	0 613	P	E2F1
204955_at	8406	1	P,A	1 670	P	SRPX
204967_at	357	1	P,A	1 552	P,A	APXL
204983_s_at	2239	1	P	1 763	P	GPC4
205013_s_at	135	1	P	1 586	P	ADORA2A
205016_at	7039	1	P,A	2 817	P	TGFA
205034_at	9134	1	P	1 521	P	CCNE2
205047_s_at	440	1	P	0 628	P	ASNS
205050_s_at	23542	1	P	0 509	P	MAPK8IP2
205068_s_at	23092	1	P,A	1 517	P	ARHGAP26
205082_s_at	316	1	P,M	0 604	P,A	AOX1, AOH1
205222_at	1962	1	P	0 599	P,A	EHHADH
205224_at	6835	1	P,A	1 556	P	SURF2
205286_at	7022	1	P,A	1 772	P	TFAP2C
205296_at		1	P	0 638	P	
205301_s_at	4968	1	P	0 609	P	OGG1
205303_at	3764	1	P	0 551	P,M	KCNJ8
205304_s_at	3764	1	P	0 543	P	KCNJ8
205357_s_at	185	1	P,M	2 281	P	AGTR1
205366_s_at	3216	1	P,A	2 016	P	HOXB6
205374_at	6588	1	P,A	1 880	P,A	SLN,
205386_s_at	4193	1	P,A	1 985	P	MDM2
205405_at	9037	1	P,A	1 619	P	SEMA5A
205479_s_at	5328	1	P,A	2 166	P	PLAU
205500_at	727	1	P	0 633	P,A	C5
205523_at	1404	1	P	0 371	P	HAPLN1
205524_s_at	1404	1	P,M	0 164	A	HAPLN1
205534_at	5099	1	P,A	1 748	P	PCDH7
205547_s_at	6876	1	P	2 453	P	TAGLN
205594_at	22834	1	P	0 561	P	KIAA0924
205625_s_at	793	1	P,A	2 737	P	CALB1
205626_s_at	793	1	P,A	1 920	P	CALB1
205657_at	23498	1	P,A	0 621	P,M,A	HAAO
205660_at	8638	1	P,A	1 771	P	OASL
205698_s_at	5608	1	P	0 652	P,M,A	MAP2K6
205780_at	638	1	P,A	2 508	P,M,A	BIK
205828_at	4314	1	P	1 721	P	MMP3

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
205829_at	3292	1	P	1.742	P	HSD17B1
205832_at	51200	1	P,A	1.678	P	CPA4
205841_at	3717	1	P	1.711	P	JAK2
205862_at	9687	1	P,M,A	1.692	P,A	GREB1
205887_x_at	4437	1	P	0.552	P,M	MSH3
205896_at	6583	1	P,A	1.656	P	SLC22A4
205924_at	5865	1	P,A	2.074	P	RAB3B
205925_s_at	5865	1	P,M	2.018	P	RAB3B
205964_at	79088	1	P	0.623	P	ZNF426
205975_s_at	3231	1	P	1.747	P	HOXD1
206056_x_at		1	P,A	0.403	P,A	SPN
206116_s_at	7168	1	P	1.924	P	TPM1
206117_at	7168	1	P,A	1.968	P	TPM1
206172_at	3598	1	P	1.525	P	IL13RA2
206201_s_at	4223	1	M,A	2.059	P,A	MEOX2
206209_s_at	762	1	P,A	1.771	P	CA4; CAIV
206259_at	5624	1	P,A	2.062	P	PROC
206290_s_at	6000	1	P	0.656	P	RGS7
206299_at	27112	1	P,A	0.618	P,A	TMEM28; TED
206300_s_at	5744	1	P,A	1.834	P	PTHLH
206314_at	55888	1	P	0.575	P	ZNF167
206343_s_at	3084	1	P,A	1.581	P	NRG1
206377_at	2295	1	P,A	1.573	P,A	FOXF2
206429_at	2150	1	P,A	3.982	P,A	F2RL1
206452_x_at	5524	1	P,A	1.543	P	PPP2R4
206461_x_at	4496	1	P,A	1.762	P	MT1H
206508_at	970	1	P,A	1.845	P	TNFSF7
206529_x_at	5172	1	P,A	1.681	P,M	SLC26A4
206543_at	6595	1	P	0.601	P	SMARCA2
206555_s_at	55623	1	P	0.568	P	THUMPD1
206615_s_at	53616	1	P	0.491	P,M	ADAM22
206695_x_at	7594	1	P	0.595	P	ZNF43
206757_at	8654	1	P	0.501	P	PDE5A
206769_at	9087	1	P,A	1.616	P	TMSB4Y
206773_at	4062	1	P,A	1.685	P	LY6H;
206825_at	5021	1	P,A	2.873	P	OXTR; OT-R
206868_at	9754	1	P,M	1.500	P	STARD8
207030_s_at	1466	1	P	1.505	P	CSRP2
207059_at	5083	1	P,M	0.654	P,A	PAX9
207068_at	7539	1	P	0.625	P	ZFP37
207145_at	2660	1	P	0.450	P,A	GDF8
207160_at	3592	1	P,A	2.213	P	IL12A
207187_at	3718	1	M,A	1.501	P	JAK3
207324_s_at	1823	1	P	0.324	P,A	DSC1
207387_s_at	2710	1	P,M	1.519	P	GK
207437_at	4857	1	P	0.436	P	NOVA1
207536_s_at	3604	1	P,A	1.767	P,A	TNFRSF9
207684_at	6911	1	P	0.660	P,A	TBX6
207765_s_at	80256	1	M,A	1.514	P,A	KIAA1539
207826_s_at	3399	1	P	1.632	P	ID3

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
207876_s_at	2318	1	P	1.533	P	FLNC
207922_s_at	10296	1	P	0.625	P	MAEA; EMP
207960_at		1	P,A	1.653	P,A	
207963_at	26236	1	P	0.649	P	C6orf54
207969_x_at	56	1	P,M,A	1.945	P	ACRV1
208016_s_at	185	1	P,A	1.587	P	AGTR1
208035_at	2916	1	P,A	2.790	P,A	GRM6
208086_s_at	1756	1	P	1.855	P	DMD
208116_s_at	4121	1	P,A	1.589	P	MAN1A1
208185_x_at		1	P	1.541	P	
208190_s_at	51599	1	P	1.504	P	LISCH7
208241_at	3084	1	P,A	1.659	P	NRG1
208250_s_at	1755	1	P,A	1.521	P	DMBT1
208511_at	26255	1	P	0.626	P	PTTG3
208514_at	3753	1	P,A	2.581	P,M,A	KCNE1
208546_x_at	8345	1	P	0.623	P	HIST1H2BH
208581_x_at	4501	1	P	2.075	P	MT1X
208588_at	59347	1	P	0.572	P,A	FKSG2
208610_s_at	23524	1	P	0.626	P	SRRM2
208637_x_at	87	1	P	1.518	P	ACTN1
208650_s_at	934	1	P	1.550	P	CD24
208690_s_at	9124	1	P	1.531	P	PDLIM1
208704_x_at	334	1	P	1.561	P	APLP2
208719_s_at	10521	1	P	0.656	P	DDX17;
208738_x_at	6613	1	P	0.583	P	SUMO2
208747_s_at	716	1	P,A	1.850	P	C1S
208782_at	11167	1	P	2.006	P	FSTL1
208789_at	22939	1	P	1.558	P	PTRF
208798_x_at	23015	1	P	0.577	P	GOLGIN-67
208902_s_at		1	P,A	0.607	P,A	FLJ46061
208937_s_at	3397	1	P	1.588	P	ID1
208944_at	7048	1	P	1.576	P	TGFBR2
208991_at		1	P	1.579	P	STAT3
208992_s_at	6774	1	P,A	1.698	P	STAT3
209040_s_at	5696	1	P	1.713	P	PSMB8
209087_x_at	4162	1	P	1.603	P	MCAM
209129_at	7205	1	P	0.616	M,A	TRIP6
209184_s_at	8660	1	P,A	1.561	P,M,A	IRS2
209189_at	2353	1	P,A	3.182	P	FOS
209193_at	5292	1	P,A	2.245	P	PIM1
209202_s_at	2137	1	P,A	1.775	P,A	EXTL3
209212_s_at	688	1	P	1.716	P	KLF5
209260_at	2810	1	P,A	1.884	P	SFN
209261_s_at	2063	1	P,A	1.565	P,M,A	NR2F6
209267_s_at	64116	1	P	1.707	P	SLC39A8
209278_s_at	7980	1	P	1.869	P	TFPI2
209283_at	1410	1	M,A	2.427	P,M,A	CRYAB
209287_s_at	10602	1	P	1.536	P	CDC42EP3
209291_at	3400	1	P	1.779	P	ID4
209293_x_at	3400	1	P	2.098	P	ID4

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
209304_x_at	4616	1	P,A	1.898	P	GADD45B
209340_at	6675	1	P	1.540	P	UAP1
209356_x_at	30008	1	P	1.500	P	EFEMP2
209438_at		1	P	0.651	P,A	
209457_at	1847	1	P	1.589	P	DUSP5
209459_s_at	57416	1	P,A	2.042	P	ABAT
209469_at	2823	1	P	0.596	P,M	GPM6A
209470_s_at	2823	1	P	0.398	P,A	GPM6A
209487_at	11030	1	P,A	2.015	P	RBPMS
209494_s_at	23598	1	P	0.602	P	ZNF278
209504_s_at	58473	1	P,M	0.654	P,A	PLEKHB1
209560_s_at	8788	1	P,A	2.255	P	DLK1
209574_s_at	753	1	P,A	1.750	P,A	C18orf1
209584_x_at	27350	1	P,A	1.576	P,A	APOBEC3C
209598_at	10687	1	P	1.566	P	PNMA2
209604_s_at	2625	1	P	1.734	P	GATA3
209631_s_at		1	P,A	1.821	P	
209656_s_at	83604	1	P	1.526	P	TM4SF10
209703_x_at	25840	1	P,A	1.854	P,A	DKFZP586A0522
209708_at	26002	1	P,A	1.579	P	MOXD1
209758_s_at	8076	1	P	1.909	P	MFAP5
209771_x_at	934	1	P	2.001	P	CD24
209803_s_at	7262	1	P	2.472	P	PHLDA2
209806_at	85236	1	P	0.652	P	HIST1H2BK
209835_x_at	960	1	A	3.576	P	CD44
209846_s_at	11118	1	P	0.620	P	BTN3A2
209875_s_at	6696	1	P	1.666	P	SPP1
209885_at	29984	1	P	1.700	P	RHOD
209904_at	7134	1	P,A	2.212	P,A	TNNC1
209908_s_at	7042	1	P,A	1.661	P,M,A	TGFB2
209936_at	10181	1	P,A	0.599	P,A	RBM5
209946_at	7424	1	P	0.340	P	VEGFC
209960_at	3082	1	P	0.531	P	HGF;
209969_s_at	6772	1	P	1.870	P	STAT1
210012_s_at	2130	1	P	0.528	P,A	EWSR1
210102_at	4013	1	P	1.573	P	LOH11CR2A
210144_at	25771	1	P,A	1.958	P,A	C22orf4
210145_at	5321	1	P,A	2.828	P,M	PLA2G4A
210162_s_at	4772	1	P,A	0.611	P,A	NFATC1
210172_at		1	P	0.537	P	SF1;
210200_at	11060	1	P,A	0.636	P,A	WWP2
210205_at	8705	1	P	0.619	P	B3GALT4
210230_at	6066	1	P,A	0.544	P,A	RNU2
210233_at	3556	1	A	1.817	P,M	IL1RAP
210234_at	2914	1	P,A	1.547	P,A	GRM4
210241_s_at	11257	1	P	0.628	P	TP53AP1
210315_at	6854	1	P,A	1.596	P,A	SYN2
210322_x_at	7404	1	P,A	1.549	P,M,A	UTY; UTY1
210355_at	5744	1	M,A	1.953	P,A	PTHLH
210385_s_at	51752	1	P	1.675	P	ARTS-1

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
210424_s_at	23015	1	P,A	0 627	P,A	GOLGIN-67
210495_x_at	2335	1	P,A	3 236	M,A	FN1
210538_s_at	330	1	P	2 377	P	BIRC3
210552_s_at	9649	1	P,M	0 631	P,A	RALGPS1
210560_at	2637	1	P,M,A	1 788	P	GBX2
210564_x_at	8837	1	P	1 754	P	CFLAR
210592_s_at		1	P	1 885	P	SAT, SSAT
210605_s_at	4240	1	P,A	1 643	P,A	MFGE8,
210612_s_at	8871	1	P,M	1 511	P	SYNJ2
210674_s_at	56137	1	P	0 635	P,M,A	PCDHA12
210715_s_at	10653	1	P	2 191	P	SPINT2
210752_s_at	6945	1	P	1 553	P	TCFL4
210809_s_at	10631	1	P	1 503	P	POSTN
210867_at	4850	1	P	0 532	A	CNOT4
210926_at		1	P	1 545	P	FKSG30
210986_s_at	7168	1	P	2 028	P	TPM1
210987_x_at	7168	1	P	2 069	P	TPM1
211017_s_at	4771	1	P	1 569	P	NF2
211043_s_at	1212	1	P	1 596	P	CLTB
211097_s_at	5089	1	P	0 579	P	PBX2
211098_x_at	54499	1	P	1 502	P	LOC54499
211160_x_at	87	1	P,A	1 619	P	ACTN1
211177_s_at	10587	1	P	1 554	P	TXNRD2
211364_at	4507	1	P	1 618	P	MTAP
211374_x_at		1	P,M,A	0 573	P,A	
211387_x_at	8732	1	P,M	0 636	P,A	RNGTT
211456_x_at		1	P,M	2 242	P	
211466_at	4781	1	P	0 618	P	NFIB
211538_s_at	3303	1	P	1 660	P	HSPA1A
211540_s_at	5925	1	P	1 506	P	RB1
211571_s_at	1462	1	P	0 460	P,A	CSPG2
211573_x_at	7052	1	P,A	2 186	P	TGM2
211593_s_at	23139	1	P	1 509	P	MAST2
211600_at		1	P	0 613	P	PTPRO
211602_s_at	7220	1	P	1 544	P	TRPC1
211668_s_at	5328	1	P,A	1 896	P	PLAU
211700_s_at	7216	1	P	0 607	P,A	TRO
211756_at	5744	1	P,A	3 128	P	PTHLH
211819_s_at	10580	1	P,A	0 558	M,A	SORBS1
211864_s_at	26509	1	P,A	2 344	P,A	FER1L3
211911_x_at	3106	1	P	1 589	P	HLA-B
211930_at		1	P	0 639	P	hnRNPA3
211990_at	3113	1	P	0 513	P,A	HLA-DPA1
212014_x_at	960	1	P,A	1 519	P,A	CD44
212061_at	23350	1	P	0 657	P	SR140
212063_at	960	1	P,A	3 062	P	CD44
212067_s_at	715	1	P	0 654	P A	C1R
212097_at	857	1	P	1 566	P	CAV1
212127_at	5905	1	P	1 742	P	RANGAP1
212143_s_at	3486	1	P	1 642	P	IGFBP3

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
212157_at	6383	1	P	0 605	P	SDC2
212172_at		1	P,M,A	0 617	P,A	AK2, ADK2
212185_x_at	4502	1	P	1 876	P	MT2A
212207_at	23389	1	P,A	1 674	P	THRAP2
212225_at	10209	1	P,A	1 556	P,A	SUI1
212230_at		1	P,A	2 188	P,A	PPAP2B
212290_at		1	P	0 656	P	SLC7A1
212294_at	55970	1	P	1 671	P	GNG12
212311_at	23231	1	P	1 602	P	KIAA0746
212314_at	23231	1	P	1 655	P	KIAA0746
212325_at	22998	1	P,A	1 815	P,M	KIAA1102
212327_at	22998	1	P,A	1 548	P,A	KIAA1102
212384_at	7919	1	P	0 463	P	BAT1
212418_at	1997	1	P	1 628	P	ELF1
212501_at	1051	1	P	0 657	P	CEBPB
212543_at	202	1	A	2 144	P	AIM1, ST4
212553_at	23248	1	P	0 597	P	KIAA0460
212587_s_at	5788	1	P	0 632	P	PTPRC
212646_at	23180	1	P,A	2 378	P,M	RAFTLIN
212651_at	9886	1	P	0 645	P	RHOBTB1
212654_at	7169	1	P	1 631	P	TPM2
212717_at	9842	1	P,A	1 815	P	PLEKHM1
212727_at	1741	1	P	0 450	P,A	DLG3
212814_at	23382	1	P	0 613	P	KIAA0828
212859_x_at		1	P,A	1 945	P	MT2A
212865_s_at	7373	1	P	0 618	P	COL14A1
212923_s_at		1	P	1 535	P	C6orf145
213014_at	9479	1	P,A	1 548	P	MAPK8IP1
213135_at		1	P	1 562	P	TIAM1
213143_at		1	P	0 493	M,A	LOC257407
213164_at		1	P	1 590	P	MRPS6
213204_at	23113	1	P	0 580	P,A	PARC
213271_s_at	23033	1	P	1 530	P	KIAA1117
213274_s_at	1508	1	P	1 679	P	CTSB
213281_at	3725	1	A	1 988	P	JUN
213290_at	1292	1	P,A	0 646	P,M,A	COL6A2
213362_at		1	P	0 651	P,M	
213403_at		1	P,A	1 807	P,A	MGC11332
213430_at	22902	1	P	0 520	P,A	RIPX
213449_at	10940	1	P	1 635	P	POP1
213456_at	25928	1	P,A	2 059	P	SOSTDC1
213496_at	9890	1	P	0 466	P	PRG1
213506_at	2150	1	P,A	3 084	P	F2RL1
213528_at	92342	1	P	0 574	P	MGC9084
213558_at	27445	1	P	1 939	P	PCLO
213618_at	116984	1	P	1 715	P	CENTD1
213624_at	10924	1	P	0 584	P	SMPDL3A
213650_at	23015	1	P	0 634	P	GOLGIN-67
213668_s_at	6659	1	P,A	1 619	P	SOX4
213764_s_at		1	P	1 971	P	MFAP5

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
213765_at		1	P	1.834	P	MFAP5
213802_at	8492	1	M,A	1.554	P,M,A	PRSS12
213803_at	3837	1	P	0.656	P	KPNB1
213810_s_at	55122	1	P,M,A	1.539	P,A	C6orf166
213835_x_at	84705	1	P	0.654	P	GTPBP3
213849_s_at	5521	1	P	0.428	M,A	PPP2R2B
213854_at	9145	1	P	1.581	P	SYNGR1
213882_at	83941	1	P,A	0.561	P,A	BBP
213929_at		1	P,A	1.557	P	
213930_at		1	P	1.601	P	APG12L
213931_at	3398	1	P	1.538	P	ID2
213932_x_at	3105	1	P	1.808	P	HLA-A
213964_x_at		1	P	0.603	P,A	
214043_at		1	P	0.637	P	PTPRD
214071_at	65258	1	P	0.644	P	GNAL
214077_x_at	4213	1	P	0.659	P	MEIS4
214079_at		1	P,A	1.527	P,A	DHRS2
214091_s_at	2878	1	P	1.630	P	GPX3
214163_at	51668	1	P	0.590	P,M,A	LOC51668
214175_x_at	8572	1	P,A	1.501	P	PDLIM4
214196_s_at	1200	1	P	0.638	P	CLN2
214209_s_at	23457	1	P,A	0.601	P,A	ABCB9
214216_s_at	23185	1	P	1.709	P	KIAA0217
214247_s_at	10530	1	P	1.807	P	DKK3
214251_s_at	4926	1	P	0.643	P,A	NUMA1
214321_at	4856	1	P,A	1.663	P	NOV
214329_x_at	8743	1	P,A	1.929	P,M,A	TNFSF10
214543_x_at	9444	1	P	1.521	P	QUAKING
214666_x_at	3658	1	P	0.622	P	IREB2
214668_at	57213	1	P	1.528	P	C13orf1
214700_x_at	26109	1	P	0.602	P	Rif1
214722_at		1	P	1.725	P	LOC376745
214734_at	23086	1	P,A	1.577	P	SLAC2-B
214761_at	23090	1	P	1.553	P	ZNF423
214776_x_at	9942	1	P	0.394	A	XYLB
214823_at	7754	1	P	0.629	P,A	ZNF204
214850_at		1	P	1.546	P,M	SMA5
214917_at		1	P,A	0.419	P,A	PRKAA1
214934_at	11071	1	P	0.529	P,A	ATP9B
214954_at	26032	1	P	1.700	P	KIAA0527
215012_at	26036	1	P	0.633	P	ZNF451
215019_x_at	84436	1	P	0.585	P	ZNF528
215034_s_at		1	A	3.141	P	TM4SF1
215073_s_at		1	P	0.585	P	NR2F2
215079_at		1	P	0.557	P,A	
215095_at		1	P	0.569	A	ESD
215114_at	26168	1	P,M	0.589	M,A	SENP3
215132_at		1	P	0.494	M,A	
215169_at	9906	1	P	0.494	P,A	SLC35E2
215189_at	3892	1	P	0.390	P,A	KRTHB6

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
215252_at		1	P,A	0.615	P,A	
215263_at	7789	1	P	1.523	P	ZXDA
215331_at	22989	1	P,A	0.610	P,A	KIAA1000
215359_x_at	7595	1	P	0.654	P	ZNF44
215410_at	5380	1	P,M,A	0.639	P,A	PMS2L2
215469_at		1	P,A	1.599	P,A	
215489_x_at	9454	1	P	1.720	P	HOMER3
215498_s_at	5606	1	P	1.597	P	MAP2K3
215506_s_at	9077	1	P,A	2.351	P	ARHI
215507_x_at		1	P	0.465	P,A	RAB22A
215551_at	2099	1	P,A	0.412	P,M,A	ESR1
215591_at	23314	1	P	0.590	P,A	SATB2
215604_x_at		1	P	0.632	P,A	
215607_x_at		1	P,A	1.648	P,A	
215643_at		1	P	0.357	P	SEMA3D
215646_s_at	1462	1	P	0.462	P	CSPG2
215660_s_at		1	P,M	0.521	P,A	MAST2
215694_at	79029	1	P	0.556	P,A	SPATA5L1
215706_x_at	7791	1	P	1.709	P	ZYX
215719_x_at	355	1	P,A	2.013	P	TNFRSF6
215760_s_at	22904	1	P	0.500	P,A	KIAA0963
215779_s_at	8339	1	P	0.592	P,A	HIST1H2BG
215834_x_at		1	P,A	1.827	P,A	SCARB1
215883_at		1	P,A	1.761	P,A	CTNNA1
215919_s_at	64963	1	P,A	0.524	P,A	MRPS11
215930_s_at	4253	1	P	0.626	P	MGEA6
215945_s_at	23321	1	P,A	1.830	P,A	TRIM2
215977_x_at	2710	1	P,A	1.942	P	GK; GKD
216049_at	22836	1	P,A	1.599	P,A	RHOBTB3
216101_at		1	P,A	0.625	P,A	
216178_x_at	3688	1	P,A	1.524	P,A	ITGB1
216217_at	23228	1	A	1.919	P,M	PLCL2
216229_x_at	80867	1	P	0.600	P,A	HCG2P7
216248_s_at	4929	1	P	1.610	P	NR4A2
216279_at	10794	1	P	1.651	P	ZNF272
216336_x_at		1	P	1.812	P	
216379_x_at	934	1	P	1.867	P	NaGLT1
216442_x_at	2335	1	P,A	2.498	P	FN1
216598_s_at	6347	1	M,A	2.128	P,M	CCL2
216604_s_at	23428	1	M,A	1.520	P,A	SLC7A8
216739_at		1	P	0.580	P,A	
216770_at		1	P,A	1.705	P,A	
216804_s_at		1	P	1.781	P	LIM
216865_at	7373	1	P	0.446	P	COL14A1; UND
216885_s_at	50717	1	P	0.658	P	H326
217019_at		1	P	0.536	P	
217028_at	7852	1	P,A	1.541	P,A	CXCR4
217052_x_at		1	P	0.634	P	TIA1
217107_at		1	P,M	0.530	P,M	
217120_s_at	9282	1	P,A	1.596	P	CRSP2

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		Normalized	Flags	Normalized	Flags	
217193_x_at	3535	1	P,A	1.517	P,A	IGL@
217212_s_at		1	P	0.545	P,A	
217218_at	23063	1	P,A	1.571	P	KIAA0261; FOE
217340_at		1	P,A	0.503	P,M,A	
217363_x_at		1	P,A	0.536	P,A	
217365_at		1	P	0.539	P,M,A	LOC65122
217550_at		1	P	0.651	P	ATF6
217554_at		1	P	0.624	P	
217602_at	5478	1	P	0.656	P,A	PPIA
217608_at	57515	1	P	0.596	P	FLJ36754
217625_x_at		1	P,A	1.751	P,M,A	
217630_at	90806	1	P,M,A	0.424	P,A	LOC90806
217653_x_at		1	P,M	0.610	P,A	
217728_at	6277	1	P,A	3.321	P	S100A6
217749_at	28831	1	P	1.668	P	COPG
217809_at	28969	1	P	1.516	P	BZW2
217813_s_at	10927	1	P	0.631	P	SPIN
217817_at	10093	1	P	1.668	P	ARPC4
217818_s_at	10093	1	P	1.763	P	ARPC4
217853_at	64759	1	P,A	2.894	P	TENS1
217866_at	79869	1	P	0.645	P	FLJ12529
217880_at	996	1	P	0.539	P	CDC27
217890_s_at	55742	1	P	1.594	P	PARVA
217896_s_at	80011	1	P	0.657	P	NIP30
217974_at	51768	1	P	0.574	P	TM7SF3
218087_s_at	10580	1	P,A	0.618	P,A	SORBS1
218278_at	54663	1	P,A	1.710	P	FLJ10439
218292_s_at	51422	1	P	1.764	P	PRKAG2
218309_at	55450	1	P	1.563	P	CaMKIINalpha
218400_at	4940	1	P	1.658	P	OAS3
218413_s_at	51193	1	P,M	0.570	A	ANC_2H01
218486_at		1	P	0.619	P	TIEG2
218532_s_at	54463	1	P,A	1.506	P,A	FLJ20152
218625_at	51299	1	P,M,A	2.530	P	NRN1
218674_at	80006	1	P	0.641	P	FLJ13611
218715_at	55813	1	P	1.512	P	HCA66
218736_s_at	54873	1	P	0.405	P	PALMD
218798_at	65095	1	M,A	0.566	P,M,A	FLJ12949
218849_s_at	10848	1	P	1.531	P	RAI
218880_at	2355	1	P,A	1.567	P,M	FOSL2
218885_s_at	79695	1	P	0.614	P	GALNT12
218902_at	54781	1	P,A	1.566	P,A	NOTCH1
218915_at	51219	1	P	1.618	P	NF2
218940_at	79609	1	P	0.588	P	C14orf138
218943_s_at	23586	1	P,A	1.648	P,A	DDX58
218980_at	80206	1	P,M	0.549	A	KIAA1695
219014_at	51316	1	P	2.403	P	PLAC8; C15
219040_at	79585	1	M,A	1.522	P,M,A	FLJ22021
219049_at	55790	1	P,A	1.503	P	ChGn
219134_at	64123	1	P	0.558	P	ETL

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		Normalized	Flags	Normalized	Flags	
219197_s_at		1	P,M	1.643	P	SCUBE2
219209_at	64135	1	P	1.847	P	MDA5
219222_at	64080	1	P,A	1.601	P,A	RBKS
219250_s_at	23767	1	P,M,A	1.582	P	FLRT3
219325_s_at	55520	1	P	0.417	P,A	ELAC1; D29
219353_at	54835	1	P	0.637	P	NHLRC2
219370_at	56475	1	A	2.475	P,M	REPRIMO
219410_at	55076	1	P	1.621	P	FLJ10134
219427_at	79633	1	P	1.522	P	FAT3
219437_s_at	29123	1	P,M	0.547	A	ANKRD11
219550_at	64221	1	P	0.532	P,A	ROBO3;
219561_at	51226	1	P,A	2.218	P,M,A	COPZ2
219603_s_at	7769	1	P	0.465	P	ZNF226
219612_s_at	2266	1	M,A	1.891	P,A	FGG
219628_at	64393	1	P	1.617	P	WIG1
219638_at	26263	1	P,A	1.590	P,M,A	FBXO22
219641_at	55070	1	P	0.634	P	DET1
219657_s_at	51274	1	P,M,A	1.686	P,A	KLF3
219683_at	7976	1	P	1.667	P	FZD3
219692_at	79412	1	P,A	1.829	P,A	KREMEN2
219694_at	54491	1	P	1.923	P	FLJ11127
219697_at	9956	1	P	2.369	P,M	HS3ST2;
219789_at	4883	1	P,A	1.835	P,A	NPR3
219800_s_at	79896	1	P	0.424	P,A	THNSL1
219813_at	9113	1	P,A	1.548	P,A	LATS1
219825_at	56603	1	P,A	1.749	P,A	CYP26B1
219869_s_at	64116	1	P,A	1.523	P	SLC39A8
219892_at	53346	1	P	1.530	P	TM6SF1
219901_at	55785	1	P	1.510	P	FLJ11183
219992_at	6866	1	P,A	2.027	P,A	TAC3
220029_at	54898	1	M,A	1.580	P,A	ELOVL2
220104_at	56829	1	P	1.752	P	ZC3HAV1
220122_at	79772	1	P	0.551	P	FLJ22344
220148_at	64577	1	P,M,A	1.633	P,M,A	ALDH8A1
220212_s_at	63892	1	P	1.512	P	THADA
220217_x_at	64663	1	P,A	2.163	P	SPANXC
220230_s_at	51700	1	P,M,A	1.643	P	CYB5R2
220321_s_at	79635	1	P	0.593	P	FLJ13646
220327_at	51159	1	P	0.589	P	FLJ38507
220358_at	55509	1	P	1.507	P	SNFT
220372_at	54943	1	P	0.611	P,A	C21orf55
220407_s_at	7042	1	P	1.668	P	TGFB2
220520_s_at	54830	1	P,A	1.699	P	FLJ20130
220544_at	60385	1	P	0.637	P,A	TSKS; TSKS1
220591_s_at	80258	1	P,A	1.992	P	FLJ22843
220643_s_at	55179	1	P	0.651	P	FAIM
220675_s_at	80339	1	P	0.657	P,A	C22orf20
220696_at	29048	1	P,A	0.586	P,A	PRO0478
220712_at		1	P,M,A	0.576	P,A	
220719_at	80079	1	P,A	1.940	P,A	FLJ13769

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
220769_s_at	79819	1	P	0.656	P,A	FLJ23129
220771_at	51152	1	P	0.602	P	LOC51152
220838_at	54932	1	P,M,A	0.635	P,A	FLJ20433
220860_at	29942	1	P	1.531	P	PURG
220922_s_at	30014	1	P,A	3.515	P	SPANXA1
220954_s_at	29990	1	P	0.642	P	PILRB
220986_s_at	81789	1	P	0.296	A	TIGD6
220991_s_at	140545	1	P	0.476	P,A	RNF32
221011_s_at	81606	1	P,A	2.132	P	LBH
221024_s_at	81031	1	P	0.415	P	SLC2A10
221059_s_at	4166	1	P	1.723	P	CHST6
221120_at	54889	1	P,A	1.576	P,A	FLJ20306
221156_x_at	9236	1	P	0.573	P	CPR8
221168_at	59336	1	P,A	1.847	P	PRDM13
221251_x_at	83444	1	P,A	1.635	P,A	HMGA1L4
221261_x_at	81557	1	P	0.494	P	MAGED4
221391_at	50840	1	P,A	1.608	P,A	TAS2R14
221419_s_at		1	P	0.282	P	
221489_s_at	81848	1	P	1.535	P	SPRY4
221594_at	84060	1	P,A	1.565	P	DKFZP564O0523
221616_s_at	51616	1	P,M,A	1.723	P,A	TAF9L
221626_at		1	P,A	1.524	P,M,A	ZNF506
221633_at	29781	1	P,A	2.103	P	384D8-2
221664_s_at	50848	1	P,A	2.047	P	F11R;
221841_s_at		1	A	2.224	P,M	KLF4
221865_at	203197	1	P	0.629	P	C9orf91
221875_x_at	3134	1	P	1.567	P	HLA-F
221898_at	10630	1	P	0.615	P	T1A-2
221958_s_at	79971	1	P,A	2.133	P	FLJ23091
221967_at	11247	1	P,A	1.529	P	NXPH4
221997_s_at	116539	1	P,A	0.639	P,A	MRPL52
222018_at	4666	1	P	0.596	P,A	NACA
222067_x_at	3017	1	P	0.481	P	H2BFB
222082_at	51341	1	M,A	1.825	P,M,A	ZBTB7
222116_s_at	54493	1	P	1.654	P	TBC1D16
222131_x_at	89941	1	P	1.527	P	RHOT2
222162_s_at	9510	1	P,A	2.423	P	ADAMTS1
222208_s_at	5439	1	P	0.621	P,M	POLR2J
222224_at		1	P,A	0.596	P,M,A	MGC71999
222229_x_at		1	P	0.604	P	
222237_s_at	7771	1	P	0.570	P	ZNF228
222313_at		1	P	0.588	P,A	
222326_at		1	P	0.522	P	
222329_x_at		1	P	0.616	P	
222361_at		1	P	0.598	P	
222366_at		1	P	0.498	P	
222370_x_at		1	P,A	1.578	P,A	
266_s_at	934	1	P	1.670	P	CD24; CD24A
32088_at	8548	1	P	0.646	P	BLZF1
32723_at	1477	1	P	0.639	P	CSTF1

Affymetrix Id	LocusLink	Day 0		Day 7		Common Name
		Normalized	Flags	Normalized	Flags	
33322_i_at	2810	1	P	1 552	P	SFN
33323_r_at	2810	1	P	1 504	P	SFN
34697_at	4040	1	P	0 625	P	LRP6
37462_i_at	8175	1	P	1 508	P	SF3A2
37892_at	1301	1	P	1 546	P	COL11A1
38037_at	1839	1	P,A	1 504	P	DTR
39548_at	4862	1	P,M,A	0 357	P,A	NPAS2
39549_at	4862	1	P,A	0 599	P,A	NPAS2
40524_at	11099	1	P,A	1 694	P	PTPN21
47105_at	54920	1	P	0 655	P	FLJ20399
48031_r_at	10826	1	P	0 578	P,A	C5orf4
53202_at	79020	1	P	0 591	P	C7orf25
60794_f_at		1	P	0 646	P	
61297_at	57513	1	P	0 592	P,A	CASKIN2
64418_at		1	P	0 658	P	AP1GBP1

Affymetrix Id	Day 0		Day 7		Common Name
	Normalized	Flags	Normalized	Flags	
AFFX-HUMISGF3A/M97935_3_at	1	P	1 851	P	STAT1
AFFX-HUMISGF3A/M97935_5_at	1	P	1 790	P	STAT1
AFFX-HUMISGF3A/M97935_MA_at	1	P	1 781	P	STAT1
AFFX-HUMISGF3A/M97935_MB_at	1	P	1 747	P	STAT1
AFFX-HUMRGE/M10098_3_at	1	P	1 922	P	
AFFX-HUMRGE/M10098_5_at	1	P	3 107	P	
AFFX-HUMRGE/M10098_M_at	1	P	1 921	P	

7 5 Appendix D – List of 93 up-regulated genes common to the BrdU 5,2`-FdU and IdU DNA Microarray Experiment

Affymetrix Id	LocusLink	Common Name
200697_at	3098	HK1
200762_at	1808	DPYSL2
200872_at	6281	S100A10
200887_s_at	6772	STAT1
201042_at	7052	TGM2
201060_x_at	2040	STOM
201149_s_at	7078	TIMP3
201464_x_at	3725	JUN
201466_s_at	3725	JUN; AP1
201502_s_at	4792	NFKBIA
201719_s_at	2037	EPB41L2
201739_at	6446	SGK
201798_s_at	26509	FER1L3
202017_at	2052	EPHX1
202284_s_at	1026	CDKN1A
202436_s_at	1545	CYP1B1
202760_s_at	11217	AKAP2
202949_s_at	2274	FHL2
203058_s_at	9060	PAPSS2
203140_at	604	BCL6
203304_at	25805	BAMBI
203665_at	3162	HMOX1
203821_at	1839	DTR
203910_at	9411	PARG1
203989_x_at	2149	F2R
204030_s_at	29970	SCHIP1
204135_at	11259	DOC1
204279_at	5698	PSMB9
204346_s_at	11186	RASSF1
204422_s_at	2247	FGF2
204475_at	4312	MMP1
204490_s_at	960	CD44
204748_at	5743	COX2
204897_at	5734	EP4
204955_at	8406	SRPX
204983_s_at	2239	GPC4
205479_s_at	5328	PLAU
205534_at	5099	PCDH7
205547_s_at	6876	TAGLN
205925_s_at	5865	RAB3B
206116_s_at	7168	TPM1
206508_at	970	TNFSF7
206773_at	4062	LY6H
207030_s_at	1466	CSRP2
207826_s_at	3399	ID3
207876_s_at	2318	FLNC
208650_s_at	934	CD24
208782_at	11167	FSTL1
208789_at	22939	PTRF
208937_s_at	3397	ID1

Affymetrix Id	LocusLink	Common Name
208944_at	7048	TGFBR2
209040_s_at	5696	PSMB8
209087_x_at	4162	MCAM
209267_s_at	64116	SLC39A8
209278_s_at	7980	TFPI2
209291_at	3400	ID4
209293_x_at	3400	ID4
209340_at	6675	UAP1
209457_at	1847	DUSP5
209771_x_at	934	CD24
209803_s_at	7262	PHLDA2
209835_x_at	960	CD44
210538_s_at	330	BIRC3
210560_at	2637	GBX2
210605_s_at	4240	MFGE8
210612_s_at	8871	SYNJ2
210986_s_at	7168	TPM1
210987_x_at	7168	TPM1
212063_at	960	CD44
212143_s_at	3486	IGFBP3
212294_at	55970	GNG12
213281_at	3725	JUN
213618_at	116984	CENTD1
214247_s_at	10530	DKK3
214722_at		LOC376745
215706_x_at	7791	ZYX
215719_x_at	355	TNFRSF6
216379_x_at	934	NaGLT1
217809_at	28969	BZW2
218625_at	51299	NRN1
218880_at	2355	FOSL2
218915_at	51219	NF2
219014_at	51316	PLAC8
219410_at	55076	FLJ10134
219612_s_at	2266	FGG
220520_s_at	54830	FLJ20130
221011_s_at	81606	LBH
221841_s_at		KLF4
266_s_at	934	CD24
38037_at	1839	DTR
AFFX HUMISGF3A/M97935_3_at		STAT1
AFFX HUMISGF3A/M97935_MA_at		STAT1
AFFX HUMISGF3A/M97935_MB_at		STAT1

7 6 Appendix E – EASE Analysis

This appendix contains part of the results generated following subjecting the 93 up-regulated genes identified as common to the BrdU, 5,2 -FdU and IdU DNA microarray experiments Due to size constraints only part of the EASE report is listed in this Appendix

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	cell death	11	71	404	10937	2.31E-04	1.20E-01	4.00E-03	330; 355; 970; 1026; 2149; 2355; 4792; 6446; 6772; 7052; 7262	200887_S_AT; 201042_AT; 201502_S_AT; 201739_AT; 202284_S_AT; 203989_X_AT; 206508_AT; 209803_S_AT; 210538_S_AT; 215719_X_AT; 218880_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	death	11	71	408	10937	2.50E-04	1.30E-01	5.00E-03	330; 355; 970; 1026; 2149; 2355; 4792; 6446; 6772; 7052; 7262	200887_S_AT; 201042_AT; 201502_S_AT; 201739_AT; 202284_S_AT; 203989_X_AT; 206508_AT; 209803_S_AT; 210538_S_AT; 215719_X_AT; 218880_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	apoptosis	10	71	379	10937	6.57E-04	3.40E-01	1.30E-02	330; 355; 970; 1026; 2149; 4792; 6446; 6772; 7052; 7262	200887_S_AT; 201042_AT; 201502_S_AT; 201739_AT; 202284_S_AT; 203989_X_AT; 206508_AT; 209803_S_AT; 210538_S_AT; 215719_X_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	programmed cell death	10	71	380	10937	6 70E-04	3 47E-01	1 40E-02	330, 355 970, 1026, 2149, 4792, 6446, 6772, 7052 7262	200887_S_AT, 201042_AT, 201502_S_AT, 201739_AT, 202284_S_AT, 203989_X_AT, 206508_AT, 209803_S_AT, 210538_S_AT, 215719_X_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	response to external stimulus	19	71	1263	10937	7 67E-04	3 97E-01	1 40E-02	355, 604, 934, 970, 1545, 2052, 2149, 2247, 2637, 4062, 4771, 4792, 5328, 5696, 5698, 5734, 5743, 6772, 7078	200887_S_AT, 201149_S_AT, 201502_S_AT, 202017_AT, 202436_S_AT, 203140_AT, 203989_X_AT, 204279_AT, 204422_S_AT, 204748_AT, 204897_AT, 205479_S_AT, 206508_AT, 206773_AT, 208650_S_AT, 209040_S_AT, 209771_X_AT, 210560_AT, 215719_X_AT, 218915_AT, 266_S_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	regulation of cellular process	9	71	323	10937	1.02E-03	5.27E-01	1.90E-02	604; 1026; 1839; 2247; 2266; 3486; 4771; 7048; 9314	202284_S_AT; 203140_AT; 203821_AT; 204422_S_AT; 208944_AT; 212143_S_AT; 218915_AT; 219612_S_AT; 221841_S_AT; 38037_AT
GO Biological Process	regulation of cell proliferation	8	71	249	10937	1.03E-03	5.33E-01	1.90E-02	604; 1026; 1839; 2247; 2266; 4771; 7048; 9314	202284_S_AT; 203140_AT; 203821_AT; 204422_S_AT; 208944_AT; 218915_AT; 219612_S_AT; 221841_S_AT; 38037_AT
GO Biological Process	regulation of biological process	9	71	328	10937	1.12E-03	5.82E-01	2.00E-02	604; 1026; 1839; 2247; 2266; 3486; 4771; 7048; 9314	202284_S_AT; 203140_AT; 203821_AT; 204422_S_AT; 208944_AT; 212143_S_AT; 218915_AT; 219612_S_AT; 221841_S_AT; 38037_AT
GO Biological Process	morphogenesis	16	71	1010	10937	1.54E-03	7.98E-01	2.30E-02	1466; 1545; 1808; 1839; 2037; 2149; 2239; 2247; 2637; 4062; 4162; 6876; 7168; 9060; 9314; 27122	200762_AT; 201719_S_AT; 202436_S_AT; 203058_S_AT; 203821_AT; 203989_X_AT; 204422_S_AT; 204983_S_AT; 205547_S_AT; 206116_S_AT; 206773_AT; 207030_S_AT; 209087_X_AT; 210560_AT; 210986_S_AT; 210987_X_AT; 214247_S_AT; 221841_S_AT; 38037_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	peptidyl-amino acid modification	3	71	17	10937	5 16E-03	1 00E+00	8 40E-02	2149, 6772 7052	200887_S_AT, 201042_AT, 203989_X_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	cell communication	29	71	2791	10937	5 40E-03	1 00E+00	8 90E-02	330, 355, 960, 970, 1808, 1839, 2149, 2247, 3486, 4162, 4240, 4792, 5099, 5328, 5734, 5865, 6281, 6772, 7048, 7052, 7791, 8406, 9411, 11186, 11217, 25805, 27122, 55970, 116984	200762_AT, 200872_AT, 200887_S_AT, 201042_AT, 201502_S_AT, 202760_S_AT, 203304_AT, 203821_AT, 203910_AT, 203989_X_AT, 204346_S_AT, 204422_S_AT, 204490_S_AT, 204897_AT, 204955_AT, 205479_S_AT, 205534_AT, 205925_S_AT, 206508_AT, 208944_AT, 209087_X_AT, 209835_X_AT, 210538_S_AT, 210605_S_AT, 212063_AT, 212143_S_AT, 212294_AT, 213618_AT, 214247_S_AT, 215706_X_AT, 215719_X_AT, 38037_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	positive regulation of cell proliferation	5	71	111	10937	5 51E-03	1 00E+00	9 10E-02	604, 1839, 2247, 2266, 7048	203140_AT, 203821_AT, 204422_S_AT, 208944_AT, 219612_S_AT, 38037_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	development	20	71	1683	10937	7 97E-03	1 00E+00	1 47E-01	1466, 1545, 1808, 1839, 2037, 2149, 2239, 2247, 2637, 3162, 3397, 3399, 4062, 4162, 6876, 7168, 7262, 9060, 9314, 27122	200762_AT, 201719_S_AT 202436_S_AT, 203058_S_AT, 203665_AT, 203821_AT, 203989_X_AT, 204422_S_AT, 204983_S_AT, 205547_S_AT, 206116_S_AT, 206773_AT, 207030_S_AT, 207826_S_AT, 208937_S_AT, 209087_X_AT, 209803_S_AT, 210560_AT, 210986_S_AT, 210987_X_AT, 214247_S_AT, 221841_S_AT, 38037_AT
GO Biological Process	signal transduction	24	71	2196	10937	8 06E-03	1 00E+00	1 48E-01	330, 355, 970 1808, 1839, 2149 2247, 3486, 4792, 5328, 5734, 5865, 6281, 6772, 7048, 7052, 7791, 9411, 11186, 11217, 25805, 27122, 55970, 116984	200762_AT, 200872_AT, 200887_S_AT, 201042_AT, 201502_S_AT, 202760_S_AT, 203304_AT, 203821_AT, 203910_AT, 203989_X_AT, 204346_S_AT, 204422_S_AT, 204897_AT, 205479_S_AT, 205925_S_AT, 206508_AT, 208944_AT, 210538_S_AT, 212143_S_AT, 212294_AT, 213618_AT, 214247_S_AT, 215706_X_AT, 215719_X_AT, 38037_AT, AFFX-HUMISGF3A/M97935_3_AT AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	cellular process	48	71	5719	10937	8 24E-03	1 00E+00	1 49E-01	330, 355, 604, 960 970, 1026, 1466, 1808, 1839, 2037, 2149, 2239, 2247, 2266, 2355, 3486, 3725, 4062, 4162 4240, 4771, 4792, 5099, 5328, 5734, 5743, 5865, 6281, 6446, 6772, 7048, 7052, 7168, 7262, 7791, 8406, 9314, 9411, 11167, 11186, 11217, 25805, 26509, 27122, 54830, 55970, 64116, 116984	200762_AT, 200872_AT, 200887_S_AT, 201042_AT, 201464_X_AT, 201466_S_AT, 201502_S_AT, 201719_S_AT, 201739_AT, 201798_S_AT, 202284_S_AT, 202760_S_AT, 203140_AT, 203304_AT, 203821_AT 203910_AT, 203989_X_AT, 204346_S_AT, 204422_S_AT, 204490_S_AT, 204748_AT 204897_AT, 204955_AT, 204983_S_AT, 205479_S_AT, 205534_AT, 205925_S_AT, 206116_S_AT, 206508_AT, 206773_AT, 207030_S_AT, 208782_AT, 208944_AT, 209087_X_AT, 209267_S_AT, 209803_S_AT, 209835_X_AT, 210538_S_AT 210605_S_AT, 210986_S_AT, 210987_X_AT, 212063_AT, 212143_S_AT, 212294_AT, 213281_AT, 213618_AT, 214247_S_AT, 215706_X_AT, 215719_X_AT, 218880_AT, 218915_AT, 219612_S_AT, 220520_S_AT, 221841_S_AT, 38037_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	muscle development	5	71	133	10937	1.03E-02	1.00E+00	1.84E-01	1466; 1839; 2247; 6876; 7168	203821_AT; 204422_S_AT; 205547_S_AT; 206116_S_AT; 207030_S_AT; 210986_S_AT; 210987_X_AT; 38037_AT
GO Biological Process	cell proliferation	14	71	1036	10937	1.33E-02	1.00E+00	2.45E-01	604; 970; 1026; 1466; 1839; 2149; 2239; 2247; 2266; 3725; 4771; 6772; 7048; 9314	200887_S_AT; 201464_X_AT; 201466_S_AT; 202284_S_AT; 203140_AT; 203821_AT; 203989_X_AT; 204422_S_AT; 204983_S_AT; 206508_AT; 207030_S_AT; 208944_AT; 213281_AT; 218915_AT; 219612_S_AT; 221841_S_AT; 38037_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT
GO Molecular Function	glycosaminoglycan binding	4	70	82	11065	1.45E-02	1.00E+00	2.44E-01	960; 1839; 2247; 11167	203821_AT; 204422_S_AT; 204490_S_AT; 208782_AT; 209835_X_AT; 212063_AT; 38037_AT
GO Biological Process	response to biotic stimulus	12	71	821	10937	1.46E-02	1.00E+00	2.72E-01	355; 604; 934; 970; 2637; 4062; 4792; 5696; 5698; 5734; 5743; 6772	200887_S_AT; 201502_S_AT; 203140_AT; 204279_AT; 204748_AT; 204897_AT; 206508_AT; 206773_AT; 208650_S_AT; 209040_S_AT; 209771_X_AT; 210560_AT; 215719_X_AT; 266_S_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Cellular Component	extracellular space	8	74	391	10787	1 63E-02	1 00E+00	1 41E-01	1839, 2247, 2266, 3486, 4312, 5328, 11167, 27122	203821_AT, 204422_S_AT, 204475_AT, 205479_S_AT, 208782_AT, 212143_S_AT, 214247_S_AT, 219612_S_AT, 38037_AT
GO Biological Process	STAT protein nuclear translocation	2	71	3	10937	1 91E-02	1 00E+00	3 40E-01	2149, 6772	200887_S_AT, 203989_X_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	blood coagulation	4	71	92	10937	2 10E-02	1 00E+00	3 85E-01	2149, 2266, 5328, 7980	203989_X_AT, 205479_S_AT, 209278_S_AT, 219612_S_AT
GO Biological Process	hemostasis	4	71	97	10937	2 41E-02	1 00E+00	4 37E-01	2149, 2266, 5328, 7980	203989_X_AT, 205479_S_AT, 209278_S_AT 219612_S_AT
GO Biological Process	immune response	10	71	682	10937	2 91E-02	1 00E+00	5 18E-01	355, 604, 934, 970, 2637, 4062, 5696, 5698, 5734, 5743	203140_AT, 204279_AT, 204748_AT, 204897_AT, 206508_AT, 206773_AT, 208650_S_AT, 209040_S_AT, 209771_X_AT, 210560_AT, 215719_X_AT, 266_S_AT
GO Molecular Function	receptor binding	8	70	494	11065	3 37E-02	1 00E+00	5 24E-01	970, 1839, 2149, 2247, 2266, 4062, 6281, 27122	200872_AT, 203821_AT, 203989_X_AT, 204422_S_AT, 206508_AT, 206773_AT, 214247_S_AT, 219612_S_AT, 38037_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	transmembrane receptor protein serine/threonine kinase signaling pathway	3	71	48	10937	3.77E-02	1.00E+00	6.13E-01	7048; 11217; 25805	202760_S_AT; 203304_AT; 208944_AT
GO Biological Process	tyrosine phosphorylation of STAT protein	2	71	6	10937	3.78E-02	1.00E+00	6.13E-01	2149; 6772	200887_S_AT; 203989_X_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	peptidyl-tyrosine phosphorylation	2	71	8	10937	5.01E-02	1.00E+00	7.23E-01	2149; 6772	200887_S_AT; 203989_X_AT; AFFX-HUMISGF3A/M97935_3_AT; AFFX-HUMISGF3A/M97935_MA_AT; AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	defense response	10	71	756	10937	5.09E-02	1.00E+00	7.29E-01	355; 604; 934; 970; 2637; 4062; 5696; 5698; 5734; 5743	203140_AT; 204279_AT; 204748_AT; 204897_AT; 206508_AT; 206773_AT; 208650_S_AT; 209040_S_AT; 209771_X_AT; 210560_AT; 215719_X_AT; 266_S_AT
GO Molecular Function	cell adhesion molecule activity	6	70	330	11065	5.46E-02	1.00E+00	7.15E-01	960; 4162; 4240; 5099; 7791; 8406	204490_S_AT; 204955_AT; 205534_AT; 209087_X_AT; 209835_X_AT; 210605_S_AT; 212063_AT; 215706_X_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Molecular Function	oxidoreductase activity\, acting on paired donors\, with incorporation or reduction of molecular oxygen\, miscellaneous	2	70	9	11065	5 48E-02	1 00E+00	7 15E-01	3162, 5743	203665_AT, 204748_AT
GO Molecular Function	heparin binding	3	70	61	11065	5 54E-02	1 00E+00	7 22E-01	1839, 2247, 11167	203821_AT, 204422_S_AT, 208782_AT, 38037_AT
GO Biological Process	organogenesis	11	71	901	10937	5 98E-02	1 00E+00	7 88E-01	1466 1545, 1808, 1839, 2247, 2637, 4062, 6876, 7168, 9060, 9314	200762_AT, 202436_S_AT, 203058_S_AT, 203821_AT, 204422_S_AT, 205547_S_AT, 206116_S_AT, 206773_AT, 207030_S_AT, 210560_AT, 210986_S_AT, 210987_X_AT 221841_S_AT 38037_AT
GO Biological Process	peptidyl-tyrosine modification	2	71	10	10937	6 22E-02	1 00E+00	7 99E-01	2149 6772	200887_S_AT, 203989_X_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	response to pest/pathogen/parasite	7	71	444	10937	6 42E-02	1 00E+00	8 12E-01	604, 934, 2637, 4062, 4792, 5743, 6772	200887_S_AT 201502_S_AT 203140_AT 204748_AT 206773_AT 208650_S_AT 209771_X_AT 210560_AT 266_S_AT AFFX HUMISGF3A/M97935_3_AT AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	protein kinase cascade	4	71	155	10937	7 69E-02	1 00E+00	8 69E-01	2149, 2247, 4792, 6772	200887_S_AT, 201502_S_AT, 203989_X_AT, 204422_S_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	posttranslational membrane targeting	2	71	14	10937	8 60E-02	1 00E+00	8 98E-01	2239, 4062	204983_S_AT, 206773_AT
GO Cellular Component	extracellular	13	74	1175	10787	9 52E-02	1 00E+00	6 87E-01	1839, 2239, 2247, 2266 3486, 4312, 4771, 5328, 7052, 7078, 7980, 11167, 27122	201042_AT, 201149_S_AT, 203821_AT 204422_S_AT, 204475_AT, 204983_S_AT, 205479_S_AT, 208782_AT, 209278_S_AT, 212143_S_AT, 214247_S_AT, 218915_AT, 219612_S_AT, 38037_AT
GO Molecular Function	oxidoreductase activity\, acting on paired donors\, with incorporation or reduction of molecular oxygen	3	70	84	11065	9 65E-02	1 00E+00	9 08E-01	1545, 3162, 5743	202436_S_AT, 203665_AT, 204748_AT
GO Biological Process	cell surface receptor linked signal transduction	11	71	991	10937	9 86E-02	1 00E+00	9 34E-01	330, 2149, 5734, 7048, 7052, 11186, 11217, 25805, 27122, 55970 116984	201042_AT, 202760_S_AT, 203304_AT, 203989_X_AT, 204346_S_AT, 204897_AT, 208944_AT, 210538_S_AT, 212294_AT, 213618_AT, 214247_S_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	regulation of cell cycle	6	71	384	10937	9 90E-02	1 00E+00	9 35E-01	1026, 2149, 2247, 3725, 4771, 6772	200887_S_AT, 201464_X_AT, 201466_S_AT, 202284_S_AT, 203989_X_AT, 204422_S_AT, 213281_AT, 218915_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT
GO Biological Process	anti-apoptosis	3	71	88	10937	1 09E-01	1 00E+00	9 49E-01	330, 355, 7052	201042_AT, 210538_S_AT, 215719_X_AT
GO Biological Process	negative regulation of apoptosis	3	71	88	10937	1 09E-01	1 00E+00	9 49E-01	330, 355, 7052	201042_AT, 210538_S_AT, 215719_X_AT
GO Molecular Function	signal transducer activity	18	70	2009	11065	1 09E-01	1 00E+00	9 30E-01	355, 960, 970, 1839, 2149, 2239, 2247, 2266 4062, 5734, 6281, 6772, 7048, 7791, 11217, 25805, 27122, 55970	200872_AT, 200887_S_AT, 202760_S_AT, 203304_AT, 203821_AT, 203989_X_AT, 204422_S_AT, 204490_S_AT 204897_AT, 204983_S_AT, 206508_AT, 206773_AT, 208944_AT, 209835_X_AT, 212063_AT, 212294_AT, 214247_S_AT, 215706_X_AT, 215719_X_AT, 219612_S_AT, 38037_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT

System	Gene Category	List Hits	List Total	Population Hits	Population Total	EASE score	Bonferroni	Bootstrap within system	Gene identifiers	Affymetrix probesets
GO Biological Process	intracellular signaling cascade	9	71	761	10937	1 12E-01	1 00E+00	9 50E-01	2149, 2247, 4792, 5734, 5865, 6772, 7052, 9411, 11186	200887_S_AT, 201042_AT, 201502_S_AT, 203910_AT, 203989_X_AT, 204346_S_AT, 204422_S_AT, 204897_AT, 205925_S_AT, AFFX-HUMISGF3A/M97935_3_AT, AFFX-HUMISGF3A/M97935_MA_AT, AFFX-HUMISGF3A/M97935_MB_AT