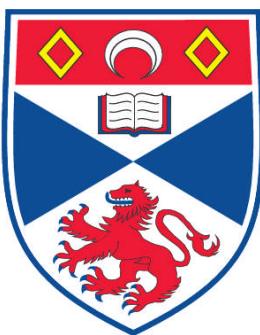


**ADENOVIRUS AND ITS INTERACTION WITH HOST CELL
PROTEINS**

Sharon Carr

**A Thesis Submitted for the Degree of MPhil
at the
University of St. Andrews**



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Adenovirus and its interaction with host cell proteins

Sharon Carr

School of Biomedical Sciences

University of St Andrews

A thesis submitted for the degree of Master of Philosophy

September 2006

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CONTENTS

Declaration.....	2
List of figures.....	16
List of tables.....	18
List of abbreviations.....	19
Acknowledgments.....	27
<u>ABSTRACT</u>	28
<u>1. INTRODUCTION</u>	29
1.1 Adenoviruses.....	29
1.1.1 Adenoviruses.....	29
1.1.2 The family adenoviridae.....	29
1.1.3 Human Adenoviruses.....	30
1.1.4 Virion structure.....	31
1.1.5 Genome organisation.....	34
1.2 E1A region.....	35
1.2.1 The E1A region.....	35
1.2.2 E1A binds p21 and related CDK inhibitors thereby stimulating cell division and growth.....	36
1.2.3 E1A binds to cyclins A and E-CDK complexes, which regulate passage into cell DNA synthesis.....	37

1.2.4	E1A binds to the p300 / CBP family of transactivators and has numerous effects.....	39
1.2.5	E1A binds to Rb / p130 family of proteins and in doing so thus releases E2F.....	42
1.2.6	E1A binds to TATA-box-binding protein (TBP) and TBP associated proteins.....	43
1.2.7	E1A is required for transformation.....	44
1.2.8	The E1A proteins induce apoptosis through a p53-dependent pathway.	45
1.2.9	The E1A region also induces apoptosis via a p53-independent pathway.	46

1.3 The E1B region

1.3.1	Proteins produced by the E1B region and their localisation patterns..	47
1.3.2	E1B-19kDa protein.....	47
1.3.3	The E1B55kDa protein is required for efficient shutoff of host cell replication.....	48
1.3.4	E1B-55kDa and E4orf6 proteins interact.....	49
1.3.5	The E1B-55kDa protein facilitates the cytoplasmic accumulation of late region viral mRNAs during infection.....	49
1.3.6	E4 Orf6 causes relocalisation of E1B-55kDa protein from the cytoplasm to the nucleus.....	51
1.3.7	E4 Orf6 and E1B-55kDa proteins form a complex and shuttle between the nucleus and the cytoplasm.....	52
1.3.8	E1B-55kDa protein can shuttle between the nucleus and the cytoplasm independently of E4 Orf6.....	52

1.3.9 E4 Orf3 also relocates E1B-55kDa protein from the cytoplasm to the nucleus.....	53
1.3.10 Identification of cellular proteins that interact with E1B-55kDa protein.....	55
1.3.11 The E1B55-kDa protein is phosphorylated.....	55
1.3.12 The E1B55kDa protein interacts with p53.....	56
1.3.13 E1B-55kDa protein regulates the levels of p53 protein.....	58
1.3.14 E1B-55kDa protein acts as a transcriptional repressor of p53.....	58
1.3.15 E1B-55kDa protein has no effect on the p53 related protein p73.....	59
1.3.16 The E1B55-kDa protein inhibits p53 acetylation by PCAF.....	60
1.3.17 The E1B-55kDa protein interacts with a mSin3A/Histone deacetylase complex.....	60
1.3.18 Degradation of p53 by E1B-55kDA and E4 Orf6 proteins occurs via a novel mechanism involving a Cullin-containing complex.....	61
1.3.19 E1B-55kDa protein interacts with Daxx.....	63
1.4 The E2 region.....	63
1.5 The E3 region.....	64
1.6 The E4 region.....	66
1.6.1 The E4 region encodes 7 different proteins.....	66
1.6.2 The E4 region is required for efficient DNA replication, late gene expression and shutoff of synthesis of host cell proteins.....	66
1.6.3 E4 Orf1.....	67

1.6.4	E4 Orf2.....	68
1.6.5	The E4 Orf3 and E4 Orf6 proteins can compensate for each other.....	68
1.6.6	E4 Orf4.....	70
1.6.7	E4 Orf3/4.....	70
1.6.8	E4 Orf6/7.....	71
1.7	Intermediate and late genes.....	71
1.8	Viral replication.....	72
1.9	Adenovirus as a viral vector.....	73
1.9.1	Why are people interested in studying adenoviruses?.....	73
1.9.2	Adenovirus as a gene therapy vector.....	74
1.9.3	Adenovirus in cancer therapy.....	74
1.10	Small Ubiquitin Modifying protein (SUMO).....	75
1.10.1	Introduction to SUMO.....	75
1.10.2	Viral proteins that are modified by SUMO.....	80
1.10.3	The Cytomegalovirus immediate-early 1 (CMV-IE1) and immediate early 2 proteins (CMV-IE2) are modified by SUMO.....	81
1.10.4	The papillomavirus E1 (PV-E1) protein is modified by SUMO.....	82
1.10.5	The Epstein-Barr virus immediate-early protein BZLF1 (EBV-Z) is modified by SUMO.....	83

1.10.6 The human herpes virus 6 immediate-early 1 (HHV6-IE1) protein is modified by SUMO.....	83
1.10.7 The adenovirus E1B-55kDa protein is modified by SUMO.....	84
1.11 Proteomics methods for studying protein interactions.....	86
1.11.1 Proteomics methods for studying adenoviruses.....	86
1.11.2 Immunoprecipitation.....	88
1.11.3 Yeast II Hybrid.....	89
1.11.4 Tandem Affinity Purification.....	89
1.11.5 Mass Spectrometry.....	90
1.11.6 Aim of this work.....	97
2. MATERIALS AND METHODS.....	99
2.1 DNA Cloning and analysis.....	99
2.1.1 Polymerase chain reaction.....	99
2.1.2 Clean-up of DNA after PCR.....	99
2.1.3 Agarose gel electrophoresis.....	99
2.1.4 Purification of DNA from agarose gels.....	99
2.1.5 Restriction digest of DNA for cloning.....	100
2.1.6 Dephosphorylation of linearised vector DNA.....	100
2.1.7 Ligation of DNA.....	100
2.1.8 Bacterial strains.....	100
2.1.9 Preparation of transformation competent <i>E. coli</i>	101
2.1.10 Preparation of electrocompetent <i>E. coli</i>	101

2.1.11 Transformation of transformation competent <i>E. coli</i>	102
2.1.12 Electroporation of electrocompetent <i>E. coli</i>	102
2.1.13 Media used for <i>E. coli</i>	103
2.1.14 Purification of plasmid DNA.....	103
2.1.15 Purification of plasmid DNA using alkali lysis miniprep method.....	104
2.1.16 Purification of plasmid DNA using alkali lysis maxiprep method....	105
2.1.17 Quantification of DNA.....	105
2.1.18 Phenol chloroform purification and ethanol precipitation of DNA....	105
2.1.19 Use of radiolabelled probes for the detection of positive colonies.....	106
2.1.20 DNA sequence analysis.....	108
2.2 Cell culture.....	108
2.2.1 Cell culture.....	108
2.2.2 Freezing down cells.....	108
2.2.3 Resuscitating cells from liquid nitrogen.....	108
2.2.4 Transient transfection of cells.....	109
2.2.5 Immunofluorescence.....	109
2.2.6 Making recombinant adenoviruses.....	110
2.2.7 Isolation of recombinant viruses by end point dilution.....	111
2.2.8 Screening of recombinant adenoviruses.....	111
2.2.9 Extraction of viral DNA using the Qiagen QIAmp DNA Blood minikit	
.....	112
2.2.10 Amplification of recombinant viruses.....	113
2.2.11 Infecting HeLa spinners with adenovirus.....	113

2.2.12	Arcton extraction of adenovirus.....	113
2.2.13	Virus purification by CsCl gradient.....	114
2.2.14	Titration of virus stocks.....	115
2.2.15	Experiment to determine the optimum concentration of puromycin required to kill HeLa cells.....	115
2.2.16	Generating HeLa stable cell lines.....	116
2.3	Protein purification and analysis.....	117
2.3.1	Quantification of protein using Bradford Assay.....	117
2.3.2	SDS-PAGE.....	117
2.3.3	Coomassie staining.....	118
2.3.4	Western Blotting.....	119
2.3.5	Tandem affinity purification method for TAP/SUMO2 cells.....	119
2.3.6	Tandem affinity purification method for TAP/E1B55k (+/- K104R)..	121
2.3.7	Dignam and Roeder method for harvesting nuclear extracts.....	123
2.3.8	TCA precipitation.....	124
2.3.9	LC-ESI-MS/MS analysis.....	124

3. GENERATION OF RECOMBINANT ADENOVIRUSES

3.1	Introduction.....	126
3.1.1	Introduction.....	125
3.1.2	Strategy for generating the recombinant viruses.....	127

3.2 Results.....	136
3.2.1 Initial strategy for cloning the left hand end of Ad5 into a standard cloning vector.....	136
3.2.2 The second strategy for cloning the left hand end of Ad5 into a standard cloning vector.....	139
3.2.3 The final strategy for cloning the left hand end of Ad5 into a standard cloning vector.....	140
3.2.4 Strategy for replacing the wild type E1B55k with the tagged E1B55k +/- K104R mutation.....	140
3.2.5 Replacing the E1B55k gene in pGEM-5zf(+) with the tagged version +/- K104R mutation using Xba1 and BsaB1 restriction sites.....	146
3.2.6 Replacing the E1B55k gene in pGEM-5zf(+) with the tagged version +/- K104R mutation using homologous recombination.....	149
3.2.7 Replacing the E1B55k gene in pGEM-5zf(+) with the tagged version using Xba1 and Kpn1 restriction sites.....	151
3.2.8 Replacing the E1B55k gene in pGEM-5zf(+) with the tagged version containing the K104R mutation using Xba1 and Mun1 restriction sites.....	153
3.2.9 Generation of the CFP/E1B55k K104R construct using E1B55k K104R from TAP/E1B55k K104R, and cloning into CFP/E1B55k suing Kpn1 and Mun1 restriction sites.....	154
3.2.10 Remaking the TAP/E1B55k and CFP/E1B55k, pGEM-5zf(+) constructs.....	155

3.2.11 Generation of the CFP/E1B55k K104R construct using E1B55k K104R from TAP/E1B55k K104R, and cloning into CFP/E1B55k using Kpn1 and Mun1 restriction sites.....	155
3.2.12 Removal of the mutations from the CFP/E1B55k K104R and TAP/E1B55k K104R constructs, using the E1B55k K104R from the original CFP/E1B55k K104R pECFP-C1 construct.....	156
3.2.13 Removal of the mutation in original CFP/E1B55k K104R pECFP-C1 construct.....	157
3.2.14 Making the pSCB-AdFL ^{-5'ITR} construct.....	158
3.2.15 Initial strategy for generating the pSCB-AdFL ^{-5'ITR} construct using the kanamycin resistance gene.....	158
3.2.16 Strategy for generating the pSCB-AdFL ^{-5'ITR} construct using the chloramphenicol resistance gene.....	159
3.2.17 Generating the recombinant viruses.....	161
3.2.18 Screening the recombinant viruses by DNA sequencing and Western blot analysis.....	161
3.2.19 Plaque purification of the recombinant viruses and generation of stocks.....	162
3.2.20 Analysis of recombinant viruses following plaque purification.....	164
3.3 Discussion.....	166

<u>4. TANDEM AFFINITY PURIFICATION OF TAP/SUMO2 FROM CELL LINES INFECTED WITH ADENOVIRUS TYPE 5</u>	170
4.1 Introduction	170
4.1.1 Introduction	170
4.1.2 Generation of TAP/SUMO-2 stable cell lines	171
4.1.3 Generation of the TAP stable cell lines	171
4.1.4 Amplification of adenovirus type 5	172
4.1.5 Experiments undertaken	173
4.1.6 Tandem affinity purification procedure	174
4.2 Results	175
4.2.1 TAP cell line	176
4.2.2 TAP-SUMO2 cell line uninfected	176
4.2.3 TAP-SUMO2 cell line infected with Ad5 at a M.O.I of 10 for 20 hours	179
4.2.4 Experiment to determine the expression levels of the E1B55k protein	182
4.2.5 TAP-SUMO2 cell line infected with Ad5 at a M.O.I of 10 for 24 hours	184
4.3 Discussion	188

5. GENERATION OF STABLE CELL LINES EXPRESSING TAP/E1B55K AND TAP/E1B55K K104R.....	192
 5.1 Introduction.....	192
5.1.1 Introduction.....	191
5.1.2 Generating the stable cell lines.....	192
5.1.3 Preparation of adenovirus <i>dl338</i>	194
5.1.4 Experiments undertaken.....	194
5.1.5 Tandem affinity purification procedure.....	196
 5.2 Results.....	197
5.2.1 Initial experiment using TAP/E1B55k K104R stable cell line (14B)...	197
5.2.2 Initial experiment using TAP/E1B55k stable cell line (311E).....	197
5.2.3 Optimisation of the tandem affinity purification procedure.....	198
5.2.4 Results from TAP/E1B55k K104R stable cell line (14B).....	200
5.2.5 Results from TAP/E1B55k stable cell line (311E).....	201
5.2.6 Results from preliminary experiments suggest inefficient lysis.....	202
 5.3 Discussion.....	203
6. FUTURE WORK.....	206
 6.1 Generation of recombinant adenoviruses.....	206
 6.2 Tandem affinity purification of TAP/SUMO2 cell lines infected with adenovirus type 5.....	207

6.3 Generation of stable cell lines expressing TAP/E1B55k and TAP/E1B55k K104R.....	208
<u>7. APPENDIX</u>	209
7.1 Details of primers used.....	209
7.2 TAP cells uninfected.....	210
7.3 TAP-SUMO2 cells uninfected (minus the TAP cells uninfected data).....	220
7.3.1 Gel chunks 1,3,7,14.....	220
7.3.2 Gel chunks 2-15.....	232
7.3.3 Gel chunks 10-13.....	260
7.4 TAP-SUMO2 cells infected with adenovirus type 5 at an M.O.I. of 10 for 24 hour (minus the TAP cells uninfected data).....	262
7.4.1 Gel chunks 1-9.....	262
7.4.2 Gel chunks 10-18.....	276
7.5 Proteins identified in adenovirus infected cells but not in uninfected cells (minus the TAP cells uninfected data).....	284
7.5.1 Gel chunks 1-9.....	284
7.5.2 Gel chunks 10-18.....	293
<u>8. BIBLIOGRAPHY.....</u>	298

List of Figures

Figure 1.1: Model of an adenovirus virion.....	32
Figure 2: The genome organisation of human adenovirus type 5.....	34
Figure 1.3: Tandem affinity purification procedure.....	91
Figure 1.4: Sequence of tandem affinity purification tag.....	92
Figure 3.1: Bacmid pSCB-AdFL.....	129
Figure 3.2: Plasmid pECFP-C1 (Clontech).....	130
Figure 3.3: Plasmid pCMV5-TAP.....	131
Figure 3.4: Generation of overlap PCR products.....	133
Figure 3.5: Homologous recombination occurs to generate full length recombinant virus.....	134
Figure 3.6: Knocking out the 5' ITR of pSCB-AdFL using chloramphenicol resistance gene.....	135
Figure 3.7: Linkers used to modify the MCS of pUC19 vector.....	138
Figure 3.8: Left hand end of adenovirus released by cleaving pSCB-AdFL with restriction enzymes <i>SalI</i> and <i>XhoI</i>	141
Figure 3.9: pGEM-5zf(+) vector (Promega).....	142
Figure 3.10: Primers used to generate the overlap PCR products.....	144
Figure 3.11: The separate stages of overlap PCR.....	145
Figure 3.12: Different primers used to sequence left hand end of adenovirus genome.....	148
Figure 3.13: Sequence of clones generated from cloning left hand end of Ad5 into pGEM-5zf(+) vector using <i>XbaI</i> and <i>BsaB1</i> restriction sites.....	150
Figure 3.14: Control used for homologous recombination.....	152

Figure 3.15: Western blot of recombinant adenoviruses generated.....	163
Figure 4.1: SDS-PAGE gel showing eluates from tandem affinity purification experiment using TAP stable cell lines.....	177
Figure 4.2: SDS-PAGE gel showing eluates from tandem affinity purification experiment using TAP-SUMO2 stable cell lines.....	178
Figure 4.3: SDS-PAGE gel showing eluates from tandem affinity purification experiment using TAP-SUMO2 cells infected with adenovirus at an M.O.I of 10 for 20 hours	180
Figure 4.4: Western blot of eluates from tandem affinity purification experiment, using TAP-SUMO2 cells infected with adenovirus at an M.O.I of 10 for 20 hours, probed with mouse serum to LAP2 β	183
Figure 4.5: Time point of TAP-SUMO2 cells infected with adenovirus type 5. Western blot of cell lysates probed with E1B55k monoclonal antibody.....	185
Figure 4.6: SDS-PAGE gel showing eluates from tandem affinity purification experiment using TAP-SUMO2 stable cell lines infected with Ad5 at an M.O.I of 10 for 24 hours.....	186
Figure 5.1: Western blot of HeLa stable cell lines expressing TAP/E1B55k and TAP/E1B55k K104R constructs.....	193
Figure 5.2: Immunofluorescence analysis of stable cell lines expressing E1B55k and E1B55k K104R.....	195
Figure 5.3: SDS-PAGE analysis of eluates from tandem affinity purification experiments carried out with stable cell lines expressing E1B55k and E1B55k K104R.....	199

List of tables

Table 1: Adenoviral proteins identified in TAP/SUMO2 cells infected with wild type adenovirus 5.....	187
Table 2: Details of primers used.....	209

List of Abbreviations

A	Adenine
ABI	Applied Biosystems
Ad2	Adenovirus type 2
Ad3	Adenovirus type 3
Ad4	Adenovirus type 4
Ad5	Adenovirus type 5
Ad12	Adenovirus type 12
ADP	Adenovirus death protein
APS	Ammonium persulphate

bp	Base pairs
BHK	Baby hamster kidney
BPV	Bovine papillomavirus
BRK	Baby rat kidney
BSA	Bovine serum albumin

CaCl ₂	Calcium chloride
cAMP	cyclic AMP
CAR	Cocksackie B and adenovirus receptor
CBP	CREB binding protein
CBP	Calmodulin binding peptide
Cdk	Cyclin dependent protein kinase
cDNA	complementary DNA

CELO	Chicken embryo lethal orphan virus
CFP	Cyan Fluorescent protein
CHO	Chinese hamster ovary
CIAP	Calf intestinal alkaline phosphatase
CO ₂	Carbon dioxide
CMV	Cytomegalovirus
CR	Conserved region
CRD	Cell cycle regulatory domain
CREB	cAMP response element-binding protein
CsCl	Caesium chloride
Cul	Cullin
DAPI	4'-6-Diamidino-2-phenylindole
DBP	DNA binding protein
dCMP	deoxycytidine monophosphate
dCTP	2'-deoxycytidine 5'-triphosphate
D-MEM	Dulbecco's modified Eagles medium
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
DTT	Dithiothreitol
E	Early
<i>E. coli</i>	<i>Escherichia coli</i>
E1	activating enzyme

E2	conjugating enzyme
E3	Ligation enzyme
EBV	Epstein-Bar virus
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol bis (2-aminoethyl ether)- N, N, N' N'-tetraacetic acid
ER	Endoplasmic reticulum
ESI	Electrospray ionisation
FACS	Fluorescence activated cell sorter
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
G	Guanine
GFP	Green Fluorescent protein
GST	Glutathione-S-transferase
HAT	Histone acetyltransferase
HeLa	Henrietta Lacks
HCMV	Human cytomegalovirus
HDAC	Histone deacetylase
HHV	Human herpes virus
HPLC	High performance liquid chromatography

HIV	Human immunodeficiency virus
hnRNP	heterogeneous nuclear ribonucleoprotein particle
IE	Immediate early
IFN	Interferon
IgG	Immunoglobulin G
ITR	Inverted terminal repeat
kDa	kilodalton
kb	kilobases
kAc	Potassium acetate
L	Litres
L	Late
LB	Luria-Bertani broth
LC	Liquid chromatography
LTR	Long terminal repeats
M	Molar
mM	millimolar
MALDI	Matrix associated laser desorption ionisation
MCS	Multiple cloning site
MDM2	Mouse double minute 2
mg	milligrammes

MgSO_4	Magnesium sulphate
mL	millilitres
mRNA	messenger RNA
MS	Mass spectrometry
MudPIT	Multidimensional protein identification technology

NaAc	Sodium acetate
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
NB(s)	Nuclear bodies
NCS	Newborn calf serum
NEDD8	Neural precursor cell expressed, developmentally down-regulated

NES	Nuclear export signal
NF	Nuclear factor
NPC	Nuclear pore complex
NLS	Nuclear localisation signal

OD	Optical density
Orf	Open reading frame
<i>ori</i>	origin of replication

PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
Pc	Polycomb

pCAF	p300/CBP associated factor
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PIAS	Protein inhibitor of activated STAT
PMF	Peptide mass fingerprinting
PML	Promyelocytic leukaemia
PMSF	Phenylmethylsulphonylfluoride
<i>pol</i>	DNA polymerase
PP2A	Protein phosphatase 2A
pRB	Retinoblastoma protein
pTP	precursor terminal protein
PV	Papillomavirus
PVDF	Polyvinylidene difluoride

R	Residue
RGD	Arginine-glycine-asparatic acid
RNA	Ribonucleic acid
RT	Room temperature

SAE	SUMO activating enzyme
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SDS	Sodium dodecyl sulphate
Ser	Serine
SILAC	Stable isotope labelling by amino acids in cell culture

<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i>
STAT	Signal transducers and activator of transcription
SUMO	Small Ubiquitin modifying protein
TAE	Tris-acetate-EDTA
TAF(s)	TBP associated proteins
TAP	Tandem affinity purification
TBP	TATA-box-binding protein
TCA	Trichloroacetic acid
TEV	Tobacco etch virus
TF	Transcription factor
Thr	Threonine
TIF	Transcription initiation factor
TNF	Tumour necrosis factor
TNFR	Tumour necrosis factor receptor
TP	Terminal protein
TRAIL	Tumour necrosis factor-alpha-related apoptosis-inducing ligand
Tris	2-amino-2-(hydroxymethyl) propane-1, 2-diol
UBL(s)	Ubiquitin like proteins
Ulp	Ubiquitin like protease
UTP	Uridine 5'-trisphosphate
VHL	Von Hippel-Lindau

v/v volume for volume

WT wild type

w/v weight for volume

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I dedicate this thesis to my dad, who is always in my thoughts.

ABSTRACT

The E1B55k gene in adenovirus type 5 was studied by generating recombinant viruses in which the E1B55k gene was N-terminally tagged with either a cyan fluorescent protein (CFP) tag or a tandem affinity purification (TAP) tag. By infecting cells with the recombinant adenovirus expressing CFP tagged E1B55k, localisation of the E1B55k gene during infection could be studied. Generation of the recombinant viruses expressing TAP tagged E1B55k would allow purification of the E1B55k protein and any cellular or viral proteins that interacted with E1B55k. By infecting different cell types and harvesting at set time points during infection, the proteins interacting with E1B55k at different time points during infection could be identified. The E1B55k protein is modified by small ubiquitin modifying protein 1 (SUMO-1) at lysine residue 104 within the SUMO consensus sequence ψ KxE, and SUMOylation can be abolished by mutating the lysine residue to an arginine (Endter et al., 2001). Therefore in order to study the effect of SUMOylation of E1B55k, on adenovirus infection, CFP and TAP tagged recombinant adenoviruses in which the E1B55k protein was mutated to contain an unmodifiable arginine at position 104 were also generated.

Stable cell lines expressing TAP tagged wild type (wt) E1B55k, and E1B55k in which lysine residue 104 had been mutated to an arginine (K104R) were also generated as an alternative method to study the interacting proteins of E1B55k. Four different isoforms of SUMO have been identified, named SUMO1-4. Stable cell lines expressing SUMO-2 were infected with wild type adenovirus type 5 and harvested at set time points in order to determine if any adenoviral proteins were modified by SUMO-2.

INTRODUCTION

1.1 Adenoviruses

1.1.1 Adenoviruses

Adenoviruses are non-enveloped double stranded DNA viruses that contain a linear genome of between 35 and 38 kilobases (kb). They contain inverted terminal repeats (ITR's) at either end of their genome (approximately 100-140bp in length). They were first discovered in 1953, by Rowe and colleagues, who isolated a virus that caused the degeneration of primary cell cultures derived from adenoid tissues (Rowe et al., 1953). In 1954, Hilleman and Werner (Hilleman and Werner, 1954) also isolated virus from respiratory secretions of military recruits and showed that it induced cytopathic changes in cultures of human cells. The viruses isolated by the two different groups were later shown to be related, and in 1956 these agents were termed adenoviruses (Enders et al., 1956). Since their discovery adenoviruses have been isolated from every class of vertebrates including fish, amphibians and reptiles.

1.1.2 The family Adenoviridae

Up until recently, the family adenoviridae only contained two genera, Mastadenovirus and Aviadenovirus, but there were certain types of adenovirus which did not fit properly into either genera. At the XIIth meeting of the International Committee on Taxonomy of Viruses (ICTV), 2000, adenoviruses were divided up into four different genera, Mastadenovirus, Aviadenovirus, Siadenovirus and Atadenovirus. Mastadenoviruses infect mammalian hosts exclusively (including humans, simians, bovines, equines, canines, porcines and

opossums) whilst aviadenoviruses have been found only in birds. Atadenoviruses were so named because of the high Adenine and Thymine (A, T) content of their genomes and they infect various mammalian, avian and reptilian hosts as well as a marsupial. The siadenovirus group are so named because they encode a putative sialidase (Davison, 2002). A sialidase is an enzyme of the hydrolase class that catalyzes the cleavage of glucosidic linkages between a sialic acid residue and a hexose or hexosamine residue at the nonreducing terminal of oligosaccharides in glycoproteins, glycolipids, and proteoglycans. The two siadenoviruses were isolated from turkey (THEV / turkey adenovirus type 3) and frog (Davison et al., 2000).

A fifth proposed genus “Ichtadenovirus” to include the fish adenovirus, which has been partially isolated from the white sturgeon, has not yet been officially proposed to the International Committee on Taxonomy of Viruses (Benko et al., 2002).

1.1.3 Human Adenoviruses

There are currently 51 different serotypes of human adenovirus identified, distinguished on the basis of their resistance to neutralisation by antisera from other known adenovirus serotypes. Type specific neutralisation results predominately from binding of the antibody to the hexon protein and the terminal knob of the fibre protein (see section 1.1.4, virion structure).

The serotypes are divided into 6 species, designated A-F, based on their ability to agglutinate red blood cells, as well as oncogenic, morphological and DNA sequencing studies. The central shaft of the fibre protein is responsible for binding

to erythrocytes and the hemagglutination can be inhibited using antisera from an adenovirus of the same species, but not of a different species (Davison et al., 2003).

Many of the human serotypes are associated with respiratory, gastrointestinal and ocular disease. Recently studies showed that species C adenoviruses persisted and could cause latent infections in approximately 80% of individuals investigated (Garnett et al., 2002). Of the human adenoviruses serotypes 2 and 5 are the most extensively studied, and they belong to species C.

1.1.4 Virion structure

Adenoviruses are non-enveloped icosahedral particles and have a diameter of 70-100nm. Each virion consists of a protein shell surrounding a DNA containing core. The protein shell (capsid) is composed of 252 subunits (capsomeres), of this 240 are hexons and 12 are pentons. Each penton contains a base, which forms part of the surface of the capsid, and a projecting fibre, whose length varies among the different serotypes. The capsid is comprised of seven known polypeptides (types II, III, IIIa, IV, VI, VIII and IX). Of the polypeptides, the hexon protein is composed of polypeptide II and is the most abundant, the fibre protein is composed of polypeptide IV, and the penton base is composed of polypeptide III. The other polypeptides are thought to act as a form of capsid cement. The core of the virion contains four known polypeptides V, VII, μ and terminal protein, and the viral genome. Polypeptide VII is the major core protein and it is thought to serve as a histone-like centre around which the DNA is wound.

Terminal protein is attached to the 5'ends of the adenovirus DNA, circularising it and acting as a primer for DNA replication.

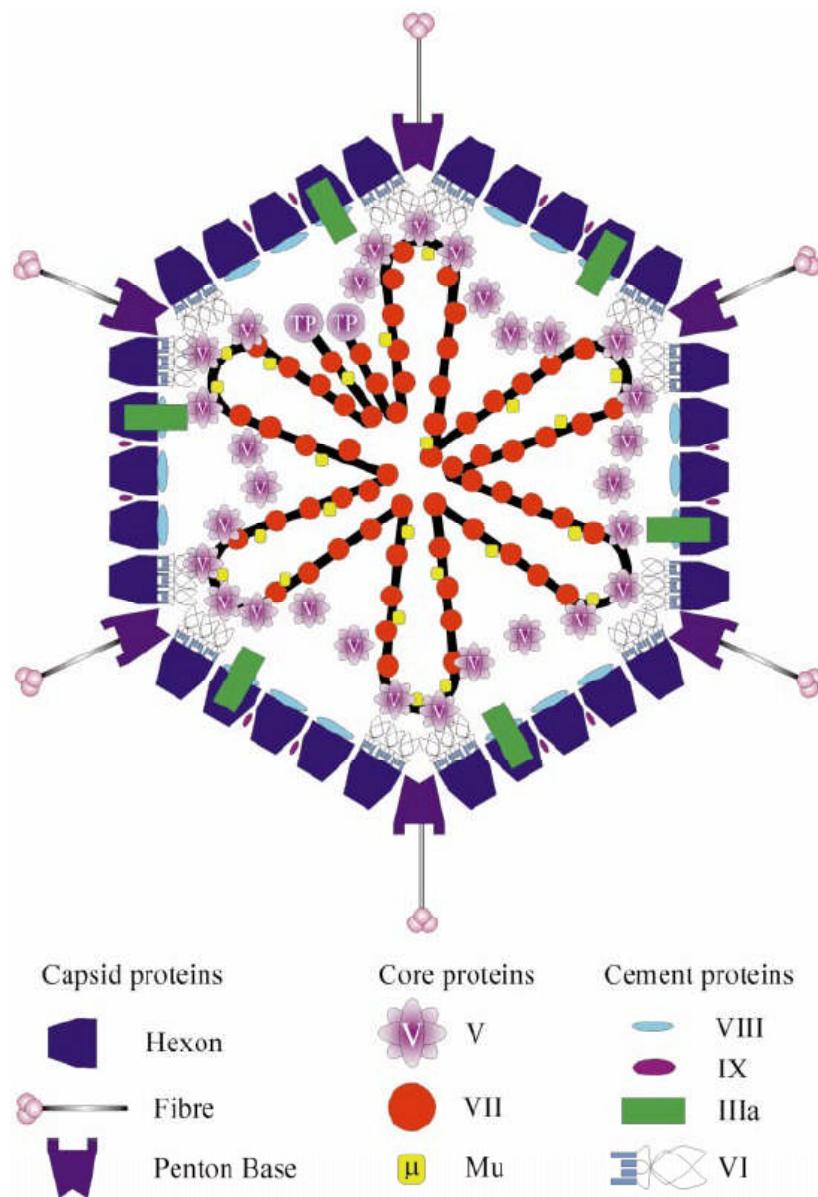


Figure 1.1: Model of an adenovirus virion. This figure was taken from the Russell, W. C., (2000). Update on adenovirus and its vectors. J Gen Virol 81, 2573-2604.

The adenovirus pentons (composed of a penton base and a fibre) are responsible for the attachment and internalisation of the adenovirus into the host cell. The N terminus of the fibre is attached to the penton base, whilst the C terminus folds into a “knob”. The “knob” is required for binding of the virion to the host cell, but it is the penton base interacting with α_v integrins on the cell surface that triggers virus internalisation and membrane permeabilisation. An arginine-glycine-aspartic acid (RGD) motif which is present within the penton base of many adenovirus serotypes serves as a recognition site for these intergrins (Shenk, 1996).

The best known adenovirus receptor is the Cocksackie B and Adenovirus Receptor (CAR), although this receptor is not used by all serotypes. Heparin sulfate glycosaminoglycans (HS-GAGs) have been demonstrated to mediate CAR-independent attachment and infection by Ad2 and Ad5 (Dechechchi et al., 2000). Adenovirus types 11, 35 and 3, which belong to subgroup B have been shown to attach to CD46, a membrane cofactor protein (Gaggar et al, 2003). Of these Ad3 has also been shown to bind to CD80 and CD86 (which are expressed on antigen-presenting cells) (Short et al, 2006). Adenovirus types 8, 19 and 37, which belong to subgroup D have been shown to infect cells after attachment to $\alpha(2-3)$ -linked sialic acid, a common carbohydrate component of glycoproteins and glycolipids (Arnberg et al, 2002).

Upon binding to the receptor, interaction between the penton base and intergrins causes the virus to be internalised in a clathrin-coated vesicle and transported to endosomes (Wickham et al, 1993). Here the acidic pH of the endosome results in partial disassembly of the capsid and escape into the cytoplasm. From here the

virion is transported to the nucleus via microtubules where the viral DNA associates with the nuclear matrix, via the terminal protein and transcription of the viral genome is initiated (Zhang and Arcos, 2005).

1.1.5 Genome organisation

The viral genome is organised into five early transcription units (E1A, E1B, E2, E3 and E4), two early delayed/intermediate transcription units (pIX and IV2a) and five late units (L1-L5). RNA splicing is very important in adenovirus replication as it allows transcription units to encode a multiple number of proteins in a relatively short DNA sequence.

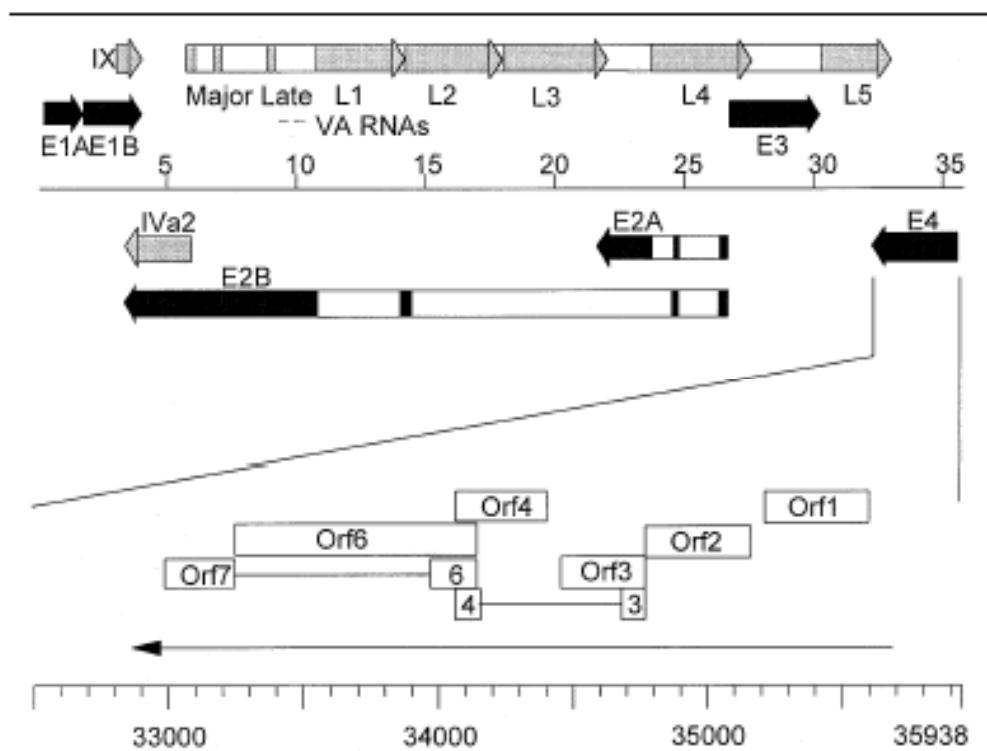


Figure 1.2: The genome organisation of human adenovirus type 5. This figure was taken from Leppard K. N., (1997). E4 gene function in adenovirus, adenovirus vector and adeno-associated virus infections. *J Gen Virol* 78 (Pt 9), 2131-2138.

1.2 The E1A region

1.2.1 The E1A region

During adenovirus infection E1A is the first viral transcription unit to be expressed, and it is essential for adenovirus mediated cell transformation (Mymryk et al., 1994; Shenk, 1996; Shenk and Flint, 1991) (Gallimore and Turnell, 2001; Zhang et al., 2004). E1A has been extensively studied in adenovirus serotype 5, and most of the published literature relates to this serotype. The two major E1A products expressed during the early phase of infection are 289R (residue) and 243R, that differ by the 46 extra amino acids that are present in 289R. These proteins are referred to as 13S and 12S respectively based on the sedimentation coefficients of their mRNAs. Three more proteins are produced later in the infectious cycle, 217R, 171R and 55R, but their function is as yet unknown (Shenk, 1996).

Comparison of E1As between different adenovirus serotypes has shown the presence of four highly conserved regions, CR1-4 (Avvakumov et al., 2002; Kimelman et al., 1985). The 243R protein lacks CR3, which transactivates expression of early viral transcription units E3 and E4, and to some extent E2 and some cellular genes (Shenk and Flint, 1991). AdE1A exerts its effect on the target cell, during both infection and transformation, by binding to and radically altering the activity of a large number of cellular proteins (Straus, 1984).

1.2.2 E1A binds p21 and related CDK inhibitors, thereby stimulating cell division and growth

For virus replication to succeed the virus must induce the host cell to enter the S-phase of the replication cycle, to provide optimal conditions for viral replication. The cell cycle can be divided up into four phases: S-phase, G2-phase, M-phase and G1-phase, which occur in this order. During S-phase the DNA is replicated and during M-phase the cell divides, with G2-phase and G1-phase being the intervals between these events. The cell cycle in normal cells is regulated by a group of enzymes called the cyclin dependent protein kinases (cdks), so named because they are activated by cyclin. Cyclin concentrations rise and fall at specific times in the cell cycle and different cyclin-cdk complexes trigger different steps of the cell cycle. As well as being affected by levels of cyclin, cdk's are also controlled by cdk-inhibitor proteins which can bind to and inhibit their function, reviewed in (Elledge et al., 1996; Hunter and Pines, 1994; Nasmyth and Hunt, 1993; Peters, 1994; Sherr, 1994; Sherr, 1996). The first cdk-inhibitor to be discovered was p21^{CIP1/WAF1}. It was discovered independently, by several different groups, as an inhibitor of cdk2 activity (Gu et al., 1993; Harper et al., 1993), as a transcriptional target of the tumour suppressor p53 protein (el-Deiry et al., 1993) and as a gene that induced senescence (Noda et al., 1994). p21^{CIP1/WAF1} has also been shown to inhibit DNA replication by binding to proliferating cell nuclear antigen (PCNA) (Flores-Rozas et al., 1994; Luo et al., 1995; Waga et al., 1994). It has also been shown to bind to and inactivate stress-activated kinases (Shim et al., 1996).

Keblusek *et al* demonstrated that adenovirus type 5 E1A could partially overcome p21^{CIP1/WAF1} induced G1 arrest. Human bone osteosarcoma epithelial (U2OS) cells were transfected with increasing amount of a plasmid expressing p21^{CIP1/WAF1} (pCMV-p21) and the number of cells arrested in the G1 phase analysed by Fluorescence Activated Cell Sorter (FACS) analysis. When co-transfected with a plasmid expressing E1A (pRSV-5E1A) the number of cells arrested in the G1 phase was reduced. Keblusek et al also demonstrated that the N-terminal domain and the CR1 region of E1A were sufficient for binding to p21^{CIP1/WAF1}. This was demonstrated by performing Glutathione-S-Transferase (GST) pulldowns, using truncated forms of E1A fused to GST (GST-E1A) and *in vitro* translated [³⁵S] methionine-labelled p21^{CIP1/WAF1}.

The binding region of E1A on the p21 protein was also determined. p21^{CIP1/WAF1} contains two separate domains, an N-terminal domain binding to cyclins and cyclin-dependent kinases and a C-terminal domain binding to PCNA (Luo et al., 1995). Results from experiments where GST fusions of the N-terminus and the C-terminus of P21 were made and incubated with cell extracts from E1A transformed cells indicate that E1A binds primarily to the N-terminus of p21^{CIP1/WAF1} (Keblusek et al., 1999).

1.2.3 E1A binds to cyclins A and E-CDK complexes, which regulate passage into cell DNA synthesis

As mentioned previously, progression through the different stages of the cell cycle is regulated by a group of enzymes called the cyclin dependent protein kinases (cdks), which are regulated by cyclin concentrations, which rise and fall during the

cell cycle. The most extensively characterised is the mitotic cyclin, cyclin B, which acts as a regulatory subunit of the serine / threonine protein kinase p32^{cdc2}, controlling entry in to M phase (Booher and Beach, 1987; Booher and Beach, 1988; Booher et al., 1989; Brizuela et al., 1989; Forsburg and Nurse, 1991; Minshull et al., 1990; Solomon et al., 1990). The role of cyclin A is less well defined, although experiments carried out demonstrated that when cyclins A and B were injected into marine invertebrate oocytes that were arrested at the G2/M border of meiosis I, M phase was induced (Swenson et al., 1986; Westendorf et al., 1989). Other reports have also demonstrated that cyclin A is required for S phase in mammalian cells (Girard et al., 1991; Pagano et al., 1992; Tsai et al., 1993; Zindy et al., 1992). As well as cyclins A and B, various other cyclins have been identified, some of which are thought to play a role in the transition from G1 to S phase (Dulic et al., 1992; Koff et al., 1991; Koff et al., 1992; Lees et al., 1992; Lew et al., 1991; Matsushime et al., 1991; Motokura et al., 1991; Xiong et al., 1991).

A number of reports have described a kinase activity associated with ElA (Giordano et al., 1991; Herrmann et al., 1991; Kleinberger and Shenk, 1991) that was able to phosphorylate two of the ElA-associated proteins, p107 and p130 (Herrmann et al., 1991). Results of experiments undertaken suggested that cyclin A was a component of the ElA-associated kinase activity (Giordano et al., 1991; Herrmann et al., 1991; Kleinberger and Shenk, 1991). Faha et al have also demonstrated that p33cdk2 is found in a stable complex with ElA (Faha et al., 1992), and that the kinase activity associated with ElA was due, almost exclusively

to p33cdk. Faha et al also demonstrated that E1A associates with cyclin E, and that the kinase activity associated with E1A consists of two kinase complexes, cyclin A-p33cdk2 and cyclin E-p33cdk2 (Faha et al., 1993).

1.2.4 E1A binds to the p300 / CBP family of transactivators and has numerous effects

CREB binding protein and p300, are two closely related proteins, which belong to a family of transcription co-activators, that can both activate or repress transcription. The transcriptional co-activators do not directly bind to DNA, but are recruited to promoters by sequence-specific DNA binding proteins, including p53 and E2F. Experiments carried out by Kung et al and Yao et al indicate that DNA binding proteins compete for limited amounts of p300 and CBP (Kung et al., 2000; Yao et al., 1998). P300 and CBP are also involved in regulation of the cell cycle and cells derived from p300/- mice have serious proliferative defects (Yao et al., 1998). Both proteins contain several conserved domains, of which one is a histone acetyltransferase (HAT) domain. Histone deacetylase (HDAC) and histone acetyltransferase (HAT) are enzymes that influence transcription by selectively deacetylating or acetylating the ε-amino groups of lysine residues located near the amino termini of core histone proteins. Chromatin acetylation correlates with transcriptional activity, whereas deacetylation correlates with gene silencing. Phosphorylation of p300/CBP by cyclin E/cdk2 results in stimulation of the HAT activity of CBP, and subsequently progression into the S-phase of the cell cycle. Perkins *et al* and Snowden *et al* discovered that the transcriptional activities of p300 and CBP are also regulated by p21^{CIP1/WAF1} (Perkins et al., 1997; Snowden et

al., 2000). Recently, a strong transcriptional repression domain, termed Cell Cycle Regulatory domain 1 (CRD1), was discovered within p300 and CBP. Expression of p21^{CIP1/WAF1} was shown to inhibit this transcriptional repression (Gregory et al., 2002; Snowden et al., 2000). Girdwood et al recently demonstrated that SUMO modification of CRD1 is required for CRD1-dependent transcriptional repression (Girdwood et al., 2003).

Both CBP and p300 bind to the N-terminal domain of the transforming E1A proteins of non-oncogenic human adenovirus 5 (Ad5) and these interactions are important for Ad5 E1A-induced cell immortalization, transformation and repression of neuronal differentiation (Boulukos and Ziff, 1993); for reviews see (Hagmeyer et al., 1995; Moran, 1994; Peper and Zantema, 1993). Dorsman et al also demonstrated that the E1A proteins of oncogenic adenovirus type 12 can also bind CBP and p300 (Dorsman et al., 1997). Both p300 and CBP can complex with another co-activator protein, P/CAF, which also has intrinsic histone acetyltransferase (HAT) activity. Reid et al demonstrated that P/CAF was able to stimulate transcription, independently of CBP/p300, and that this was due to its intrinsic HAT activity (Reid et al., 1998). The E1A protein was shown to displace P/CAF from the p300/CBP co-activator complex (Yang et al., 1996). Reid et al demonstrated that E1A does this by directly binding to P/CAF, independently of CBP, and that residues within the E1A conserved region 1 are required for this. Binding of E1A to P/CAF inhibits its activity as a transcriptional activator (Reid et al., 1998).

Chakravarti et al demonstrated that the carboxy terminus of E1A can inhibit the HAT activity of p300 *in vitro* by functioning as an acetylase inhibitor. p300/CBP have been demonstrated to acetylate p53, thus regulating both its DNA binding and transcriptional activities (Gu and Roeder, 1997; Gu et al., 1997). Chakravarti et al demonstrated that E1A inhibited p300-mediated acetylation of p53, and that this was due to it interacting directly with the HAT domain of p300. E1A was also shown to bind directly to the HAT domain on P/CAF, thus inhibiting its transcriptional activity. Together these data demonstrate that the HAT inhibitor activity of E1A is important for its ability to inhibit p300-dependent transactivation *in vivo* (Chakravarti et al., 1999).

The cAMP dependent transcription factor (CREB), upon phosphorylation, interacts strongly with CBP, and CREB function is dependent on the HAT activity of CBP (Torchia et al., 1997; Yao et al., 1998). The interferon- α dependent transcription factor STAT1 also binds to CBP, and is dependent on its HAT activity for function (Korzus et al., 1998). Perissi et al also demonstrated that E1A inhibits the HAT activity of CBP by binding to the C/H3 domain of CBP (Perissi et al., 1999).

The signal transducer and activator of transcription (STAT) family of proteins are latent transcription factors that are activated by tyrosine phosphorylation in response to cytokine or growth factor stimulation of cells. So far, seven members have been identified in mammalian cells, named 1-7. STAT1 is involved in mediating interferon (IFN) driven gene transcription and can be activated by both type I IFN (α/β) and type II IFN (γ), which are involved in the innate immune response to pathogen infection. Look et al demonstrated that mutant forms of E1A,

which could no longer bind CBP/p300 could still interact directly with STAT1 and block IFN γ -driven, STAT1-dependent gene activation. This interaction occurred between the N-terminal of E1A and the C-terminus region of STAT1 (Look et al., 1998).

1.2.5 E1A binds to Rb / p130 family of proteins and in doing so thus releases E2F

The E2F family of transcription factors, of which there are 7, numbered 1-7, are extremely important in cell cycle regulation and they can be divided up into 4 distinct groups. E2Fs 1-3 are transcriptional activators, and E2Fs 4-5 are transcriptional repressors (Dyson, 1998; Mann and Jones, 1996; Pierce et al., 1998; Trimarchi and Lees, 2002; Wang et al., 2000; Wu et al., 2001). E2F6 and E2F7 both form their own groups and both are thought to be transcriptional repressors (Cartwright et al., 1998; de Bruin et al., 2003; Di Stefano et al., 2003; Gaubatz et al., 1998; Morkel et al., 1997; Trimarchi et al., 1998). E2F1 was the first family member to be discovered as a cellular transcription factor that binds to a specific DNA sequence in the adenovirus E2 promoter (Kovesdi et al., 1986). The E2F-binding sequence has since been found in the promoters of many genes required for DNA replication. DNA binding proteins, DP1 and DP2/3 are required by E2F family members and together they form a heterodimer of E2F and DP (Dyson, 1998).

E2Fs 1-5 activity is regulated by the retinoblastoma RB-family of proteins, which include retinoblastoma protein pRB, p107 and p130. The RB family of proteins can prevent E2F transcription factors from interacting with transcription coactivators thereby inhibiting their transactivation function (Mundle and

Saberwal, 2003). They can also act by recruiting chromatin modifying enzymes to silence transcription, pRB has also been demonstrated to recruit Histone Deacetylase 1 (HDAC1) (Brehm et al., 1998).

In normal cells, cell cycle dependent phosphorylation of pRB by cyclin-cdk complexes releases E2F1 to activate transcription of target genes required for the S-phase of the cell cycle (Harbour and Dean, 2000; Harbour et al., 1999; Lundberg and Weinberg, 1998).

1.2.6 E1A binds to TATA-box-binding protein (TBP) and TBP associated proteins (TAFs)

For mRNA synthesis to occur, RNA polymerase II must assemble in a complex with the necessary transcription factors (TFs) to form a pre-initiation complex (PIC). The initial step of this is the binding of the TIF IID, a complex consisting of TBP and TAFs, to core promoter sequence elements.

Geisberg et al demonstrated that the CR3 region of adenovirus E1A binds to TBP and that this interaction was required for transactivation by E1A (Geisberg et al., 1994).

Mazzarelli et al demonstrated that a region within the C terminus of TAF_{II}135 can bind to the CR3 domain of adenovirus E1A and block its ability to stimulate transcription of an E1A inducible promoter (Mazzarelli et al., 1997).

1.2.7 E1A is required for transformation

All human adenoviruses that have been tested can oncogenically transform cultured rodent cells. Rodent cells do not support lytic infection of adenovirus, but are transformed due to the integration and expression of the early region E1A and E1B genes. This results in cell proliferation, immortalisation, and altered cell morphology. However, only a number of adenoviruses have the ability to directly induce the formation of tumours within rodents. Species A adenoviruses, including serotypes 12, 18 and 31 are highly tumorigenic, whereas species E (serotype 4) and C (serotypes 1, 2, 5 and 6) adenoviruses are not known to be tumour inducing.

Transformation can be induced in human cell lines, as well as rodent cell lines, by transfection with plasmids encoding E1A and E1B genes. Expression of E1A alone is sufficient to immortalise cells, although E1B-19kDa and E1B-55kDa proteins are required to convert cells to a fully transformed tumorigenic phenotype (Graham et al., 1977; Shenk, 1996).

It has also been demonstrated that two early region 4 (E4) gene products of adenovirus type 5, E4 Orf6 and E4 Orf3 proteins, can also co-operate with E1A and E1A plus E1B to substantially enhance transformation (Moore et al., 1996; Nevels et al., 1997; Nevels et al., 1999a; Nevels et al., 1999b).

Originally it was thought that transformed cells continually expressed the viral E1 genes, and that this was how the transformed phenotype was maintained (Graham et al., 1977; Hutton et al., 2000; Zantema et al., 1985). Nevels et al recently demonstrated that transfection of primary baby rat kidney (BRK) cells, with plasmids expressing adenovirus type 5 E1A with either Ad5 E4 Orf3 or E4 Orf6,

resulted in their transformation. The transformed cell lines were then screened by immunoblot analysis and PCR analysis for the presence of viral proteins and viral DNA respectively. The results demonstrated that the viral DNA/viral proteins were not retained after transformation. Although, when plasmids were co-expressed with a plasmid encoding E1B, E1A and E4 proteins were retained. The transformed cell lines, which did not express viral DNA or viral proteins, were then shown to induce tumours when injected into athymic mice. This suggests that transient expression of the viral genes for E1A and E4 Orf6 or E4 Orf3 was enough to induce the fully transformed phenotype. Mutagenesis assays demonstrated E1A to be mutagenic and that both E4 Orf3 and E4 Orf6, enhanced the mutagenic frequency compared to E1A alone (Nevels et al., 2001). These data correlate with results from Shen et al, who reported that cells transformed by combinations of E1A with the HCMV IE1 and IE2 genes, only transiently expressed the HCMV proteins, but accumulated mutations in the cellular p53 gene (Shen et al., 1997).

1.2.8 The E1A proteins induce apoptosis through a p53-dependent pathway

Expression of E1A has been shown to cause an increase in the level of p53 protein and induce p53 dependent apoptosis (Lowe and Ruley, 1993; Querido et al., 1997b; Teodoro et al., 1995). This is due to binding of E1A to pRB and p300, causing release of the E2F transcription factors, which in turn up regulate expression levels of p53 (see section 1.2.5).

Lowe et al demonstrated levels of p53 protein were stabilised in Ad5-transformed cells as well as untransformed cells expressing E1A alone. The increased levels of

p53 in cells expressing E1A alone were found to induce apoptosis. Cells that survived were shown to no longer express E1A and p53 levels in these cells had returned to normal. Apoptosis was not induced in cells that were expressing both the E1A and E1B regions. These results indicate that the E1A region induces p53 dependent apoptosis, which is prevented by the E1B region.

Both 289R and 243R proteins are able to induce apoptosis through a p53 dependent pathway (Lowe and Ruley, 1993).

1.2.9 The E1A region also induces apoptosis via a p53-independent pathway

E1A can induce apoptosis independently of p53 or other adenoviral proteins. Putzer et al demonstrated by FACS analysis that E1A could induce apoptosis in p53 null (SK-OV-3) cells, as Western blot analysis confirmed that Caspase-3, which plays a very important role in apoptosis, was cleaved into its active form. Putzer et al also demonstrated that this apoptotic activity required both p300 and CBP, and it could be inhibited by the E1B 19k protein and pRB (Putzer et al., 2000).

Marcellus et al showed that in Ad5 a product of the E4 region was essential for E1A-induced p53 independent apoptosis. It is the 289R protein of E1A that is responsible for inducing p53 independent apoptosis (Teodoro et al., 1995). In studies using p53 null mouse cells constitutively expressing the E1A region, apoptosis was observed, when cells were infected with an adenovirus lacking E1A and E1B regions, but expressing E2, E3 and E4 regions. The E4 region was implicated when cells were infected with an adenovirus, which expressed the E1B region but lacked the E4 region, did not die of apoptosis to the same extent. An

adenovirus vector lacking both the E1 and E4 regions was unable to induce apoptosis in E1A-expressing cell lines (Marcellus et al., 1996).

1.3 The E1B region

1.3.1 Proteins produced by the E1B region and their localisation patterns

Two proteins are encoded by the E1B region, E1B-55kDa and E1B-19kDa proteins, and they cooperate with the E1A products to induce cell growth. Zantema et al studied the localisation of the E1B proteins by immunofluorescence using cells transformed with the complete E1 region of Ad5. Monoclonal antibodies raised against the E1B-55kDa and E1B19k proteins, showed the E1B19k protein to have a perinuclear localisation, with the E1B-55kDa protein localised to the cytoplasm, as well as discrete cytoplasmic bodies. The p53 protein was also observed in these cytoplasmic bodies and in the nucleus, but not in the cytoplasm (Zantema et al., 1985).

1.3.2 E1B-19kDa protein

The E1B-19kDa protein is capable of cooperating with the E1A gene products in the transformation of primary rodent cells (White and Cipriani, 1990), although it shares no sequence homology with other transforming proteins. E1B-19kDa protein is found associated with both cytoplasmic and nuclear envelope membranes, as well as the intermediate filament cytoskeleton (White and Cipriani, 1989; White et al., 1984). Expression of E1B-19kDa protein destroys the intermediate filament cytoskeleton by affecting the organisation of both vimentin-type filaments in the cytoplasm and the lamin filament network in the nucleus

(White and Cipriani, 1989; White and Cipriani, 1990). As well as modifying the morphology of the infected cell, the E1B-19kDa protein also maintains the integrity of both cellular and viral DNA within the nucleus and negatively regulates viral gene expression and replication (Pilder et al., 1984; Takemori et al., 1984; White et al., 1988; White et al., 1986; White et al., 1984). White et al first proposed that the role of the E1B-19kDa protein was to prevent E1A induced apoptosis (White et al., 1991). In support of this idea, the E1B-19kDa protein was found to be a potent inhibitor of the cytotoxic action of tumour necrosis factor α (TNF α) or anti-Fas antibodies (Gooding et al., 1991a; Hashimoto et al., 1991; White et al., 1992), both of which were known to induce apoptosis (Itoh et al., 1991; Lester et al., 1988). Rao et al demonstrated that expression of the E1A region induced programmed cell death, which could be inhibited by coexpression of the E1B-19kDa protein or the cellular Bcl-2 proto-oncogene (Rao et al., 1992). E1B-19kDa protein is a homologue of Bcl-2.

1.3.3 The E1B55kDa protein is required for efficient shutoff of host cell replication

Babiss and Ginsberg, using Ad5 viruses with mutations in the E1B region affecting both the E1B-55kDa and E1B-19kDa proteins, demonstrated that the E1B-55kDa protein was required to shut off host cell protein synthesis. It was also suggested that a 25kDa E4 protein (E4 Orf6) was required for this to occur (Babiss and Ginsberg, 1984).

1.3.4 E1B-55kDa and E4orf6 proteins interact

Sarnow et al developed a monoclonal antibody, 2A6, which recognises the E1B-55kDa protein from Ad5 and Ad2 (Sarnow et al., 1982). Initial immunoprecipitation experiments undertaken with the monoclonal antibody, using Ad5 infected HeLa and BHK cells, demonstrated that the E1B-55kDa protein interacted with an E4 protein that was 25kDa (E4 Orf6) (Sarnow et al., 1984). Rubenwolf et al, using immunoprecipitation and immunoblotting, mapped the domains in E1B-55kDa protein required for interaction with E4 Orf6 protein in lytically infected A549 cells.

Linker insertion mutations at amino acid 143 and in the central domain of E1B-55kDa eliminated the binding of the E4orf6 protein. Coimmunoprecipitation assays also demonstrated that p53 is not required for the E1B-55kDa and E4 Orf6 protein interaction in adenovirus-infected cells. The region of the E4orf6 protein required for E1B-55kDa interaction was located to the amino-terminal 55 amino acid residues (Rubenwolf et al., 1997).

1.3.5 The E1B-55kDa protein facilitates the cytoplasmic accumulation of late region viral mRNAs during infection

Using an Ad5 mutant (H5dl338) which contained a deletion within the E1B region, preventing expression of E1B-55kDa protein but not the E1B-19kDa protein, Pilder et al demonstrated a 100 fold reduction in adenovirus yield, compared to wild type, during infection of HeLa cells. This mutant virus also failed to transform rat cells at normal efficiency. The levels of the cytoplasmic mRNA were monitored, using Northern analysis, at different time points in cells

infected with either wild type virus or mutant virus H5*dl*338. Compared to the wild type, infection with virus H5*dl*338 was shown to result in reduced levels of mRNAs encoded by the major late transcription unit. In cells infected with H5*dl*338, the amount of cellular actin mRNA was only slightly reduced, whereas infection with wild type virus caused a several fold reduction. By measuring the RNA synthesised in isolated nuclei using [³²P]UTP, it was found that 20-24 hours post infection the transcription rates for the late mRNA families were reduced 3-4 fold in H5*dl*338 virus infected cells, compared to wild type virus. The cellular actin gene as found to be transcribed at similar rates in both mutant and wild type infected cells, suggesting that actin levels were not affected at the level of transcription. Therefore, in order to determine the rate at which newly synthesised mRNA accumulated in the cytoplasm, cultures were labelled continuously with [³H]uridine 12 hours after infection, and the rate of appearance of labelled RNA in the infected-cell cytoplasm monitored by hybridisation. Late viral mRNAs were shown to accumulate in the cytoplasm 2-3 fold less with H5*dl*338 infected cells, compared to wild type infected cells. Accumulation of actin mRNA in the cytoplasm was maintained at all time points monitored in H5*dl*338 infected cells. In cells infected with wild type virus however, accumulation of actin mRNA in the cytoplasm was reduced at 12 hours post infection and terminated at 16 hours post infection. These results showed that the E1B-55kDa protein was essential for the accumulation of viral mRNA in the cytoplasm and to prevent the accumulation of host cell mRNAs. Pilder et al also generated mutant viruses, which did not express the E4 Orf6 protein, and these were shown to display lytic growth characteristics similar to H5*dl*338 (Pilder et al., 1986).

Horridge and Leppard have since shown that the E1B-55kDa protein has RNA-binding activity, which maps to a region of the protein with homology to a family of RNA-binding proteins, and which has been shown previously to be essential for functionality of the protein *in vivo* (Horridge and Leppard, 1998).

1.3.6 E4 Orf6 causes relocalisation of E1B-55kDa protein from the cytoplasm to the nucleus

Goodrum et al determined the localization of the adenovirus type 5 E4 Orf6 and E1B-55kDa proteins in the absence of other adenovirus proteins. When expressed by transfection in human, monkey, hamster, rat, and mouse cell lines, the E1B-55kDa protein was found to be predominantly cytoplasmic, whereas the E4 Orf6 protein accumulated in the nucleus. When coexpressed by transfection in human, monkey, or baby hamster kidney cells, the E1B-55kDa protein colocalised in the nucleus with the E4 protein. This failed to occur in rat or mouse or CHO hamster cell lines. These results suggested that a primate cell-specific factor mediates the functional interaction of the E1B-55kDa and E4 Orf6 proteins of adenovirus (Goodrum et al., 1996).

1.3.7 E4 Orf6 and E1B-55kDa proteins form a complex and shuttle between the nucleus and the cytoplasm

Dobbelstein et al demonstrated that the E1B-55kDa and E4 Orf6 proteins form a complex and together, shuttle between the nucleus and the cytoplasm. The E4 Orf6 protein was found to contain a nuclear export signal (NES), 10 amino acids long, similar to sequences found in the retroviral proteins rev and rex. This sequence was totally conserved between adenovirus types 2 and 5. Mutational analysis of this region greatly reduced shuttling of the E1B55-kDa / E4 Orf6 complex. The E1B55-kDa protein was essential for shuttling of both wild type and mutant E4 Orf6. A nuclear localisation signal (NLS) was also discovered to be present within the C-terminus of E4 Orf6, which was required for the retention of the protein in the nucleus. The retention of E4 Orf6 in the nucleus was abolished by mutation of this sequence or the association with E1B-55kDa protein (Dobbelstein et al., 1997).

Weigel and Dobbelstein discovered by mutational analysis that an NES within the E4 Orf6 protein is essential for the E1B-55kDa/E4 Orf6 complex to leave the nucleus and that virus production was severely reduced in viruses lacking the NES (Weigel and Dobbelstein, 2000).

1.3.8 E1B-55kDa protein can shuttle between the nucleus and the cytoplasm independently of E4 Orf6

Kratzer et al demonstrated that the E1B-55kDa protein shuttled independently from E4 Orf6 and p53, and contained a C-terminal nuclear localization signal (NLS) as well as a N-terminal leucine rich nuclear export signal (NES). The

localization of E1B-55kDa was generally cytoplasmic, but became nuclear in the presence of inhibitors that targeted the CRM1-mediated nuclear export pathway. The E1B-55kDa protein was shown to directly participate in the export of mRNA from the nucleus to the cytoplasm, by accompanying RNA from the nucleus, similar to the retroviral shuttling proteins rev and rex (Kratzer et al., 2000). Dosch et al confirmed that the E1B-55kDa protein was also exported from the nucleus by the CRM1-dependent pathway in adenovirus-infected cells, whereas E4 Orf6 appeared to be transported via a nonspecific mechanism. It was also confirmed that export of E1B-55kDa was inhibited by treatment with Leptomycin B (an inhibitor of the CRM1-dependent export pathway), and by mutation of the essential leucine residues in the NES. The NES was also demonstrated to be transferable to heterologous proteins (Dosch et al., 2001).

1.3.9 E4 Orf3 also relocates E1B-55kDa protein from the cytoplasm to the nucleus

Konig et al demonstrated that E1B-55kDa protein is normally localised to the cytoplasm, in discrete cytoplasmic bodies along with p53 protein. The E4 Orf6 protein caused relocation of E1B-55kDa from the cytoplasm to the nucleus, where both proteins were found in Promyelocytic leukaemia (PML) associated tracks. The p53 protein, no longer bound to the E1B-55kDa protein, was found distributed over the nucleoplasm, where it was free to activate transcription. When co-expressed with E4 Orf3 and E4 Orf6, the E1B-55kDa protein preferentially localised with E4 Orf6. Irrespective of the presence of E4 Orf3, E1B-55kDa and E4 Orf6 caused the inhibition and degradation of p53. In cells that

were infected with virus expressing E4 Orf3 and not E4 Orf6, E1B-55kDa was localised to the nucleus along with E4 Orf3, and p53 was present in the nucleoplasm. These results suggested that during adenovirus infection, the E4 Orf3 protein which is expressed first, binds to E1B-55kDa, liberating p53. Subsequent expression of E4 Orf6 leads to the inactivation and degradation of p53 (Konig et al., 1999).

Leppard and Everett demonstrated an increase in the biochemical interaction between E1B-55kDa and E4 Orf3 in the absence of Orf6, the E1B-55kDa association with E4 Orf3 in Nuclear Domain 10 (ND10) was also found to increase in the absence of Orf6. The E4 Orf3 is known to associate with and cause the reorganisation of cell nucleus structures known as ND10 (Ascoli and Maul, 1991), which contain PML and other proteins such as Daxx, that are involved in a wide range of cellular activities. The E4 Orf3 protein associates with ND10 causing their components to redistribute into tracks (Carvalho et al., 1995).

Different isoforms of PML have been shown to exist, including Small Ubiquitin Modifying (SUMO) protein modified forms. Leppard and Everett demonstrated that the number of PML isoforms was increased in adenovirus infected cells, and that this was E4 Orf3 dependent (Leppard and Everett, 1999).

Corbin-Lickfett and Bridge demonstrated that the region of E4 Orf6 that is essential for proteasome-mediated degradation of p53 was also required to promote viral late gene expression in a complementation assay. Proteasome inhibitors abrogated the E4 Orf6- induced viral late gene expression in the complementation assay, suggesting that the proteasome is required for late viral gene expression (Corbin-Lickfett and Bridge, 2003).

1.3.10 Identification of cellular proteins that interact with E1B-55kDa protein

Ornelles and Shenk proposed that the E1B-55kDa/E4 Orf6 complex facilitated the transport and accumulation of viral mRNAs, while simultaneously blocking the transport of host cell mRNAs, by relocating a cellular factor required for nucleocytoplasmic transport of mRNAs. This correlates with the observation that cellular splicing factors and heterogeneous nuclear ribonucleoprotein particle (hnRNP) proteins are redistributed to the sites of viral RNA transcription and DNA accumulation during the late phase of infection (Jimenez-Garcia et al., 1993). Gabler et al isolated a human gene encoding a novel nuclear RNA-binding protein of the hnRNP family, that associates with the Ad5 E1B-55kDa protein. The regions of E1B55-kDa protein that are required for binding to this protein were mapped and found to overlap with the regions that bound p53, and that interaction between the two proteins is modulated by the E4 Orf6 protein. Expression of E1B-55kDa associated protein interfered with Ad5 E1A/E1B-mediated transformation of primary rat cells. Furthermore, stable expression of E1B-55kDa associated protein in Ad-infected cells overcame the E1B-55kDa dependent inhibition of cytoplasmic host mRNA accumulation (Gabler et al., 1998).

1.3.11 The E1B55-kDa protein is phosphorylated

The E1B55-kDa protein was shown to be phosphorylated (Sarnow et al., 1982; Yee et al., 1983) at both serine and threonine residues (Malette et al., 1983). Teodoro et al mapped two phosphorylation sites to the extreme carboxy terminus of the E1B55-kDa protein at Ser-490 and Ser-491. Mutation of these sites to

alanine residues was shown to greatly reduce the transforming activity and partially blocked repression of p53 dependent transcription by the E1B-55kDa protein, although the mutant was demonstrated to bind p53 at normal levels. (Teodoro et al., 1994). Subsequently, Teodoro et al demonstrated that phosphorylation occurred exclusively at residues Ser-490, Ser-491 and at threonine residue (Thr-495). No phosphorylated forms of E1B55-kDa protein were detected when these sites were mutated to alanine residues, and the result was the complete abrogation of both transcriptional repression and transformation activities of the E1B55-kDa protein (Teodoro and Branton, 1997b).

Teodoro and Branton also demonstrated that the E1B55-kDa protein blocks E1A-induced p53 dependent apoptosis, and that this is dependent on the phosphorylation of E1B-55kDa protein (Teodoro and Branton, 1997a).

1.3.12 The E1B55kDa protein interacts with p53

Sarnow et al, using monoclonal antibody 2A6, raised against E1B-55kDa protein, found that p53 protein was co-precipitated with the E1B-55kDa protein, in immunoprecipitation experiments undertaken in transformed mouse and hamster cells (Sarnow et al., 1982).

Modulation of the cell cycle is also regulated by the protein p53, which was first discovered in 1979 by DeLeo et al, Lane and Crawford, and Linzer et al, (DeLeo et al., 1979; Lane and Crawford, 1979; Linzer et al., 1979) respectively. p53 is a tumour suppressor protein which is present at low levels during normal cell division. Situations such as hypoxia, or DNA damage induced by ionising or UV radiation, lead to the activation of stress signals in the cell, causing increased

levels of p53, resulting in inhibition of cell growth and induction of apoptosis.

Approximately 50% of cancers in man are a result of loss of normal p53 function.

The regulation of p53 is critical in allowing maintenance of the balance between normal cell division and tumour suppression.

MDM2 protein is a key component in regulating p53, and it acts as a ubiquitin ligase (Honda, 1997), binding to it and targeting it for ubiquitination and subsequent degradation by the proteasome (Bottgar et al, 1997; Haupt et al, 1997; Kubbutat et al, 1997). MDM2 is also self-regulating, through autoubiquitination, targeting itself for degradation by the proteasome (Honda and Yasuda, 2000; Fang et al, 2000; Midgley et al, 2000). MDM2 is also a transcriptional target of p53, so as levels of p53 are increased, so too are levels of MDM2, resulting in a decrease in levels of p53. The importance of MDM2 was demonstrated in an experiment where mice were generated lacking MDM2 and the result was embryonic lethality. This lethality was also demonstrated in mice lacking MDMX, a protein related to MDM2 (Jackson and Berberich, 2000). Phosphorylation of p53 by DNA-dependent protein kinase decreases the interaction between p53 and MDM2, resulting in increased levels of p53. The p19ARF protein also increases p53 levels by interacting with MDM2, inhibiting its activity as a ubiquitin ligase for p53 (Foster and Lozano, 2002).

P53 is a sequence-specific DNA-binding protein and a transcription factor, capable of both activating and repressing transcription of cellular genes. An increase in p53 levels results in increased transcription of cellular genes responsible for cell cycle arrest (p21) and apoptosis (Bax) (Xu et al, 1998).

1.3.13 E1B-55kDa protein regulates the levels of p53 protein

Teodoro and Branton have shown that the E1B-55kDa protein blocks p53 dependent apoptosis and that this was dependent on the phosphorylation of E1B-55kDa protein (Teodoro and Branton, 1997b).

Querido et al demonstrated that the E1A 243R protein induced an increase in p53 levels resulting in p53-dependent apoptosis, in the absence of E1B-55kDa and E1B-19kDa proteins. Mutational analysis indicated that the increase in p53 levels was dependent on 243R forming a complex with p300 or pRB. The E1A 243R mutants which could not bind p300 or pRB could not induce increased levels of p53.

Increased level of p53 correlated with the induction of apoptosis (Querido et al., 1997b).

Querido et al showed that the 289R E1A protein did not induce increased levels of p53, and in the presence of E1B-55kDa protein, blocked that induced by E1A 243R. This inhibition was shown to require E4 Orf6, produced as a result of transactivation by the 289R E1A protein, and E1B-55kDa proteins, suggesting that interactions involving p53, E1B-55kDa and E4 Orf6 proteins may play a role in preventing E1A 243R-induced accumulation of p53 (Querido et al., 1997a).

1.3.14 E1B-55kDa protein acts as a transcriptional repressor of p53

The E1B-55kDa protein binds to the amino terminus of p53 (Kao et al., 1990) and blocks its ability to activate transcription (Yew et al., 1994), thus acting as a transcriptional repressor.

Yew et al demonstrated this by creating a fusion protein of E1B-55kDa protein and a GAL4 DNA binding domain, which was shown to repress transcription from

a variety of promoters with engineered upstream GAL4-binding sites. Mutations that affected the transforming activity of E1B55-kDa protein and its ability to inhibit p53-mediated trans-activation, also reduced the repression activity of the E1B55-kDa/GAL4 fusion protein. These results demonstrated that the E1B55-kDa protein functions as a direct transcriptional repressor that is targeted to p53-responsive genes by binding to p53. Several domains within the E1B55-kDa protein contribute to the stable association with p53, with the essential region lying between residues 224 and 354 (Yew et al., 1994).

1.3.15 E1B-55kDa protein has no effect on the p53 related protein p73

Roth et al demonstrated that although the E1B-55kDa and E4 Orf6 Proteins bind and inactivate the p53 tumour suppressor protein, they had no effect on the cellular protein p73, which shows extensive similarities to p53 in both structure and function. The E1B-55kDa protein did not bind p73 nor did it inhibit p73-induced transcription, and neither E1B-55kDa protein or E4 Orf6 promoted degradation of p73. The switching of 5 amino acids near the amino terminus, between the p53 and p73 proteins, rendered the p53 protein resistant and the p73 protein susceptible to E1B-55kDa/E4 Orf6 induced degradation (Roth et al., 1998).

Higashino et al, subsequently went on to demonstrate that the E4 Orf6 protein interacted with p73, inhibiting p73-mediated transcriptional activation (Higashino et al., 1998).

1.3.16 The E1B55-kDa protein inhibits p53 acetylation by PCAF

The transactivation activity of p53 is dependent on its ability to bind to specific DNA sequences near the promoter region of target genes.

It has been demonstrated that the acetylases p300, CREB binding protein (CBP) and PCAF acetylate p53 protein, enhancing its sequence-specific DNA-binding activity(Gu and Roeder, 1997; Gu et al., 1997; Liu et al., 1999; Sakaguchi et al., 1998). Liu et al demonstrated that the E1B-55kDa protein specifically inhibited p53 acetylation by PCAF *in vivo* and *in vitro*, and that the DNA binding activity of p53 was diminished in cells expressing E1B-55kDa. Data suggested that the E1B55-kDa protein interfered with the physical interaction between PCAF and p53 (Liu et al., 2000).

1.3.17 The E1B-55kDa protein interacts with a mSin3A/Histone deacetylase complex

Within the cell, DNA molecules are wound around structures known as core histones, and the reversible acetylation of these histones is an important regulatory mechanism for controlling gene expression. Histone acetylation is associated with activated transcription and is carried out by histone acetyltransferases (HATs). Deacetylation is associated with transcriptional repression and is carried out by histone deacetyltransferases (HDACs). In mammalian cells HDAC1 and HDAC2 are found in a complex with mSin3A (a transcription corepressor protein). Punga and Akusjarvi showed the E1B-55kDa protein interacts with HDAC1 and mSin3A, both in an adenovirus-transformed 293 cell line and during a lytic adenovirus infection. It was also demonstrated that the E1B-

55kDa/HDAC1/mSin3A complex was enzymatically active, catalyzing deacetylation of a histone substrate peptide (Punga and Akusjarvi, 2000).

1.3.18 Degradation of p53 by E1B-55kDa and E4 Orf6 proteins occurs via a novel mechanism involving a Cullin-containing complex

It is known that E4 Orf6 and E1B-55kDa proteins inhibit transcriptional activation by the tumour suppressor protein p53. Several different groups have shown that these proteins function together to reduce the half-life of p53 and induce p53 degradation (Cathomen and Weitzman, 2000; Moore et al., 1996; Nevels et al., 2000; Querido et al., 1997a; Shen et al., 2001; Steegenga et al., 1998).

Querido *et al* demonstrated that this degradation of p53 occurred independently of MDM2 and p19ARF, but required an extended region of E4 Orf6 which seemed to possess three separate biological functions; interaction with E1B-55kDa, interaction with two cellular proteins 19kDa and 14kDa in size, and a nuclear localization signal (NLS). Degradation was also significantly blocked by the 26S proteasome inhibitor MG132 (Querido et al., 2001b).

Querido et al have since determined the mechanism of adenovirus-induced p53 degradation through identification of E4 Orf6-associated cellular proteins. The E4 Orf6 protein was demonstrated to assemble in a multi-protein complex consisting of E1B-55kDa, Elongins B and C, Cul5 and the RING-H2 finger protein Rbx1, and that this complex functioned as an E3 ubiquitin ligase that promoted the ubiquitination and subsequent degradation of p53 *in vivo*. This complex is very similar to other E3 ubiquitin ligase complexes including the von Hippel-Lindau

(VHL) tumor suppressor and SCF (Skp1–Cul1/Cdc53–F-box) E3 ubiquitin ligase complexes. The E4 Orf6 complex was shown to be capable of stimulating ubiquitination of p53 *in vitro* in the presence of E1 activating and E2 conjugating enzymes. In order to determine if the Cullins were essential for adenovirus-induced p53 degradation, experiments were undertaken in Chinese hamster ovary (CHO) cells that are temperature sensitive for the NEDD8 pathway, as Cullins are activated via the NEDD8 pathway. Ubiquitin mediated degradation of p53 did not occur at the nonpermissive temperature indicating that the Cullins are essential for this to occur (Querido et al., 2001a).

Harada et al immunopurified an E1B-55kDa-associated complex from Ad5 infected HeLa cells. SDS-PAGE and mass spectrometric analysis identified these proteins to include Cullin-5, Rbx1 and Elongins B and C, pp32 and importin- α 1. Harada et al also demonstrated that this complex was capable of causing the polyubiquitination of p53 *in vitro*, and that the components existed together as a single high-molecular-weight complex *in vivo* (Harada et al., 2002).

Blanchette et al demonstrated that the E4 Orf6 protein contained two conserved BC-box motifs, that are present in Elongin C-interacting proteins, and that these mediated interactions with Elongins B and C during complex formation. These motifs were shown to be required for E1B-55kDa/E4 Orf6 induced degradation of p53. Data suggested that the E1B-55kDa protein did not directly bind to E4 Orf6 but bound it as part of a complex with Cul5 and Elongins B and C (Blanchette et al., 2004).

1.3.19 E1B-55kDa protein interacts with Daxx

Using the Yeast II Hybrid system Zhao et al discovered the E1B-55kDa protein interacted with Daxx. Mutation of three potential phosphorylation sites (S489/490 and T494 to alanine) within the E1B 55-kDa protein did not affect its interaction with Daxx, although such mutations were previously shown to inhibit E1B-55kDa's ability to repress p53-dependent transcription and to enhance transformation. Daxx coimmunoprecipitated with E1B-55kDa protein in 293 cell extracts, and immunofluorescence studies showed that Daxx colocalised with E1B-55kDa protein within discrete nuclear dots, along with p53 protein (which were distinct from ND10). Overexpression of PML dramatically increased the colocalisation of Daxx and PML, and reduced the colocalisation of Daxx, p53 and E1B-55kDa. Expression of the E1B-55kDa protein in Saos2 osteosarcoma cells led to a reduction in the number of ND10, leading to the suggestion that E1B55kDa and PML compete for the available Daxx within the cell (Zhao et al., 2003).

1.4 The E2 region

The E2 region, divided into E2A and E2B regions, encodes three different proteins, all of which are directly involved in DNA replication. The gene product of E2A is the abundant nuclear DNA-binding protein (DBP), with the E2B region encoding the precursor terminal protein (pTP) and the viral DNA polymerase (*pol*). E2A expression is mediated by the E1A region (Shenk and Flint, 1991). Viral DNA replication requires all three E2 proteins, plus two cellular proteins,

nuclear factor I (NFI) and nuclear factor III (NFIII) (Challberg and Rawlins, 1984).

Replication of adenoviral DNA is initiated at the origin of DNA replication (*ori*), which is located within the terminal 51bp of the ITRs (Hay and Russell, 1989). A complex of two viral proteins, pTP and *pol* bind here. The pTP protein is synthesised as an 80kDa protein, which is active in initiation of replication (Challberg et al., 1980; Stillman et al., 1981). During virus assembly it is processed by proteolysis to generate a 55kDa fragment, TP, that is covalently attached to the 5' end of the adenoviral genome, where it is thought to be an additional *cis*-acting component of *ori* (Challberg and Kelly, 1981). The *pol* protein contains both 5' to 3' polymerase and 3' to 5' exonuclease activity that probably serves as a proofreading function during polymerisation (Field *et al.*, 1984). The pTP and *pol* proteins bind to the *ori* as a heterodimer. NFI and NFIII also bind within this 51bp region and act by stabilising binding of the pTP-*pol* complex.

The pTP protein acts as a primer for DNA replication (Rekosh et al., 1977), preserving the integrity of the terminal sequence of the viral chromosome during subsequent rounds of DNA replication. DNA replication is initiated by a protein priming mechanism in which a covalent linkage is formed between the α -phosphoryl group of the terminal residue, dCMP, and the β -hydroxyl group of a serine residue in pTP, a reaction catalysed by *pol* (Challberg et al., 1980). Chain elongation then requires *pol* and DBP. The role of DBP was established by the generation of viruses with temperature-sensitive mutations in the DBP gene that

were unable to replicate viral DNA at non-permissive temperatures (Ensinger and Ginsberg, 1972).

1.5 The E3 region

The E3 region, which is dispensable for the replication of adenovirus, encodes a range of proteins that subvert the host immune response. The E3 promoter contains NF κ B-binding sites that can be induced by cytokines such as tumour necrosis factor (TNF) (Deryckere et al., 1995).

The adenovirus E3-19kDa glycoprotein (gp19k) is a glycoprotein localised in the membrane of the endoplasmic reticulum (ER), where it binds to major histocompatibility complex (MHC) class I antigens, preventing their export from the ER to the cell surface. It is the presence of a lysine based motif (KKXX) that is responsible for retention in the ER (Jackson et al., 1993).

The E3-10.4kDa/14.5kDa complex (RID α / β) also inhibit TNF α and Fas ligand-induced cell death by internalising their receptors (TNFR1, Fas and TNF-related apoptosis-inducing ligand (TRAIL)-R1) (Elsing and Burgert, 1998; Gooding et al., 1991b; Shisler et al., 1997; Tollefson et al., 1998) and promoting their degradation in lysosomes (Elsing and Burgert, 1998; Shisler et al., 1997; Tollefson et al., 1998). RID α / β localise to the plasma membrane, Golgi and ER and to vesicles within the cell (Tollefson et al., 1998).

The E3-14.7kDa protein (14.7k) inhibits apoptosis through the TNFR, Fas and TRAIL pathways (Chen et al., 1998b; Gooding et al., 1988; Krajcsi et al., 1996), with the effect on Fas being less pronounced. The E3-14.7kDa protein is localised in the cytosol and nucleus (Li *et al.*, 1998) and functions by binding to cellular

proteins that mediate apoptosis including NEMO/IKK γ . Apoptosis induced by transfecting cells with NEMO/IKK γ , is reversed by 70% in the presence of E3-14.7kDa protein (Li et al., 1999). The E311.6kDa adenovirus death protein (ADP) is the only E3 protein not involved in subverting the host cell immune response. It is produced late in infection and induces cell death, resulting in the release of progeny virus from the cell.

1.6 The E4 region

1.6.1 The E4 region encodes 7 different proteins

Transcripts from the E4 region are subject to alternate splicing events, leading to the production of approximately 18 distinct mRNAs, which are predicted to encode 7 different proteins named Open Reading Frame 1 (Orf1), Orf2, Orf3, Orf4, Orf3/4, Orf6, Orf6/7 (Virtanen et al., 1984). All, except Orf3/4 have been demonstrated to exist in infected cells. To date, all of the adenoviruses sequenced appear to have an E4 region, with the exception of chicken embryo lethal orphan virus (CELO), which is an avian adenovirus (Chiocca et al., 1996).

1.6.2 The E4 region is required for efficient DNA replication, late gene expression and shutoff of synthesis of host cell proteins

Halbert et al confirmed the requirement of early region 4 for efficient DNA replication, late gene expression and shutoff of synthesis of host cell proteins by generating mutant Ad5 viruses carrying defined lesions in the E4 region. Mutant virus *dl366*, which lacked the majority of the E4 region was severely defective,

and could only be propagated on stable Vero (monkey kidney) cells expressing the E4 region. Virus *dl355*, which lacked 14bp within the segment encoding the E4 Orf6 protein, showed a delayed onset of viral DNA synthesis. Expression of late viral proteins was reduced in both *dl355* and *dl366*, although more severely in *dl366*. Shutoff of host protein synthesis was also less efficient with the mutant viruses, more so with virus *dl366* (Halbert et al., 1985).

Attempts to identify the specific role of each of the E4 proteins, by mutational analysis of each of the E4 Orfs, have been unsuccessful. This was due to the fact that only a mutation in the E4 Orf6 region demonstrated any phenotypic effect on virus growth, and this effect was minimal compared to deletion of the entire E4 region.

1.6.3 E4 Orf1

The E4 Orf1 protein, in a variety of different adenovirus serotypes, is thought to be involved in transformation, but only when expressed at sufficient levels (Javier, 1994; Ohman, 1995). Orf1 sequences appear to be related to dUTPase enzymes, although they lack an essential conserved dUTPase motif and are inactive in dUTPase assay (Weiss et al., 1997). Although it has been shown that the avian adenovirus CELO has a putative dUTPase gene in a location analogous to E4 Orf1 (Chiocca et al., 1996). The CELO E4 Orf1 gene contains the essential conserved dUTPase motif and is active in a dUTPase assay (Weiss et al., 1997). The role of Orf1 in lytic infection has yet to be determined. E4 Orf1 deficient Ad5 shows no growth defect in HeLa cells (Leppard, 1997).

1.6.4 E4 Orf2

There is at present no functional information regarding the E4 Orf2 protein, although it has been shown to be localised to the cytoplasm in infected HeLas, is produced at early times during adenovirus infection, and is not detected in a complex with other proteins (Dix and Leppard, 1995).

1.6.5 The E4 Orf3 and E4 Orf6 proteins can compensate for each other

The role of E4 Orf3 proteins and E4 Orf6 proteins has been discussed earlier in relation to E1B-55kDa and p53 proteins.

Bridge and Ketner demonstrated that the E4 products Orf3 and Orf6 could compensate for each other. By generating a series of Ad5 mutants with deletions in the E4 region it was demonstrated viral late protein synthesis was essentially normal in viruses that were unable to express either Orf3 or Orf6. In mutants that could not express both Orf3 and Orf6, late protein synthesis was dramatically reduced and plaques formed with an efficiency less than 10^{-6} that of wild type virus. Mutants that lacked Orf3 or Orf6 formed plaques with only slightly less efficiency than wild type virus (Bridge and Ketner, 1989).

Huang and Hearing also generated mutant Ad5 viruses in order to assign specific function to each of the E4 gene products. Mutant viruses which expressed Orf6 but lacked Orf3, behaved like wild type virus. Mutant viruses which expressed Orf3 but lacked Orf6 displayed a delay in the onset of viral replication, reduced levels of viral late protein synthesis and inefficient shutoff of host cell protein synthesis, and a 10 to 20 fold reduction in virus yield. Mutant viruses lacking both Orf3 and Orf6 displayed a significant lag in the onset of viral replication, a dramatically

reduced level of viral late protein synthesis, no obvious shutoff of host cell protein synthesis and up to a 10^5 reduction in virus yield (Huang and Hearing, 1989).

Viruses that lack the E4 region show a number of severe phenotypes, including defects in viral mRNA accumulation, transcription, splicing, late protein synthesis, host cell shutoff and viral DNA replication. This defect is due in part to the production of genome concatemers (Weiden and Ginsberg, 1994), caused by the covalent joining of viral genomes, and resulting in molecules that exceed the packaging capacity of the capsid. Concatemerisation is mediated by the host cell, through a Mre11/Rad50/Nbs1 complex (Boyer et al., 1999; Stracker et al., 2002). In Ad5 expression of E4 Orf6 and E1B-55kDa proteins results in the proteasome-mediated degradation of the Mre11 complex members (Stracker et al., 2002). The E4 Orf3 protein is able to exclude Mre11, Rad50 and Nbs1 from the viral replication centres.

Analyses of infections with serotypes Ad4 and Ad12 demonstrated that the degradation of Mre11/Rad50/Nbs1 proteins is a conserved feature of the E1b55K/E4orf6 complex. The transfection of expression vectors for the E4orf3 proteins of Ad4 and Ad12 did not alter the localization of Mre11 complex members (Stracker et al., 2005).

1.6.6 E4 Orf4

E4 Orf4 also regulates protein phosphorylation in the infected cell by binding to protein phosphatase 2A (PP2A) (Muller, 1992), resulting in the selective hypophosphorylation of some proteins, including E1A. The hypophosphorylated E1A residues have been mapped, and at least one of these residues is a known target for mitogen-activated protein kinase (Whalen et al., 1997). Bondesson et al demonstrated that E4 Orf4 negatively regulates both E4 and E1A transcription, dependent on PP2A activity, and represses E1A-mediated activation of the E4 promoter (Bondesson et al., 1996). These data suggest the presence of a regulatory loop in which E1A activates expression of the E4 region. Expression of the E4 Orf4 protein results in the negative regulation of both E1A and E4 regions (Leppard, 1997).

Mannervik et al demonstrated that adenovirus E1A activation of the viral E2 promoter was abrogated by coexpression of the E4 Orf4 protein. The abrogation of E1A activation of the E2 promoter was deemed to occur through the E2F DNA binding sites present on the E2 promoter, demonstrated by the fact that E4 Orf4 inhibited E2F-1/DP-1 mediated transactivation, as well as E2 mRNA expression during virus growth (Mannervik et al., 1999).

1.6.7 E4 Orf3/4

This protein is predicted to exist, based on analysis of Ad2 mRNA structure in HeLa cells (Virtanen et al., 1984), although the protein has not yet been detected in infected cells.

1.6.8 E4 Orf6/7

E4 Orf6/7 dimerises to link two E2F molecules, thus facilitating binding at two E2F sites present in the Ad2 promoter, and activating transcription (Cress and Nevins, 1994; Huang and Hearing, 1989; Obert et al., 1994). O'Connor and Hearing showed that the E4 Orf6/7 gene product induces binding of the cellular transcription factor E2F to the viral E2A promoter region, which is directly correlated with the transcriptional activation of the E2A promoter *in vivo*. The E4 Orf6/7 protein acts by functionally compensating for the E1A proteins. In the absence of E1A, expression of E4 Orf6/7 is sufficient to displace the retinoblastoma protein family members from E2Fs, leaving E2Fs free to activate transcription of the E2A promoter (O'Connor and Hearing, 2000).

1.7 Intermediate and late genes

As well as the five early transcription units, the adenovirus genome contains two delayed early units (IX and IVa2) and one major late unit which is processed to generate five families of late mRNAs (L1-L5), all of which are transcribed by polymerase II. There are also two viral-associated (VA) RNAs, transcribed by polymerase III, that play a role in combating cellular defence mechanisms (Mathews and Shenk, 1991).

After the onset of virus DNA replication, the IVa2 and IX genes are expressed at high levels, activating transcription of the major late promoter (MLP) and resulting in expression of the late genes, which are expressed under the control of the MLP (Lutz et al., 1997; Tribouley et al., 1994).

The proteins encoded by the late region include all of the structural proteins (II – IX, see section 1.1.4), and the proteins involved in their production and assembly.

1.8 Viral replication

As stated in section 1.1.4, the adenovirus binds to the host cell via a receptor, the best known of which is the Cocksackie B and Adenovirus receptor (CAR).

Binding is mediated via the adenovirus pentons, and interactions with α_v integrins on the cell surface result in virus internalisation, in clathrin-coated vesicles, where they are transported to endosomes. Here the acidic pH of the endosome results in partial disassembly of the capsid and escape into the cytoplasm. From here the virion is transported to the nucleus via microtubules where the viral DNA associates with the nuclear matrix, via the terminal protein, and transcription of the viral genome is initiated (Zhang and Arcos, 2005).

During DNA replication the E1B-55kDa protein functions in complex with E4 Orf6 to stimulate the cytoplasmic accumulation and translation of viral mRNAs, which is accompanied by shut off of host protein synthesis and host mRNA nuclear export (Babiss and Ginsberg, 1984; Pilder et al., 1986). After their production, hexon and penton capsomeres accumulate in the nucleus where assembly of the virion occurs. Assembly appears to begin with the formation of an empty capsid (Philipson, 1984; Sundquist et al., 1973) and, subsequently, a viral DNA molecule enters the capsid (Hasson et al., 1992; Hasson et al., 1989). The DNA-capsid recognition event is mediated by the packaging sequence, a *cis*-acting DNA element present near the left end of the viral chromosome (Grable and

Hearing, 1992; Hammarskjold and Winberg, 1980; Hearing et al., 1987; Tibbetts, 1977).

After assembly of the virions, their release is mediated via two mechanisms. Expression of the E1B-19kDa protein destroys the intermediate filament cytoskeleton by affecting the organisation of both vimentin-type filaments in the cytoplasm and the lamin filament network in the nucleus (White and Cipriani, 1989; White and Cipriani, 1990). Late in the infectious cycle, the viral proteinase cleaves the cellular cytokeratin protein K18, rendering it ineffective at polymerising to form filaments (Chen et al., 1993). Disruption of the filaments combined with expression of the adenovirus death protein (ADP) which induces cytolysis, results in the release of virus from the cell (Tollefson et al., 1996a; Tollefson et al., 1996b).

1.9 Adenovirus as a viral vector

1.9.1 Why are people interested in studying adenoviruses

The growth characteristics of adenovirus make it ideal to use as a gene therapy vector. These include the ability to infect both dividing and non-dividing cells. Adenoviruses deliver their genome to the nucleus of the host cell where the viral genome will exist as an episome, but will rarely integrate into the host cell chromosome. They also infect a wide variety of different cell types including highly differentiated tissues such as skeletal muscle, brain and heart. Adenovirus is also relatively easy to work with and can easily be grown to high titres.

1.9.2 Adenovirus as a gene therapy vector

Packaging restraints of the adenovirus genome limit the amount of foreign DNA that can be added to approximately 2kb. In order to increase the amount of foreign DNA packaged, some of the viral genome must be deleted.

The first generation of adenovirus vectors had the E1 and E3 regions deleted. Removal of the E1 region rendered the virus replication incompetent and these viruses had to be propagated on transcomplementing cell lines such as 293, 911 or PER.C6 cells (Graham et al., 1977; Fallaux et al., 1996 and Fallaux et al., 1998, respectively). As the E3 region is responsible for modulating the host cell immune response these vectors elicited a strong inflammatory and immune response, resulting in transient transgene expression. These vectors were also shown to replicate, producing replication competent adenovirus as a result of “leaky” expression. Recently it was discovered in cells lacking E1A, that the E4ORF6/7 protein could functionally compensate for E1A (O'Connor and Hearing, 2000), which could explain the occurrence of replication competent virus.

1.9.3 Adenovirus in cancer therapy

dl520 (ONYX-015) is an adenovirus with the E1B-55kDa gene deleted, which has been demonstrated to have anti-tumoural activity in patients with recurrent head and neck cancer. In adenovirus infected cells E1B-55kDa functions by targeting the tumour suppressor protein for degradation. In a large percentage of tumour cells the p53 gene is mutated and does not function, therefore, it was expected that ONYX-015 would selectively replicate in tumour cells lacking normal p53, and target them for degradation (Bischoff et al., 1996). Although there has been some

success in phase II clinical trials, it is now realised that replication is not limited to p53 negative cells (Hay et al., 1999; Vollmer et al., 1999), and that combinations of gene therapy and chemotherapy may be required for total regression (Heise et al., 1997).

dl922-947 is an E1A deleted adenovirus that has been demonstrated to replicate specifically in cancer cells. During adenoviral infection the E1A gene activates cell proliferation, by binding to and inactivating the pRB protein, which prevents progression from the G1-phase to the S-phase of the cell cycle. In normal quiescent cells, adenovirus lacking normal E1A function cannot induce cell proliferation and viral replication. In tumour cells proliferation is uncurbed, allowing E1A deleted adenovirus to replicate. This mutant adenovirus has demonstrated relatively potent antitumoural activity *in vivo* against a wide variety of tumours (Fueyo et al., 2000; Heise et al., 2000).

1.10 Small Ubiquitin Modifying protein (SUMO)

1.10.1 Introduction to SUMO

Small Ubiquitin Modifying protein (SUMO) is a ubiquitin-related protein that covalently binds to other proteins using a mechanism analogous to, but distinct from, ubiquitin. Whereas ubiquitin targets proteins to the proteasome for degradation, SUMO conjugation results in a diverse range of effects. Since the discovery of ubiquitin, it has emerged that there is a whole family of ubiquitin-like proteins (UBLs). In 1992 Loeb and Haas discovered the first of the family members ISG15, a protein covalently conjugated to cellular proteins upon induction by interferon. It was not until 1996 that SUMO-1 was discovered

independently by several different groups and is therefore also known as sentrin, GMP1, UBL1, and PIC1 (Boddy et al., 1996; Matunis et al., 1996; Meluh and Koshland, 1995; Okura et al., 1996; Shen et al., 1996).

SUMO is present in all eukaryotes and equivalent proteins are also present in yeast, Smt3 in *Saccharomyces cerevisiae* and Pmt3 in *Schizosaccharomyces pombe*. Deletion of Smt3 in *S. cerevisiae* is lethal, whereas deletion of Pmt3 is *S. pombe* results in poor growth. So far four different isoforms of SUMO have been identified termed SUMO-1, SUMO-2, SUMO-3 (Chen et al., 1998a) and SUMO-4 (Bohren et al., 2004). When comparing sequences, SUMO 2 and 3 are 97% identical to each other but only approximately 50% identical to SUMO-1. SUMO-4 has approximately 86% sequence identity with SUMO-2. When compared with ubiquitin, SUMO-1 has only 18% sequence similarity, although both proteins have remarkably similar three-dimensional structures. The main differences between the two are firstly that SUMO-1 has a flexible 20-residue N-terminal extension which is absent in ubiquitin, and secondly the surface charge distribution of the two proteins is dramatically different. Under normal conditions SUMO-1 seems to be the most prominently conjugated isoform (with a molecular weight of 11kD), whereas SUMO-2 and SUMO-3 appear to be preferentially conjugated under stress conditions (Saitoh and Hinchey, 2000). There are exceptions however where SUMO-2 and SUMO-3 have been shown to modify substrates under normal physiological conditions (Azuma et al., 2005; Eaton and Sealy, 2003). SUMO-2, SUMO-3 and SUMO-4, unlike SUMO-1, have a SUMO attachment consensus site and have been shown to form SUMO chains *in vitro* and *in vivo* (Bohren et al., 2004; Tatham et al., 2001).

Studies of SUMO modified proteins have demonstrated that SUMO modification occurs at the Lysine residue (K) within the consensus sequence ψ KxE, where ψ represents a hydrophobic amino acid such as L, I, V or F, x represents any amino acid and E is Glutamate. This sequence can be transferred to non SUMO-modified proteins, making them substrates for SUMO *in vitro*, although a nuclear localisation signal is also required for them to be modified *in vivo* (Rodriguez et al., 2001).

SUMOylation is a reversible process and it is thought that the SUMOylation/deSUMOylation process is highly dynamic. SUMO modification is similar to ubiquitination, in that they both require an E1 activating enzyme and an E2 conjugating enzyme and an E3 ligase, although SUMOylation can occur *in vitro* without the requirement for an E3 ligase.

SUMO is synthesized as a precursor with a short C-terminal extension, which must be removed before it can be conjugated to substrates. This is done by SUMO specific proteases, which cleave four residues from the C-terminal end, exposing the carboxyl group of Gly97.

The first SUMO specific proteases were discovered in *S. cerevisiae* and were termed Ulp1 and Ulp2 (Ubiquitin-Like modifier Proteases) (Hochstrasser, 2000; Li and Hochstrasser, 1999; Schwienhorst et al., 2000). Ulp1 is a cysteine protease whose active site appears to be structurally related to those of adenoviral processing proteases. Experiments demonstrated that Ulp1, like other SUMO specific proteases acts as dual function enzyme, acting as a protease to process the SUMO precursor into its active form, and acting as an isopeptidase to release SUMO from its conjugated substrate, enabling it to re-enter the conjugation

pathway (Li and Hochstrasser, 1999). Ulp2 has been demonstrated to function as mainly as a deSUMOlyating enzyme with some processing activity (Li and Hochstrasser, 2000; Schwienhorst et al., 2000). Ulp1 is distributed in the nuclear periphery, whereas Ulp2 displays uneven nuclear distribution. *S. pombe* has only one SUMO specific protease, SpUlp1, which carries out both activities. The distribution of SpUlp1 appears to be regulated by the cell cycle. Localisation is in the nuclear envelope during S and G2 phases, and nuclear during mitosis (Taylor et al., 2002). Comparison of sequence analysis has led to the suggestion that there are six Ulp genes in mammals, although so far only four have been characterised. These include SENP3/SMT3IP1 (Nishida et al., 2000) shown to be localised to the nucleolus, SENP6/SUSP1 which is localised to the cytoplasm (Kim et al., 2000), SENP1/SuPr-2 which is detected in the nucleus (Bailey and O'Hare, 2004; Gong et al., 2000) and finally SENP2/Axam and its isoforms produced as a result of alternative splicing (Best et al., 2002; Nishida et al., 2001). All SUMO-specific proteases share a conserved C-terminal sequence of approximately 200 residues, which contains the catalytic domain, whereas the N-termini are very divergent.

Activation of SUMO is ATP dependent, and involves the E1 activating enzyme, which is a heterodimer of SAE2-SAE1(Desterro et al., 1999). The SUMO activation enzymes are both nuclear proteins, with a diffuse localisation (Azuma et al., 2001). In *S. cerevisiae*, both subunits of E1 are essential for viability consistent with an essential function of SUMO modification (Dohmen et al., 1995; Johnson et al., 1997). Activation results in SUMO being linked by a thioester to a cysteine residue in SAE2. The activated SUMO is then transferred, in a transesterification reaction, to a cysteine residue of the E2 conjugating enzyme Ubc9. In most species

studied the genes encoding Ubc9 are essential, the exception being again *S. pombe*, in which SUMO conjugation is not required for viability (Ho and Watts, 2003; Johnson, 2004; Jones et al., 2002; Seufert et al., 1995). As with the SUMO activating enzyme, Ubc9 is a predominantly nuclear protein (Seufert et al., 1995). Ubc9 proteins are well conserved with approximately 56% identity between the mammalian and *S. cerevisiae* orthologues. Ubc9, in conjunction with the E3 ligase then conjugates SUMO to the substrate protein. So far three different types of E3 ligases have been identified, PIAS (Protein Inhibitor of Activated STAT), RanBP2 and PC2 (a polycomb group protein). To date, no SUMO E3 activity has been found to be essential in yeast.

RanBP2 was actually one of the first SUMO targets identified, although the first SUMO modified protein identified was RanGAP1, a cytosolic protein which is targeted to the cytoplasmic filaments of the nuclear pore complex (NPC) upon modification by SUMO-1. Here it interacts with the RanBP2, which acts as a binding site for Ran, and as a SUMO E3 ligase (Lee et al., 1998; Mahajan et al., 1997; Mahajan et al., 1998; Matunis et al., 1996; Matunis et al., 1998; Pichler et al., 2002). This interaction results in activation of the GTPase activity of the cytosol/nucleus shuttling factor Ran, resulting in the release of the cargo from exportins that exit the nucleus in a complex with RanGTP. This in turn allows the released GDP to form complexes with importins and their cargo, which can then be imported into the nucleus. *In vitro* data suggests that SUMO-dependent localisation of RanGAP to the NPC is critical for nuclear import (Mahajan et al., 1997).

SUMOylation and deSUMOylation is a dynamic process, resulting in only a percentage of substrates being SUMOylated at any one time, and hence it is likely that only a small proportion of SUMO substrates have been identified. However the number of SUMO substrates identified is rapidly increasing, due to the awareness of this modification, the use of proteomics methods for studying protein-protein interactions, and the application of procedures preventing rapid deSUMOylation upon cell lysis (Johnson and Blobel, 1999). The proteins that have been identified as SUMO targets so far are involved in a wide variety of cellular functions from signal transduction and transcriptional replication to DNA damage repair and chromosome segregation. SUMO modification has been demonstrated to alter the activity of proteins, their ability to interact with other proteins and their subcellular localisation (Melchior, 2000).

1.10.2 Viral proteins that are modified by SUMO

Since the discovery of SUMO, a large number of viral proteins have been shown to be modified by SUMO-1, although the role of this remains unclear. There are currently six viral proteins known to be SUMOylated across three different DNA virus families; Adenovirus, Papillomavirus and Herpesvirus. All of these proteins are early gene products with important regulatory roles in viral transcription or replication. There are also three viral families that encode proteases related to the SUMO proteases (Adenovirus, Asfarvirus and Poxvirus).

1.10.3 The Cytomegalovirus immediate-early 1 (CMV-IE1) and immediate early 2 proteins (CMV-IE2) are modified by SUMO

The first viral protein discovered to be SUMOylated was the human Cytomegalovirus immediate-early 1 protein (CMV-IE1). The SUMOylated form was observed as a higher molecular weight version of the protein (Muller and Dejean, 1999). CMV-IE1 is a multifunctional viral kinase that phosphorylates itself and several other substrates, effecting gene regulation, cell cycle regulation and preventing apoptosis. (McElroy et al., 2000; Pajovic et al., 1997; Stenberg and Stinski, 1985; Zhu et al., 1995). It is also responsible for the disruption of the Promyelocytic Leukaemia Nuclear Bodies (PML NBs) during infection (Ahn and Hayward, 1997; Kelly et al., 1995; Korioth et al., 1996). The SUMOylation site has been mapped to lysine 450 (Spengler et al., 2002; Xu et al., 2001), and mutational analysis where the lysine has been changed to an arginine, have shown that the mutant has the same distribution and activity as the wild type protein (Xu et al., 2001).

The second viral protein discovered to be SUMOylated, was human Cytomegalovirus immediate-early 2 protein, when SUMO-1, SUMO-3 and Ubc9 were pulled down in a yeast two hybrid screen (Hofmann et al., 2000). Hofmann et al also demonstrated that CMV-IE2 was modified by SUMO during the course of a lytic infection. In 2001, Ahn *et al* mapped the SUMO conjugation site to lysine residues K175 and K180, and demonstrated that CMV-IE2 was SUMOylated *in vitro*. CMV-IE2 acts as a transcriptional promoter for early viral proteins, and for other heterologous promoters (Hagemeier et al., 1992; Klucher et al., 1993; Malone et al., 1990). It also acts as a transcriptional repressor for its own promoter

(Hermiston et al., 1990; Pizzorno et al., 1988). Studies using double mutants, where both lysines were mutated to arginines, demonstrated SUMOylation has no effect on cellular localisation or protein stability, although it does reduce the protein's ability to act as a transcriptional activator (Ahn et al., 2001; Hofmann et al., 2000).

1.10.4 The papillomavirus E1 (PV-E1) protein is modified by SUMO

The papillomavirus E1 protein (PV-E1) was the third viral protein discovered to be SUMO modified. It is an ATP-dependent DNA helicase that binds to specific sequences in the viral DNA, unwinds it, and initiates its replication (Wilson et al., 2002). It also recruits cellular factors required for DNA replication such as DNA polymerase- α (Bonne-Andrea et al., 1995; Conger et al., 1999; Masterson et al., 1998; Park et al., 1994). The initial discovery of human Ubc9 resulted from a yeast II hybrid screen using human papillomavirus type 16 E1 protein (Yasugi and Howley, 1996). In 2000, Rangasamy and Wilson demonstrated that Bovine papillomavirus E1 also interacted with Ubc9.

The major SUMO-1 attachment site of BPV-E1 is at K514, which lies in the sequence IKAP, differing slightly from the accepted SUMO consensus sequence. (Rangasamy et al., 2000).

In vitro transfection experiments using Green Fluorescent Protein (GFP) fusions of wild type or K514R mutants demonstrated that SUMOylation affects localisation but not stability of the protein. Wild type BPV-E1 accumulated in the nucleus, whereas the K514R mutant accumulated at the periphery of the nucleus. Upon co-transfection into mammalian cells, a plasmid expressing wild type BPV-E1 could

stimulate expression of a BPV-origin containing plasmid, whereas a plasmid expressing K514R mutant of BPV-E1 could not (Rangasamy and Wilson, 2000).

1.10.5 The Epstein-Bar virus immediate-early protein BZLF1 (EBV-Z) is modified by SUMO

The Epstein-Bar virus immediate-early protein BZLF1 (EBV-Z) is one of the first proteins expressed during the lytic form of viral infection, and acts as a transcriptional activator inducing expression of the EBV early genes (Kieff, 2001). It also inhibits cell cycle progression (Cayrol and Flemington, 1996), activates the p38 and c-Jun signalling cascades (Adamson et al., 2000) and alters CBP and p53 function (Adamson and Kenney, 1999; Zhang et al., 1994), respectively. Adamson and Kenney demonstrated that EBV-Z is SUMOylated during lytic infection and mapped the SUMO modification site to lysine residue 12 (Adamson and Kenney, 2001). Mutation analysis of residues 12 and 13 impair the replicative function of this protein (Sarisky et al., 1996).

1.10.6 The human herpes virus 6 immediate-early 1 (HHV6-IE1) protein is modified by SUMO

The human Herpes virus immediate-early genes (IE) are the first viral products synthesised upon viral infection. Similar to other human IE herpesvirus proteins, herpes virus 6 immediate-early 1 (HHV6-IE1) induces the transcription of heterologous promoters. Two variants of this protein are produced termed variants A and B, and they vary in their ability to transactivate the HHV-LTR promoter (Gravel et al., 2002). Gravel was the first to demonstrate that the HHV6-IE1

proteins are SUMOylated *in vivo* by performing coimmunoprecipitation experiments in infected mammalian cells. This was confirmed by Stanton et al (Stanton et al., 2002).

1.10.7 The adenovirus E1B-55kDa protein is modified by SUMO

The E1B-55kDa protein of adenovirus type 5 was first discovered to be SUMO modified in 2001 by Endter et al. Upon discovering the presence of the SUMO consensus sequence Endter et al undertook experiments to determine if in fact E1B-55kDa was SUMOylated. Plasmids encoding wild type E1B-55kDa or E1B-55kDa K104R were transfected into H1299 cells along with a plasmid encoding SUMO-1. Total cell extracts were then prepared and immunoblotted, with appropriate antibodies. In cells transfected with only E1B-55kDa, a major band of 55kDa, corresponding to the E1B-55kDa was detectable. In cells co-transfected with E1B-55kDa and SUMO-1, an additional band of approximately 75kDa was observed corresponding to SUMO conjugated E1B-55kDa. This 75kDa band was absent in cells co-transfected with SUMO-1 and E1B-55kDa K104R, demonstrating that lysine 104 is the SUMO modification site. Endter et al also demonstrated the presence of the 75kDa form of E1B-55kDa in adenovirus type 5 infected cells, indicating the E1B-55kDa is SUMOylated *in vivo*.

To determine if SUMOylation of E1B-55kDa affected localisation, Endter et al generated baby rat kidney (BRK) cells stably expressing either wild type E1B-55kDa or E1B-55kDa K104R. In both of these cell lines the distribution of E1B-55kDa was cytoplasmic. Both cell lines were then transfected with a plasmid expressing SUMO-1. In the cells expressing wild type E1B-55kDa the E1B-55kDa

was directed to the nucleus where it co-localised with SUMO-1 in either track-like or dot-like structures. In contrast, the cells expressing E1B-55kDa K104R retained the E1B-55kDa in the cytoplasm.

Endter et al also demonstrated that SUMOylation of adenovirus type 5 E1B-55kDa is required for complete transformation of BRK cells and inhibition of p53 transactivation. Transformation assays were carried out on BRK cells. Co-transfection of plasmids encoding E1A and wild type E1B-55kDa resulted in a more than seven fold increase in foci formation, compared with E1A alone. In comparison the E1B-55kDa K104R mutant did not aid focus formation (Endter et al., 2001).

Lethbridge et al demonstrated that adenoviruses that did not express E4 Orf6 during infection, showed enhanced SUMOylation of E1B-55kDa protein. The amount of SUMO-1 modified E1B-55kDa protein was not related to its association with ND10. It is known that the SUMOylation of PML is required for it to form ND10 structures (Muller, 1998). Duprez et al demonstrated that PML is modified by SUMO-1 both *in vivo* and *in vitro* (Duprez et al., 1999). It is possible that the SUMOylation of E1B-55kDa protein is related to its localisation at the nuclear matrix (Lethbridge et al., 2003).

1.11 Proteomics methods for studying protein interactions

1.11.1 Proteomics methods

Studying the sequences of different adenovirus genomes provides useful information on how adenoviruses have evolved. Although it is important to determine the sequence of the different adenovirus genomes, in order to fully understand how adenoviruses replicate, it is necessary to determine how each of the viral proteins interact with each other and with the host cell proteins during infection and subsequent replication.

Proteomics methods are being deployed to help understand these interactions.

Proteomics refers to the study of all of the proteins (or proteome) expressed by an organism.

There are two different types of proteomics, expression proteomics and interaction proteomics. In expression proteomics, the total protein complement of the cell is examined under two different physiological states (e.g. normal and diseased), and the differences in protein expression are examined. This can be done using several different methods, including 2D gel electrophoresis, Stable Isotope Labelling by Amino acids in Cell culture (SILAC), Multidimensional protein identification technology (MudPIT) or shotgun proteomics followed by mass spectrometric analysis.

Stable Isotope Labelling by Amino acids in Cell culture (SILAC) involves growing mammalian cell lines in media lacking a standard essential amino acid, but supplemented with a non-radioactive, isotopically labelled form of that amino acid. Growth of cells maintained in this media is no different from cells grown in normal media, and the labelled amino acids become totally incorporated into the

cells. Protein populations from control and experimental samples are mixed and identified by mass spectrometric analysis. From the results it is possible to determine if the levels of a specific protein have increased or decreased in the experimental sample by comparing the relative signal intensity of labelled and unlabelled peptide pairs. (Ong et al., 2002).

Multidimensional protein identification technology (MudPIT) is a technique for the separation and identification of complex protein mixtures that does not require the protein mixture to be separated by SDS-PAGE (Graumann et al., 2004).

MudPIT relies on digestion in solution of the protein mixture to be analysed and separation of the resulting complex peptide mixture by multidimensional capillary chromatography connected on-line to an ion trap or Q-TOF mass spectrometer (Link et al., 1999; McCormack et al., 1997). This technique was developed in an attempt to alleviate the problems associated with SDS-PAGE including limits of dynamic range of detection, variable elution efficiencies of peptides from the SDS-PAGE gel and potential selection against proteins with properties that impede analysis by SDS-PAGE (Gavin et al., 2002; Ho et al., 2002).

Shotgun proteomics is a gel-free approach, based on multidimensional liquid chromatography separation of complex peptide mixtures, coupled to mass spectrometry. A protein population is first prepared from a biological source using protein purification strategies including chromatographic or electrophoretic fractionation, or affinity purification. Enzymatic or chemical digestion then takes place to generate a mixture of peptides, which are subsequently separated using multidimensional chromatographic techniques before being introduced into a mass spectrometer (Swanson and Washburn, 2005).

Interaction proteomics is used to study what other proteins interact with a specific protein. There are three different methods of studying interaction proteomics, immunoprecipitation, yeast II hybrid system and affinity purification where the protein is fused to an affinity tag and is immunoprecipitated with an antibody specific to the tag, or purified using a column that the tag binds to (e.g. calmodulin or GST). Since purification of the protein of interest is solely dependent on the tag used, it is important to choose the best tag. One tag can be coupled to the protein for single-step affinity purification, for example the FLAG tag, or two tags can be coupled to the protein for tandem affinity purification. Tandem affinity purification reduces the amount of contaminants purified. The tandem affinity purification tag, consisting of a calmodulin binding peptide (CBP) fused to a Protein A binding site via a tobacco etch virus (TEV) protease cleavage site, was developed by Rigaut et al (Rigaut et al., 1999).

1.11.2 Immunoprecipitation

Immunoprecipitation requires antibodies specific to the protein of interest. Cell extracts are incubated with the specific (primary) antibody. The antibody and bound protein are then purified using immunoaffinity or protein A or G column chromatography. For immunoaffinity the column is packed with beads that are coated with a secondary antibody that binds to the primary antibody. In column chromatography the primary antibody will bind directly to the protein A or G within the column. Following binding, the column is washed to release any unbound material. The protein of interest is then eluted along with any interacting

proteins, which can then be analysed by SDS-PAGE and mass spectrometric analysis.

1.11.3 Yeast II Hybrid

This method involves the generation of a cDNA library. The protein of interest is expressed in yeast, as a fusion to the DNA binding domain of a transcription factor, lacking a transactivation domain. This yeast strain also encodes one or more reporter genes with binding sites for the DNA binding domain. A plasmid library that expresses cDNA encoded proteins fused to a transcription activation domain is then introduced into the yeast strain. The cDNA encoded proteins that interact with the protein of interest will activate the transcription factor and cause expression of the reporter gene, allowing cells containing the interacting proteins to be identified (Fields and Song, 1989).

1.11.4 Tandem Affinity Purification

Tandem Affinity Purification (TAP) was developed by Rigaut et al in 1999 and it allows purification of specific proteins expressed at their physiological levels. TAP expression vectors have been created in which the protein of interest can either be C or N terminally tagged. The TAP tag vector contains a multiple cloning site (MCS), a calmodulin binding peptide (CBP), a tobacco etch virus (TEV) protease cleavage site and a protein A IgG binding unit of *Staphylococcus aureus*. These tags were selected because they showed highly efficient recovery of proteins that were present at low levels. The gene of interest is cloned into the MCS of the vector so that it is expressed as a TAP tag fusion protein. This

construct is then introduced into the host cell or organism, and cells that are expressing the fusion protein at, or close to, its natural level are selected for. The specified protein and any host cell proteins that interact with it are then recovered by affinity purification.

In the first affinity purification step, cell extracts are put through a column containing an IgG matrix. Fusion proteins bind the IgG via the Protein A site. After washing, TEV protease is added to release the bound material. The eluate is then incubated with calmodulin-coated beads in the presence of calcium, thus allowing binding of the fusion protein to the calmodulin beads via the CBP. After washing the bound material is released by native elution with EGTA (see figures 3 and 4). Proteins can then be analysed using SDS-PAGE and mass spectrometry. The advantages of this system are that proteins can be purified, without prior knowledge of their composition, activity or function. The proteins are also expressed at, or close to their natural levels, and they can be purified even if they are present at low concentrations. The other advantage is that all proteins that interact with the protein of interest, could in theory, be identified in a single experiment. The results if interpreted properly may also give an indication of the relative proportions of identified proteins (Rigaut et al., 1999).

1.11.5 Mass Spectrometry

Recent advances in the field of mass spectrometry, as well as the availability of large databases containing the known mass and amino acid sequence of many proteins, have made identification of proteins much easier than it was previously.

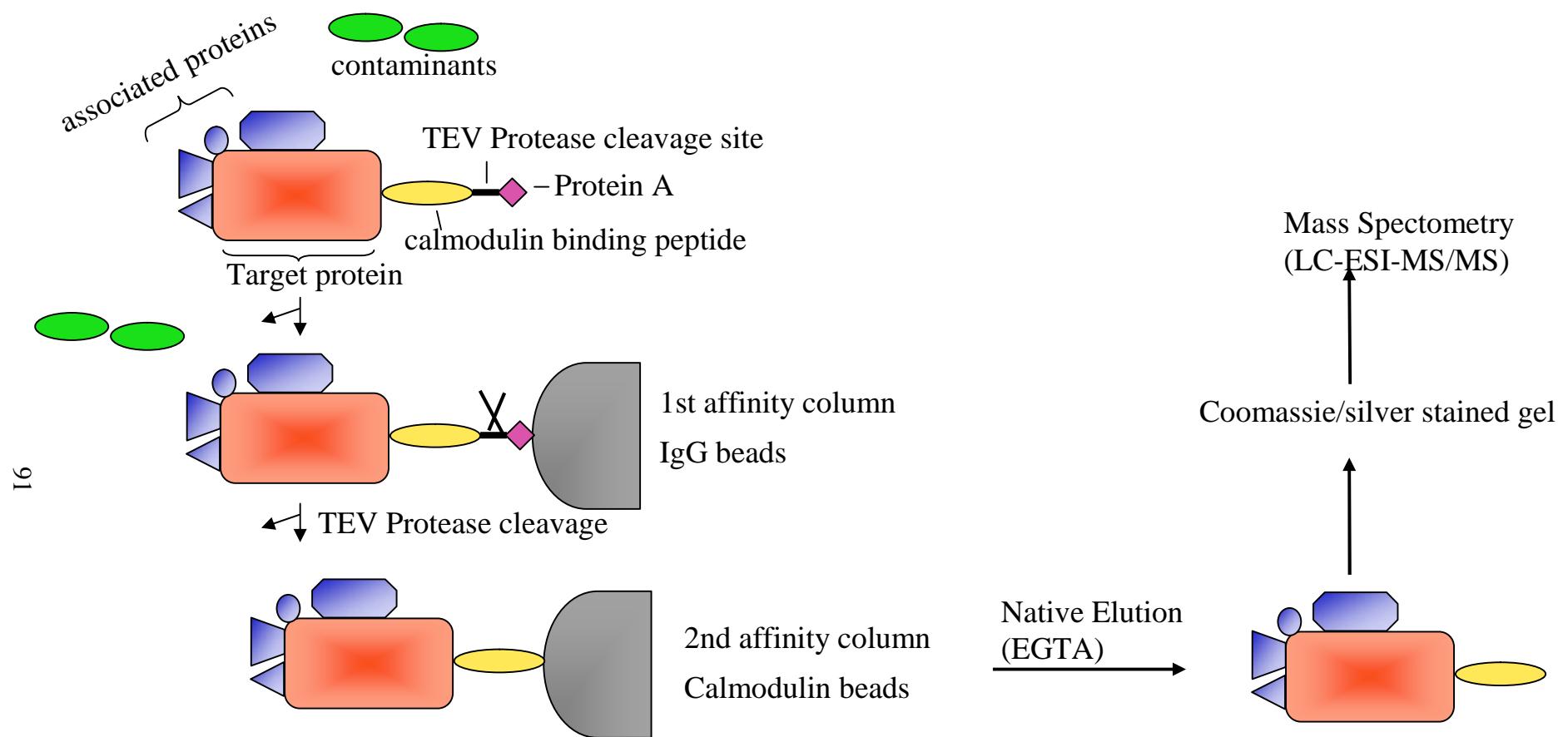


Figure 1.3: Tandem Affinity Purification Procedure. The Tandem Affinity Purification (TAP) tag and the target protein are produced as a fusion protein. The TAP tag consists of a calmodulin binding peptide, a TEV protease cleavage site and a protein A binding site. Cell extracts are passed through the first affinity column which consists of IgG beads, to which the protein A binds. The column is washed to eliminate contaminating proteins. TEV protease is then added to the column, releasing the target protein (which is still fused to the calmodulin binding peptide) and any interacting proteins. The eluates are then passed through a second affinity column consisting of calmodulin beads, to which the calmodulin binding peptide binds. Again contaminating proteins are washed away. The target protein and any interacting proteins are then eluted using EGTA, and analysed by SDS-PAGE and Mass Spectrometric analysis.

92

Kozak

gaa ttc gcc ctt **gcc acc** atg gac aca agt gcc cac gat gaa gcc gta gac aac aaa ttc
 E F A L A T M D T S A H D E A V D N K F
 aac aaa gaa caa caa aac gcg ttc tat gag atc tta cat tta cct aac tta aac gaa gaa
 N K E Q Q N A F Y E I L H L P N L N E E
 caa cga aac gcc ttc atc caa agt tta aaa gat gac cca agc caa agc gct aac ctt tta
 Q R N A F I Q S L K D D P S Q S A N L L
 gca gaa gct aaa aag cta aat ggt gct cag gcg ccg aaa gta gac aac aaa ttc aac aaa
 A E A K K L N D A Q A P K V D N K F N K
 gaa cga caa aac gcg ttc tat gag atc tta cat tta cct aac tta aac gaa gaa caa cga
 E Q Q N A F Y E I L H L P N L N E E Q R
 aac gcc ttc atc caa agt tta aaa gat gac cca agc caa agc gct aac ctt tta gca gaa
 N A F I Q S L K D D P S Q S A N L L A E
 Gct aaa aag cta aat ggt gct cag gcg ccg aaa gta gac gcg aat tca gcg ggt aag
 A K K L N G A Q A P K V D A N S A G A
 Agc gag aac ttg tac ttc cag ggc gga aga tcg atg gaa aag aga aga tgg aaa aag aat
 S E N L Y F Q G G R S M E K R R W K K N
 ttc ata gcc gtc tca gca gcc aac cgc ttt aag aaa atc tca tcc tcc ggg gca ctt gat
 F I A V S A A N R F K K I S S S G A L D
 tat gat att cca act gct ctg gaa gtt ctg ttc cag ggg ccc ctg **gga tcc** aga tct ggt
 Y D I P T A L E V L F Q G P L G S R S G
 acc acg cgt atc gat aag ctt gca tgc ctg cag gtc gac tct aga gta tcg cggt gca
 T T R I D K L A C L Q V D S R V S R V A
 tcc ctg tga
 S L *

Figure 1.4: Sequence of Tandem affinity purification tag. The sequence of the TAP tag is shown with the protein A sequence in red, the TEV protease cleavage site in green, the calmodulin binding peptide in blue and the polylinker containing the MCS in pink. The BamH1 site that the E1B55k gene (+/-K104R mutation) was cloned into is underlined. The Kozak sequence prior to the start codon is also highlighted.

Prior to the development of mass spectrometry, Edman degradation was used to determine the sequence of proteins, this method relied on the identification of amino acids, as they were chemically cleaved from the amino end of the protein. Any proteins, which were modified at their amino terminus, could not be sequenced using this method. Therefore, mass spectrometry has become the technique of choice, used to identify proteins resulting from proteomics experiments. Mass spectrometry involves the ionisation of molecules, and the measurement of their mass, which is calculated based upon their specific trajectories in a vacuum system. Compared to Edman degradation, mass spectrometry is much more sensitive, quicker and does not require proteins to be purified to homogeneity.

In techniques such as tandem affinity purification, hundreds of proteins, can be co-purified with the protein of interest, leading to a complex mixture of proteins in the resulting eluates. In order to reduce the complexity of the sample, the eluates are generally first separated according to molecular weight by SDS-PAGE. The lane containing the sample is then excised in different gel slices, which are then subjected to in-gel digestion and LC-ESI-MS/MS separately.

Even though it is possible to measure the mass of intact proteins, there are several reasons why proteins are first cleaved into peptides. One of the main reasons is that proteins are not always soluble under the same conditions, and by cleaving the proteins into peptides there is no issue regarding solubility. Although it is possible to use detergent to solubilise proteins, detergents can interfere with mass spectrometry, as they can ionise well, and are in huge excess relative to the

proteins. Proteins can be also be modified, for example by phosphorylation, which can increase their mass, making it more difficult to identify a specific protein, if there are different forms of the same protein within the sample. Therefore, obtaining the accurate mass of an intact protein is insufficient to allow its identification. The most reliable way of identifying a protein is by cleaving it into peptides. As mass spectrometry is most efficient for determining the mass of peptides up to twenty residues long accurately, sequence specific proteases are used to cleave the proteins into peptides. The most common protease used is trypsin, although others can be used such as Lys-C, Asp-N and Glu-C. Trypsin cleaves proteins on the carboxy terminus of arginine and lysine residues. Less sequence-specific proteases are avoided as they divide the peptide signal into many overlapping species and generate unnecessarily complex mixtures.

Proteomics experiments generally result in complex peptide mixtures. Therefore, prior to mass spectrometric analysis, it is necessary to separate the peptides, usually by reverse phase chromatography to reduce the complexity at any one analysis point. This can be done using a microscale capillary high-performance liquid chromatography (HPLC) column. In electrospray ionisation (ESI) MS/MS the HPLC is coupled to the mass spectrometer and column eluates are analysed on-line. Although matrix assisted laser desorption (MALDI) ionisation does not allow direct on-line coupling to HPLC, LC fractions can be spotted onto a target plate and subsequently analysed (off-line) (see below for description of ionisation techniques). In HPLC the peptides are eluted from the column using a solvent gradient, which caused them to be eluted in terms of their hydrophobicity. As the signal intensity in the mass spectrum is directly proportional to the analyte

concentration, the peptides are eluted in as small a volume as possible. Upon reaching the end of the column, the peptide mixture has to be transferred into the gas phase and ionised. Two new “soft” ionisation techniques that were developed in the late 1980’s, first allowed the practical analysis of biomolecules; these techniques were MALDI and ESI. In MALDI (Karas and Hillenkamp, 1988), the analyte is mixed with a large excess of ultraviolet-absorbing matrix, which is normally a low molecular weight aromatic acid. On irradiation with a focused laser beam of the appropriate wavelength, the excess matrix molecules cause the transfer of the embedded non-volatile analyte molecules into the gas phase. After numerous ion-molecule collisions in the plume of ions and molecules, singly protonated analyte ions are formed, which are accelerated by electric potential into the mass spectrometer. MALDI mass spectrometers are primarily used to determine the accurate masses of the peptides in the mixture. In ESI (Fenn et al., 1989), when a peptide arrives at the end of the column, it flows through the tip of a metal needle, held at a high electrical potential, causing it to be electrostatically dispersed. Highly charged droplets are generated, and once the droplets become airborne the solvent evaporates, resulting in a decrease in size and an increase in charge density. Droplet fission eventually results in each droplet containing on average one ion, which can then be analysed using a mass spectrometer.

There are several different types of mass spectrometers including Quadrupole, Time of Flight (TOF), Quadrupole ion traps, standard ion traps and Fourier-transform ion cyclotron resonance (FTICR).

Quadrupole mass spectrometers work by applying different sinusoidal potentials with each one allowing the passage of ions with a specific m/z ratio. In FTICR

ions are confined within the high magnetic field of a super-conducting-magnet, where they circle with frequencies that are inversely proportional to their m/z ratio. This circling induces a current which constitutes a frequency spectrum of the ion motion. This is converted by Fourier transformation into a mass spectrum.

TOF is based on the time it takes ions to travel through an electric-field-free flight tube. In the ion source, all ions are accelerated to the same kinetic energy. As kinetic energy is a function of mass, the lighter ions fly faster than the heavier ions and therefore reach the detector sooner. In ion traps, the ions are first caught in a dynamic electric field, and are then ejected onto the detector with the aid of another electric field, according to their m/z ratio. In addition there are also mass spectrometers that combine principles such as Quadrupole Time of Flight (Q-TOF) mass spectrometers. Each of these instruments generates a mass spectrum, which is a recording of the signal intensity of the mass-to charge (m/z) ratio, of each ion. Individual protein samples can be identified by peptide mass fingerprinting (PMF) but for mixtures it is necessary to generate sequence information from peptides. This is done by tandem MS, also known as MS/MS. In tandem MS a particular peptide ion is isolated and fragmented, by bombardment with an inert gas, such as nitrogen, argon or helium, resulting in an MS/MS spectrum. As each peptide fragment in a series differs from its neighbour by one amino acid, this allows the amino acid sequence to be determined, by considering the mass difference between neighbouring peaks in a sequence. MS/MS data can be generated from ions generated by either ESI or MALDI.

The resulting MS/MS spectra are then searched against a protein database, using a programme such as the Mascot search engine. The proteins, which could result

from the peptides are then listed in terms of probability, along with details such as percentage protein coverage. Proteins for which there are several high scoring MS/MS spectra, are likely to be true hits, whereas proteins with lower scores may need to have their presence confirmed using other techniques such as Western blotting. The identification of a protein is dependent on several factors including the amount of protein, the degree of post-translational modifications, and whether or not the protein has previously been registered on the database (Steen and Mann, 2004).

1.11.6 Aim of Work

The aim of this work was to generate four recombinant replication competent adenoviruses, using plasmid pSCB-AdFL, which expressed the full length adenovirus type 5 genome. The recombinant viruses were designed express Tandem Affinity Purification (TAP) tagged or Cyan Fluorescent Protein (CFP) tagged E1B55k, with tags located on the N-terminus. As E1B55k protein is modified by SUMO-1 at lysine residue 104, recombinant viruses were also generated in which the lysine residue was mutated to an arginine residue, thus abolishing SUMOylation of the E1B55k protein. The tandem affinity purification tag was to allow the identification of interacting proteins (both viral and cellular), and the CFP tag would allow localisation to be studied using live cell imaging. By comparison of results generated in which the E1B55k protein could be modified by SUMO, with those in which it could not, it was hoped to identify the importance of SUMOylation during adenovirus infection.

Stable HeLa cell lines were also generated, which expressed TAP tagged E1B55k and TAP tagged E1B55k K104R. By tandem affinity purifying these stable cell lines both uninfected, and infected with wild type adenovirus 5, it would be possible to determine cellular and viral proteins that interact with the E1B55k protein. This method was established as an alternative method of generating results, without the recombinant adenoviruses.

Stable HeLa cell lines expressing TAP tagged SUMO2 protein, generated in the laboratory by Dr Akihiro Nakamura, were also tandem affinity purified, both uninfected, and infected with wild type adenovirus 5. The aim of this work was to determine if any of the adenoviral proteins were modified by SUMO2 protein.

2. MATERIALS AND METHODS

2.1 DNA cloning and analysis

2.1.1 Polymerase chain reaction

DNA was amplified using Pfu DNA polymerase (Promega UK, or produced in-house), using a GeneAmp PCR system 2400.

2.1.2 Clean-up of DNA after PCR

PCR products were separated by agarose gel electrophoresis, to allow separation from any contaminating products. Appropriate bands were then excised from the gel and DNA was purified using the High Pure PCR product purification kit (Roche) according to the manufacturer's instructions.

2.1.3 Agarose gel electrophoresis

Agarose gels were made by melting powdered agarose (Biogene) in TAE buffer (0.04M Tris-acetate, 0.001M EDTA, pH 8.0) to achieve the desired percentage. Ethidium bromide was added to a concentration of 0.125µg/mL and gels were cast in an H1-set gel rig or a Midi Excel gel rig (both Bioscience Services). Gels were run at a constant voltage of 60V in TAE buffer.

2.1.4 Purification of DNA from agarose gels

The DNA band to be purified was excised from the gel and purified using the High Pure PCR product purification kit (Roche) according to the manufacturer's instructions.

2.1.5 Restriction digest of DNA for cloning

1 μ g of DNA was incubated with 10U of restriction enzyme(s) in the appropriate buffer (Promega UK, New England Biolabs, UK), in a total volume of 10 μ L at the appropriate temperature for 2-4 hours.

2.1.6 Dephosphorylation of linearised vector DNA

1 μ g of DNA was incubated with 1U of Calf Intestinal Alkaline Phosphatase (CIAP) in the buffer supplied with the CIAP (Promega, UK) at 37°C for 40 minutes, and DNA purified using the Roche High Pure PCR product purification kit, according to manufacturer's instructions, prior to ligation.

2.1.7 Ligation of DNA

Vector and insert DNA were prepared by digestion with appropriate restriction enzymes and mixed with T4 DNA ligase and buffer supplied with the ligase (New England Biolabs) in a total reaction volume of 20 μ L Reactions were incubated at 16°C overnight or for 2 hours at room temperature.

2.1.8 Bacterial strains

Escherichia coli (*E. coli*) strain DH5 α were used in the preparation of DNA (genotype: ϕ 80dlacZ Δ M15, *rec A1*, *end A1*, *gyr A96*, *thi-1*, *hsd R17* (r_k-, m_k+), *s_p E44*, *rel A1*, *deoR*, Δ (*lacZYA-argF*)_169).

Electrocomp™ GeneHogs® *E. coli* strain DH10 α (Invitrogen) were used in the preparation of plasmid pSCB-AdFL DNA (genotype: F-*mcrA(mrr-hsdRMS-*

mcrBC) φ80lacZΔM15 ΔlacX74 deoR recA1 araD139Δ(ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG fhuA::IS2 (confers phage T1 resistance).

E. coli strain BJ5183 were used in the preparation of plasmid pSCB-AdFL-^{5'ITR} (genotype: , *end A1, sbc BC, rec BC, gal K, met, thi-1, bio T, hsd R (str^R)*).

2.1.9 Preparation of transformation competent *E. coli*

A 250mL culture of DH5α was grown up from a glycerol stock, in Luria-Bertani broth (L-broth, see section 2.1.13) at 37°C, until the Optical Density (O.D) at 600nm was between 0.6 and 0.7. Cells were then transferred to prechilled 50mL Falcon tubes and incubated on ice at 4°C for 30 minutes. Cells were then spun at 2,800rpm for 5 minutes at 4°C. The cell pellet was then resuspended in 12.5mL of 100mM CaCl₂ and 12.5mL of freshly made 40mM MgSO₄ and incubated on ice for 30 minutes. Cells were then spun at 2,800rpm for 5 minutes at 4°C. The cell pellet was then resuspended in 2.5mL of 100mM CaCl₂ and 2.5mL 40mM MgSO₄. Cells were incubated on ice at 4°C overnight. Glycerol was then added to a final concentration of 10%, cells were dispensed into 200μL aliquots, snap frozen in liquid nitrogen and stored at -70°C.

CaCl₂, MgSO₄, and glycerol solutions were sterilised by autoclaving and were prechilled on ice prior to use.

2.1.10 Preparation of electrocompetent *E. coli*

A 1L culture of DH5α was grown up from a glycerol stock, in L-broth at RT, until the O.D. at 600nm was 0.5. Cells were then transferred to 4 x 250mL centrifuge pots and chilled on ice, at 4°C overnight. Cells were then spun at 4,000 rpm for 15

minutes at 4°C. The cell pellet was resuspended in a total volume of 1L 1mM Hepes pH7.0. Cells were spun at 4,000 rpm for 15 minutes at 4°C. The cell pellet was then resuspended in a total volume of 500mL 1mM Hepes pH7.0. Cells were respun at 4,000 rpm at 4°C. The cell pellet was resuspended in 10% glycerol, transferred to a 15mL Falcon tube and spun at 3,000rpm for 15 minutes at 4°C. The cell pellet was then resuspended in 2mL 10% glycerol, dispensed into 200µL aliquots, snap frozen in liquid nitrogen and stored at -70°C. Hepes and glycerol solutions were sterilised by autoclaving and prechilled on ice prior to use.

2.1.11 Transformation of transformation competent *E. coli*

DNA ligation reactions, or 100ng of plasmid DNA, was mixed on ice with 50µL of transformation competent *E. coli* and incubated on ice for 20 minutes. The sample was heat shocked at 42°C for 50 seconds then placed on ice for 2 minutes. Following this, L-broth was added (900µL) and sample incubated at 37°C for 1 hour. After centrifugation at 14,000rpm for 1 minute the cell pellet was resuspended in 100µL L-broth and plated onto an L-agar plate containing the appropriate antibiotic to select for the plasmid. The plates were then incubated at 37°C overnight.

2.1.12 Electroporation of electrocompetent *E. coli*

Prior to electroporation, ligation reactions or DNA samples, were dialysed by being placed on 0.025µm, 13mm Millipore dialysis discs (VSWP01300), on a petri dish of sterile water, for 1 hour at RT. Samples were then added to 2mm

electroporation cuvettes (Equibio, ECU-102), prechilled on ice, and 50 μ L electrocompetent *E. coli* added and mixed. The sample was incubated on ice for 20 minutes and electroporated using an Easyject Prima Electroporator (Equibio) at 2,500 volts. L-broth (900 μ L) was then added and the sample incubated at 37°C for 1 hour. The sample was then centrifuged at 14,000rpm for 1 minute to pellet cells. The cell pellet was resuspended in 100 μ L of L-broth and plated onto an L-agar plate containing the appropriate antibiotic to select for the plasmid. The plates were incubated at 37°C overnight.

2.1.13 Media used for *E. coli*

E. coli cultures were grown in L-broth (10g tryptone, 5g yeast extract, 10g NaCl made up in 1 litre water). Concentrations of antibiotics used were as follows:

Antibiotic:	Stock solution:	Concentration used at:
Ampicillin	100mg/mL (H ₂ O)	100 μ g/mL
Kanamycin	50mg.mL (H ₂ O)	50 μ g/mL
Tetracyclin	10mg/mL (80% ethanol)	10 μ g/mL
Chloramphenicol	20mg/mL (80% ethanol)	20 μ g/mL

The ampicillin, kanamycin and tetracyclin were all purchased from Sigma, and the chloramphenicol from Duchefa.

2.1.14 Purification of plasmid DNA

In general plasmids were transformed or electroporated into *E. coli* DH5 α , then plated onto L-agar plates containing the appropriate concentration of antibiotic,

and incubated at 37°C overnight. Individual colonies were then grown up in L-broth, containing the appropriate antibiotic, in the shaker incubator at 37°C overnight. The cells were then lysed and DNA was purified from cultures using the Qiagen miniprep spin kit or the Qiagen maxiprep kit according to manufacturer's instructions.

Plasmids pSCB-AdFL and pSCB-AdFL^{-5'ITR} were electroporated into Electrocomp™ GeneHogs® *E. coli* (Invitrogen), plated onto L-agar plates containing the appropriate antibiotic and incubated overnight at 30°C. Individual colonies were then grown up in L-broth, containing the appropriate antibiotic, in a shaker incubator at 30°C overnight.

DNA was then purified using the alkali lysis method (see below).

2.1.15 Purification of DNA using alkali lysis miniprep method

1mL of the bacterial culture was pelleted by spinning at 13,000rpm for 1 minute. The cell pellet was resuspended in 100µL of ice cold Solution I (50mM glucose, 25mM Tris-HCl pH8.0, 10mM EDTA pH8.0). Freshly prepared Solution II (200µL, 0.2M NaOH, 1% SDS) was then added and mixed by inverting. 150µL of ice cold Solution III (3M Potassium acetate (KAc) pH5.5) was then added, mixed by inverting and placed on ice for 5 minutes. The sample was spun at 13,000rpm for 5 minutes and the supernatant phenol/chloroform purified and ethanol precipitated (see later for details). The resulting DNA was then resuspended in 50µL sterile water.

2.1.16 Purification of DNA using alkali lysis maxiprep method

500mL of bacterial culture was pelleted by spinning at 6,000rpm for 15 minutes. The cell pellet was resuspended in 60mL of Solution I (15mM Tris-HCl pH8.0, 10mM EDTA, 100µg/ml Ribonuclease A (Sigma). Freshly prepared Solution II (60mL of 0.2M NaOH, 1% SDS) was then added, mixed and incubated at RT for 5 minutes. Solution III (60mL of 3M KAc pH5.5) was then added and incubated on ice for 10 minutes. The sample was spun at 6,000rpm for 10 minutes at 4°C and the supernatant added to 160mL of isopropanol and incubated on ice for 10 minutes. The sample was spun at 6,000rpm for 15 minutes at 4°C, supernatant was discarded and pellet resuspended in 5mL of 70% ethanol. The sample was then spun at 6,000rpm for 5 minutes, supernatant discarded and the DNA pellet resuspended in 500µL sterile water, dispensed into 20µL aliquots and stored at -20°C.

2.1.17 Quantification of DNA

DNA was quantified by measuring the absorbance at 260nm using a spectrophotometer equipped with a quartz cuvette. The concentration in µg/mL is equal to the absorbance at 260nm x 50 x the dilution factor. The quality of the DNA can be analysed by measuring the ratio of A260nm/A280nm (good quality DNA should be between 1.7 and 1.9).

2.1.18 Phenol chloroform purification and ethanol precipitation of DNA

To the DNA sample was added an equal volume of phenol:choloroform:isoamylalcohol (25:24:1) which was mixed by vortexing. The

sample was spun at 13,000rpm for 5 minutes and the top layer dispensed into a fresh tube. To this was added an equal volume of chloroform, which was mixed by vortexing. The sample was spun at 13,000rpm for 5 minutes and the top layer dispensed into a fresh tube. To this was added 0.1 volumes of 3M NaAc pH5.2 and 2 volumes of 100% ethanol, and incubated on ice for 30 minutes. The sample was spun at 13,000rpm for 10 minutes and the cell pellet resuspended in 200 μ L of 70% ethanol. The sample was then spun at 13,000rpm for 10 minutes, the supernatant discarded and the pellet air dried. The DNA pellet was then resuspended in an appropriate volume of sterile water.

2.1.19 Use of radiolabelled probes for the detection of positive colonies

Normally colonies resulting from cloning were screened by restriction digest analysis. But when large numbers of colonies were to be screened, this was done using a radiolabelled probe. A gene sequence known to be present in the “insert” DNA was amplified by PCR and purified by agarose gel electrophoresis. The PCR product was then labelled with [α -³²P]dCTPs using the Ready-To-Go DNA labelling beads (-dCTP) from Amersham Biosciences.

DNA (25-50ng) was added to 44 μ L sterile water and denatured at 100°C for 3 minutes then immediately placed on ice for 2 minutes. The DNA was then added to one of the Ready-To-Go DNA labelling beads. When dissolved, 5 μ L (50 μ Ci) of [α -³²P]dCTP was added and mixed by pipetting, and incubated at 37°C for 15 minutes. The PCR product was then labelled and ready to use.

Colonies that were to be screened were first plated onto 2 separate plates of L-agar with the appropriate antibiotic. A gridded membrane was placed in one of the

plates, and each grid numbered (Hybond N⁺ 82mm gridded discs, Amersham Biosciences). The other plate was gridded and numbered identically. Colonies were plated onto specific grids on each plate, and plates incubated at 37°C overnight. Membranes, on which the colonies had grown, were then screened using the radiolabelled probe.

The membranes were placed in 10% SDS for 3 minutes, followed by Denaturing solution (0.5M NaOH, 1.5M NaCl) for 5 minutes, then Neutralisation solution (1.5M NaCl, 0.5M Tris pH7.4) for 5 minutes, and finally 20 x SSC for 5 minutes. This process lysed the colonies, releasing the DNA onto the membrane. The membranes were then placed between 2 pieces of Whatman 3MM filter paper and placed in a vacuum oven at 80°C for 2 hours, in order to fix the DNA onto the membrane. The membranes were then placed in roller bottles and incubated in 10mL of hybridisation buffer (PerfectHybTM Plus hybridisation buffer, Sigma) containing 50µL of labelled probe, in the hybridisation oven at 65°C overnight. The membranes were then washed at 65°C in a total volume of 2L 2 x SSC (preheated to 65°C) over a period of 4 hours, with SSC being changed regularly. The membranes were then placed in a cassette along with a preblanked screen for 1 hour prior to being read on the Phosphorimager (Fujix BAS1000, MacB software).

Colonies that were identified as positives by hybridisation, could then be picked from the duplicate plate and screened by restriction digest and sequence analysis.

2.1.20 DNA sequence analysis

DNA sequencing was either carried out in house by Alex Houston using an automated ABI PRISM™ DNA sequencer or was analysed by the University of Dundee DNA sequencing service using an ABI 3730 capillary DNA sequencer.

2.2 Cell culture

2.2.1 Growth and maintenance of cells

HeLas (Human cervical carcinoma) and 293 (Human embryonic kidney cells) cell lines were maintained in exponential growth in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Calf Serum (FCS) and 2mM glutamine. Cell monolayers were grown in humidified incubators at 37°C, in 5% CO₂.

HeLa spinners and 293 spinners were maintained in exponential growth in Joklik medium supplemented with 10% Newborn Calf Serum (NCS). Spinner cultures were grown in suspension, at 37°C.

2.2.2 Freezing down cells

Cells were frozen down in 40% appropriate growth media, 40% FCS/NCS, 20% DMSO, in sterile screw capped vials. Cells were first stored at -70°C for up to 1 week prior to being transferred to liquid nitrogen.

2.2.3 Recovering cells from liquid nitrogen

Cells were thawed on ice for 10 minutes then at 37°C for 5 minutes. When thawed, 1mL of FCS was added to the cells and 10mL of growth medium. The cells were

spun at 1,200rpm for 5 minutes at RT. The cell pellet was then resuspended in 7mL of appropriate growth medium, transferred to a 25cm² flask and placed in the incubator, at 37°C, until confluent.

2.2.4 Transient transfection of cells

Cell monolayers were grown to approximately 50-80% confluency and were transfected using FuGENE 6 transfection reagent (Roche) at a 3μL:1μg ratio of FuGENE 6: DNA, according to the manufacturer's instructions. DNA and FuGENE 6 were mixed in serum free OptiMEM®1 GlutaMAX™1 medium (Gibco) in 100μL total volume.

2.2.5 Immunofluorescence

For immunofluorescence analysis, coverslips were sterilised in 100% ethanol and air dried, then placed in 12 or 24 well plates. The cells were then added to plates and grown until appropriate confluency, then fixed and analysed as follows:

The media was removed and cells washed 3 times in 1mL Phosphate Buffered Saline (PBS)/ 1mM MgCl₂ /0.5mM CaCl₂. The cells were then fixed in 3% w/v paraformaldehyde/ PBS for 10 minutes, then washed 3 times in PBS. The cells that were transiently transfected with plasmids expressing Cyan Fluorescent Protein (CFP) were mounted onto microscope slides using 6μL of movial/4'-6-Diamidino-2-pheylindole (DAPI) and viewed at this stage. Otherwise cells were washed 2 times in 100mM Glycine/ PBS for 10 minutes then washed 3 times in PBS. At this point the coverslips could be stored in PBS at 4°C overnight. The cells were washed once in 0.2% TritonX-100/PBS for 10 minutes, then washed 3 times in

PBS. The cells were washed 3 times in 0.2% Bovine Serum Albumin (BSA)/PBS over a 5 minute period. The primary antibody at 1/200 dilution was then added in 0.2% BSA/PBS for 45 minutes, then cells were washed 3 times in 0.2% BSA/PBS, over a 5 minute period. The secondary antibody (anti-mouse Fluorescein isothiocyanate (FITC) conjugate, Santa Cruz Biotechnology, Germany) was then added at a 1/500 dilution in 0.2% BSA/PBS and incubated in darkness for 30 minutes. The cells were then washed 3 times in 0.2% BSA/PBS, then washed twice in PBS. The coverslips were then mounted onto microscope slides using 6 μ L of movial/DAPI and viewed with a Nikon Microphot-FXA immunofluorescence microscope.

2.2.6 Making recombinant adenoviruses

Plasmids pSCB-Ad5FL-^{5'ITR} linearised with I-SceI, and pGEM + LHendAd5 linearised with Sal1, were transfected simultaneously into a 25cm² flask of 80% confluent 293 cells using FuGENE 6 transfection reagent. Both DNA samples were purified by agarose gel electrophoresis after restriction digest. Transfection reactions contained 1 μ g of each linearised DNA sample, 6 μ L FuGENE 6 and the volume was made up to 100 μ L with OptiMEM®1 GlutaMAX™1 medium. After transfection the growth medium was changed every two days until plaques were visible, approximately day ten post transfection.

The viruses were then harvested. The cells were scraped into the media and pelleted by centrifugation at 1,200rpm for 5 minutes. The supernatant was aliquoted and kept, and the cell pellet was resuspended in 100 μ L PBS and freeze

thawed 3 times in dry ice and methanol to release virus from cells. All samples were then frozen at -70°C.

2.2.7 Isolation of recombinant viruses by end point dilution

Each well of a 96 well plate was seeded with HeLa cells in DMEM supplemented with 10%FCS and 2mM glutamine. When cells were approximately 60% confluent and ready to infect, a series of 10 fold dilutions of recombinant virus was prepared (100µL of virus in 900µL of media) from 10^{-1} down to 10^{-12} . The medium was then removed from the 96 well plate and the pre-prepared virus dilutions added in columns of 8, from 10^{-2} down to 10^{-12} , with medium only being added to the last column as a control. The plate was then returned to 37°C, 5% CO₂. The plates were monitored daily for evidence of plaque formation and the medium changed every three days, or sooner if it became acidic. After ten days the virus was harvested from the end point wells. Cells were place in a sterile screw capped vial and were freeze thawed 3 times in dry ice and methanol then stored at -70°C. This whole procedure was then repeated another two times using the cell lysate from one well, chosen at random (with the other two being stored at -70°C as back up).

2.2.8 Screening of recombinant adenoviruses

Plaques picked were used to infect 1x75cm² flask of 80% confluent HeLas. After two to three days, when plaques became visible, the virus was harvested by arcton extraction (see below). DNA was then isolated and purified from the arcton

extracted virus using the Qiagen QIAamp DNA Blood minikit (see below). Viral DNA was then subject to PCR and DNA sequence analysis.

Recombinant viruses were also screened using Western blot analysis. A 1x25cm² flask of HeLas cells was infected with virus. After 48 hours cells were harvested by scraping the cells into the media. The cells were pelleted by centrifugation at 1,200rpm for 5 minutes. The cells were then washed in PBS, repelleted, then lysed in 200µL of RIPA buffer (25mM Tris pH8.2, 50mM NaCl, 0.5% NP40, 0.5% deoxycholate, 0.1% SDS, 0.1% sodium azide). The RIPA buffer had protease inhibitors added to it just prior to use; one complete mini (EDTA free) protease inhibitor tablet (Roche) was dissolved in 2mL of RIPA buffer, then 1mL of SDS sample buffer (0.15M Tris-HCl pH6.7, 5% SDS, 30% glycerol plus 0.1% bromophenol blue) and 60µL of 500mM iodoacetamide was added. The RIPA buffer (without the protease inhibitors and the SDS sample buffer) could be stored at 4°C. The SDS sample buffer could be stored at RT.

β-mercaptoethanol was added to each sample (10µL) and samples sonicated briefly (at 5mA for 30 seconds). The samples were then boiled and run on a 10% SDS-PAGE gel, transferred onto Immobilon-P membrane overnight then analysed by Western blot analysis using E1B55k monoclonal antibody 2A6 (a kind gift from Dr Keith Leppard).

2.2.9 Extraction of viral DNA using the Qiagen QIAamp DNA Blood minikit

To arcton extracted virus from 0.5x 75cm² flask was added 100µL of RNase A (10mg/mL) and incubated at 37°C for 30 minutes. After this 62.5µL of protease and 500µL of buffer AL were added and the sample incubated at 70°C for 10

minutes. The sample was then loaded onto a column and spun through at 13,000rpm for 1 minute. The column was then washed through with 500µL of buffer AW1, then 500µL of buffer AW2, spinning at 13,000rpm after each wash. The DNA was then eluted from the column in 50µL sterile water.

2.2.10 Amplification of recombinant viruses

From the plaque pick, virus was scaled up into 1x75cm² flask of HeLas, then 20x75cm² flasks of HeLas, then into 5L of HeLa spinners. The virus was arcton extracted between each scale up.

2.2.11 Infecting HeLa and 293 spinners with adenovirus

As the volume of the spinner cell culture was so large, to ensure that all of the cells came into contact with the virus during infection, the cells were centrifuged at 4,000rpm for 30 minutes to pellet them. Cells were then resuspended in 500mL of serum free Joklik medium, the virus was added to this, and cells incubated, with spinning at 37°C for 1 hour. The volume was then made back up to 5L with Joklik/2% NCS and incubated spinning at 37°C until the cells showed signs of infection.

2.2.12 Arcton extraction of adenovirus

Arcton (1,1,2-Trichlorotrifluoroethane, Aldrich) was used to extract virus from the cytoplasmic membrane of cells. Cells infected with adenovirus were pelleted by centrifugation at 1,200rpm for 5 minutes. The cell pellet was then washed in PBS and repelleted. The cell pellet was then resuspended in an appropriate amount of

PBS and an equal volume of arcton added. This was mixed by shaking vigorously for 30 minutes at RT, after which the sample was spun at 4,000rpm for 15 minutes at RT. The aqueous (top) phase containing the virus was removed, dispensed into aliquots and stored at -70°C. This can be further purified by Caesium chloride (CsCl) gradient.

2.2.13 Virus purification by CsCl gradient

Prior to CsCl purification the arcton extracted virus was spun at 4,000rpm for 10 minutes at RT to pellet any cell debris. The CsCl gradient was then set up by gently layering 2mL of 2M CsCl onto 3mL of 3M CsCl in an ultracentrifuge tube for either an SW40Ti rotor or an SW41Ti rotor. The tubes were presterilised prior to setting up the gradient by spraying with 70% ethanol and allowing to air dry. The virus was then gently layered onto the gradient, taking care not to disrupt any of the layers. The samples were then spun in an ultracentrifuge at 30,000rpm for 2 hours at RT in either SW40Ti or SW41Ti rotor. After spinning, the virus was present as an opalescent band between the 3M and 2M solutions. The cell debris and empty virus particles were visible above the actual viral band. The viral band was removed using a 19 gauge needle and 2mL syringe, by piercing the side of the tube. The virus was then placed in a Slide-A-Lyzer cassette (Pierce) and dialysed in 2L of dialysis buffer (1mM MgCl₂, 135mM NaCl, 10mM Tris-HCl pH7.8, 10% glycerol) at 4°C for 4 hours. The dialysis buffer was then replaced and virus dialysed overnight. The virus was then aliquoted into sterile screw capped vials and stored at -70°C. CsCl was made up in STE buffer (100mM NaCl, 10mM Tris-

HCl pH7.8, 1mM EDTA) and was autoclaved prior to use. Dialysis buffer was also autoclaved prior to use.

2.2.14 Titration of virus stocks

Viruses were titrated using end point dilution. Each well of a 96 well plate was seeded with HeLa cells in DMEM supplemented with 10%FCS and 2mM glutamine. When cells were approximately 60% confluent and ready to infect a series of 10 fold dilutions of recombinant virus was prepared (100µL of virus in 900µL of media) from 10^{-1} down to 10^{-12} . The medium was then removed from the 96 well plate and the pre-prepared virus dilutions added in columns of 8, from 10^{-1} down to 10^{-12} , down the plate, with medium only being added to the last column as a control. The plate was then returned to 37°C, 5% CO₂. The plates were monitored daily for evidence of plaque formation and the medium changed every three days, of sooner if it became acidic. After ten days each well of the 96 well plate was scored +/- for the presence of plaques.

2.2.15 Determination of the optimum concentration of puromycin required to kill HeLa cells

Puromycin (Melford) was made up to a stock concentration of 50mM in sterile water. This was then added to 9x50mL aliquots of growth media (DMEM supplemented with 10% FCS and 2mM glutamine) to give concentrations of 25µM, 20µM, 15µM, 12.5µM, 10µM, 7.5µM, 5µM, 2.5µM and 1µM. Twelve well plates of HeLa cells were set up in normal growth medium. When the cells were 80% confluent, media containing puromycin at the different concentrations

was added to each well. This was performed in duplicate. Some of the wells were maintained in normal growth medium only as a control. The media was changed regularly for 7 to 10 days, with media containing the appropriate concentration of puromycin being added to the appropriate well. At this point the optimum concentration of puromycin required to kill the HeLa cells was determined to be 5 μ M.

2.2.16 Generating HeLa stable cell lines

Stable cell lines were generated using the pEFIRES-PURO construct (Hobbs et al., 1998), which was a kind gift from Dr Pablo De Felipe. By cloning the gene of interest into the pEFIRES-PURO vector and transfecting into cells, which are then grown in puromycin containing media, cells expressing the construct are selected for.

Approximately 5 μ g of the pEFIRES-PURO construct, expressing TAP/E1B55k or TAP/E1B55k K104R, was transfected into a 75cm² flask of 80% confluent HeLa cells, using FuGENE 6 transfection reagent (Roche). Twenty four hours later the flask was trypsinised and the cells split between 4 petri dishes (150mm diameter), with 2 dishes each containing 45% of the cells and the remaining 2 containing 5% of the cells. The cells were grown in normal growth medium overnight before the addition of growth medium containing puromycin at a concentration of 5 μ M. The medium was changed regularly over a 10 to 14 day period, at which point puromycin resistant colonies of cells had developed. Individual colonies were picked using a 1mL pipette and transferred to 1 well of a 12 well plate, where they were expanded in medium containing puromycin. Colonies were then screened by

immunofluorescence and Western blot analysis for expression of the TAP/E1B55k or TAP/E1B55k K104R genes, using a monoclonal antibody to the E1B55k gene as a primary antibody. Colonies that were deemed to be expressing recombinant protein at sufficient levels, were expanded and stocks frozen down in liquid nitrogen (see section 2.2.2 for details).

2.3 Protein purification and analysis

2.3.1 Quantification of protein using Bradford Assay

Protein concentrations were determined using Bradford's method (Bradford, 1976). Protein samples were mixed with 1mL of Bradford reagent (BioRad) for 10 minutes at RT, then the absorbance measured at 595nm using a spectrophotometer. Protein absorbance was converted to mg/mL concentrations using a standard curve constructed by measuring the absorbance of a range of different concentrations of BSA.

2.3.2 SDS-PAGE

To make a standard 10% gel the following recipe was used. The TEMED and 10%APS were added just prior to the gels being poured. BioRad Protean II gel rigs were used.

Separating gel:

Separating gel buffer (1.5M Tris-HCl pH 8.9, 0.4% SDS)	3.95mL
30% polyacrylamide/0.8% bis-acrylamide	4mL
H ₂ O	6.9mL

10% APS	150µL
TEMED	10µL

Stacking gel:

Stacking gel buffer (0.5M Tris-HCl pH6.7, 0.4% SDS)	1.776mL
30% polyacrylamide/0.8% bis-acrylamide	1.2mL
H ₂ O	4mL
10% APS	112µL
TEMED	20µL

Samples to be analysed by SDS-PAGE were boiled at 100°C for 5 minutes in SDS boiling mix prior to loading (0.15M Tris-HCl pH6.7, 5% SDS, 30% glycerol plus 0.1% bromophenol blue). The gel was run in 1x SDS running buffer (25mM Tris, 200mM Glycine, 0.35mM SDS) at 150 volts for approximately 1 hour. Prestained marker was run alongside the protein samples. Gels were then either Coomassie stained or used in western blot analysis.

2.3.3 Coomassie staining

Coomassie stain was used to detect proteins. The gel was stained in Coomassie stain (40% v/v methanol, 10% v/v glacial acetic acid, 0.1% w/v Coomassie brilliant blue R-250) for approximately 10 minutes. Excess stain was then removed from the gel by soaking in destain (20% v/v methanol, 10% v/v glacial acetic acid). The gel was then transferred into ultrapure water.

2.3.4 Western blotting

Proteins resolved on SDS-PAGE gel were transferred to Immobilon-P (PVDF membrane, Sigma) presoaked in methanol. The proteins were transferred using a wet transfer system (BioRad) in Transfer buffer (200mM Tris-HCl, 185mM Glycine, 20% v/v methanol) at 400mA for 2 hours or at 40mA overnight. The membranes were then blocked in blocking buffer (5% w/v skimmed milk powder, 0.1% Tween 20 in PBS) at RT for 1 hour. The primary antibody (E1B55k mouse monoclonal antibody), at 1/500 dilution, was added to membrane, diluted in blocking buffer and incubated at RT for 1 hour. The secondary antibody (Horseradish peroxidase conjugated anti-mouse IgG, Amersham) was added at 1/3000 dilution, diluted in blocking buffer and incubated at RT for 1 hour. The membranes were washed in 0.1% Tween 20 in PBS in between blocking and addition of antibodies, and after addition of the secondary antibody. Western blotting was then performed using Enhanced Chemiluminescent (ECL) detection.

2.3.5 Tandem Affinity Purification method for TAP/SUMO2 cells

120 petri dishes (150mm in diameter) of TAP-SUMO2 or TAP HeLa stable cell lines were harvested when 100% confluent and subjected to tandem affinity purification. The cells were harvested in batches of 5 plates. The media was removed and the plate washed twice in approximately 15mL PBS, then SDS lysis buffer added (50mM Tris-HCl pH8.0, 2% w/v SDS, 10mM iodoacetamide, 1mM EDTA, plus 1x complete EDTA free protease inhibitor tablet (Roche) per 100mL). The petri dish was rocked to ensure even coverage of the lysis buffer then cells were scraped into the lysis buffer using a sterile cell scraper (5mL of lysis buffer

was used for each set of 5 plates). Cell lysates were then frozen in 4x50mL Falcon tubes at -70°C or the procedure continued. If the cell lysates were frozen at -70°C, they were thawed in a waterbath at RT, then placed on ice. The contents of each Falcon tube were then sonicated approximately 10x for 15 seconds at 10mA to shear the genomic DNA. The samples were then spun at 14,000rpm for 5 minutes at RT to pellet any cell debris. The cells were then split evenly between 2x1L durans of Renaturation buffer (50mM Tris-HCl pH8.0, 1M NaCl, 1% NP40, 10mM iodoacetamide, 0.5mM EDTA, 1mM PMSF). Each 1L sample was then passed through a 5mL column containing 1.2mL of IgG sepharose beads, pre-washed in Renaturation buffer. The samples were passed through the columns using gravity flow, and took approximately 4 hours to pass through the column. The columns were then washed with 5 bed volumes of Renaturation solution, then 50mL of Tobacco Etch Virus (TEV) protease cleavage buffer (50mM Tris-HCl pH8.0, 1M NaCl, 1% NP40, 1mM DTT, 0.5mM EDTA) using gravity flow. Each set of beads was then resuspended in 900µL TEV cleavage buffer and 50µL of TEV protease at 1mg/mL added. The samples were incubated, rotating on a daisy wheel at 4°C overnight.

The TEV protease cleaved beads were then transferred to 2x5mL columns and the sample eluted from the beads with 7mL of calmodulin binding buffer (50mM Tris-HCl pH8.0, 1M NaCl, 1mM DTT, 1mM magnesium acetate, 1mM imidazole, 1% NP40, 2mM CaCl₂), which was collected in 2x15mL Falcon tubes. Simultaneously 2x5mL columns were set up with 1mL of calmodulin beads in each, and washed with 10mL of calmodulin binding buffer. The beads were then transferred into the 15ml Falcon tubes and the volume made up to 15mL with calmodulin binding

buffer. The samples were then incubated, rotating on a daisy wheel at 4°C for 4 hours. The beads were then transferred back into the 2x5mL columns and washed through with 30mL of calmodulin binding buffer.

Protein was then eluted from the calmodulin beads using calmodulin elution buffer (50mM Tris-HCl pH8.0, 1M NaCl, 1mM DTT, 1mM Magnesium acetate, 1mM imidazole, 1% NP40, 10mM EGTA). Each of the columns were plugged prior to the addition of 1.5mL of calmodulin elution buffer, and incubated at 4°C for 10 minutes. Protein was then allowed to elute by gravity flow and this process was repeated. All 4x1.5mL aliquots were then stored at -70°C until ready to analyse by SDS-PAGE and Mass Spectrometric analysis. Eluates were then thawed on ice and TCA precipitated (see below).

2.3.6 Tandem affinity purification method for TAP/E1B55k (+/-K104R)*

60 petri dishes (150mm diameter) of TAP/E1B55k or TAP/E1B55k K104R HeLa stable cell lines were harvested when 100% confluent and subjected to tandem affinity purification. The cells were harvested in batches of 10 plates. The media was removed and plates were washed twice in approximately 15mL PBS. The cells from each plate were then scraped into 2mL PBS, transferred to a 50mL Falcon tube and pelleted by centrifugation at 1,200rpm for 2 minutes. The cell pellets were then resuspended in a total volume of 60mL lysis buffer (50mM Tris-HCl pH7.4, 150mM NaCl, 1mM EDTA, 1% NP40, 15% glycerol, 1mM PMSF), and incubated at 4°C for 30 minutes. The lysis buffer was made up in 100mL volume and contained 1x complete EDTA free protease inhibitor tablet (Roche). The cell lysates were then spun at 14,000rpm for 15 minutes at 4°C to pellet any

cell debris. The supernatant was then split evenly between 6x 15mL Falcon tubes and 200 μ L of IgG sepharose (Sigma) added to each tube, and samples were incubated with rotation at 4°C for 4 hours. The IgG beads were then pelleted by centrifugation at 2,000rpm for 2 minutes at 4°C. The beads were then washed 3x in TBS/0.05% Tween-20 with the beads being pelleted in between washes. The beads were then washed in TEV cleavage buffer (10mM Tris pH8.0, 150mM NaCl, 0.1% NP40, 0.5mM EDTA, 1mM DTT) before being resuspended in a total volume of 200 μ L of TEV cleavage buffer. TEV protease was added (20 μ L at 1mg/mL concentration) and the sample incubated at 4°C, overnight, with rotation, in an eppendorf tube.

The beads were pelleted by centrifugation at 13,000rpm for 3 minutes at RT. The supernatant was transferred to a clean tube, to which was added 3 volumes of calmodulin binding buffer and 3 μ L of 1M CaCl₂ (per mL of supernatant). Calmodulin beads (200 μ L) were then transferred to a disposable 2mL polystyrene column (Pierce) and washed in 5mL of calmodulin binding buffer (10mM β -mercaptoethanol, 10mM Tris pH8.0, 150mM NaCl, 1mM Magnesium acetate, 1mM imidazole, 2mM CaCl₂, 0.1% NP40). The supernatant was then added to the washed calmodulin beads and incubated at 4°C for 2 hours, with rotation. After incubation the sample was added to disposable 2mL polystyrene columns and allowed to drain by gravity flow. The beads were then washed in approximately 30mL of calmodulin binding buffer. Proteins were then eluted into 1.5mL of calmodulin elution buffer (10mM β -mercaptoethanol, 10mM Tris pH8.0, 150mM NaCl, 1mM magnesium acetate, 1mM imidazole, 2mM EGTA, 0.1% NP40). The column was plugged prior to the addition of 1.5mL of elution buffer, then

incubated at 4°C for 10 minutes. The protein was then eluted by gravity flow. The protein eluates were stored at -70°C until ready to analyse by SDS-PAGE and Mass Spectrometric analysis. Eluates were then thawed on ice and TCA precipitated.

* This is the basic protocol before any modifications were made in order to optimise the tandem affinity purification procedure. Optimisation of this protocol is discussed in results chapter 3.

2.3.7 Dignam and Roeder method for harvesting nuclear extracts

Cells were scraped into PBS and are pelleted by centrifugation at 1,500rpm at 4°C. The cell pellet is then resuspended in 3 volumes of Dignam A buffer (10mM Hepes-KOH pH7.9, 1.5mM MgCl₂, 10mM KCl, 0.5mM DTT, 0.5mM PMSF) and incubated on ice for 15 minutes. The cells are lysed by pushing several times through a 25 gauge needle. The nuclei are then pelleted by centrifugation at 14,000rpm for 30 minutes, and are resuspended in 1.5 volumes of Dignam C buffer (20mM Hepes pH7.9, 25% glycerol, 0.42M NaCl, 1.5mM MgCl₂, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF), by incubation at 4°C for 30 minutes, with rotation. The cell membranes are pelleted by centrifugation at 15,000rpm for 30 minutes, and the supernatant, containing the nuclear extract, is dialysed against Dignam buffer D (20mM Hepes pH7.9, 20% glycerol, 0.1M KCl, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF).

2.3.8 TCA precipitation

Protein samples were aliquoted into 1mL aliquots, then 1/3 volume (0.33mL) of 100% w/v TCA/ 0.5% sodium deoxycholate was added to each aliquot and incubated on ice for 15 minutes. The sample was then centrifuged at 14,000rpm for 20 minutes at 4°C and the supernatant discarded. Each pellet was then washed twice in 1mL of neat acetone and respun at 14,000rpm for 2 minutes. Protein pellets were then air dried in the fume hood and resuspended in a total volume of 30µL of SDS boiling mix.

The sample were then run on a 10% SDS-PAGE gel at 100 volts for 1.5 hours, Coomassie stained, then destained. The lane of the gel containing the sample was then cut into separate “chunks”, approximately 10-15 chunks per track, which were then subjected to in-gel digestion and LC-ESI-MS/MS analysis.

2.3.9 LC-ESI-MS/MS analysis

The gel track was excised into approximately 10-15 sections. Each section was cut into 1mm cubes and the gel cubes for each section were split between 2 wells of the 96 well plate if necessary. These were then subjected to in-gel digestion, using a ProGest Investigator in-gel digestion robot, using standard protocols. Briefly the gel cubes were destained by washing with acetonitrile and subjected to reduction and alkylation before digestion with trypsin at 37°C. The peptides were extracted with 10% formic acid and concentrated down to 20µL (SpeedVac, ThemoSavant). They were then separated using an UltiMate nanoLC (LC Packings, Amsterdam) equipped with a PepMap C18 trap and column. The eluent was sprayed into a Q-Star Pulsar XL tandem mass spectrometer (Applied Biosystems, Foster City, CA) and analysed in Information Dependent Acquisition (IDA) mode. MS/MS data for

doubly and triply charged precursor ions was converted to centroid data, without smoothing, using the Mascot Daemon 2.1 (Matrix Science, London) data import filter for Sciex Analyst. The MS/MS data file generated was analysed using the Mascot search engine against MSDB July 2005. The data was searched with tolerances of 0.2Da for the precursor and fragment ions, trypsin as the cleavage enzyme, one missed cleavage, carbamidomethyl modification of cysteine as a fixed modification and methionine oxidation selected as variable modifications.

3. GENERATION OF RECOMBINANT ADENOVIRUSES

3.1 Introduction

3.1.1 Introduction

Four recombinant adenoviruses expressing tagged E1B55k were generated. The adenoviruses were constructed from plasmid pSCB-AdFL which contains the full length wild type adenovirus 5 genome, to allow the study of this protein. The recombinant adenoviruses expressed E1B55k that was N-terminally tagged with either Cyan Fluorescent Protein (CFP), or Tandem Affinity Purification (TAP) tag. The CFP tag would allow the localisation of the E1B55k gene to be studied during infection, using the Delta Vision microscope, which can be used for live imaging. The TAP tag would enable the purification of the E1B55k protein along with any interacting host cell or viral proteins, which could then be identified using SDS-PAGE and LC-ESI/MS/MS. As discussed previously, the E1B55k protein is modified by SUMO protein (Endter et al, 2001), and it is a lysine residue at amino acid 104, that lies within the SUMO consensus sequence ΨKxE , that is essential for SUMO modification. By mutating the lysine to an arginine, modification of E1B55k by SUMO is abolished but the charge remains unchanged (Endter et al, 2001). Therefore, E1B55k K104R versions of the recombinant adenoviruses were made, to study the impact of SUMO modification on the E1B55k protein, both in terms of localisation, and interacting proteins, during the infection process.

The four recombinant adenoviruses generated were:

- TAP/E1B55k
- TAP/E1B55k K104R
- CFP/E1B55k
- CFP/E1B55k K104R

3.1.2 Strategy for generating the recombinant viruses

The construct pSCB-AdFL was used to generate the recombinant adenoviruses, which consists of a bacmid backbone, and the full length wild type adenovirus 5 genome flanked by I-Sce1 restriction sites. This construct was a kind gift from Philip Moullier (Inserm, Nantes). See figure 3.1.

The strategy for making the recombinant viruses involved cloning the left hand end of the adenovirus genome, containing the E1B55k gene, into a standard cloning vector. Thus, by dramatically reducing the size of the DNA fragment, this would allow the E1B55k gene to be replaced with the tagged version plus or minus the K104R (+/- K104R) mutation, using standard molecular biology techniques. In order to simplify the cloning procedure, the left hand end of the adenovirus genome was cloned in such a way, that unique restriction sites (Xba1 and BsaB1) would be present either side of the E1B55k gene, allowing it to be replaced easily, with the tagged version +/- K104R mutation.

Cyan fluorescent protein (CFP) tagged E1B55k and E1B55k K104R constructs were a kind gift from Tony Vaughan. These constructs had been generated by cloning the E1B55k and E1B55k K104R genes into the EcoR1/ BamH1 restriction sites of the multiple cloning site (MCS) of vector pECFP-C1 (Clontech). The E1B55k genes had been amplified by PCR, and the primers designed to have the necessary restriction sites, to facilitate cloning into the pECFP-C1 vector (see figure 3.2).

TAP tagged E1B55k and E1B55k K104R were generated by amplifying the E1B55k genes from the CFP constructs and cloning into vector pCMV5/TAP, a kind gift from Maria Deak (University of Dundee). The PCR primers used were designed to have BamH1 restriction sites, to allow cloning into the BamH1 site of the MCS of pCMV5/TAP (see figure 3.3).

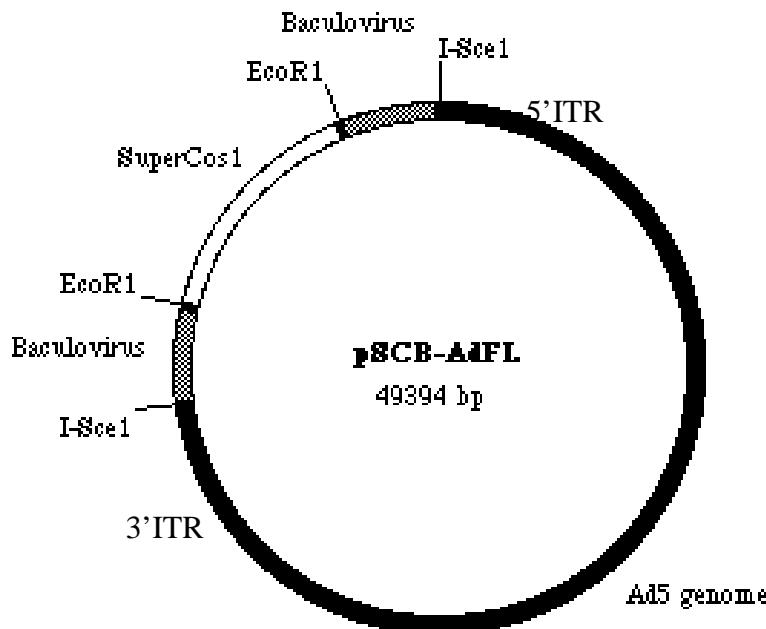


Figure 3.1: Bacmid pSCB-AdFL. This map illustrates Bacmid pSCB-AdFL containing the full length wild type adenovirus 5 genome, flanked by I-Sce1 restriction sites.

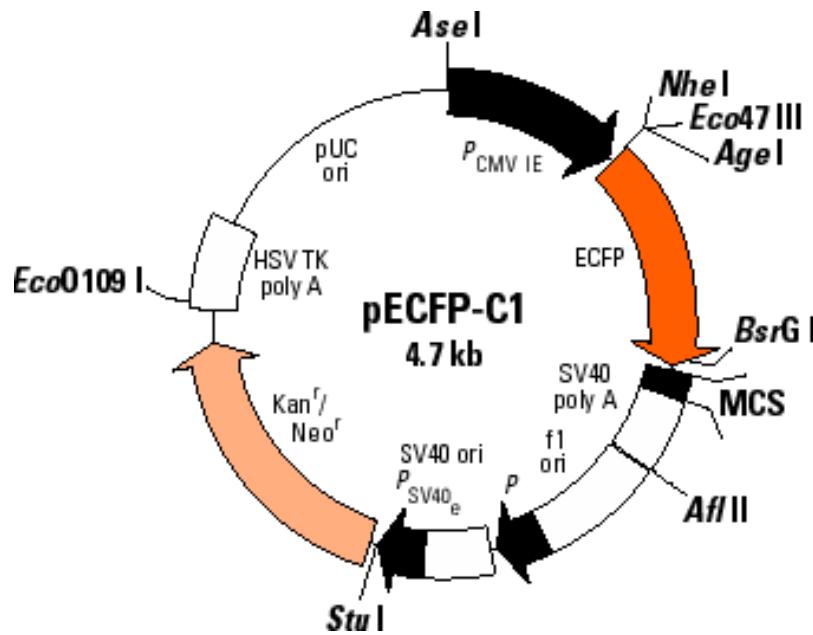
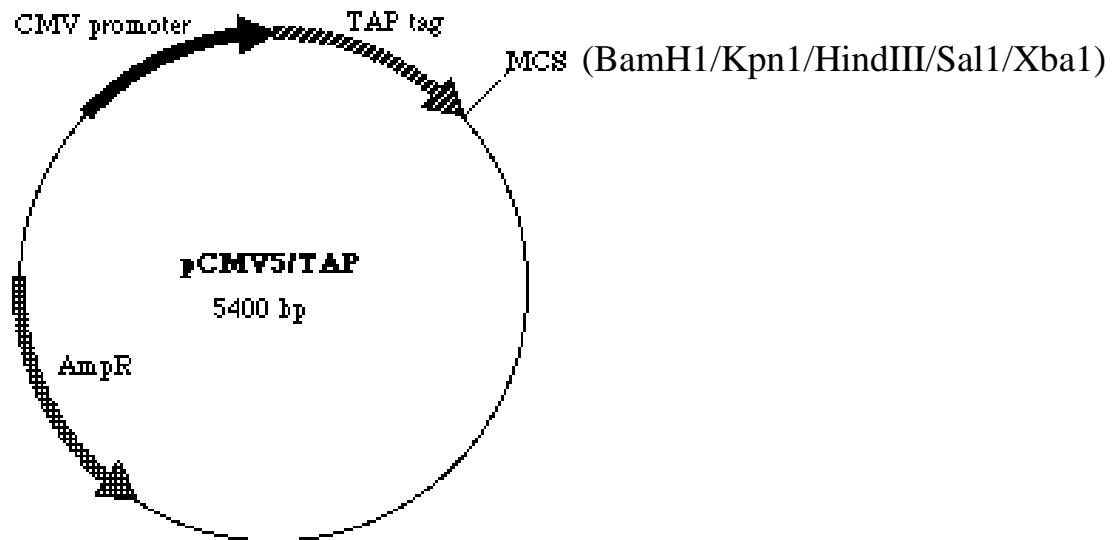


Figure 3.2: Plasmid pECFP-C1. This figure illustrates plasmid pECFP-C1 into which the E1B55k gene, and E1B55k K104R gene, were cloned by Tony Vaughan. The E1B55k genes were cloned between EcoR1 and BamH1 restriction sites of the MCS.



131

Figure 3.3: Plasmid pCMV5/TAP. The genes encoding E1B55k and E1B55k K104R were amplified from plasmid pECFP-C1 using primers p9 and p10A which were designed to include BamH1 restriction sites. PCR products were digested and cloned into the BamH1 site in the MCS, of plasmid pCMV5/TAP.

After generation of the TAP tagged and CFP tagged versions of E1B55k, +/- K104R mutation (TAP-E1B55k, TAP-E1B55k K104R, CFP-E1B55k and CFP-E1B55k K104R), overlap PCR was used to flank each of the tagged proteins, with adenovirus type 5 DNA homologous to the regions either side of wild type E1B55k. During the original cloning, the left hand end of Adenovirus type 5 had been cloned into the standard cloning vector in such a way, that unique restriction sites were present either side of the E1B55k gene. The overlap PCR products was designed to incorporate these restriction sites, allowing the wild type E1B55k gene to be replaced with the tagged versions +/- K104R mutation (see figure 3.4).

Generation of the recombinant adenoviruses was achieved by co-transfection of two different constructs, one containing the left hand end of Ad5 and the other containing the remainder of the Ad5 genome, with both plasmids having a homologous region of adenoviral DNA, allowing homologous recombination to occur (see figure 3.5).

The second construct required was generated by knocking out the left hand Inverted Terminal Repeat (ITR) of plasmid pSCB-AdFL, using homologous recombination (see figure 3.6). Overlap PCR was used to generate an antibiotic resistance gene, flanked by regions homologous to either side of the region to be knocked out. Co-electroporation of the overlap PCR product and pSCB-AdFL (linearised with I-Sce1) into recombinogenic BJ5183 cells, resulted in homologous recombination and generation of positive colonies, which could be selected for by growing on plates containing the appropriate antibiotic.

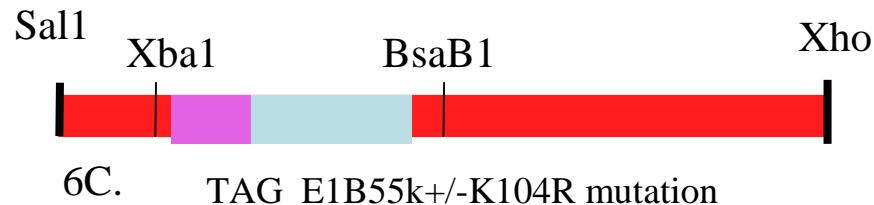
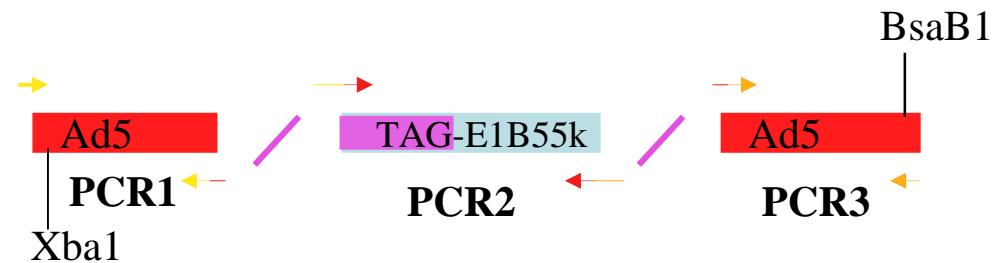
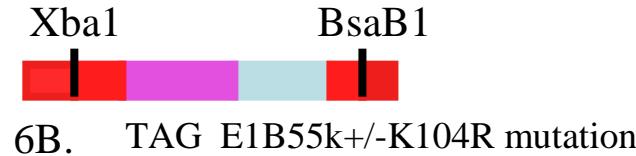
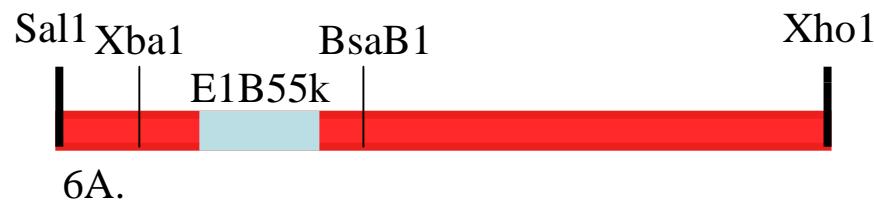


Figure 3.4: Generation of overlap PCR products. This diagram illustrates how overlap PCR was used to replace E1B55k in the left hand region of Ad5 with the tagged version, +/- the K104R mutation. The region of PSCB-AdFL that was cloned into the pGEM-5zf(+) vector is shown in A. The overlap PCR product used to generate tagged versions of E1B55k +/- the K104R mutation, flanked on either side by the appropriate adenovirus type 5 DNA (containing the unique restriction sites Xba1 and BsaB1), are shown by B. The resulting plasmids generated by replacing the E1B55k with the tagged version +/- K104R mutation are illustrated by C.

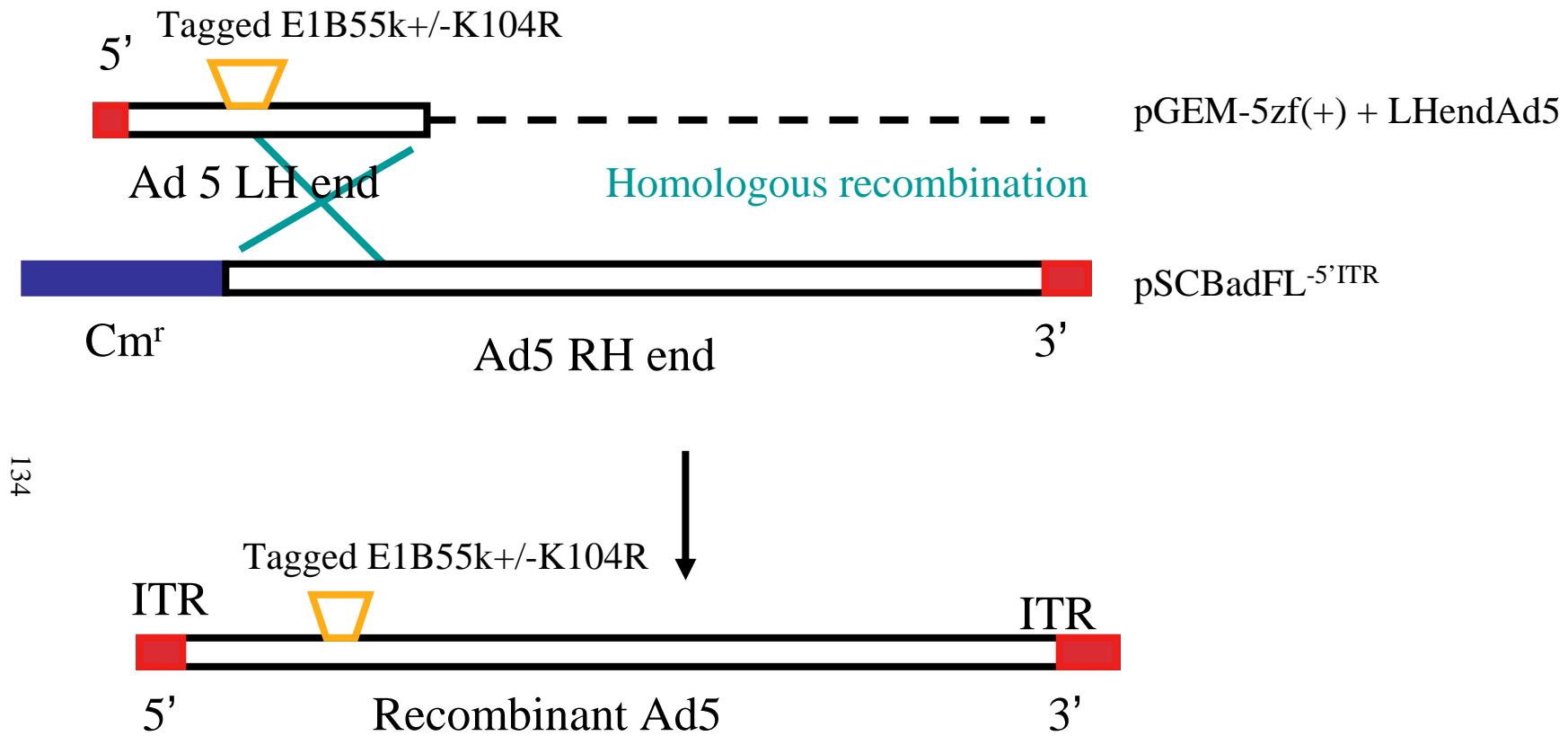


Figure 3.5: Homologous recombination occurs to generate full length recombinant virus. Linearised plasmids pGEM-5zf(+) + LHendAd5 and pSCBAdFL^{-5'ITR} are co-transfected into permissive tissue culture cells. Homologous recombination results in the generation of recombinant adenovirus.

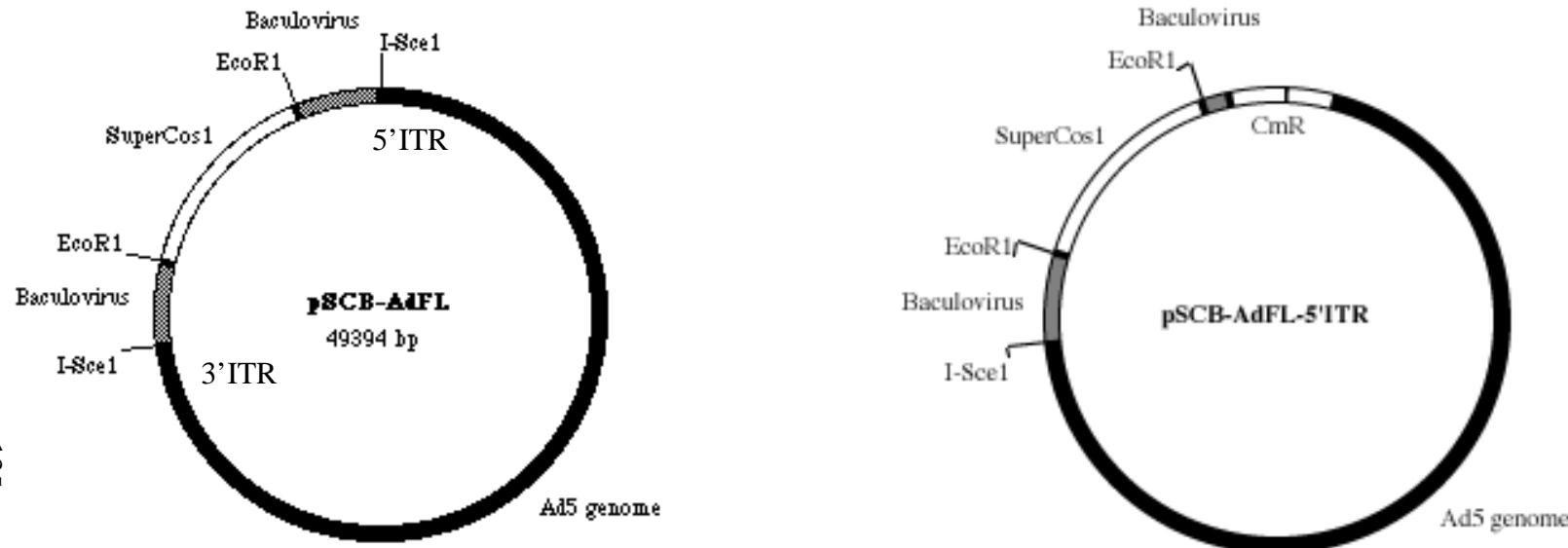


Figure 3.6: Knocking out the 5' ITR of pSCB-AdFL using chloramphencil resistance gene. The 5' ITR was knocked out of plasmid pSCBAdFL and replaced with the Chloramphenicol resistance gene from plasmid pACYC184 (New England Biolabs). Overlap PCR was used to generate the chloramphenicol resistance gene flanked by the appropriate Baculovirus and Ad5 DNA either side. Plasmid pSCBAdFL was then partially digested with restriction enzyme I-SceI (to cleave it at the 5'ITR only) and gel purified. Linearised plasmid was then co-electroporated into recombinogenic BJ5183 cells along with purified PCR product. Homologous recombination resulted in formation of plasmid pSCBAdFL-^{-5'ITR}.

Recombinant adenovirus was generated by co-transfected each of the linearised constructs into tissue culture cells, permissive for adenovirus growth. Homologous recombination occurring between segments of homologous DNA in each of the plasmids resulted in the formation of recombinant adenovirus (see figure 3.5). This was indicated by the appearance plaques on the cell monolayer, approximately ten days post-transfection.

3.2 Results

3.2.1 Initial strategies for cloning the left hand end of Ad5 into a standard cloning vector

The original cloning strategy for making the recombinant adenoviruses was devised using a basic restriction map of plasmid pSCB-AdFL, as there was initially no sequence information available.

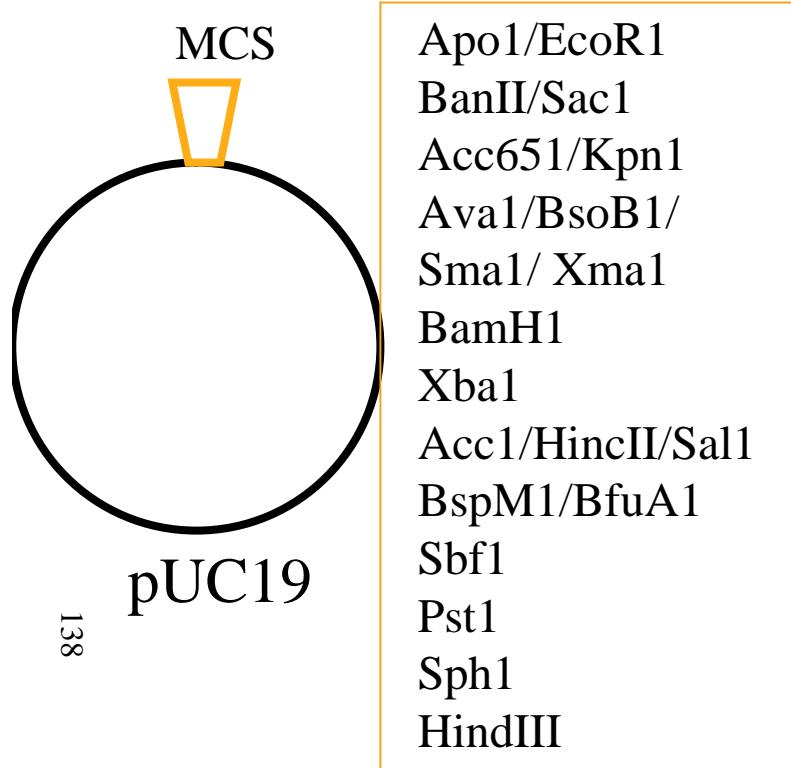
The original strategy for cloning the left hand end of the adenovirus genome into the pUC19 vector required modification of the MCS. This was achieved by using linkers L3 and L4, which were designed to include an I-Sce1 restriction site, and knock out the Xba1 site in the MCS of pUC19. Linkers L1 and L2 which had been originally been designed for the same purpose, had an error which prevented the Xba1 site from being knocked out. Linkers L3 and L4 were cloned successfully into pUC19, verified by restriction digest and sequence analysis, using primers pUC19F and pUC19R, which were designed to sequence through the MCS.

Plasmid pSCB-AdFL was cut with restriction enzymes I-Sce1 and Sal1 generating 5 fragments of 19.4kb, 13kb, 8.8kb, 7.3kb and 0.3kb, with the 8.8kb band being the desired fragment. This 8.8kb DNA fragment was purified by agarose gel

electrophoresis, to remove any contaminating DNA bands, and ligated into the modified pUC19 vector also digested with Sal1 and I-Sce1. The modified pUC19 vector, after restriction digest, had also been purified by agarose gel electrophoresis to remove any contaminating DNA bands. Prior to ligation DNA was quantified by agarose gel electrophoresis, to ensure there was sufficient DNA present for the ligation reaction to occur.

All initial cloning attempts resulted in empty vector suggesting that one of the restriction enzymes was not cutting properly. Restriction digests were carried out first with I-Sce1, as this restriction enzyme requires a 6bp overhang, followed by Sal1, which requires a 3bp overhang. A control ligation reaction was also set up, with modified pUC19 vector cut with I-Sce1 and Sal1, to ensure that the vector was not religating, which could occur if only one of the restriction enzymes had cut.

PCR of the insert DNA, using primers to the E1B55k gene (P9 and P10A), confirmed that the correct fragment was being used for the cloning. After ligation the DNA samples were transformed into transformation competent DH5 α , but all resulting colonies screened consisted of empty vector. Subsequent attempts at electroporating the DNA into electrocompetent DH5 α also resulted in colonies consisting of empty vector. The MCS of the modified pUC19 plasmid was reanalysed by sequence analysis, and found to be mutated, explaining why the cloning attempts had proved unsuccessful (see figure 3.7).



5' G'AATTCTAGGGATAACAGGGTAAT A'CTAGA 3'
3' CTTAA'G ATCCCTATTGTCCCATTATGATC'T 5'

EcoR1 I-Sce1 Xba1

5' AATTCTAGGGATAACAGGGTAAT A 3'
3' G ATCCCTATTGTCCCATTATGATC 5'

EcoR1 I-Sce1 ~~Xba1~~

Figure 3.7: Linkers used to modify the MCS of pUC19 vector. Linkers L3 and L4 were designed to incorporate an I-Sce1 restriction site into the MCS, but knock out the Xba1 restriction site. Linkers L3 and L4 were designed to be cloned into pUC19 digested with EcoR1 and Xba1. After initial cloning, sequence analysis confirmed that the sequence of the MCS was as it should have been. When sequenced subsequently, after cloning attempts had failed, the sequence of the MCS was found to read EcoR1 - I-Sce1 - unidentifiable sequence - EcoR1 - I-Sce1 - Sal1 - Xba1, suggesting that some sort of mutation had occurred.

3.2.2 The second strategy for cloning the left hand end of Ad5 into a standard cloning vector

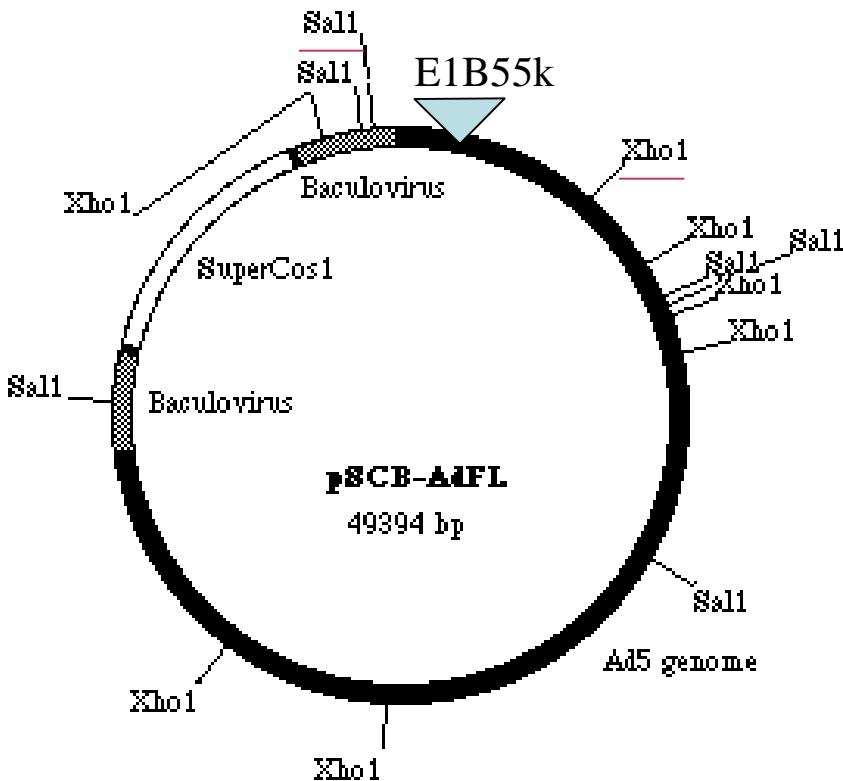
The second attempt at cloning the left hand end of Ad5 was based on sequence data of pSCB-AdFL, which had kindly been sent by Christophe Darmon. The second strategy was to clone the left hand end of Ad5 into pUC19 by cutting plasmid pSCB-AdFL with Sal1 only, and ligating the fragment containing the left hand end of Ad5 into the Sal1 site of unmodified pUC19. Digestion of pSCB-AdFL with Sal1 generated 6 fragments of 20.8kb, 10.4kb, 10.2kb, 6.9kb, 0.3kb, 0.2kb, of which the 10.2kb band was the desired fragment. The 10.2kb fragment containing the left hand end of Ad5 was purified by agarose gel electrophoresis, and ligated into the pUC19 vector. The pUC19 vector had been digested with Sal1, purified by agarose gel electrophoresis, and treated with Calf-intestinal alkaline phosphatase (CIAP) to prevent religation. The ligation reactions were then transformed into DH5 α , and plated onto ampicillin plates. Resulting colonies were screened by hybridisation using the E1B55k PCR product (amplified using primers P9 and P10A) labelled with ^{32}P . This method proved unsuccessful. Although colonies were generated, all colonies screened consisted of religated bacmid backbone. This was caused by contamination of the 10.2kb band with 10.4kb band, which was the bacmid backbone, and which was ampicillin resistant.

3.2.3 Final strategy for cloning the left hand end of Ad5 into a standard cloning vector

The final strategy for cloning the left hand end of Ad5 involved digesting plasmid pSCB-AdFL with Xho1, generating fragments of 17.6kb, 14.5kb, 7.8kb, 4.9kb, 2.4kb, 1.4kb and 1kb. This time the 7.8kb band was well separated from other bands and was purified by agarose gel electrophoresis, then digested with Sal1 yielding fragments of 6.5kb, 1.3kb and 0.2kb, of which the 6.5kb band was the desired fragment, containing the left hand end of the adenovirus genome. The 6.5kb band was purified by agarose gel electrophoresis to remove any contaminating DNA bands and cloned into pGEM-5zf(+) vector (see figures 3.8 and 3.9). The pGEM-5zf(+) vector had been digested with Sal1, purified by agarose gel electrophoresis and CIP treated, to prevent religation (Sal1 and Xho1 restriction enzymes have compatible overhangs). Following ligation, samples were transformed into DH5 α and plated onto ampicillin plates. Resulting colonies were screened by hybridisation using E1B55k PCR product labelled with ^{32}P . Cloning using this strategy was successful.

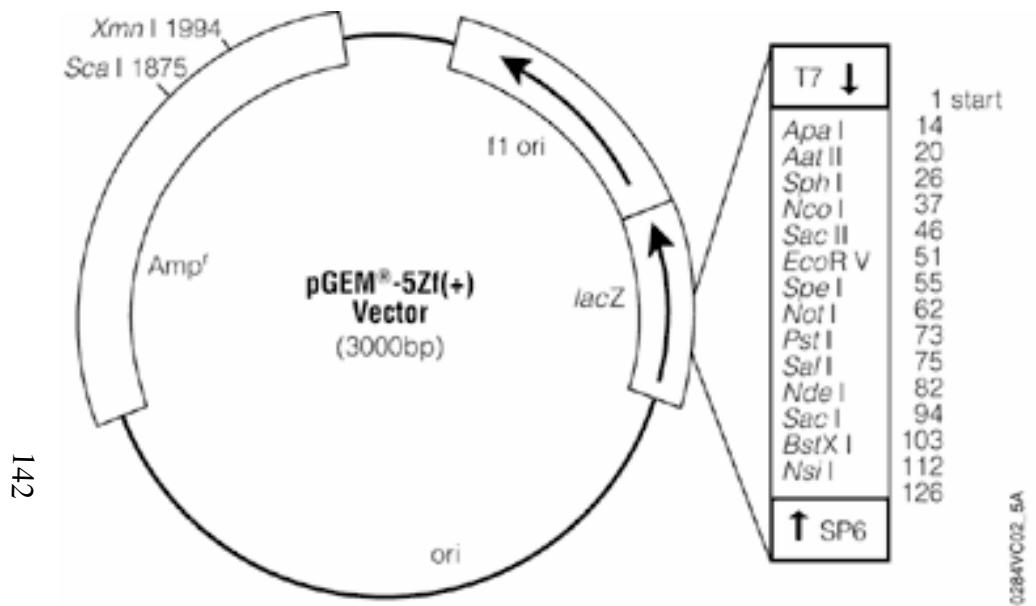
3.2.4 Strategy for replacing wild type E1B55k with the tagged E1B55k +/- K104R mutation

Overlap PCR was used to generate the tagged E1B55k +/- K104R mutation, flanked on either side by Ad5 DNA, homologous to the regions on either side of the wild type E1B55k. The strategy for cloning the left hand end of the adenovirus genome was designed so that unique restriction sites would be present on either side of the E1B55k, allowing it to be replaced easily with the tagged



141

Figure 3.8: Left hand end of adenovirus released by cleaving pSCB-AdFL with restriction enzymes Sal1 and Xho1. Plasmid pSCB-AdFL was digested with Xho1 to yield fragments of 7.7kb, 2.3kb, 1.6kb, 1.1kb, 14.6kb, 4.7kb and 17.4kb, with the 7.7kb band containing the left hand end of the Ad5 genome and part of the Bacmid backbone. The 7.7kb band was gel purified and further digested with Sal1 to yield fragments of 6.5kb, 1.3kb and 0.2kb. The 6.5kb fragment (between the underlined Sal1 and Xho1 restriction sites), containing the left hand end of the Ad5 genome and part of the bacmid backbone was gel purified and ligated into the pGEM-5zf(+) vector.



Sal1 restriction site: **G'TCGAG**
CAGCT'G

Xho1 restriction site: **C'TCGAG**
GAGCT'C

Sal1/Xho1: **G'TCGAG**
CAGCT'G

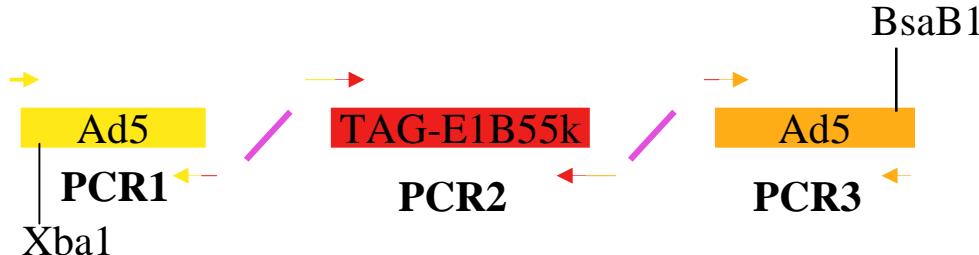
Figure 3.9: The pGEM-5zf(+) vector (Promega). The 6.5kb gel purified fragment from pSCB-AdFL containing the left hand end of the adenovirus genome contained Sal1 / Xho1 overhangs and was cloned into the Sal1 restriction site of pGEM-5zf(+). Sal1 and Xho1 restriction sites have compatible overhangs.

version +/- K104R mutation. Hence the overlap PCR product was designed to include the regions homologous to the regions either side of the wild type E1B55k, including these unique restriction sites (see figure 3.10). The following plasmids were used as templates during generation of the overlap PCR product; TAP/E1B55k, TAP/E1B55k K104R, CFP/E1B55k, CFP/E1B55k K104R and pGEM-5zf(+) + LHendAd5 (the Ad5 regions either side of the E1B55k gene were amplified from this plasmid).

In the initial round of overlap PCR each of the three DNA regions that make up the final product were amplified separately. These were the region downstream of the E1B55k gene (product 1, using primers P1 and P2, or P1 and P3), the tagged E1B55k gene plus or minus the K104R mutation (product 2, using primers P4 and P6, or P5 and P6), and the region upstream of the E1B55k gene (product 3, using primers P7 and P8). The primers used differed, depending on whether they were used for the amplification of the CFP or the TAP tagged E1B55k genes. The PCR products were purified by agarose gel electrophoresis to remove any contaminating products, prior to being used in the second round of overlap PCR.

In the second round, products 1 and 2 were combined in equal quantities and amplified using primers P1 and P6, to generate product 4. The DNA concentration was assessed by agarose gel electrophoresis. Products 2 and 3 were also combined in equal quantities and amplified using primers P4 and P8, or P5 and P8, to generate product 5. Again both of the resulting PCR products were purified by agarose gel electrophoresis prior to being used in the final round of overlap PCR.

In the final round of amplification products 4 and 5 were combined in equal quantities and amplified using primers P1 and P8 (see figure 3.11).



Primers

5' CGC TCTAGA GAATGCAATAGTAGTACGGATAGCTGTGAC 3' (P1)
Xba1
PCR1 3' CTCAAAATATTCCATT CGGTGG TAC CTGTGTTCACGGGTG 5' TAP (P2)
3' AAAATATTCCATTCGGTGG TAC CACTCGTCCCGCTCCTC 5' CFP (P3)

5' GAGTTTATAAAGGATAA GCCACC ATG GACACAAGTGCCCAC 3' TAP (P4)
5' GAGTTTATAAAGGATAA GCCACC ATG GTGAGCAAGGGCGAG 3' CFP (P5)
PCR2 3' TCGCTACTTCTATGTCTAACT CCATGACTTACACACCCGCA 5' (P6)

5' AGCGATGAAGATACAGAT TGA GGTACTGAAATGTGTGGCGT 3' (P7)
PCR3 3' TTTGGTCTGAGACAAACCTAAACCTAGTTCGTCACAGA 5' (P8)
BsaB1

Figure 3.10: Primers used to generate the overlap PCR products. The primers used to replace E1B55k with the tagged E1B55k gene plus or minus the K104R mutation, differed depending on whether they were used to amplify the TAP/E1B55k genes or the CFP/E1B55k genes. Primers P1, P2, P4, P6, P7 and P8 were used to generate the TAP tagged E1B55k overlap PCR products. Primers P1, P3, P5, P6, P7 and P8 were used to generate the CFP tagged E1B55k overlap PCR products.

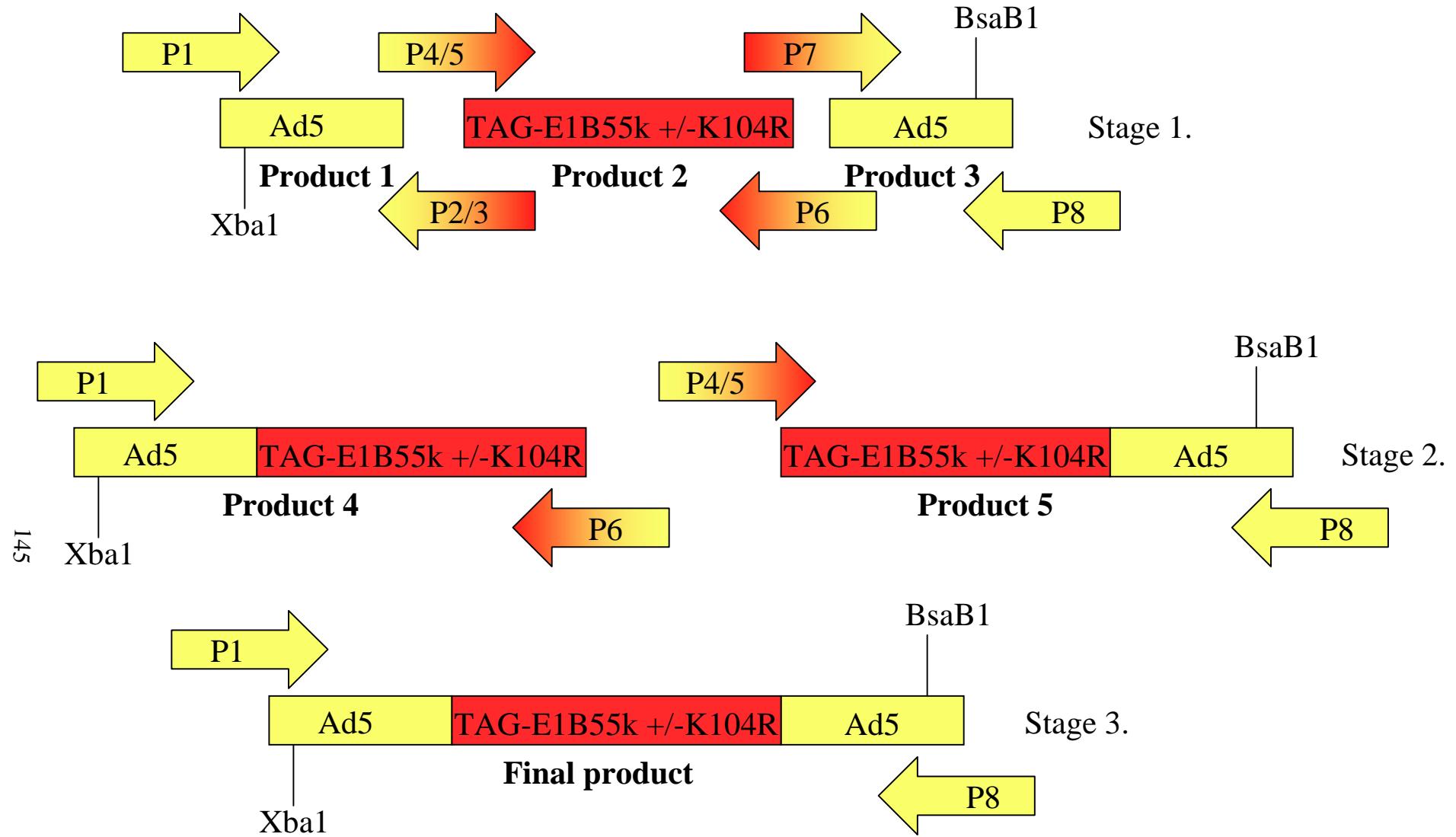


Figure 3.11: The separate stages of overlap PCR. In Stage 1, each of the products were amplified separately using appropriate primers, generating three separate products 1,2 and 3. In Stage 2, products 1 and 2 were combined in equal quantities and amplified using primers P1 and P6 to generate product 3. Products 2 and 3 were combined in equal quantities and amplified using primers P4 or P5, and P8 to generate product 4. In Stage 3 products 4 and 5 were combined in equal quantities and amplified using primers P1 and P8 to generate the final product.

The final PCR product was purified by agarose gel electrophoresis, then subsequently cleaved with restriction enzymes Xba1 and BsaB1, ready to be cloned into the pGEM-5Zf(+) + LHendAd5 vector.

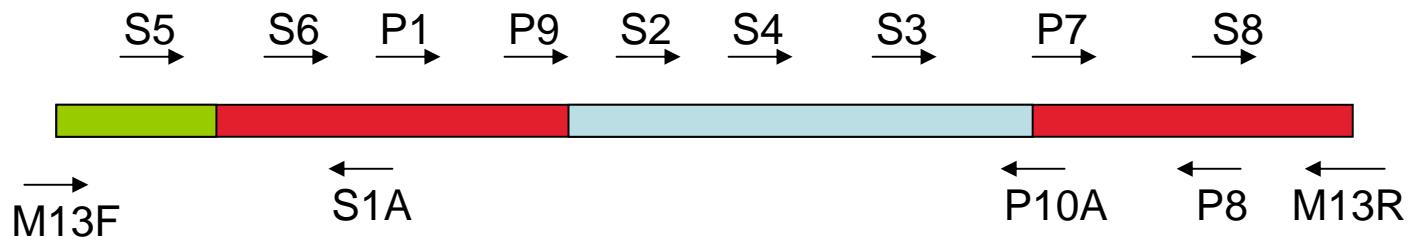
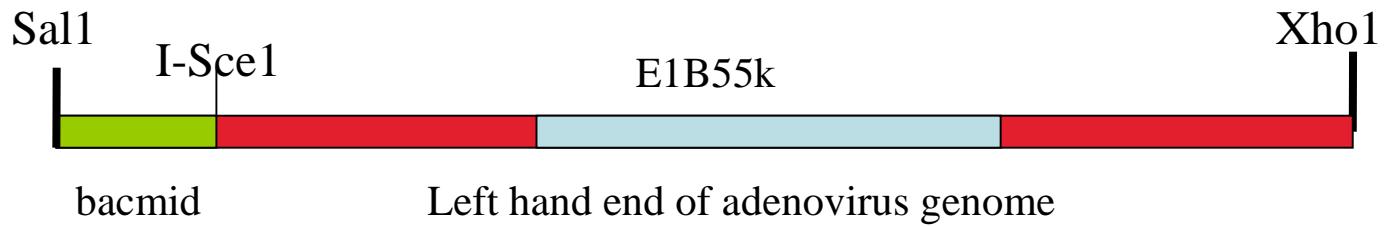
3.2.5 Replacing the E1B55k gene in pGEM-5zf(+) with the tagged version +/- K104R mutation using Xba1 and BsaB1 restriction sites

Problems were encountered when cutting both overlap PCR products and vector DNA with the restriction enzymes Xba1 and BsaB1. The Xba1 enzyme cleaved normally, but when DNA was digested with the BsaB1 enzyme and loaded onto the agarose gel, the DNA did not run into the gel upon application of the electrical current, but remained in the well. Various different methods were tried in order to solve this problem, including adding 0.25% SDS, treating the DNA with Proteinase K, heat inactivating the enzyme, denaturing the enzyme and purifying the DNA from the restriction digest using the Roche High Pure PCR product purification kit, prior to running it on the agarose gel. This problem was eventually resolved by setting up the restriction digests in 50 μ L reaction volumes, thus reducing the concentration of the DNA, and cutting for 30 minutes to 1 hour, as opposed to 20 μ L reaction volumes for 1-2 hours.

When the pGEM-5zf(+) + LHendAd5 vector was successfully cleaved with Xba1 and BsaB1, the fragment that released was 1.6kb, instead of the expected 2.7kb. In order to determine if this was due to the BsaB1 enzyme affecting how the DNA ran into the agarose gel, or if it was due to the presence of another BsaB1 restriction site within the pGEM5zf(+) + LHendAd5 plasmid, the entire fragment of

DNA which had been cloned into the pGEM-5zf(+) vector was sequenced. Fourteen different primers were used to sequence the entire 6587bp region (see figure 3.12). The expected sequence was put together as a DNA Strider file. The sequence data generated was then aligned with the expected sequence using the BLAST sequence alignment programme. Three mutations were detected in the entire sequence; at 85bp the sequence read *tc* instead of *ct* (no restriction sites were introduced), at 795bp there was a *c* missing (this introduced an SfaN1 site), at 5747bp there was a *c* instead of a *g* (no restriction sites were introduced). The mutations at 85bp and 795bp were present in the bacmid backbone and not in the adenovirus sequence. The third mutation lay outwith the E1B55k region (the Xba1 site lay at 2133bp, the BsaB1 site at 4855bp and the E1B55k gene was from 2814 to 4304bp). The restriction enzyme Xba1 has no isoschizomers, and the enzyme BsaB1 has six (Bsc81, Bsh13651, BsiB1, BsrBR1, Mam1), and not SfaN1. Therefore the restriction pattern generated by the pGEM-5zf(+) vector when digested with Xba1 and BsaB1 could not be explained by the introduction of the SfaN1 site.

Although the restriction pattern did not look correct, the sequence information did not highlight any mutations, therefore the cloning was attempted. The overlap PCR products digested with Xba1 and BsaB1, were ligated into the pGEM-5Zf(+) vector digested with Xba1 and BsaB1. The ligation reaction was then dialysed, prior to electroporation into DH10 α s. Resulting colonies were screened by hybridisation using ^{32}P labelled TAP PCR product (amplified using primers P19 and P21). The colonies resulting from cloning the CFP E1B55k genes could



148

Figure 3.12: Different primers used to sequence the left hand end of the adenovirus genome. This figure shows the approximate location of the primers that were used to sequence the fragment of plasmid pSCB-AdFL that was cloned into the pGEM-5zf(+) vector, containing the left hand region of the adenovirus type 5 genome.

not be screened as primers had not yet been designed to amplify the CFP gene. Two colonies that appeared positive by hybridisation (clone 32 and clone 134), were analysed by DNA sequence analysis using primers P2, P4, P6 and P7. Sequencing with primer P2 should generate sequence homologous to the region downstream of E1B55k, primer P4 should generate TAP sequence, primer P6 should generate E1B55k sequence and the P7 primer should generate sequence homologous to the region upstream of E1B55k. The results of the sequencing by primers P4 and P6 were very unexpected, as sequence data generated by P4 was not only TAP sequence, but also E1B55k sequence for clone 32, and E1B55k as well as adenovirus type 5 sequence for clone 134 (see figure 3.13).

3.2.6 Replacing the E1B55k gene in pGEM-5zf(+) + LHendAd5 with the tagged version +/- K104R mutation using homologous recombination

In order to rule out any possibilities that this could be due to the restriction enzymes used, the cloning was attempted by homologous recombination. A unique Kpn1 site present within the E1B55k gene in the pGEM-5zf(+) + LHendAd5 vector, was used to linearise the DNA. The linearised DNA was then co-electroporated, with the overlap PCR product into recombinogenic BJ5183 cells. Homologous recombination should occur between the overlapping regions of Ad5 DNA, upstream and downstream from the E1B55k, generating tagged E1B55k +/- K104R mutation. Initial attempts at cloning proved unsuccessful. In order to increase the cloning efficiency DNA concentrations were varied, and the competency of the electrocompetent cells was checked and found to be sufficient at $\sim 1 \times 10^8$ cfu/ μ g. A control for the homologous

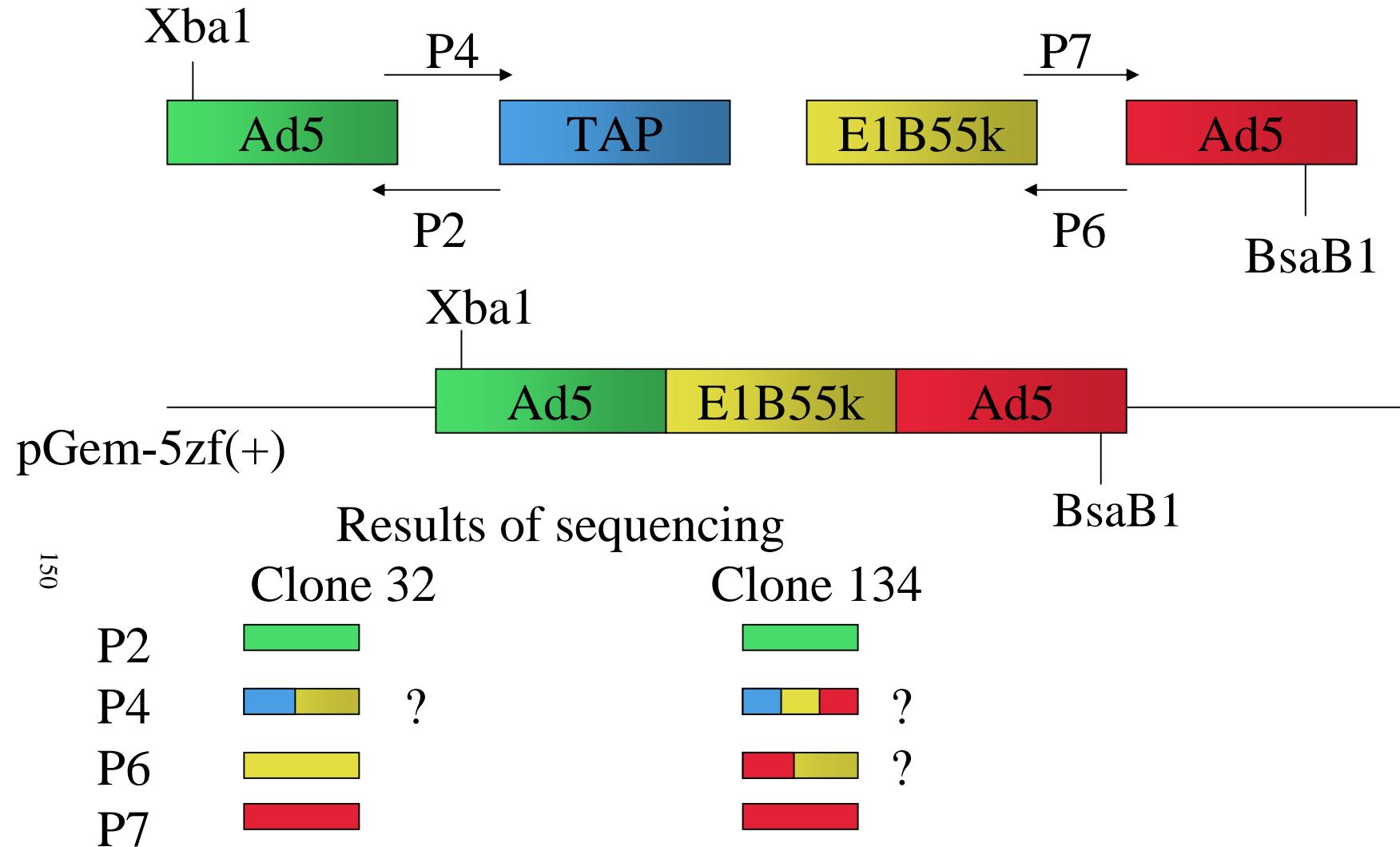


Figure 3.13: Sequence of clones generated from cloning the left hand end of Ad5 into pGEM-5zf(+) vector using Xba1 and BsaB1 restriction sites. Sequencing results of two clones (32 and 134) resulting from the cloning of the TAP/E1B55k gene into pGEM-5zf(+) + LhendAd5 using Xba1 and BsaB1 restriction sites. Sequencing with primer P4 should generate TAP sequence only. Sequencing clone 32 with P4 has generated both TAP and E1B55k sequence. Sequencing clone 134 with P4 has generated TAP, E1B55k And Ad5 sequence. Sequencing with primer P6 should only generate E1B55k sequence, whereas clone 134 sequenced with P6 has generated E1B55k and Ad5 sequence.

recombination was also developed using the pFastBac vector (see figure 3.14). For the control reaction the ampicillin gene was removed from the pFastBac vector using NaeI and SacII restriction sites. This was then co-electroporated into BJ5183 cells along with the vector DNA, with the ampicillin gene removed using BspH1 restriction sites. Regions of homology between the NaeI and the BspH1 restriction site, and the BspH1 and the SacII restriction site, were sufficient for homologous recombination to occur, and the ampicillin gene to be cloned back into the vector. During subsequent attempts at cloning, the control reaction generated recombinant colonies, whereas the actual cloning resulted in no colonies.

3.2.7 Replacing the E1B55k gene in pGEM-5zf(+) + LHendAd5 with the tagged versions using Xba1 and Kpn1 restriction sites

Although cloning using this method proved unsuccessful, it led to the realisation that the Kpn1 site could be used for the generation of the CFP and TAP tagged versions of pGEM-5zf(+) + LHendAd5, by digestion of both the overlap PCR products and the vector DNA with restriction enzymes Xba1 and Kpn1. The Kpn1 restriction site lay just after the start of the E1B55k gene, prior to lysine 104. Vector DNA and overlap PCR products were digested with Xba1 and Kpn1, purified by agarose gel electrophoresis, ligated, dialysed and electroporated into DH10 α s. Resulting colonies were screened using 32 P labelled TAP or CFP PCR products, generated using primers P19/P21 and P22/P23 respectively. Positive colonies were verified by DNA sequence analysis.

Areas of homology
BspH1-SacII 1224bp
Nae1-BspH1 407bp

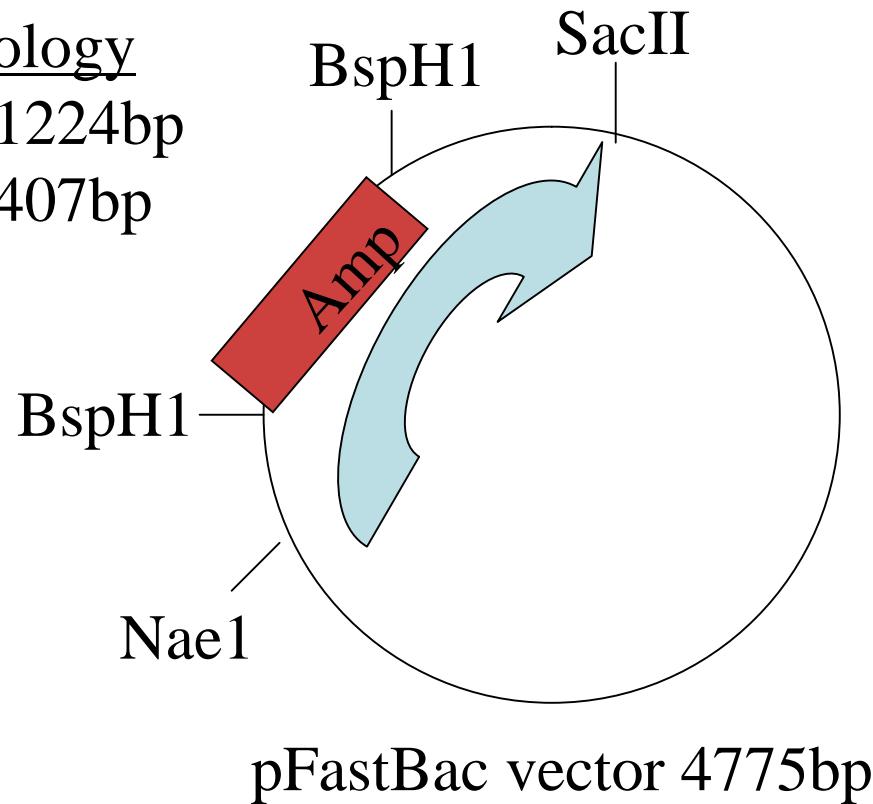


Figure 3.14: Control used for homologous recombination. In order to generate a control reaction for the overlap PCR product containing the tagged E1B55k gene, plus or minus the K104R mutation, and plasmid pGEM-5zf(+)LHendAd5 linearised with Kpn1, the ampicillin resistance gene was removed from pFastBac using restriction enzymes Nae1 and SacII. This was then co-electroporated into recombinogenic BJ5183 cells along with pFastBac vector digested with BspH1 and gel purified. Homologous recombination would result in formation of the complete plasmid, which could be selected for on ampicillin plates.

3.2.8 Replacing the E1B55k gene in pGEM-5zf(+) + LHend Ad5 with the tagged versions containing the K104R mutation, using Xba1 and Mun1 restriction sites

Possible ways of generating the CFP/E1B55k K104R and TAP/E1B55k K104R plasmids were explored by analysing the restriction map of vector pGEM-5zf(+) + LHendAd5. Fortunately, a unique Mun1 restriction site located at 3942bp within the Ad5 region (with the E1B55k gene lying between base pairs 2019 and 3509, and the BsaB1 site at 4061bp. This unique restriction site would not have been present, had the original fragment been cloned, from the I-Sce1 site to the Sal1 site. The presence of this unique Mun1 site meant that the original overlap PCR products could still be used to generate the last two constructs, but they and the vector DNA would be digested with Xba1 and Mun1, rather than Xba1 and BsaB1. After restriction digest, products were purified by agarose gel electrophoresis, ligated, dialysed and electroporated into DH10 α s. Colonies were screened by hybridisation using 32 P labelled TAP or CFP PCR products. The TAP/E1B55k K104R construct was successfully generated using this cloning strategy, and verified by DNA sequence analysis. The CFP/E1B55k K104R construct was also generated but sequence analysis indicated the presence of a mutation within the E1B55k gene, which affected an amino acid, changing a tyrosine (tac) to a histidine (cac). The overlap PCR product was regenerated from the beginning and the cloning repeated but the mutation was still present in colonies screened.

3.2.9 Generation of the CFP/E1B55k K104R construct using E1B55k K104R from TAP/E1B55k K104R, and cloning into CFP/E1B55k using Kpn1 and Mun1 restriction sites

In order to generate the CFP/E1B55k K104R construct, it was decided to remove the E1B55k K104R gene from TAP/E1B55k K104R construct, using Kpn1 and Mun1 restriction sites, and clone it into the CFP/E1B55k construct, also cut with Kpn1 and Mun1. Restriction digest of the CFP/E1B55k construct revealed a problem, as the product released should have been 1.8kb, but was approximately 3kb. All of the other constructs generated so far were then screened by restriction digest with Kpn1 and Mun1. pGEM-5zf(+) + LHendAd5 generated a 3kb fragment as did the TAP-E1B55k construct, whereas the TAP/E1B55k K104R construct generated a product of the correct size (1.8kb). These results indicated that the problem had arisen when the LHend of Ad5 had originally been cloned into the pGEM-5zf(+) vector. As the entire insert had been sequenced, all 6587bp, this sequence was rechecked by eye, rather than using the BLAST sequence alignment programme. It was discovered that the sequence data generated from one of the primers aligned perfectly with the E1B55k gene, and then this alignment ended abruptly. The remaining half of this sequence data was good quality data, but it did not align with the E1B55k gene, or any other part of the expected sequence. This sequence was then searched against the BLAST database and was found to be homologous to Insertion Sequences and Transposons, one of which was the *E. coli* K12 transposon. Sequencing of the other constructs, which also dropped out a 3kb fragment upon digestion with Kpn1 and Mun1, confirmed the presence of a

transposon. The exact location of the transposon in the constructs was mapped by restriction digest and DNA sequence analysis.

3.2.10 Remaking the TAP/E1B55k and CFP/E1B55k, pGEM-5zf(+) constructs

The TAP/E1B55k and CFP/E1B55k constructs were remade. The overlap PCR products were regenerated from the beginning and used to replace the E1B55k gene in the pGEM-5zf(+) + LHendAd5 vector using Xba1 and Mun1 restriction sites. As the transposons were present within the E1B55k gene of the original constructs, digestion with Xba1 and Mun1 ensured removal of the transposon.

3.2.11 Generation of the CFP/E1B55k K104R construct using E1B55k K104R from TAP/E1B55k K104R, and cloning into CFP/E1B55k using Kpn1 and Mun1 restriction sites

Three of the four constructs were now generated, TAP/E1B55k, CFP/E1B55k and TAP/E1B55k K104R. As stated previously, when trying to generate the CFP/E1B55k K104R construct, using the overlap PCR product, a mutation was detected within the E1B55k gene, changing a tyrosine (tac) to a histidine (cac). This mutation occurred even when the PCR product was made from the beginning. Therefore, generation of the CFP/E1B55k K104R construct was achieved by removing the E1B55k K104R gene from the TAP/E1B55k K104R construct, using Kpn1 and Mun1 restriction enzymes and cloning it into the CFP/E1B55k vector also cleaved with Kpn1 and Mun1. Colonies were screened by hybridisation using ^{32}P labelled E1B55k PCR product. Positive colonies were checked by restriction digest and DNA sequence analysis. Sequence analysis highlighted the presence of

a mutation with the E1B55k gene of the CFP/E1B55k K104R construct, where an amino acid was changed from an arginine (cgg) to a leucine (ctg). The TAP/E1B55k K104R construct was also checked and found to contain the same mutation, which had been missed when the construct was checked by sequence analysis previously.

3.2.12 Removal of the mutations from the CFP/E1B55k K104R and TAP/E1B55k K104R constructs, using the E1B55k K104R from the original CFP/E1B55k K104R pECFP-C1 construct

In order to remove the mutation from both constructs it was decided to cut the E1B55k K104R gene from the original CFP/E1B55k K104R pECFP-C1 construct, which was generated by Tony Vaughan. Restriction sites Kpn1 and HindIII were used to replace the mutated E1B55k K104R in the TAP construct, and HindIII to replace the E1B55k K104R in the CFP construct. The CFP construct required HindIII only, due to the presence of a HindIII restriction site, just upstream from the EcoR1 site and BamH1 site in the pECFP-C1 vector into which the E1B55k genes had been cloned.

This cloning strategy was successful and colonies that were positive by hybridization were verified by restriction digest and DNA sequence analysis. Unfortunately, sequence analysis indicated that both of the constructs generated had a mutation in the E1B55k gene, where an amino acid was changed from a tyrosine (tac) to a histidine (cac). This was the exact same mutation as that detected when trying to make the construct using the overlap PCR product,

explained by the fact that this original construct was used as a template to make the overlap PCR product.

3.2.13 Removal of the mutation in the original CFP/E1B55k K104R pECFP-C1 construct

The original CFP/E1B55k K104R pECFP-C1 construct generated by Tony Vaughan was sequenced again and the raw sequence data checked by eye. At the point of the mutation two peaks were visible, one for a thymidine and the other a cytosine, indicating a mixed population of DNA. Other than this one point mutation the rest of the sequence was exactly as it should have been, and the sequence data generated was good quality.

The original CFP/E1B55k K104R pECFP-C1 DNA was transformed into DH5 α . The resulting colonies were screened by DNA sequence analysis using primer P9. Approximately 20 colonies were screened and all but one had the tyrosine to histidine mutation. The colony not containing the mutation was picked and grown in L-broth containing kanamycin and DNA was extracted using the Qiagen maxiprep kit.

This DNA was then used to generate the CFP/E1B55k K104R and the TAP/E1B55k K104R constructs. The original constructs containing the tyrosine to histidine mutation were digested with Kpn1 and Mun1 and the E1B55k K104R gene replaced, with the non-mutated version. These constructs were then sequenced with primer P9 to verify that the tyrosine to histidine mutation was not present and that amino acid 104 was an arginine. This was confirmed and these constructs were then used to generate the recombinant viruses.

3.2.14 Making the pSCB-AdFL^{-5'ITR} construct

When designing the overlap PCR primers to knock out the 5' ITR using an antibiotic resistance gene, the sequence data was not yet available for plasmid pSCB-AdFL. As the Ad5 sequence is available online, it was only necessary to sequence the bacmid backbone region upstream of the 5' ITR. The initial primer designed to sequence this region (S1) gave poor sequence data, which was due to it binding to the ITR. A second primer (S1A) was designed to bind further back from the ITR, and produced good quality sequence data.

3.2.15 Initial strategy for generating the pSCB-AdFL^{-5'ITR} construct using the kanamycin resistance gene

The initial strategy for knocking out the 5' ITR of pSCB-AdFL involved using the Kanamycin resistance gene from plasmid pET30a+ (Novagen). The overlap PCR products were generated using plasmids pET30a+ and pSCB-AdFL as templates. Each of the three separate parts of the overlap PCR product were initially amplified separately. Primers P14 and P15 were used to amplify the bacmid backbone region, generating product 1. Primers P16 and P17 were used to amplify the Kanamycin resistance gene, generating product 2. Primers P18 and P8 were used to amplify the Ad5 region downstream of the wild type E1B55k gene, generating product 3. Products were then purified by agarose gel electrophoresis prior to commencing with the second round of amplification. Here products 1 and 2 were combined in equal quantities, and amplified using primers P14 and P17, generating product 4. Products 2 and 3 were also combined in equal quantities and amplified using primers P16 and P8, generating product 5. Products 4 and 5 were

then purified by agarose gel electrophoresis, combined in equal quantities, and amplified using primers P14 and P8. The final PCR product was purified by agarose gel electrophoresis and co-transformed into recombinogenic BJ5183 cells, along with plasmid pSCB-AdFL, and plated onto Kanamycin plates incubated at 37°C overnight. All of the colonies isolated were analysed by DNA sequence analysis and were found to contain no adenoviral DNA. It was discovered that there was a neomycin resistance gene present within the bacmid backbone. Homologous recombination between the ITR's was likely to have caused circularisation of the bacmid backbone, resulting in the adenovirus DNA being excised. Therefore in order to carry out the cloning successfully it would be necessary to use a different antibiotic resistance gene.

3.2.16 Strategy for generating the pSCB-AdFL^{-5'ITR} construct using the chloramphenicol resistance gene

It was decided to use the chloramphenicol resistance gene from plasmid pACYC184 (New England Biolabs). Primers P15, P16, P17 and P18 had to be redesigned to amplify the Chloramphenicol resistance gene, rather than the Kanamycin resistance gene, these primers were named P15a, P16a, P17a and P18a respectively. Overlap PCR was carried out as previously, this time using plasmids pACYC184 and pSCB-AdFL as templates. The overlap PCR product was co-transformed into transformation competent, recombinogenic BJ5183 cells, along with plasmid pSCB-AdFL, and plated onto chloramphenicol plates to allow selection for positive colonies. This method proved unsuccessful, therefore electroporation was tried using electro-competent BJ5183 cells, in order to

increase the efficiency of DNA penetration (electroporation is several times more efficient than transformation). In order to increase the efficiency of homologous recombination, it was decided to linearise the pSCB-AdFL DNA. As there were no unique restriction sites present that would allow linearization of the DNA within the correct region, the DNA was partially digested with restriction enzyme I-Sce1, of which there were two sites within the plasmid. Partial digestion of the DNA was found to be optimal using a 1 in 10 dilution of enzyme and with DNA being digested for 10-15 seconds. When partially digested pSCB-AdFL (in ng quantities) was co-electroporated with the overlap PCR product (in µg quantities) into electrocompetent BJ5183 cells, plated onto chloramphenicol plates and grown at 30°C overnight, 1 colony was generated. The colony was also confirmed to be ampicillin resistant, by plating onto ampicillin plate and incubating at 30°C overnight. A colony was picked from the ampicillin plate and grown in L-broth containing chloramphenicol for 2 to 3 hours at 30°C. DNA was extracted using alkaline lysis DNA extraction, transformed into DH5 α , plated onto an ampicillin plate and incubated at 30°C overnight. The aim of this was to get the DNA out of the recombinogenic bacteria and into stable bacteria, before any mutations could occur. A colony was then picked from this plate and grown up in L-broth containing chloramphenicol overnight at 30°C. DNA was extracted using the alkaline lysis method. The construct was then analysed by restriction digest and sequence analysis and was found to be correct, pSCB-AdFL^{-5'ITR}.

3.2.17 Generating the recombinant viruses

Generation of the recombinant viruses was achieved by co-transfection of the pGEM-5zf(+) construct expressing TAP/E1B55k, CFP/E1B55k, TAP/E1B55k K104R or CFP/E1B55k K104R, along with plasmid pSCB-AdFL^{-5'ITR}. Both constructs were linearised with SalI prior to transfection into HeLa cells. Homologous recombination occurring between both plasmids, resulted in the generation of full length recombinant adenovirus type 5, which was evident by the appearance of plaques approximately 10 days post-infection.

3.2.18 Screening of recombinant viruses by DNA sequencing and Western blot analysis

When the recombinant viruses expressing TAP/E1B55k and CFP/E1B55k were made, they were only sequenced across the junctions between the tag and the E1B55k gene, using primers P2 and P4, or P3 and P5 respectively. Only this region was sequenced, as the DNA constructs that had been used to generate the viruses had been sequenced comprehensively, with the TAP/E1B55k construct having been sequenced with primers P1, P2, P4, P9, P10A and P7, and the CFP/E1B55k construct sequenced with primers P1, P3, P5, P9, P10A and P7. No mutations were found in either of the constructs.

In Western blot analysis of these recombinant viruses, the tagged E1B55k fusion protein appeared to be truncated. The tagged E1B55k protein, when expressed should be approximately 76kDa. But when cells that were infected with recombinant virus expressing TAP/E1B55k were lysed and analysed by Western blot analysis using a monoclonal antibody to E1B55k, there were two products of

approximately 32kDa and 25kDa. When cells that are infected with recombinant adenovirus expressing CFP/E1B55k were lysed and analysed by Western blot analysis using the same monoclonal, there were two products of approximately 45kDa and 32.5kDa (see figure 3.15).

When the TAP/E1B55k K104R and CFP/E1B55k K104R viruses were made, they were sequenced with primers P4, P9 and P10A, or P5, P9 and P10A respectively. Again this was due to the fact that the constructs that had been used to generate the viruses had been sequenced comprehensively. Sequencing of both viruses with these primers gave good quality sequence data and no mutations appeared to be present.

In Western blot analysis of these recombinant viruses however, the tagged E1B55k K104R fusion protein appeared to be truncated. The tagged E1B55k K014R protein, when expressed should be approximately 76kDa. But when cells that were infected with recombinant virus expressing TAP/E1B55k K104R were lysed and analysed by Western blot using a monoclonal antibody to E1B55k, there were two products of approximately 32kDa and 25kDa. When cells that were infected with recombinant adenovirus expressing CFP/E1B55k K104R were lysed and analysed by Western blot using the same monoclonal, there were two products of approximately 45kDa and 32.5kDa.

3.2.19 Plaque purification of the recombinant viruses and generation of stocks

Although by Western blot analysis, the tagged E1B55k gene appeared truncated in all of the recombinant viruses, sequencing analysis indicated that there were no

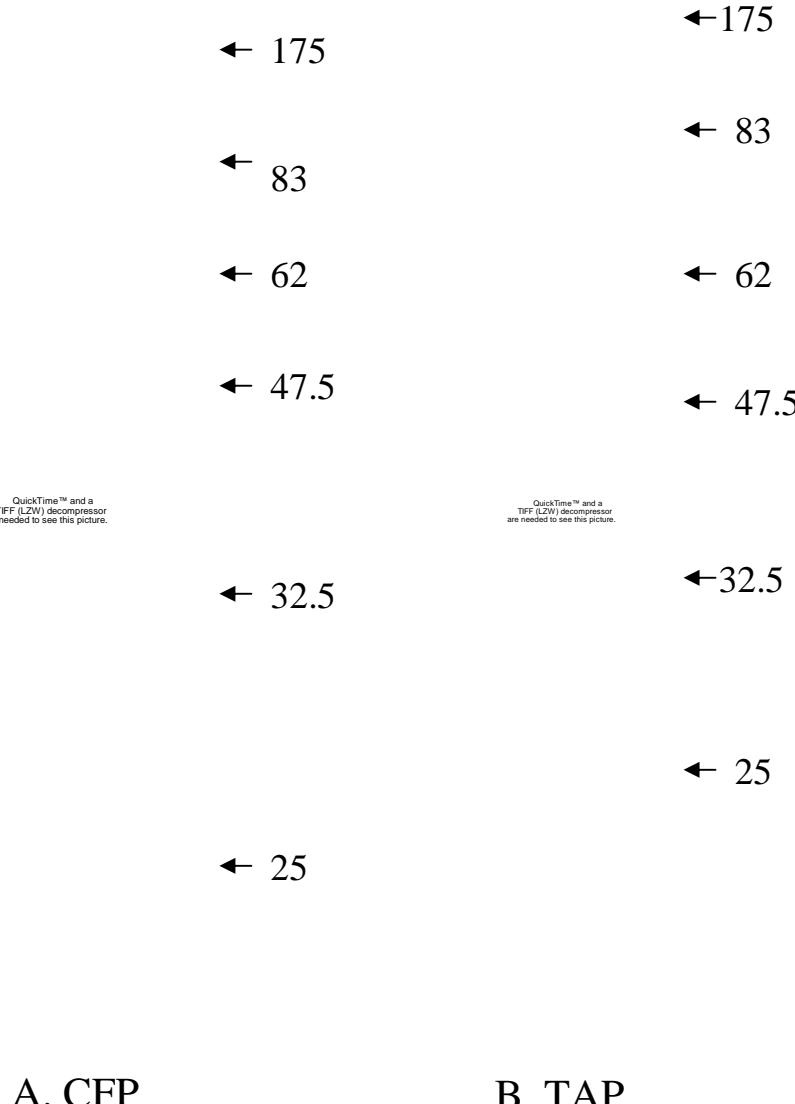


Figure 3.15: Western blots of recombinant adenoviruses generated. The first round plaque purifications of the recombinant adenoviruses were probed with E1B55k monoclonal antibody. Figure A shows CFP-E1B55k K104R recombinant adenovirus, expressing products of approximately 40 and 32.5kDa. Figure B shows a TAP-E1B55k K104R recombinant adenovirus, expressing products of approximately 32.5 and 25kDa. The tagged E1B55k proteins should be approximately 76kDa in size.

mutations present. Therefore viruses were plaque purified 3 times by end point dilution. Stocks of recombinant virus were grown up initially in 20x150cm² flasks of HeLa cells, which was then scaled up to 5L cultures of HeLa spinner cells. Virus was then purified by arcton extraction and caesium chloride purification. All virus stocks were stored at -80°C.

3.2.20 Analysis of recombinant viruses following plaque purification

After plaque purification, the recombinant viruses were again analysed by Western blot analysis, and again the products appeared truncated, as previously shown. In order to confirm that no mutations had been introduced, the sequencing that had been performed previously was reanalysed, and further sequencing was carried out.

On closer inspection, the sequencing of both recombinant E1B55k K104R viruses with primer P9, revealed the presence of a mutation within the E1B55k K104R gene. This sequence was towards the end of the sequence data generated with primer P9, where the quality of the sequence data had degenerated. An extra guanine had been introduced within the sequence, which resulted in the remainder of the E1B55k gene being thrown out of frame, and the introduction of a stop codon further downstream, within the E1B55k gene. Sequence analysis carried out using primers S2 and S4, which sequence within the E1B55k gene, confirmed the presence of this mutation.

The truncated products shown by Western blot analysis could be explained by the introduction of the stop codon, within the E1B55k K104R gene, of both recombinant viruses.

In order to try to determine the source of this mutation, the sequence data of the constructs used to generate these viruses was reanalysed.

The E1B55k K104R gene from the CFP/E1B55k K104R pECFP-C1 construct had been used to generate the TAP/E1B55k K104R and CFP E1B55k K104R constructs, which had subsequently been used to generate the recombinant viruses. This construct, kindly supplied by Dr Tony Vaughan, had been sequenced comprehensively prior to use, using primers P1, P3, P5, P9, P10A and P7, and was found to be completely mutation free, with one exception. Analysis of the raw sequence data had indicated that at one specific base pair within the sequence of the E1B55k gene, two separate peaks identical in height were present. One peak represented cytosine and the other thymidine, which seemed to indicate a mixed population of DNA. When this construct had been used to generate the overlap PCR product, the final PCR product was found to contain a mutation. At this specific base pair, only one peak was present for cytosine, which resulted in this base and the following two bases encoding the amino acid histidine (*cac*) instead of tyrosine (*tac*). In order to eliminate this problem, which seemed to be due to a mixed population of DNA, the DNA was retransformed into *E. coli* and individual colonies were screened by sequence analysis. A colony was selected which only had one peak at this specific base pair, which was a thymidine. The E1B55k K104R gene from this construct was then used to generate the TAP/E1B55k K104R and CFP/E1B55k K104R constructs that were used to make the recombinant viruses. When this colony was picked and analysed by sequence analysis, only the first part of the E1B55k gene was sequenced. This was due to

the fact that the entire 2.1kb had been sequenced previously and appeared to be exactly as it should have been.

In order to determine if this construct had been the source of the mutation, the remainder of the E1B55k gene was sequenced using primers to provide comprehensive coverage. The results confirmed that this construct had been the source of the mutation present in the recombinant viruses, as an extra guanine was found at exactly the same location as in the recombinant viruses.

Reanalysis of the sequence data for the TAP/E1B55k and CFP/E1B55k recombinant viruses has failed to indicate any mutations which could be causing truncated expression of the E1B55k gene. Further sequence analysis of the plaque purified viruses also failed to reveal any mutations. This discrepancy between the sequence data and the Western blot result may be explained by further sequence analysis of viral DNA extracted from the recombinant viruses. It is possible that a mutation may have been introduced during the homologous recombination, which has not yet been detected.

3.3 Discussion

The aim of generating these recombinant viruses was to be able to study the localisation of the E1B55k protein, and to identify which viral and cellular proteins interact with the E1B55k protein, during adenovirus infection, and to determine the effect of SUMOylation.

Different cell types would be infected with the recombinant viruses expressing the CFP tagged E1B55k protein and live imaging carried out using the Delta Vision

microscope. This would have allowed the localisation of the E1B55k protein to be determined at specific time points during infection. As two different versions of the recombinant virus were generated, one in which the E1B55k protein could be modified by SUMO (E1B55k), and one which could not (E1B55k K104R), this would allow the effect of SUMOylation on localisation to be determined.

By generating the recombinant viruses expressing the TAP tagged E1B55k (+/- K104R) protein, cell lines infected with the recombinant viruses could be harvested at set time points, and tandem affinity purification would identify the cellular proteins and viral proteins that interacted with E1B55k at these set times during infection. Comparison of the results from the recombinant viruses expressing E1B55k and E1B55k K104R, would provide information as to whether SUMOylation alters the proteins that interact with E1B55k.

When undertaking such studies it was essential that no mutations were present within the E1B55k gene, as any mutations could influence the results.

Unfortunately the discovery of several mutations during the making of these recombinant viruses has hindered progress dramatically, therefore no data has yet been generated using these viruses.

Although the recombinant viruses expressing TAP/E1B55k and CFP/E1B55k have been made and plaque purified 3 times, Western blot analysis of cells infected with the recombinant viruses, show the tagged E1B55k protein to be truncated. DNA sequence analysis however, has not indicated the presence of any mutations that could be causing this, although further sequence analysis has to be undertaken in order to confirm this.

The recombinant viruses expressing TAP/E1B55k K104R and CFP/E1B55k K104R have also been generated and plaque purified 3 times. DNA sequence analysis of these viruses however, has demonstrated the presence of a mutation resulting in the introduction of a stop codon within the E1B55k gene. Western blot analysis of cells infected with these viruses, show the tagged E1B55k gene to be truncated, which can be explained by the introduction of the stop codon. However, a comprehensive strategy has been developed for the generation of these recombinant viruses and many technical issues have been resolved. Therefore provided a source of the E1B55k K104R gene can be found which is mutation free, remaking the recombinant viruses should be straightforward.

One of the major technical problems encountered, was the insertion of a transposon into several of the constructs. Attempts to locate the source of the transposon by DNA sequence analysis proved unsuccessful, as transposons have very high sequence homology. It was suspected that the source of the problem may have been the competent cells, therefore all competent cell were discarded and fresh stocks made.

The mutation present within the CFP/E1B55k K104R pECFP-C1 construct was unfortunately not discovered at an earlier stage. When the mutation was discovered, DNA sequence analysis of the E1B55k gene indicated a mixed population of DNA, due to the fact that two peaks were present at one specific base pair (one for cytosine and one for thymidine). When the DNA sample was transformed and colonies were screened by DNA sequence analysis, a colony chosen that only had the one peak corresponding to thymidine. It was assumed that the remainder of the E1B55k sequence was as it should be, as when the

CFP/E1B55k K104R pECFP-C1 construct had been sequenced originally, no other mutations had been detected. This assumption led to the generation of recombinant viruses containing a mutation within the E1B55k gene.

The source of the mutation within the TAP/E1B55k K104R construct is unknown, although it may have arisen during PCR amplification, as no mutations were detected in the original pCMV5-TAP/E1B55k K104R construct generated.

In conclusion it is very important to be diligent when analysing sequence data, especially when sequencing such large DNA fragments, as it is very easy to overlook mutations that are present.

4. TANDEM AFFINITY PURIFICATION OF TAP/SUMO2 CELL LINES INFECTED WITH ADENOVIRUS TYPE 5

4.1 Introduction

4.1.1 Introduction

A stable HeLa cell line expressing Tandem Affinity Purification (TAP) tagged SUMO-2 protein was generated by Dr Akihiro Nakamura, a former member of the group. The stable cell line was generated in order to identify cellular proteins that are modified by SUMO-2 protein. Proteins, upon modification by SUMO, become covalently attached to the SUMO protein. To ensure that only proteins modified by SUMO were purified during the tandem affinity purification procedure, the TAP procedure was modified by Dr Akihiro Nakamura, to include a harsh cell lysis (see materials and methods). This would disrupt any unspecific interactions with the SUMO-2 protein, allowing only proteins that are true substrates of SUMO-2 to be identified.

The aim was to carry out tandem affinity purification of the TAP/SUMO-2 cells, infected with wild type adenovirus 5, and compare the results generated from tandem affinity purification carried out in uninfected cells. By comparing the two sets of data it would be possible to determine if infection of cells with adenovirus type 5 affected the SUMOylation of cellular proteins by SUMO-2 protein.

Stable HeLa cell lines expressing the TAP tag alone, were also generated by Dr Akihiro Nakamura, as a negative control. Tandem affinity purification was also carried out with this cell line.

4.1.2 Generation of TAP/SUMO-2 stable cell lines

As stated previously, the TAP/SUMO-2 HeLa cells were generated by Dr Akihiro Nakamura. The SUMO-2 gene was amplified by PCR, using primers designed with the appropriate restriction sites, to allow cloning into the MCS of the pCMV5/TAP vector, and generation pCMV5 TAP/SUMO-2. TAP/SUMO-2 was then amplified by PCR, using primers designed with appropriate restriction sites, to allow cloning into the MCS of the pEFIRE-S-P construct (Hobbs *et al*, 1998). The pEFIRE-S-P construct contains a puromycin resistance gene, allowing for the selection of cells which are expressing the construct, by growing in medium containing puromycin.

The pEFIRE-S-P construct, expressing the TAP/SUMO-2 fusion protein, was transfected into HeLa cells using FuGENE 6 transfection reagent (Roche). Cells expressing the construct were selected for using puromycin in the cell media. Puromycin resistant colonies of cells were then selected and expanded. Expression of TAP/SUMO2 from selected cell colonies was confirmed by Western blot analysis. A clone that was expressing TAP/SUMO2 at consistent levels was used to generate the stable cell lines. The cells were grown up in media containing puromycin, and stocks frozen down in liquid nitrogen.

4.1.3 Generation of the TAP stable cell lines

As discussed above, the TAP tag stable cell lines were generated by Dr Akihiro Nakamura. The TAP gene was amplified from the pCMV5/TAP vector using primers designed to have appropriate restriction sites to allow cloning into the MCS of the pEFIRE-S-P vector. The pEFIRE-S-P construct, expressing the TAP

protein was then transfected into HeLa cells using FuGENE 6 transfection reagent (Roche). Cells expressing the construct were selected for by the presence of puromycin in the cell media and resistant cell colonies were then selected and expanded. Expression of the TAP protein was confirmed by Western blot analysis and a clone that was expressing TAP at consistent levels was used to generate the stable cell lines. Cells were grown up in media containing puromycin, and stocks frozen down in liquid nitrogen (see section 2.2.2 for method).

4.1.4 Amplification of adenovirus type 5

One vial, containing 1mL of wild type Ad5, produced by John Nicholson in the laboratory, and dated 18/6/02, was used to inoculate 2x5L cultures of 293 spinners at approximately 80% confluency. After 4 days, each of the 5L cultures was split equally into 2x5L cultures as the media was becoming acidic, generating 20L of cells in total.

Six days from the day of inoculation, all cells showed signs of viral infection and were harvested by arcton extraction and caesium chloride purification. The virus was titrated, in triplicate, using end point dilution. The titre of the purified virus was 2×10^{11} pfu/mL, but the total volume was only 900 μ L, much less than would have been expected. The virus was aliquoted and stored at -80°C.

In order to generate more virus, 4x100 μ L aliquots of this stock, were used to inoculate 2x5L cultures of 293 spinners. After 3 days the cells were split equally into 3x5L cultures, as the media was becoming acidic. Four days post inoculation, all 15L were harvested by arcton extraction and caesium chloride purification. The

virus was then titrated, in triplicate, using end point dilution, and found to be 2.3×10^{12} pfu/mL. The virus was aliquoted and stored at -80°C.

4.1.5 Experiments undertaken

For the experiments undertaken using the stable cell lines, one vial of cells was recovered from liquid nitrogen in a 25cm² flask, which was then scaled up to 1x75cm² flask, to 12x75cm² flasks, and finally to 120 petri dishes (150mm diameter). Cells were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% FCS, 2mM glutamine and puromycin (2μg/mL). From the initial vial that was resuscitated, 10 vials of cells were frozen down so as not to deplete stocks. Upon completion of each separate experiment, remaining cells were thrown away and a fresh vial used for any new experiments undertaken. This was to prevent the expression levels of the protein from dropping, by passaging the cells too many times.

Four separate TAP experiments were undertaken, once conditions had been optimised and the experimental procedures were shown to be working. For each separate experiment 120 petri dishes (150mm diameter) of cells were used.

1. Uninfected TAP/SUMO-2 cells
2. TAP/SUMO-2 cells infected with Ad5 at a M.O.I of 10 for 20 hours
3. TAP/SUMO-2 cells infected with Ad5 at a M.O.I of 10 for 24 hours
4. TAP cells, uninfected as a negative control

4.1.6 Tandem Affinity Purification procedure

For each of the experiments undertaken, all 120 petri dishes (150mm diameter) were harvested when 100% confluent, by scraping the cells directly into the lysis buffer. The cell extracts (approximately 120mL) were then stored at -80°C until they were subjected to Tandem Affinity Purification.

The cells were then thawed in a water bath at room temperature, place on ice, then sonicated in order to shear the genomic DNA. Any cell debris was pelleted by centrifugation, to allow the sample to pass through the affinity columns more easily. The sample was then split evenly between 2x1L solutions of renaturation buffer, and passed through the first affinity purification column, using gravity flow.

The TAP tag consists of two IgG binding domains of *Staphylococcus aureus* protein A, and a calmodulin binding peptide separated by a TEV protease cleavage site.

The first affinity column contains IgG sepharose, to which the protein A of the TAP tag binds. Any contaminating proteins are eluted when the column is washed through with renaturation solution. The protein of interest and any interacting proteins, are then cleaved from the IgG sepharose using TEV protease. The cleaved proteins are eluted from the IgG sepharose into calmodulin binding buffer (containing calcium), to which calmodulin beads are added. The calmodulin binding peptide of the TAP tag binds to the calmodulin beads, with the calcium acting as a co-factor. Any contaminating proteins are eluted when the column is washed through with calmodulin binding buffer. The protein of interest and any

interacting proteins are then eluted in calmodulin elution buffer, containing EGTA, which acts by chelating the calcium (see figure 1.3).

After elution the proteins are concentrated into a smaller volume, to allow the sample to be loaded into one lane of an SDS-PAGE gel, this is done by TCA precipitation. Once the proteins have been separated according to molecular weight, by SDS-PAGE, the proteins are visualised by Coomassie staining of the gel. Protein bands are then excised from the gel and are subjected to in-gel digestion followed by analysis by LC-ESI-MS/MS.

Each gel slice that was analysed by LC-ESI-MS/MS produced its own set of data. Where possible, all of these data were combined to include all the gel slices from the entire lane of the gel, and then searched against the MSDB database using the Mascot search engine. This was done in order to obtain an overall list, encompassing all proteins identified during the experiment. In some cases though, the data sets were too large. In these cases the data was split into two or three different sets, and then searched against the Mascot database.

4.2 Results

The Mascot programme generates a list of predicted protein hits. Each protein is allocated an overall score, which is based on the number of peptides that matched the protein and how many MS/MS fragment ions are matched for each peptide, with the most probable hits having the highest overall scores, and proteins that are questionable as being true hits having the lowest scores.

4.2.1 TAP cell line

The purpose of carrying out the Tandem Affinity Purification, using TAP stable HeLas, was as a means of identifying false positives. These are proteins that interact with the TAP tag only, and not the SUMO2 protein. The list of proteins resulting from this experiment was subtracted from the list of proteins generated in subsequent experiments. The Coomassie stained SDS-PAGE gel on which the TCA precipitated eluates from the TAP procedure were run can be seen in figure 4.1. The list of proteins generated from this experiment can be seen in appendix (7.2). The proteins that have been identified in this experiment may interact directly with part of the TAP tag, or else they may be particularly “sticky” proteins, present in abundance, that have not been separated from the TAP tag during the washing stages.

4.2.2 TAP-SUMO2 cell line uninfected

After TCA precipitation of the TAP eluates, the sample was resuspended in SDS boiling mix and boiled prior to running on the gel. When the sample had been loaded there still seemed to be material present in the bottom of the eppendorf tube. Therefore more SDS boiling mix was added, and the sample boiled and loaded onto the next lane of the SDS-PAGE gel (see figure 4.2). As it is very costly to have whole lanes analysed by LC-ESI-MS/MS, all of the first lane, and only part of the second lane containing sample was analysed. In the second lane most of the proteins evident by Coomassie staining were above the 47.5KDa molecular weight, and below this very little protein was visible except for the SUMO2 protein, which was also very abundant in the first lane. Hence only the

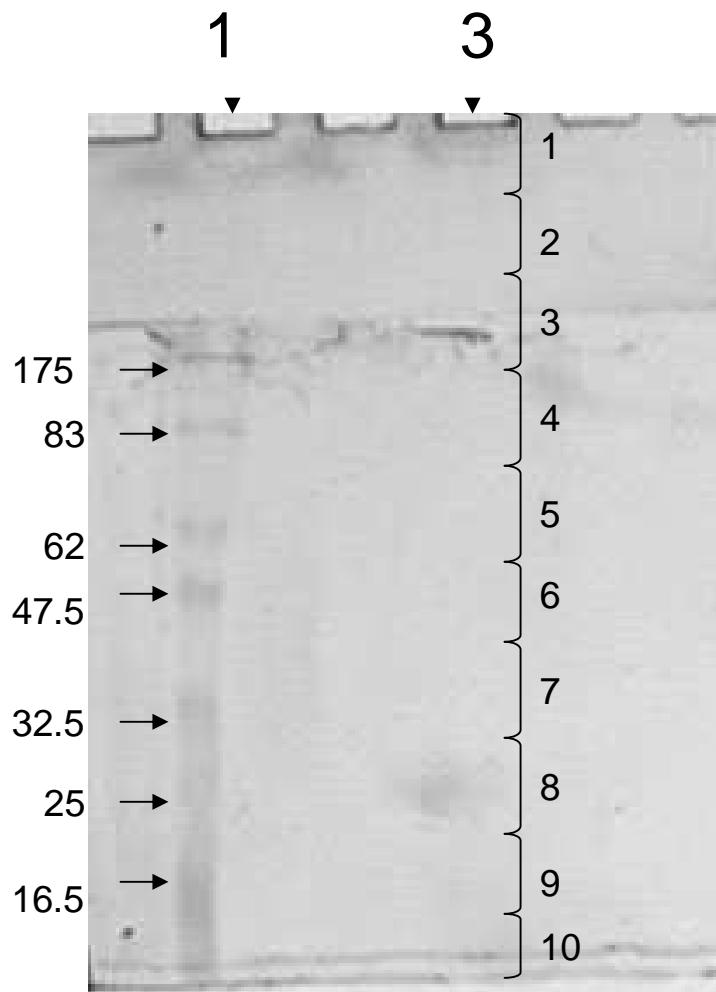


Figure 4.1: SDS-PAGE gel showing eluates from Tandem affinity purification experiment using TAP stable cell lines. 120 petri dishes (150mm diameter) of TAP-HeLa cells were grown to 100% confluence, lysed and subjected to Tandem Affinity Purification. Protein eluates were then TCA precipitated, resuspended in SDS boiling mix and run on a 10% SDS-PAGE gel (Coomassie stained). The lane containing the sample (3) was excised from the gel into 10 separate chunks and subjected to LC-ESI-MS/MS.

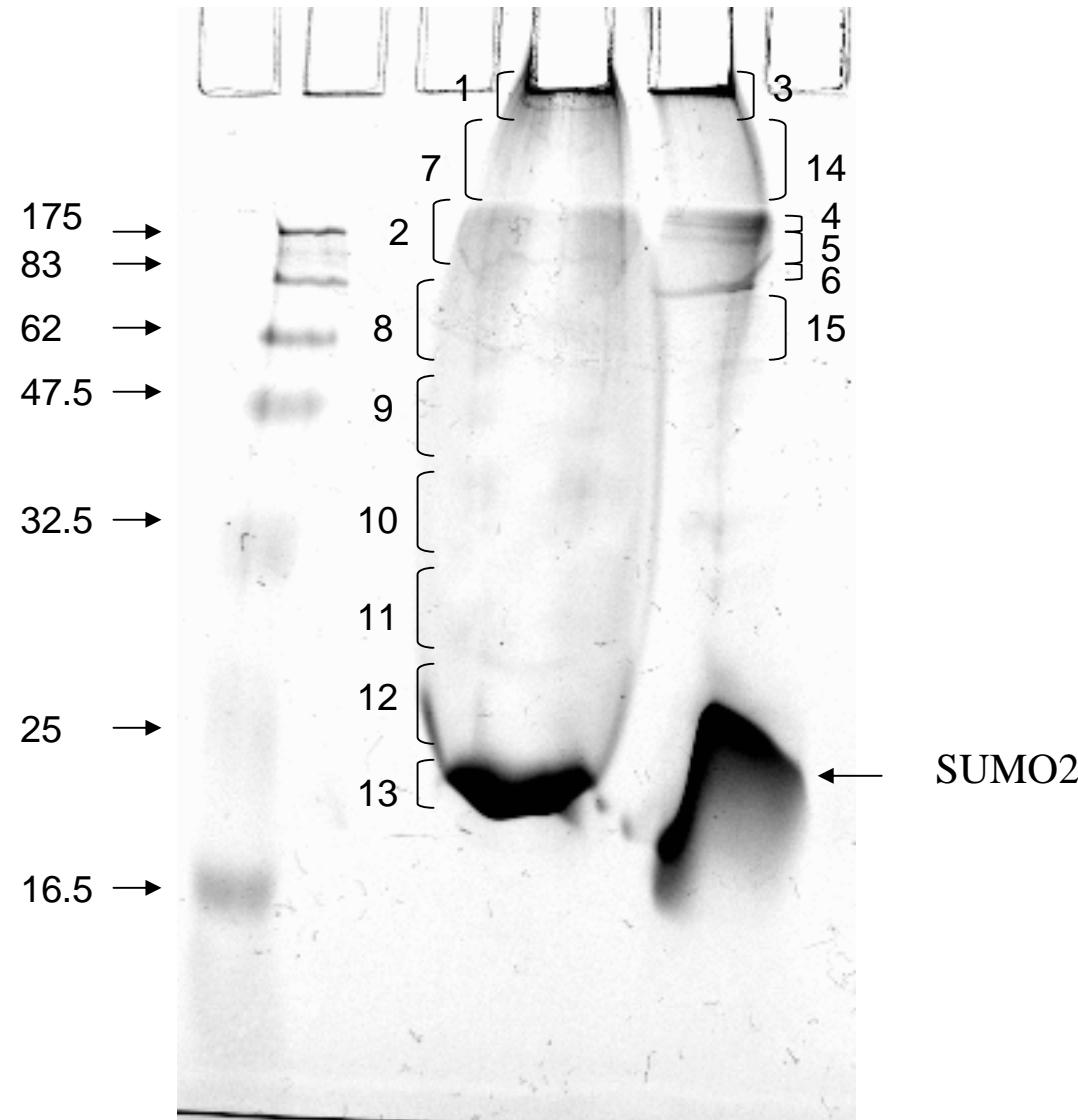


Figure 4.2: SDS-PAGE gel showing eluates from Tandem affinity purification of TAP-SUMO2 stable cell lines. A TAP pulldown was carried out using cell extracts from 120 petri dishes (150mm diameter) of TAP-SUMO2 cells. Eluates were TCA precipitated, resuspended in SDS boiling mix and run on a 10% SDS-PAGE gel, which was Coomassie stained. The bands indicated by brackets were excised and proteins identified by LC-ESI-MS/MS.

proteins above the 47.5KDa marker were excised.

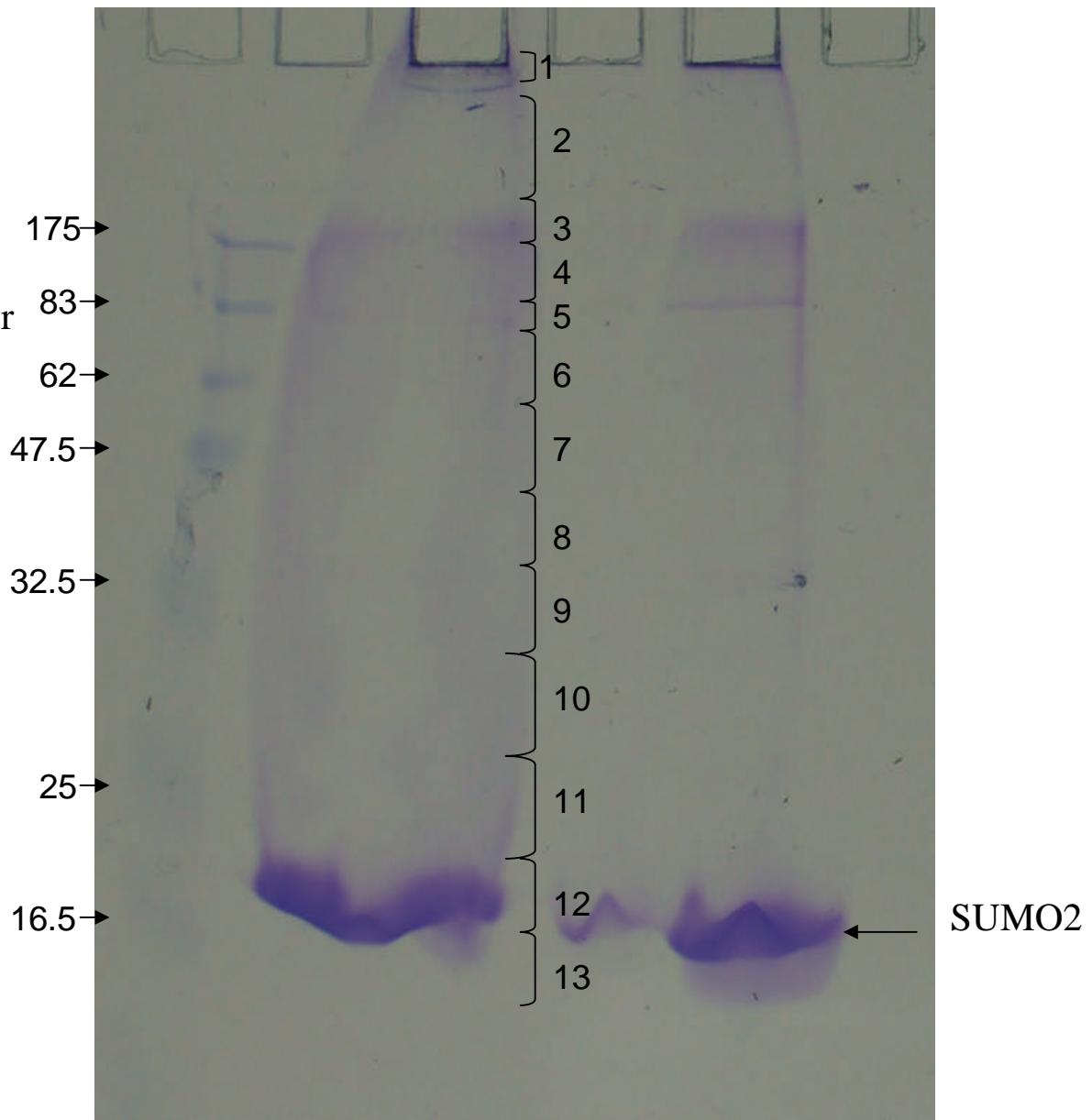
This experiment generated a large amount of data, and subsequently the data generated could not be combined into one file for searching against the MSDB database. Instead the data sets were split into three comprising of the top, middle and bottom regions of the SDS-PAGE gel. Data set one consisted of gel slices 1,3,7 and 14; data set two consisted of gel slices 2,4,5,6,8 and 15; data set three consisted of gel slices 10, 11, 12 and 13. The data generated from the TAP HeLa stable cell lines was subtracted from all three sets of data. See Appendix (7.3). The data generated consisted of 1566 proteins, after subtraction of the TAP HeLa data.

4.2.3 TAP-SUMO2 cell line infected with Ad5 at a M.O.I of 10 for 20 hours

The TAP-SUMO2 cells were infected with a M.O.I of 10 to ensure that all of the cells would come into contact with virus and become infected simultaneously. The time point of 20 hours post infection was chosen as the early viral genes would be expressed, but the late viral genes would not. The aim of the experiment was to determine the effect of the expression of the early viral genes on SUMOylation of cellular proteins. After TCA precipitation of the TAP eluates, the sample was resuspended in SDS boiling mix and boiled prior to running on the gel. When the sample had been loaded there still seemed to be material present in the bottom of the eppendorf tube. Therefore more SDS boiling mix was added, and the sample boiled and loaded onto the next lane of the SDS-PAGE gel (see figure 4.3). Only the first lane containing the sample was excised and analysed by LC-ESI-MS/MS as bands seemed in equal proportions to those in the second lane, and protein

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Figure 4.3: SDS-PAGE gel showing eluates from Tandem affinity purification of TAP-SUMO2 cells infected with Ad5 at an M.O.I of 10 for 20 hours. 120 petri dishes (150mm diameter) of TAP-SUMO 2 cells were grown to 80% confluence and infected with wild type Ad5 at a M.O.I. of 10, for 20 hours. Cells extracts were then Tandem affinity purified and eluates TCA precipitated, resuspended in SDS boiling mix and run on a 10% SDS-PAGE gel which was coomassie stained. Bands indicated were excised from the gel and the proteins identified using LC-ESI-MS/MS.



levels appeared to be abundant enough to be identified by mass spectrometric analysis. As this experiment generated less data than anticipated, as a result all of the gel slices could be combined and searched against the Mascot database. After subtraction of TAP HeLa data, the data set consisted of only 255 proteins (data not shown).

One of the proteins identified however, was the Lamina Associated Polypeptide 2 isoforms beta (lap2 β) and gamma (lap2 γ) protein with an overall score of 92. Isoforms beta and gamma had also previously been identified in the experiment using the uninfected TAP-SUMO2 stable cell lines, in data set two, with an overall score of 189. There are three different isoforms of lamina associated polypeptide, α , β and γ , which have identical N-termini, sharing their first 189 amino acids. Peptides that were identified included peptides that were present in all three isoforms, as well as peptides that were unique to lap2 α and lap2 β . Although the peptides that were identified did not contain a SUMO consensus sequence themselves, three possible SUMO consensus sequences were identified in both lap2 α and lap2 β . All three possible SUMO consensus sequences were shared between both lap2 α and lap2 β , LKSE beginning at amino acid 16, and LKLE, beginning at amino acid 94. The third possible SUMO consensus sequence VKSE began at amino acid 400 in lap2 α and at amino acid 291 in lap2 β . It should be noted that proteins with a score of 60 or above were considered as being a genuine hit. In order to determine if this was really a true positive, as only one lane of the gel had been excised and the rest of the gel remained intact, proteins were transferred onto PVDF membrane and analysed by Western blot, using mouse serum raised against lap2 β . A band of the correct molecular weight indicated that

lap2 β was pulled out during the tandem affinity purification, indicating that it is probably modified by SUMO2 protein (see figure 4.4).

When the LC-ESI-MS/MS data was searched against the viral database, no adenoviral proteins were identified. This was surprising as it is known that E1B55k protein is SUMOylated, although, experiments that demonstrated this, were undertaken using SUMO1 (Endter et al., 2001). In order to determine if E1B55k protein was actually being expressed after 20 hours, when the TAP-SUMO2 cells were infected with a M.O.I of 10, a time point experiment was undertaken to monitor expression levels of the E1B55k protein.

4.2.4 Experiment to determine expression levels of the E1B55k protein

A total of 24 petri dishes (150mm diameter) were set up of TAP-SUMO2 cells, which were grown to 80% confluency, then half of the dishes were infected with Ad5 at an M.O.I of 10. At specific time points, 1 infected Petri dish and 1 uninfected Petri dish were harvested. The medium was removed and cells washed in PBS, then 5mL of lysis buffer was added to each plate and cells scraped into the lysis buffer using a sterile cell scraper. Samples were then sonicated in order to shear genomic DNA, and centrifuged to pellet any cell debris. Equal amounts of each sample were then loaded onto SDS-PAGE gels. After running, proteins were transferred to PVDF membranes, and analysed by Western blot analysis using E1B55k monoclonal antibody. The levels of E1B55k protein were monitored at 0, 5, 7, 9, 11, 13, 15, 17, 19, 20, 22 and 24 hours post infection (see figure 4.5). Time points were only taken up to 24 hours as the aim was to harvest the cells prior to the expression of the late viral genes. It was deemed that after 24 hours post

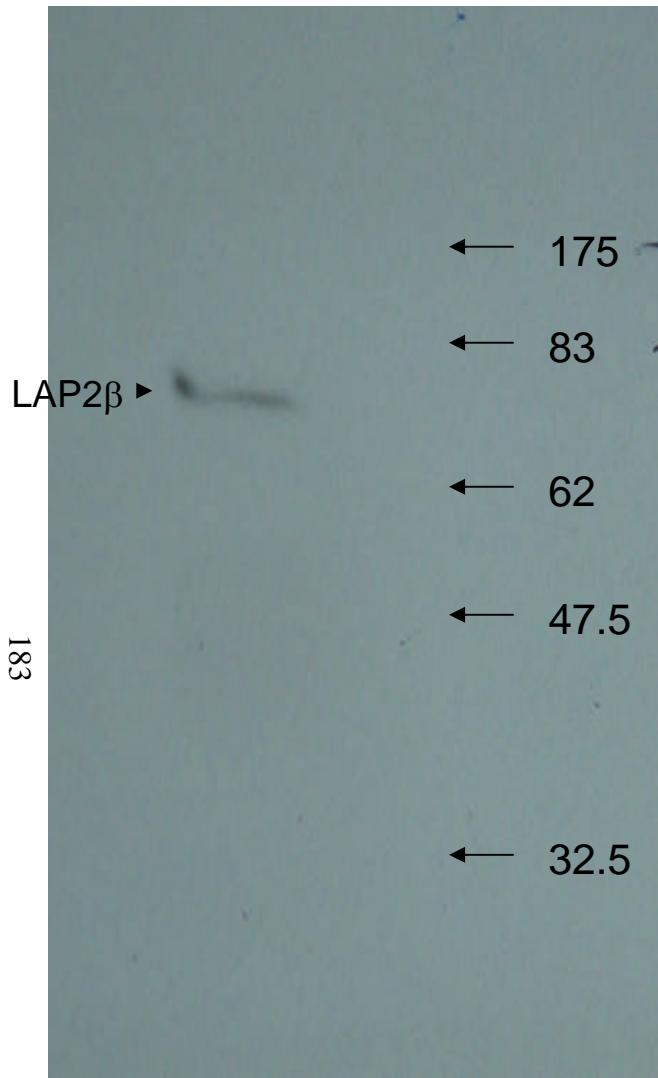


Figure 4.4: Western blot of eluates from tandem affinity purification experiment using TAP-SUMO2 stable cell lines infected with wild type Ad5 at an M.O.I of 10, for 20 hours, probed with LAP2 β antibody. The eluates resulting from Tandem affinity purification of 120 petri dishes (150mm diameter) of TAP-SUMO2 Cells, infected with Ad5, were TCA precipitated and run on an SDS-PAGE gel. Bands were excised from one lane for mass spectrometric analysis, as shown in figure 4. The other lane of the gel was transferred to PVDF membrane and probed with mouse serum against LAP2 β .

infection the viral late genes would be being expressed. From the data it is evident that at 22 hours post infection, the levels of E1B55k expression appear to be very similar to the expression levels at 20 hours post infection. Therefore it was decided to repeat the tandem affinity purification experiment, this time harvesting the cells at 24 hours post infection, when levels seemed to be substantially higher.

4.2.5 TAP-SUMO2 cell line infected with Ad5 at a M.O.I of 10 for 24 hours

Following TCA precipitation of the TAP eluates, the protein pellet was resuspended in SDS boiling mix and loaded onto one lane of an SDS-PAGE gel. This time no sample appeared to be remaining in the bottom of the eppendorf tube. The lane of the gel containing the sample was cut into 18 slices which were analysed by LC-ESI-MS/MS. Far more data was generated than in the previous experiment where the cells were harvested 20 hours post infection. The data set was too large to combine and search against the MSDB database. Therefore the data set was split into two, data set 1 consisting of gel slices 1-9 and data set 2 consisting of gel slices 10-18 (see figure 4.6). The data generated from the TAP HeLa stable cell lines was subtracted from both sets of data. The data generated consisted of 735 proteins, after subtraction of the TAP HeLa data (see Appendix (7.4)). When the data was searched against the MSDB database, lap2 β protein was present in both data sets. In data set 1, with an overall score of 152, and in data set 2, with an overall score of 63. Lap2 β is 454 amino acids long, with a molecular weight of 50.5kDa. Therefore, when SUMO modified, the molecular weight of lap2 β is expected to be approximately 75kDa, as it appears in figure 4.4. Lap2 β protein is present in both data sets, 1-9 which includes proteins with a

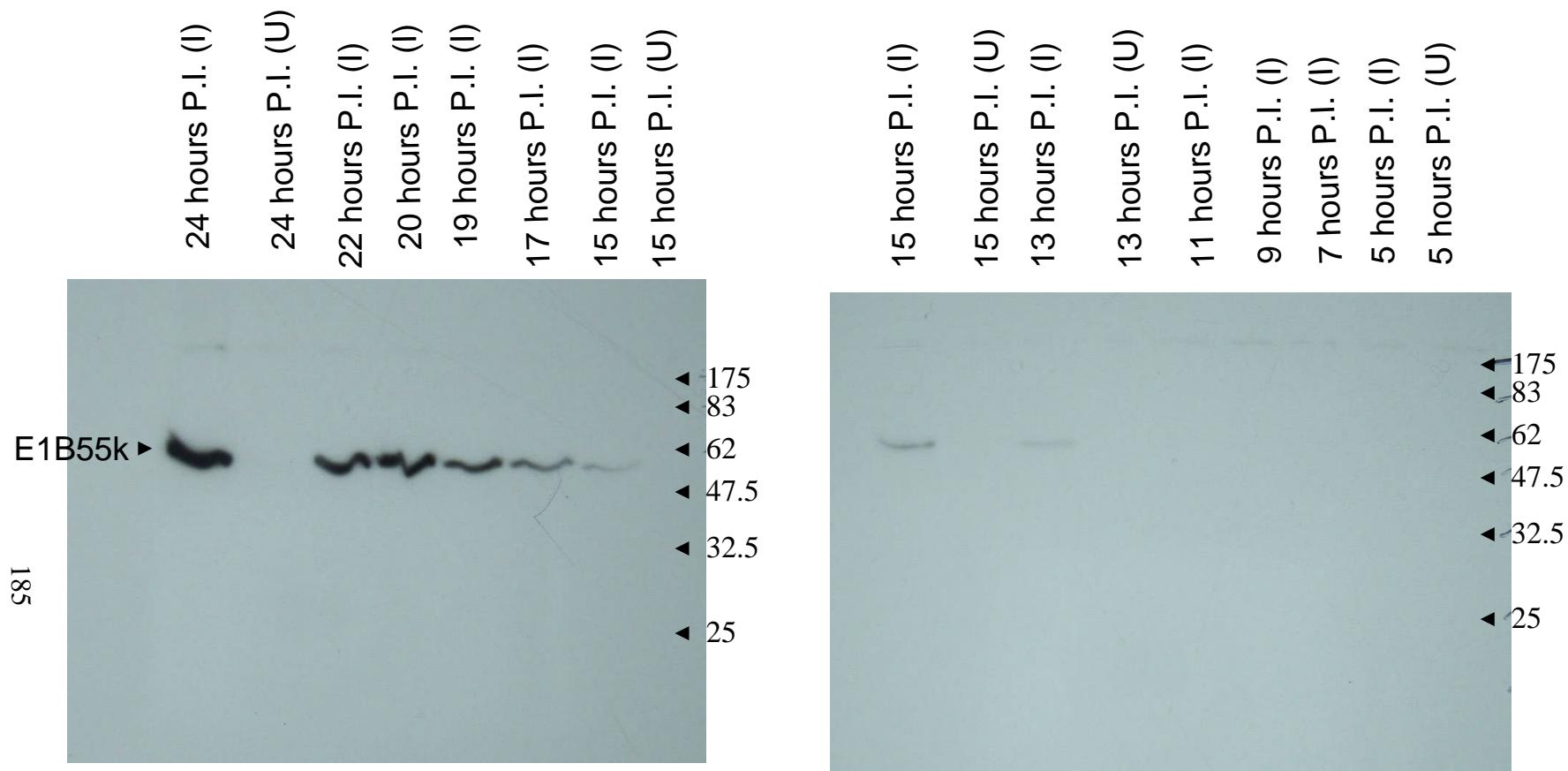


Figure 4.5: Time point of TAP-SUMO2 cells infected with adenovirus type 5. Western blot of cell lysates probed with E1B55k monoclonal. 150mm diameter petri dishes of TAP-SUMO2 HeLa's were grown to 80% confluence then infected with wild type Ad5 at a M.O.I of 10. Cell extracts were harvested from petri dishes at set time points in SDS lysis buffer, separated on 10% SDS-PAGE gels, transferred onto PVDF membranes and analysed by Western blot analysis using E1B55k monoclonal antibody. Time points are indicated as hours post infection (P.I.), infected (I) or uninfected controls (U). From the data it is evident that E1B55k expression was maximal at the 24 hour time point.

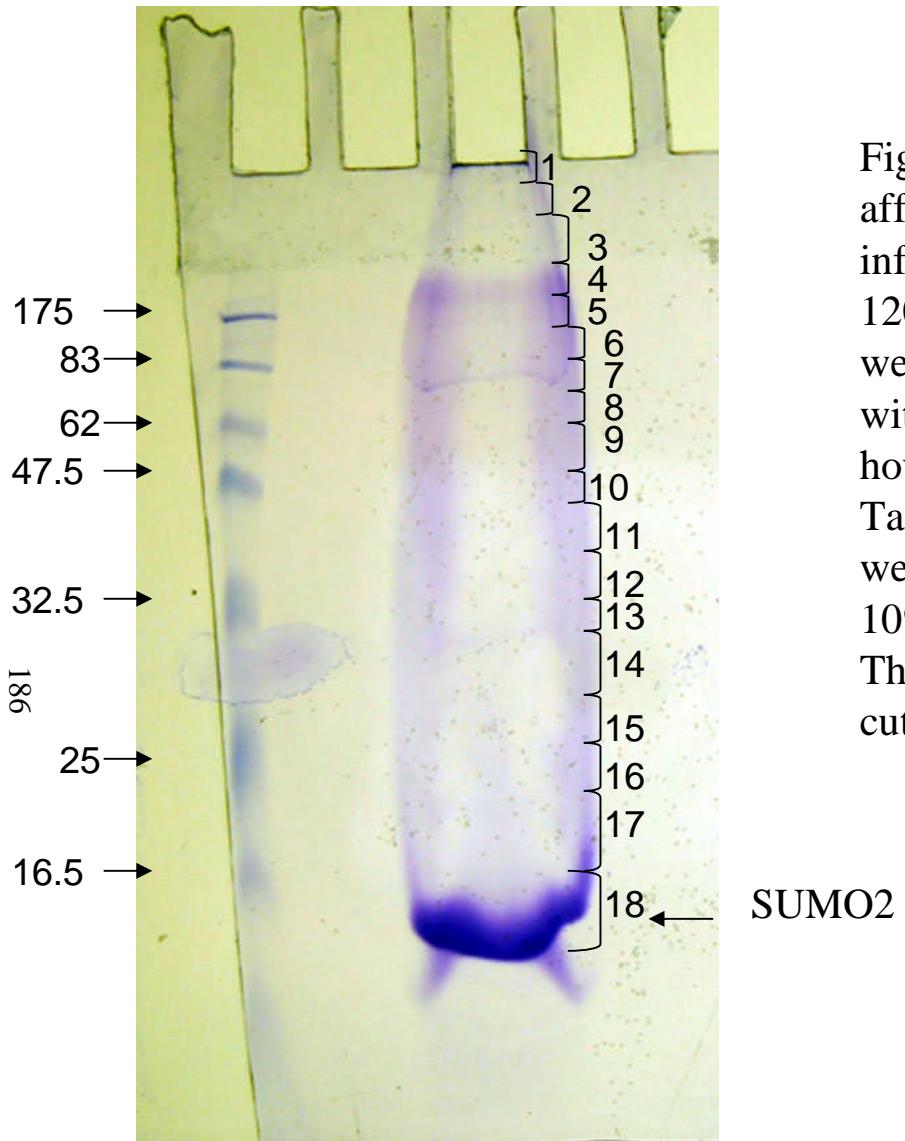


Figure 4.6: SDS-PAGE gel showing eluates from tandem affinity purification experiment using TAP/SUMO21 cells infected with Ad5 at and M.O.I of 10 for 24 hours.
120 petri dishes (150mm diameter) of TAP-SUMO2 cells were grown to 80% confluence, then infected with wild type Ad5 at an M.O.I. of 10. After 24 hours cells were harvested and subjected to Tandem Affinity Purification. Protein eluates were then TCA precipitated and run on a 10% SDS-PAGE gel and Coomassie stained. The bands indicated by brackets were cut out and proteins identified by LC-ESI-MS/MS.

molecular weight greater than 47.5kDa, and data set 10-18 which includes all proteins with a molecular weight less than 47.5kDa. This is likely due to degradation of the SUMOylated protein. When the data was searched against the viral database, this time adenovirus proteins were identified, see below:

<u>Gel slice</u>	<u>accession number</u>	<u>adenoviral protein identified*</u>
1	FOADM2	minor core protein pV – human adenovirus 2, 297 (33%)
3	AAC13964	L19443 NID: Human adenovirus F, 39 (8%)
4	Q6VGU4_9ADEN	E2A DNA binding protein – human adenovirus C, 67 (4%)
5	Q6VGU4_9ADEN	E2A DNA binding protein – human adenovirus C, 111 (7%)
6	Q9IIH5_ADEF1	Hexon – Frog adenovirus 1, 38 (4%)
8	Q6VGU4-9ADEN	E2A DNA binding protein – human adenovirus C, 70 (4%)

Table 1: Adenoviral protein identified in TAP/SUMO2 cells infected with wild type adenovirus 5. (*The numbers given are the overall score, followed by percentage peptide coverage).

Although no E1B55k protein was identified, the E2A DNA binding protein and pV from adenovirus were identified. As the E1B55k protein has only been demonstrated to be modified by SUMO1 (Endter et al, 2001), this result is as expected. The adenovirus DNA binding protein (DBP), has not been shown to be modified by SUMOylation, therefore this result needs to be confirmed by repeating the tandem affinity purification, and analysing the eluates by western blot analysis using an antibody to DBP. Protein V (pV) has previously been shown to be modified by SUMO (Rodriguez, Desterro and Hay, unpublished results).

4.3 Discussion

A large number of proteins have been demonstrated to be modified by SUMO2 *in vitro*. These include proteins involved in transcriptional regulation, mRNA metabolism, chromatin remodelling, nucleocytoplasmic transport, signal transduction, SUMOylation, ubiquitination, DNA replication, vesicle trafficking, DNA repair, translation, cell cycle and cell division, cytoskeletal regulation, energy metabolism as well as molecular chaperone proteins (see appendices 1-3). As well as identifying proteins that have already been confirmed as SUMO2 substrates, these experiments have also demonstrated that Lamina associated polypeptide 2 β (LAP2 β) protein is a substrate of SUMO2. LAP2 β is a lamin-binding protein of the LAP2 family, of which there are 6 different isoforms. It is a type II integral protein of the inner nuclear membrane, which appears to be involved in the spatial organisation of the interface between nucleoplasma, lamina and nuclear envelope (Dreger et al., 1999). It binds to lamin B and the DNA-bridging protein barrier-to-autointegration factor (BAF), and may link the nuclear

membrane to the underlying lamina and provide docking sites for chromatin (Dechat et al., 2000).

When comparing the data from both sets of TAP experiments, lots of proteins were identified in the adenovirus infected TAP/SUMO2 cells that were not identified in the uninfected cells (430 proteins in total). For proteins to be taken as true hits, the criteria chosen was that they should have an overall Mascot score of above 60. These proteins were compared to data generated previously by Dr Akihiro Nakamura who had undertaken experiments using uninfected TAP/SUMO2 cells and heat shocked TAP/SUMO2 cells, and had used similar amounts of cells (unpublished results). Of the proteins with Mascot scores above 60, only 5 proteins had previously been identified in experiments undertaken by Dr Akihiro Nakamura. These were; dna-K type molecular chaperone, accession number A27077; L-Lactate dehydrogenase chain H, accession number DEHULH; Alpha enolase, accession number ENOA_HUMAN; phosphoglycerate kinase, accession number KIHUG; Glyceraldehyde-3-phosphate dehydrogenase, accession number G3P1_HUMAN.

Proteins that were identified in only the adenovirus infected TAP/SUMO2 cells include;

General transcription factor 3, accession number AAF19786; Threonyl-tRNA synthetase, accession number A47363; DNA recombination and repair protein, accession number AAD10197; Ribosomal protein S3, accession number R3HUS3. Although similar types of proteins have been previously identified in experiments undertaken by Dr Akihiro Nakamura including; Transcription factor IIIC box B-binding chain, accession number I38414; Leucyl t-RNA synthetase, accession

number Q9NSE1; Post-replication repair protein hRAD18, accession number AAA70430; Ribosomal protein S5, accession number S55916. So in order to determine if the proteins identified during these experiments are SUMOylated specifically upon adenovirus infection the experiments would need to be repeated and Western blot analysis of the TAP eluates carried out with antibodies specific to the proteins of interest. This would confirm that the LC-ESI-MS/MS was identifying proteins present in the eluates, but in order to confirm that the protein was actually SUMOylated, a SUMOylation assay would have to be undertaken. The purified protein would be incubated with SUMO2, and the essential components of the SUMO pathway. SDS-PAGE and Western blot analysis would then confirm if SUMOylated forms of the protein became apparent following incubation with SUMO2. Sequence analysis would allow determination of possible SUMOylation sites, and mutation analysis could be undertaken to identify the specific SUMO modification site and the effect of SUMOylation on the protein. The effect of SUMOylation on localisation could be determined by generation of fluorescently tagged mutant and wild type forms of the protein, and analysis by microscopy. Another way of determining whether certain proteins were SUMOylated upon adenovirus infection would be to undertake SILAC experiments with uninfected and infected cells. By comparing the results from TAP-SUMO2 cells that were uninfected, from TAP-SUMO2 cells infected with adenovirus type 5, the relative amounts of specific proteins in each could be compared. Therefore if a protein was present at double the concentration in adenovirus infected cells, it can be taken that adenoviral infection increases the amount of that specific protein that is SUMOylated.

In conclusion, a lot of data has been generated by these experiments, however a lot more work has to be undertaken in order to validate the results.

5. GENERATION OF STABLE CELL LINES EXPRESSING TAP/E1B55K AND TAP/E1B55K K104R

5.1 Introduction

5.1.1 Introduction

Stable cell lines expressing tandem affinity purification (TAP) tagged E1B55k and E1B55k K104R were generated, with the aim of undertaking tandem affinity purification experiments in both uninfected cells, and in cells infected with adenovirus type 5 lacking E1B55k (*dl338*). The aim of these experiments was to identify both host cell and adenoviral proteins that interact with E1B55k. This was devised as an alternative method for identifying such hits, due to the problems experienced generating the recombinant adenoviruses (see chapter 3).

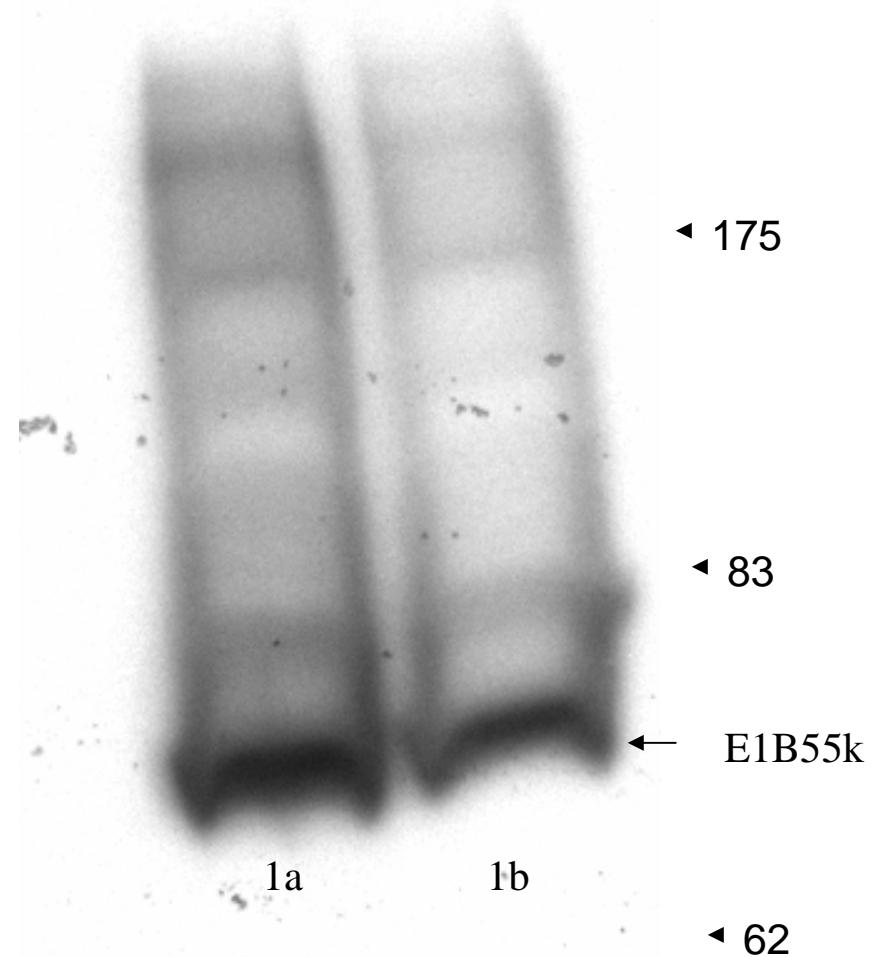
5.1.2 Generating the stable cell lines

The pCMV5-TAP vectors expressing E1B55k and E1B55k K104R that were generated in results chapter 1, were used as templates in PCR reactions. The TAP/E1B55k and TAP/E1B55k K104R genes were amplified using primers P19 and P20, which were designed to include *Xho*1 and *Xba*1 restriction sites respectively. The PCR products were then digested with *Xho*1 and *Xba*1 and cloned into the MCS of the pEFIRES-PURO vector, a kind gift from Dr Pablo De Felipe. The constructs were then verified by DNA sequence analysis. Western blot analysis, using a monoclonal antibody to E1B55k, confirmed expression of the tagged E1B55k proteins from both constructs (see figure 5.1).

The stable cell lines were generated, by transfecting the pEFIRES-PURO constructs, expressing TAP/E1B55k and TAP/E1B55k K104R, into petri dishes

Figure 5.1: Western blot of HeLa cells expressing TAP/E1B55k and TAP/E1B55k K104R constructs. A monoclonal antibody to the E1B55k protein was used in the western blots. Figure 1a illustrates TAP/E1B55k and figure 1b illustrates TAP/E1B55k K104R. Both products appear to be the correct size (approximately 76kDa).

193



(150mm diameter) of HeLa cells. The presence of puromycin in the growth medium selected for cells that were expressing the constructs. After 1 to 2 weeks, puromycin resistant colonies were picked and expanded in 24 well plates. Clones were screened by immunofluorescence (see figure 5.2) and Western blot analysis for expression of TAP/E1B55k and TAP/E1B55k K104R. Clones that were expressing at the highest levels were chosen for use in tandem affinity purification experiments.

5.1.3 Preparation of adenovirus *dl338*

A 20L culture of 293 spinner cells was infected with Ad5 *dl338* (supplied by John Nicholson in the laboratory) and harvested by arcton extraction and caesium chloride purification, when cells showed signs of infection. The virus was aliquoted and stored at -80°C. The titre of the virus was subsequently determined using end point dilution and found to be 1×10^{12} pfu/ml.

5.1.4 Experiments undertaken

For each experiment, one vial of cells was resuscitated from liquid nitrogen and the population expanded to 60 petri dishes (150mm diameter). Cells were grown in Medium supplemented with puromycin. The aim was to undertake four experiments, but only the first two experiments were carried out.

1. Uninfected TAP/E1B55k stable cell lines
2. Uninfected TAP/E1B55k K104R stable cell lines
3. TAP/E1B55k stable cell lines infected with Ad5 *dl338*
4. TAP/E1B55k K104R stable cell lines infected with Ad5 *dl338*

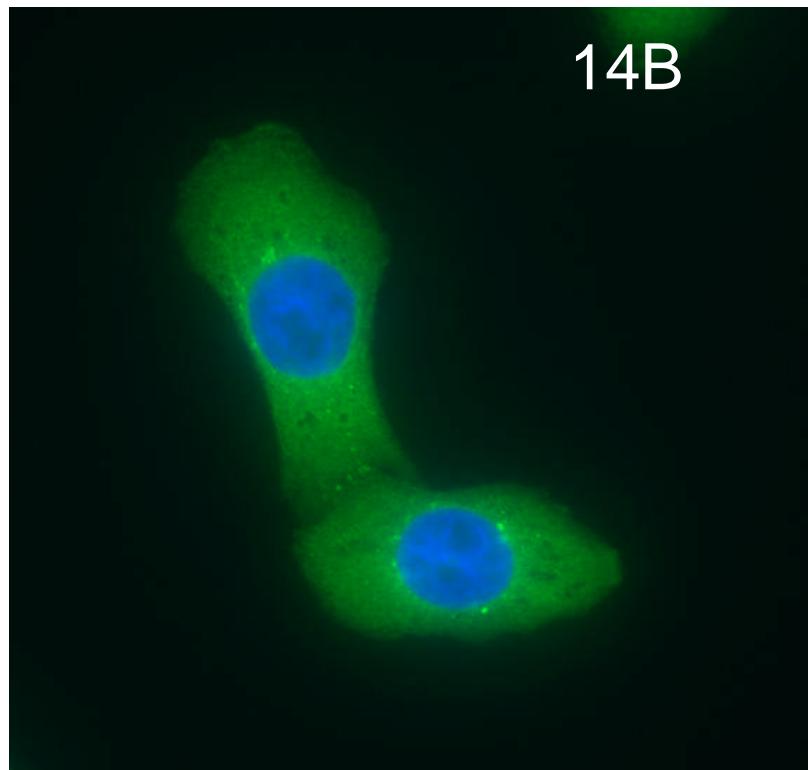


Figure 27a: Clone 14B, expressing TAP/E1B55k K104R.

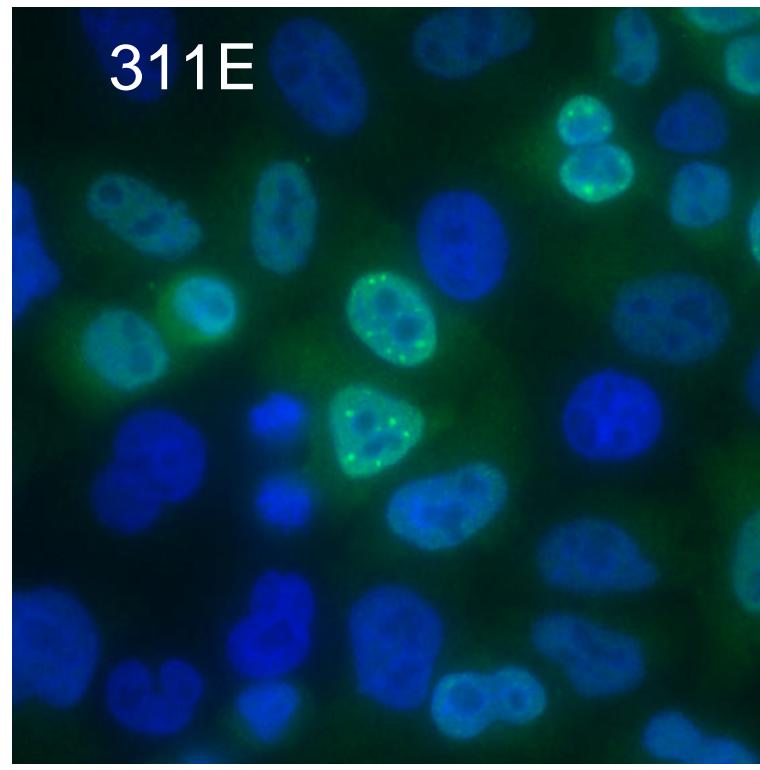


Figure 27b: Clone 311E, expressing TAP/E1B55k.

Figure 5.2: Immunofluorescence analysis of stable cell lines expressing E1B55k and E1B55k K104R. Stable HeLa cell lines were generated, expressing TAP/E1B55k (Figure 27a) and TAP/E1B55k K104R (figure 27b). Immunofluorescence was carried out using an E1B55k monoclonal antibody, and anti-mouse FITC conjugated secondary antibody.

5.1.5 Tandem Affinity Purification procedure

For each experiment, all 60 petri dishes (150mm diameter) were harvested when 100% confluent, by scraping the cells directly into PBS. The cell extracts were then pelleted and resuspended in lysis buffer. After lysis the cells were centrifuged to pellet any cell debris. IgG sepharose was then added to supernatant, to which the protein A of the TAP tag binds. After incubation the IgG beads were pelleted by centrifugation and washed in TBS/Tween. The protein of interest and any interacting proteins, were then cleaved from the IgG beads using TEV protease. Following cleavage, the samples were centrifuged to pellet the IgG beads. The supernatant was removed and to it was added calmodulin binding buffer (containing calcium), and calmodulin beads. The protein of interest and any associated proteins, bind to the calmodulin beads via the calmodulin binding peptide of the TAP tag. The calmodulin beads were then washed with calmodulin binding buffer to remove any contaminants, and proteins were eluted from the beads in calmodulin elution buffer containing EGTA, which acts by chelating the calcium.

After elution the proteins were concentrated into a smaller volume, to allow the sample to be loaded into one lane of an SDS-PAGE gel, by TCA precipitation. Once proteins have been separated according to molecular weight, by SDS-PAGE, the proteins were visualised by Coomassie staining of the gel. Protein bands were then excised from the gel, subjected to in-gel digestion and analysis by LC-ESI-MS/MS.

The MS/MS data file generated for each gel slice, was analysed using the Mascot search engine. Where possible all of these data were combined to include all of the

gel slices from the entire lane of the gel, and then searched against the MSDB database, using the Mascot search engine. This was done in order to acquire an overall list, encompassing all of the proteins purified during the experiment.

5.2 Results

5.2.1 Initial experiment using TAP/E1B55k K104R stable cell line (14B)

The first experiment was carried out using 60 petri dishes of uninfected TAP/E1B55k K104R stable cell line. Following tandem affinity purification, TCA precipitation and SDS-PAGE, no proteins were visible by Coomassie staining (data not shown). When part of the lane was analysed by mass spectrometry only keratin was detected. However, when the calmodulin beads which had been used in the experiment, were boiled and run on an SDS-PAGE gel, E1B55k protein was confirmed as being present by mass spectrometric analysis. This indicated that the E1B55k protein was not being eluted from the calmodulin beads.

5.2.2 Initial experiment using TAP/E1B55k stable cell line (311E)

The second experiment was carried out using 60 petri dishes of uninfected TAP/E1B55k stable cell line. Following tandem affinity purification, TCA precipitation and SDS-PAGE, no proteins were visible by Coomassie staining. When the lane containing the sample was analysed by mass spectrometry, only keratin was detected. However, Western blot analysis had confirmed that E1B55k protein was present in the eluates prior to TCA precipitation, indicating that the proteins had been lost during the TCA precipitation.

5.2.3 Optimisation of the tandem affinity purification procedure

The tandem affinity purification procedure was optimised in order to minimise protein loss. At key stages of the protocol, protein loss was minimised by making the following changes:

- A substantial amount of the E1B55k protein appeared not to bind to the IgG sepharose. The incubation time with the IgG sepharose was extended from 4 hours to 5 hours in order to increase binding.
- To ensure that all of the bait protein was cleaved from the IgG sepharose, the amount of TEV protease used was increased from 100 units to 500 units.
- To ensure elution of the protein from the calmodulin beads the amount of EGTA in the elution buffer was increased from 2mM to 10mM.
- Extra care was taken when TCA precipitating the samples and all supernatant removed was stored at -80°C as back up.

Also, in order to minimise the level of keratin contamination, eluates were run on a precast NuPAGE® Novex 4-12% Bis-Tris gel, on an XCell *SureLock*™ gel rig using NuPAGE® MOPS SDS Running Buffer (Invitrogen). The gel rig, precast gels and premade running buffer were specifically purchased for use in tandem affinity purification procedures, and used solely for this purpose.

After the tandem affinity purification procedure had been optimised the experiments using the uninfected stable cell lines were repeated and, this time, proteins were visible by Coomassie staining for each of the experiments (see figure 5.3). Bands were excised from the gel and subjected to in-gel digestion,

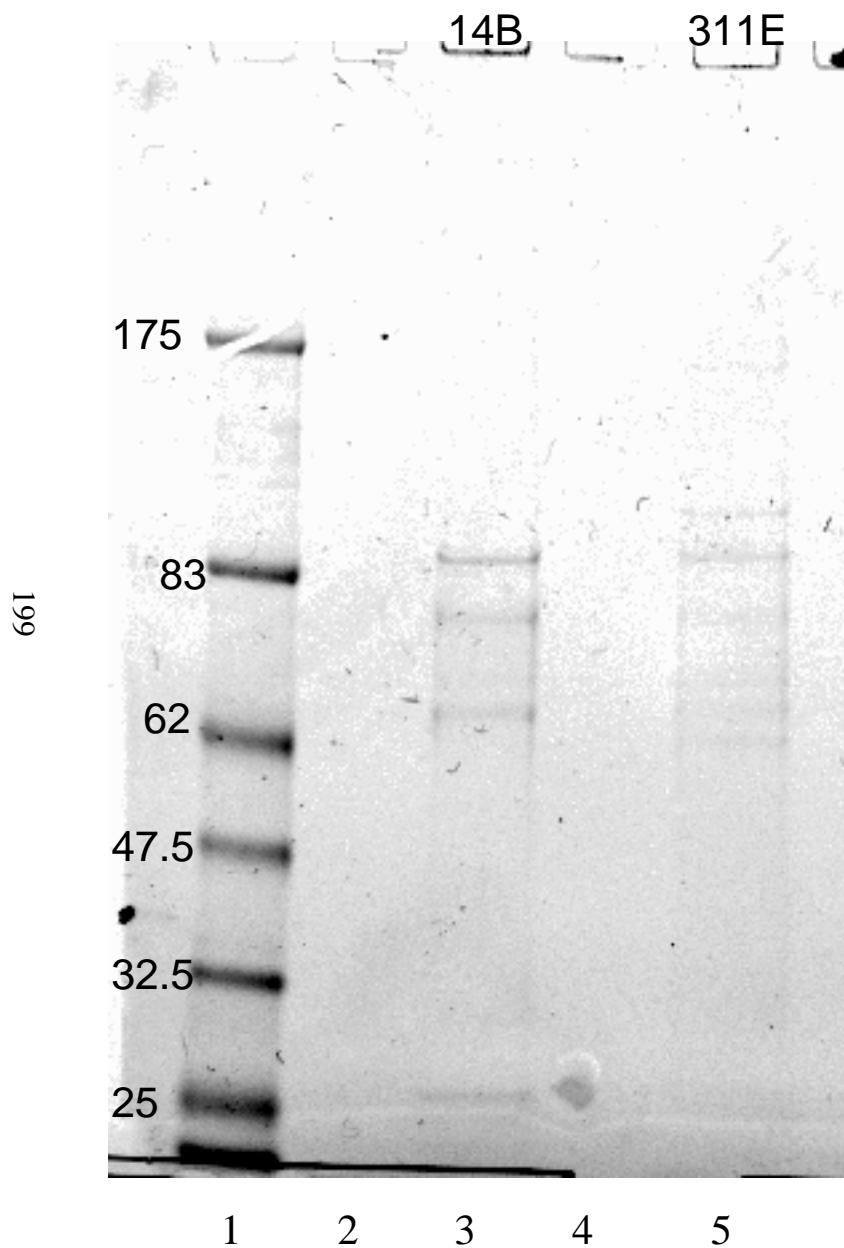


Figure 5.3: SDS-PAGE analysis of eluates from Tandem affinity purification experiments carried out with stable cell lines expressing E1B55k and E1B55k K104R. 60 petri dishes (150mm diameter) of each stable cell line were grown to 100% confluence, lysed and subjected to tandem affinity Purification. Protein eluates were TCA precipitated, resuspended in SDS boiling mix and run on a 10% SDS-PAGE gel (Coomassie stained). Lane 3 contains the eluates from the TAP/E1B55k K104R stable cell line (clone 14B) and lane 5 contains the eluates from the TAP/E1B55k stable cell line (clone 311E). The lanes containing the samples were excised from the gel and subjected to tryptic digestion and LC-ESI-MS/MS.

followed by LC-ESI-MS/MS analysis. The data generated was then searched using the Mascot search engine, to generate a list of proteins that possibly interact with E1B55k. Each protein is allocated an overall score, which is based on the quality of the individual MS/MS spectra and the number of peptides that matched the protein, with the most probable hits having the highest overall scores, and proteins that are questionable as being true hits having lower scores. The criteria chosen for proteins to be considered as a true hit, was that the overall score should be higher than 60. As a means of ensuring that the experiments had worked efficiently, the data was first checked for the presence of E1B55k protein.

5.2.4 Results from TAP/E1B55k K104R stable cell line (14B)

The lane containing all proteins interacting with E1B55k, non-SUMO modifiable, was divided up into 16 separate fragments, which were searched individually against the human database and the all species database. The first hit in nearly of all of the samples was keratin, and the highest overall Mascot score was 619. In fact almost all of the high scoring proteins were identified as keratin, with the exception of dna-K type molecular chaperone protein, which had an overall Mascot score of 512. When searched against the all species database, only one of the fragments contained peptides that matched the E1B55k protein from human adenovirus type 1 (accession number Q71BY4).

These results were not as anticipated, as it was expected that the E1B55k protein would be present in abundance. In order to look in more detail for the adenovirus

E1B55k protein, the data generated for each of the 16 fragments was searched individually against the virus database.

Of the 16 separate fragments, E1B55k protein from human adenovirus type 5 (Q1AD55) was found in fragments 7, 9, 11, 12, 13 and 14 with an overall Mascot score of 31, 108, 39, 46, 49 and 55 respectively. Although the score of 108 appears high, further scrutiny showed that for this overall score, 6 peptides matched, with the highest score for an individual peptide being 51. Of the peptides that were identified, only 3 had scores which identified the protein as being E1B55k. The remaining peptides had scores that indicated only homology with E1B55k protein.

5.2.5 Results from TAP/E1B55k stable cell line (311E)

The lane containing this sample was divided up into 22 separate fragments, which were searched individually against the human database. Again, the first hit in nearly of all of the samples was keratin, and the highest overall Mascot score for keratin being 300. In fact almost all of the high scoring proteins were identified as keratin, with the exception of dna-K type molecular chaperone protein, which had an overall Mascot score of 498. The results were also combined and searched against the human database and against the all species database. E1B55k from human adenovirus type 5 (Q1AD55) was detected in the combined search against the all species database, with an overall score of 90 with 7 peptides matching, although the highest score for an individual peptide was 45.

In this experiment the E1B55k protein from adenovirus type 5 had been identified, although the overall score was much lower than expected. In order to look in more detail for E1B55k protein, the data generated for each of the 22 fragments was

searched individually against the virus database. Of the 22 separate fragments, E1B55k protein from adenovirus type 5 was detected in fragments 12, 13, 19, 20 and 21 with an overall score of 35, 29, 45, 28 and 31 respectively. The highest score for any individual peptide was 45. In these results only one of the peptides had a score which identified the protein as being E1B55k. The remaining peptides had a score which indicated only homology with E1B55k protein.

5.2.6 Results of preliminary experiments suggest inefficient cell lysis

Although the tandem affinity purification procedure was initially demonstrated to work, it appeared to be very inefficient, even with the steps implemented to try to optimise purification of the tagged proteins.

Some preliminary experiments were undertaken to ensure that the cells were being lysed efficiently prior to tandem affinity purification. This was due to the fact that the E1B55k protein had lower overall scores in the stable cell line expressing wild type E1B55k, where the localisation was nuclear, compared to the stable cell line expressing E1B55k K104R, where the localisation was cytoplasmic. A sonication step was added to the existing tandem affinity purification protocol, in order to increase disruption of cell membranes. It was decided not to alter the salt concentration in the lysis buffer as this could disrupt interaction between the E1B55k protein and any interacting host cell or viral proteins. The aim was to directly compare this modified method to the Dignam and Roeder method for purifying nuclear cell extracts. By comparing these methods for the TAP/E1B55k stable cell lines, where the localisation of the E1B55k protein is nuclear, it would

be possible to determine if the cell nucleus was lysed sufficiently to allow its contents to be released.

Prior to the completion of these experiments, this project was ceased, in order to focus on other aspects of the project.

5.3 Discussion

Stable cell lines expressing TAP/E1B55k and TAP/E1B55k K104R have been successfully generated and the tandem affinity purification procedure has been demonstrated to work, although it requires further optimisation. Western blot analysis of washes and eluates taken throughout the TAP procedure illustrated that a considerable proportion of the E1B55k protein was lost during the initial binding to IgG beads. Thus the incubation time for this step was increased from 4 to 5 hours. This may not solve the problem though as the inefficient binding could also be due to the conformation of the N-terminally TAP tagged E1B55k protein. The tertiary structure of the protein may prevent the Protein A of the TAP tag from binding efficiently to the IgG beads. To investigate whether this is a problem, a C-terminally TAP tagged E1B55k stable cell line could be generated and compared, to see if there is a difference in binding efficiency. The Western blot results also demonstrated that a small amount of the protein was lost due to inefficient cleavage of the TEV protease, preventing release of the E1B55k protein from the IgG beads. This was overcome by increasing the amount of TEV protease used from 100 units to 500 units. Finally in order to ensure complete elution of the protein from the calmodulin beads the amount of EGTA in the elution buffer was increased from 2mM to 10mM.

Keratin contamination from skin and hair was also highlighted as a problem during these tandem affinity purification experiments. Steps were taken to reduce keratin contamination including; wearing gloves, ensuring the work area was cleaned prior to the start of the experiment, soaking the gel rig in detergent prior to use, making the buffers and solutions required using new glassware ordered specifically for this purpose and storing them in sterile cell culture flasks rather than in Durans and using a separate stock of chemicals to make up the buffers and not the general lab stock. During the course of these experiments an XCell *SureLock*TM gel and precast NuPAGE[®] Novex 4-12% Bis-Tris gels were ordered specifically for these experiments, along with NuPAGE[®] MOPS SDS Running Buffer (Invitrogen). By having a specially designated gel rig only to be used for tandem affinity purification and using precast gels and premade running buffer it was hoped that keratin contamination would be dramatically reduced.

Although E1B55k protein was detected in the tandem affinity purification experiments undertaken in each of the cell lines, the E1B55k protein did not appear to be in abundance. This could be caused by inefficient lysis of the cells. In order to determine if the lysis procedure was sufficient to release the contents of both the nucleus and the cytoplasm, it was planned to compare results, by Western blot, between, the standard lysis method, the lysis method with an added sonication step, and the Dignam and Roeder method for lysing nuclear extracts. The plan was to do these experiments using the TAP/E1B55k stable cell line, where the localisation of the E1B55k protein is nuclear. Unfortunately work was ceased on this project prior to completion of these experiments.

It should also be noted that when run on a SDS-PAGE gel, the tagged E1B55k protein would be expected to run between the 83kDa and 62kDa markers. In both stable cell lines however, following TAP purification, the tagged E1B55k protein appeared to be present in gel slices excised that lay between the 83kDa and 25kDa markers, indicating that the tagged E1B55k protein was being degraded. In Western blot analysis of the pEFIRE-S-PURO constructs used to generate the stable cell lines however, the tagged proteins appeared to be the correct size (approximately 76kDa). Perhaps the E1B55k protein is being degraded during the TAP procedure, this could be possibly be prevented by increasing the amount of protease inhibitors used.

TAP experiments undertaken with the TAP-SUMO2 stable cell lines, showed that when 120 petri dishes (150mm diameter) were used, many protein bands were visible by Coomassie staining upon SDS-PAGE analysis of eluates. By increasing the amount of petri dishes used in these experiments up to 120, far more proteins that interact with E1B55k could be identified.

In conclusion, by optimising a few more of the parameters, it would seem likely that these experiments could yield data.

6. FUTURE WORK

6.1 Generation of the recombinant adenoviruses

The recombinant adenoviruses expressing TAP/E1B55k and CFP/E1B55k have been generated, plaque purified 3x, and stocks have been frozen down at -80°C. In western blot analysis of these recombinant viruses however, the tagged E1B55k protein appears to be truncated, although DNA sequencing has not indicated the presence of any mutations within the DNA sequence which would cause this. The recombinant viruses have only been partially sequenced therefore, before any experiments are undertaken the remainder of the tagged E1B55k gene should be sequenced to ensure that no mutations are present.

Provided that both recombinant viruses are mutation free tandem affinity purification experiments can be undertaken with the virus expressing TAP/E1B55k. Different cell lines could be infected with the recombinant virus and harvested at different time points during infection, in order to determine what cellular and viral proteins interact with E1B55k at specific time during infection. Live cell imaging could be undertaken with different cell types infected with the recombinant virus expressing CFP/E1B55k. This would allow the localisation of the E1B55k gene to be monitored throughout infection, and if localisation patterns differed between different cell types.

The recombinant adenoviruses expressing TAP/E1B55k K104R and CFP/E1B55k K104R, where the E1B55k gene cannot be modified by SUMO, will need to be remade. It is essential that the E1B55k K104R gene used to generate the constructs is mutations free. As a method has been developed for cloning the constructs required to generate the recombinant viruses, remaking the viruses should be

straight forward. Generation of these viruses will allow a direct comparison between the recombinant viruses, where the E1B55k gene can be modified by SUMO. This would enable a better understanding of the effect of SUMOylation on the E1B55k protein.

6.2 Tandem affinity purification of TAP/SUMO2 stable cell lines infected with adenovirus type 5

A large amount of data has been generated from both the uninfected TAP/SUMO2 cells, and from TAP/SUMO2 cells infected with adenovirus type 5. The list of proteins resulting from the tandem affinity purification of 120 petri dishes of TAP/SUMO2 cells, totals 1566 cellular proteins. The list of proteins generated from tandem affinity purification of 120 petri dishes of TAP/SUMO2 cells infected with Ad5 at a M.O.I of 10 for 24 hours, totals 427 cellular proteins. This is a huge number of possible SUMO2 substrates, although a substantial number of the proteins indicated to be SUMO2 substrates in this experiment have previously been confirmed as substrates. In order to confirm if any further proteins identified in these experiments are real substrates of SUMO2, the experiments will need to be repeated and western blot analysis carried out on eluates, using an antibody to the protein of interest. This method has already been used to confirm that lamina associated polypeptide (Lap) 2 β is modified by SUMO2.

6.3 Generation of stable cell lines expressing TAP/E1B55k and TAP/E1B55k K104R

Stable cell lines expressing TAP/E1B55k and TAP/E1B55k K104R have been successfully generated and the tandem affinity purification procedure has been demonstrated to work, although it requires further optimisation. Measures taken to increase the efficiency of the TAP procedure have included increasing incubation time with IgG beads, increasing the amount of TEV protease, and increasing the amount of EGTA in the elution buffer.

The inefficient binding to the IgG beads may however be due to the tertiary structure of the TAP/E1B55k fusion protein, preventing the protein A binding domain from binding to the IgG beads. It would be interesting to generate C-terminally tagged E1B55k stable cell lines to compare the binding efficiencies.

In order to determine if the lysis procedure is sufficient to release the contents of both the nucleus and the cytoplasm a comparison should be undertaken between the standard lysis method, the lysis method with an added sonication step, and the Dignam and Roeder method for lysing nuclear extracts. This should be done using the TAP/E1B55k stable cell line, where the localisation of the E1B55k protein is nuclear.

The experiments should also be repeated with double the amount of cells (120 petri dishes instead of 60 petri dishes), as experiments undertaken with this quantity of TAP/SUMO2 cells gave vast quantities of data.

Lastly these experiments when optimised should be repeated on the stable cell lines infected with adenovirus *dl338*. This would allow identification of any adenoviral proteins that interact with E1B55k, as well as cellular proteins.

APPENDIX

7.1 Details of primers used (Table 2: Sequence of primers used for sequence analysis, PCR and overlap PCR)

<u>Primer name</u>	<u>Sequence (5'-3')</u>	
L1	AATTCTAGGGATAACAGGGTAATT	
L2	AGCTAATTACCCCTGTTATCCCTAG	
L3	AATTCTAGGGATAACAGGGTAATA	<i>Linkers for inserting I-SceI site into pUC19</i>
L4	CTAGTATTACCCCTGTTATCCCTAG	
S1	CCCACGCCCGCGCACGTCACAAACTCCA	<i>Primers for sequencing bacmid backbone of pSCB-AdFL</i>
S1A	CACATCCGTCGCTTACATGTGTTCCG	
S2	CTTGTGAGGCTAC	
S3	CCTGTGATGCTGG	
S4	GGCCAATACCAAC	
S5	CCATTGTAATGAGACGC	<i>Primers for sequencing left hand end of Ad5 cloned into pGEM-5zf(+)</i>
S6	GATCGAAGAGGTACTGG	
S7	GTAGATGATCCAGTCGTAG	
S8	CGTAGTTACCCTCACAG	
P1	CGCTCTAGAGAAATGCAATAGTAGTACGGATAGCTGTGAC	
P2	GTGGGCACTTGTGTCATGGTGGCTTATCCTTATAAAACTC	
P3	CTCCTCGCCCTTGCTCACCATGGTGGCTTATCCTTATAAAA	
P4	GAGTTTATAAAGGATAAGCCACCATGGACACAAGTGGCAC	
P5	GAGTTTATAAAGGATAAGCCACCATGGTGAGCAAGGGCGAG	
P6	ACGCCCCACACATTCAGTACCTCAATCTGTATCTTCATCGCT	
P7	AGCGATGAAGATAACAGATTGAGGTACTGAAATGTGTTGGCGT	
P8	AGACACTTGCTTGTACCAAATCCAAACAGAGTCTGGTTT	
P9	AATGGATCCGAGCGAAGAAACCCATC	
P10A	CCGGGATCTCAATCTGTATCTTCAT	<i>Primers for sequencing the pCMV5-TAP vector</i>
P11	GCCGTCCTCAGCAGCCAACCGC	
P12	TTAACGAAAGAACACGAAAC	
P13	CTGAGCATCTTAGCTTTT	
P14	ACCGCTGCGCATAGTTTCTGTAATTACAACAGTGCT	
P15	TTCCCGTTGAATATGGCTCATATCAGCAACTATATATTGATA	
P16	TATCAATATATAGTTGCTGATATGAGCCATATTCAACGGAA	
P17	GGCTGCTGCAAAACAGATACAGAAAAACTCATCGAGCATCAA	
P18	TTGATGCTCGATGAGTTTCTGTATCTGTTGCAGCAGCC	
P15	ACCGATCAACGCTCTCATTTCGATCAGCAACTATATATTGATA	<i>Overlap PCR products for knocking out the 5' ITR of pSCB-AdFL</i>
P16	ATATCAATATATAGTTGCTGATCGAAAATGAGACGTTGATCGG	
P17	AGGCTGCTGCAAAACAGATACATTACGCCCGCCCTGCCACTC	
P18	AGAGTGGCAGGGCGGGCGTAATGTATCTGTTGCAGCAGCC	
PUC19F	CCGCACAGATGCGTAAGGAG	
PUC19R	CAGCTGGCACGACAGGTTTC	<i>Primers for sequencing the MCS of pUC19</i>
P19	GTTTCTCGAGGCCCTTGCCACC	
P20	GTATTCTAGAGTCGACCTGCAG	<i>Primers designed to amplify TAP-E1B-55kDa</i>
P21	CTGGAACAGAACTTCCAG	<i>3' primer for amplifying TAP only</i>
P22	GTAAACGGCCACAAGTTC	
P23	CTTGTACAGCTCGTCCATG	<i>Primers designed to amplify CFP</i>

7.2 TAP CELL LINES UNINFECTED (see figure 4.1)

Accession number, name of protein and overall Mascot score

- 1.[AAG41947](#) Keratin 1 (2300)
- 2.[KRHU2](#) keratin 1, type II, cytoskeletal (1509)
- 3.[Q8N175_HUMAN](#) Keratin 10 (1454)
- 4.[ABHUS](#) serum albumin precursor [validated] (1443)
- 5.[Q96CL4_HUMAN](#) Keratin 6A (1336)
- 6.[CAA19927](#) OTTHUMP00000016001 protein (1318)
- 7.[Q7RTN9_HUMAN](#) Type II keratin K6h (1315)
- 8.[K2C6C_HUMAN](#) Keratin, type II cytoskeletal 6C (Cytokeratin 6C) (1307)
- 9.[K2C6E_HUMAN](#) Keratin, type II cytoskeletal 6E (Cytokeratin 6E) (1300)
- 10.[KRHUEB](#) keratin 6b, type II (1260)
- 11.[K1CP_HUMAN](#) Keratin, type I cytoskeletal 16 (Cytokeratin 16) (1239)
- 12.[K2C6F_HUMAN](#) Keratin, type II cytoskeletal 6F (Cytokeratin 6F) (CK 6F) (K6f keratin)(1184)
- 13.[K1CN_HUMAN](#) Keratin, type I cytoskeletal 14 (Cytokeratin 14) (1167)
- 14.[CAA82315](#) Cytokeratin 9 (1163)
- 15.[Q6PI71](#) Keratin 5 (856)
- 16.[A44861](#) keratin, 67K type II epidermal (756)
- 17.[Q7L3S5_HUMAN](#) JUP protein (721)
- 18.[AAA36145](#) Keratin K5 (607)
- 19.[K1CQ_HUMAN](#) Keratin, type I cytoskeletal 17 (Cytokeratin 17) (591)
- 20.[IJHUG1](#) desmoglein 1 precursor (444)
- 21.[TFHUP](#) transferrin precursor [validated] (379)
- 22.[ATBOG](#) actin gamma - bovine (tentative sequence) (376)
- 23.[S60712](#) band-6-protein (371)
- 24.[AAH59367](#) Transferrin (365)
- 25.[UQBO](#) ubiquitin - bovine (tentative sequence)(348)
- 26.[BAA23486](#) Polyubiquitin (338)
- 27.[BAB71634](#) CDNA FLJ25308 fis, clone SYN00939, highly similar to Ig gamma immunoglobulin heavy chain (332)
- 28.[CAA23759](#) Unnamed protein product (324)
- 29.[BAC04926](#) CDNA FLJ39691 fis, clone SMINT2010672, highly similar to Homo sapiens immunoglobulin lambda heavy chain (318)
- 30.[BAD08204](#) anti-HBs antibody heavy chain (317)
- 31.[Q9NSA9_HUMAN](#) Cytokeratin (313)
- 32.[HBCZ](#) hemoglobin beta chain – chimpanzee (311)
- 33.[BAC85237](#) Unnamed protein product (307)
- 34.[AAH66642](#) AcOrf-12 peptide (304)
- 35.[Q6PJ95_HUMAN](#) Hypothetical protein (303)
- 36.[CAA75030](#) IMMUNOGLOBULIN KAPPA HEAVY CHAIN PRECURSOR (303)
- 37.[AAH67091](#) IGHG1 protein (303)
- 38.[BAC85529](#) Unnamed protein product (302)
- 39.[BAC04226](#) CDNA FLJ36487 fis, clone THYMU2017844, highly similar to Homo sapiens immunoglobulin lambda heavy chain (301)
- 40.[BAC85171](#) Unnamed protein product (301)

- 41.[G2HU](#) Ig gamma-2 chain C region (292)
 42.[1C3TA](#) 1d8 ubiquitin mutant YES (291)
 43.[BAC85202](#) Unnamed protein product (262)
 44.[KRHU5](#) keratin 15, type I, cytoskeletal
 45.[1BUWB](#) Chain B, Crystal Structure Of S-Nitroso-Nitrosyl Human Hemoglobin A (257)
 46.[JE0242](#) Ig kappa chain NIG26 precursor (254)
 47.[Q6V0K9_HUMAN](#) Mutant hemoglobin beta chain (Fragment) (253)
 48.[Q6FH51_HUMAN](#) SFN protein (244)
 49.[BAC85434](#) Unnamed protein product (240)
 50.[MYLK2_HUMAN](#) Myosin light chain kinase 2, skeletal/cardiac muscle (EC 2.7.1.117) (MLCK2) (232)
 51.[Q96KX8_HUMAN](#) MGC27165 protein (226)
 52.[Q701L8_HUMAN](#) Keratin 3 (221)
 53.[1IGAA](#) iga1 chains a and b, heavy, chains c and d, light, chain A (219)
 54.[BAC86513](#) Unnamed protein product (216)
 55.[Q7Z3Y8_HUMAN](#) Type I inner root sheath specific keratin 25 irs3 (215)
 56.[BAC85432](#) Unnamed protein product (213)
 57.[Q5XKE5_HUMAN](#) Keratin 6L (206)
 58.[CAC10254](#) Immunoglobulin heavy chain (Fragment) (205)
 59.[1B56](#) fatty acid binding protein (205)
 60.[BAC01698](#) Immunoglobulin kappa light chain VLJ region (Fragment) (202)
 61.[CAC10219](#) Immunoglobulin heavy chain (Fragment) (201)
 62.[K1CM_HUMAN](#) Keratin, type I cytoskeletal 13 (Cytokeratin 13)(198)
 63.[CAD48782](#) Sequence 29 from Patent EP1229047 (197)
 64.[Q96PQ8_HUMAN](#) Factor VII active site mutant immunoconjugate (196)
 65.[1MCOH](#) Immunoglobulin g1 (igg1) (mcg) with a hinge deletion, chain H (195)
 66.[Q6GMW0_HUMAN](#) Hypothetical protein (190)
 67.[Q8TC63_HUMAN](#) IGHG4 protein (191)
 68.[Q5XG83_HUMAN](#) Hypothetical protein (Fragment) (190)
 69.[CAC10224](#) Immunoglobulin heavy chain (Fragment) (183)
 70.[CAB58611](#) SEQUENCE 1 FROM PATENT WO9318147 (181)
 71.[Q9HB00_HUMAN](#) Desmocollin 1b (180)
 72.[CAC21968](#) Sequence 2 from Patent WO0073481 (Fragment) (179)
 73.[1FVDB](#) Fab fragment of humanized antibody 4d5, version 4, chain B (178)
 74.[Q5SP17_HUMAN](#) Heat shock 70kDa protein 1A (177)
 75.[CAD19028](#) Sequence 33 from Patent EP1158004 (175)
 76.[BAC01711](#) Immunoglobulin kappa light chain VLJ region (Fragment) (175)
 77.[1AD0B](#) Ig heavy chain Fab fragment, antibody a5b7, chain B (175)
 78.[BAA82105](#) Anti-Entamoeba histolytica immunoglobulin kappa light chain (Fragment) (165)
 79.[AAD29608](#) Kappa 1 immunoglobulin light chain (165)
 80.[AAS85992](#) Immunoglobulin heavy chain (157)
 81.[Q6FGZ8_HUMAN](#) TUBB protein (Fragment) (145)
 82.[ITHU](#) alpha-1-antitrypsin precursor [validated] (143)
 83.[AAS85981](#) Immunoglobulin heavy chain (141)

- 84.Q7Z795_HUMAN Keratin 2p (141)
 85.AAR32408 Immunoglobulin heavy chain variable region (138)
 86.AAH42911 LGAL7S protein (138)
 87.CAI18215 Heat shock 10kDa protein 1-like (137)
 88.Q8NA87_HUMAN Hypothetical protein FLJ35741 (137)
 89.BAC86537 Unnamed protein product (136)
 90.A24903 tubulin alpha-1 chain - Chinese hamster (134)
 91.AAR02477 Immunoglobulin heavy chain variable region (134)
 92.EF2_HUMAN Elongation factor 2 (EF-2) (132)
 93.Q7RTT2_HUMAN Keratin 5b (132)
 94.PSHUAM 14-3-3 protein zeta (132)
 95.UBRTA tubulin alpha chain – rat (131)
 96.GTP_HUMAN Glutathione S-transferase P (EC 2.5.1.18) (GST class-pi)
 (GSTP1-1) (129)
 97.AAS86018 Immunoglobulin heavy chain (129)
 98.AAR32428 Immunoglobulin heavy chain variable region (129)
 99.S25740 Ig lambda chain (128)
 10.S14675 Ig lambda chain (125)
 101.BAA82101 Anti-Entamoeba histolytica immunoglobulin gamma heavy
 chain (Fragment) (125)
 102.AAR32393 Immunoglobulin heavy chain variable region (125)
 103.BAB18260 Anti HBs antibody heavy-chain Fab (Fragment) (124)
 104.AAS85965 Immunoglobulin heavy chain (124)
 105.AAA20163 IMMUNOGLOBULIN LIGHT CHAIN (FRAGMENT) (123)
 106.AAS85983 Immunoglobulin heavy chain (122)
 107.AAS86007 Immunoglobulin heavy chain (121)
 108.S23624 Ig heavy chain V region (fragment) (121)
 109.C3HU complement C3 precursor [validated] (121)
 110.AAR32411 Immunoglobulin heavy chain variable region (119)
 111.AAA16946 IMMUNOGLOBULIN KAPPA CHAIN (FRAGMENT) (118)
 112.AAP23227 Antibody light chain (Fragment) (118)
 113.BAC85347 Unnamed protein product (118)
 114.Q8WU19_HUMAN K-ALPHA-1 protein (118)
 115.CSHUA peptidylprolyl isomerase (EC 5.2.1.8) A (117)
 116.1B3EA serum transferrin n-terminal lobe (117)
 117.BAC86532 Unnamed protein product (117)
 118.A36040 Ig heavy chain V-III region (ART)
 (fragments) (117)
 119.A36898 maspin (116)
 120.AAR02542 Immunoglobulin heavy chain variable region (116)
 121.AAR32417 Immunoglobulin heavy chain variable region (116)
 122.Q8WZ42_HUMAN Titin (115)
 123.1A8JL immunoglobulin lambda light chain dimer (mcg), chain L (115)
 124.S25754 Ig lambda chain (fragment) (115)
 125.AAR32418 Immunoglobulin heavy chain variable region (115)
 126.Q7KZS6_HUMAN Tubulin, beta, 4 (112)
 127.A49444 Ig gamma-1 heavy chain (New)(fragment) (111)
 128.AAR32424 Immunoglobulin heavy chain variable region (111)

- 129.[AAA35597](#) Beta-globin (109)
 130.[Q9BYX7_HUMAN](#) FKSG30 (109)
 131.[AAR88369](#) anti-HIV-1 gp120 immunoglobulin 48d heavy chain (109)
 132.[AAF05685](#) Anti-phospholipid immunoglobulin heavy chain VH-D-J
 region (fragment) (108)
 133.[AAR02563](#) Immunoglobulin heavy chain variable region (108)
 134.[1VGEH](#) tr1.9 fab fab fragment of a igg1 kappa autoantibody, chain H
 (108)
 135.[AAR32532](#) Immunoglobulin heavy chain variable region (108)
 136.[AAS54980](#) anti-HIV-1 gp120 immunoglobulin X5 heavy chain (108)
 137.[AAR02564](#) AY393691 NID (108)
 138.[HACZ](#) hemoglobin alpha chain – chimpanzee (108)
 139.[AAA87670](#) IMMUNOGLOBULIN HEAVY CHAIN FD REGION V-D-J-
 CH1 (FRAGMENT) (107)
 140.[AAR32526](#) Immunoglobulin heavy chain variable region (107)
 141.[AAS86010](#) Immunoglobulin heavy chain (107)
 142.[3FCTB](#) metal chelatase catalytic antibody fab fragment, chain B (106)
 143.[BAA82104](#) Anti-Entamoeba histolytica immunoglobulin gamma heavy
 chain (Fragment) (106)
 144.[AAS85976](#) Immunoglobulin heavy chain (106)
 145.[S01713](#) tubulin beta-7 chain – chicken (106)
 146.[AAS68223](#) Immunoglobulin heavy chain (106)
 147.[AAG30516](#) Immunoglobulin heavy chain (Fragment) (105)
 148.[AAA52865](#) IMMUNOGLOBULIN HEAVY CHAIN PRECURSOR
 (FRAGMENT) (104)
 149.[1AV1A](#) apolipoprotein a-i lipid-binding domain mutant N-TERMINAL
 MET, DEL(1-43), chain A (103)
 150.[Q9NXG7_HUMAN](#) Hypothetical protein FLJ20261 (102)
 151.[BAA00861](#) Immunoglobulin lambda light chain (100)
 152.[CAC10747](#) Immunoglobulin heavy chain variable region (Fragment) (99)
 153.[OQHU](#) hemopexin precursor [validated]
 154.[CAA85581](#) IMMUNOGLOBULIN HEAVY CHAIN VARIABLE
 REGION (FRAGMENT) (96)
 155.[Q7RTS7_HUMAN](#) Keratin 5c (95)
 156.[AAA69737](#) (CLONE A5VL) IMMUNOGLOUBLIN LIGHT CHAIN
 mRNA, V-REGION (FRAGMENT) (93)
 157.[AAQ05732](#) Ig heavy chain variable region (92)
 158.[CAF31289](#) Immunoglobulin heavy chain variable region (92)
 159.[K2C8_HUMAN](#) Keratin, type II cytoskeletal 8 (Cytokeratin 8) (92)
 160.[ANXA2_HUMAN](#) Annexin A2 (Annexin II) (Lipocortin II) (Calpastatin I
 heavy chain) (Chromobindin 8) (p36) (Protein I) (90)
 161.[K3HUB6](#) Ig kappa chain V-III region (B6) (tentative sequence) (88)
 162.[AAH69647](#) Keratin, hair, basic, 4 (87)
 163.[AAF61432](#) Keratin 12 (87)
 164.[AAH33679](#) Beta-tubulin 1, class VI (86)
 165.[Q6S380_HUMAN](#) Plectin 6 (86)
 166.[Q6S379_HUMAN](#) Plectin 7 (84)
 167.[Q7Z3M3_HUMAN](#) Hypothetical protein DKFZp686L04275 (Fragment)

(82)

- 168.[CAD97642](#) Hypothetical protein (81)
169.[G02520](#) plectin (80)
170.[Q6S376_HUMAN](#) Plectin 11 (79)
171.[AAA99717](#) Complement C4B precursor (79)
172.[AAC08331](#) MONOCLONAL ANTIBODY RL1 IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION (FRAGMENT) (79)
173.[AAQ05356](#) Ig heavy chain variable region, VH3 family (78)
174.[Q10466_HUMAN](#) Titin, heart isoform N2-B (EC 2.7.1.-) (Connectin) (76)
175.[AAT96423](#) Immunoglobulin variable region VL kappa domain (75)
176.[Q9UE12_HUMAN](#) Type I hair keratin 1 (75)
177.[CAF31287](#) Immunoglobulin heavy chain variable region (75)
178.[AAQ05367](#) Ig heavy chain variable region, VH3 family (75)
179.[CAD89465](#) Immunoglobulin gamma heavy chain variable region (Fragment) (75)
180.[CAF28440](#) Immunoglobulin heavy chain variable region (74)
181.[CAA06862](#) ANTI-(ED-B) SCFV (FRAGMENT) 74
182.[CAA00206](#) 1-ALPHA-1-ANTITRYPSIN (FRAGMENT)(74)
183.[AAL59372](#) Anti-cardiolipin immunoglobulin heavy chain (Fragment) (73)
184.[CAF00450](#) Unnamed protein product (73)
185.[CAD88649](#) Immunoglobulin heavy chain (Fragment) (73)
186.[Q7RTM4_HUMAN](#) Spectrin-like protein of the nuclear envelope and Golgi (71)
187.[SYNE1_HUMAN](#) Nesprin 1 (Nuclear envelope spectrin repeat protein 1) (Synaptic nuclear envelope protein 1) (71)
188.[CAF14734](#) Unnamed protein product (71)
189.[Q7Z3Y9_HUMAN](#) Type I inner root sheath specific keratin 25 irls2 (70)
190.[A38973](#) beta-catenin (69)
191.[AAB59530](#) Fibrinogen gamma-prime chain (69)
192.[HSHUB1](#) histone H2B.1 (69)
193.[DEHUG3](#) glyceraldehyde-3-phosphate (69) dehydrogenase (phosphorylating) (EC 1.2.1.12) [validated] (69)
194.[I37459](#) keratin Ha3-II, type I, hair (fragment) (68)
195.[AAP87378](#) Immunoglobulin gamma heavy chain (Fragment) (66)
196.[Q8WW89_HUMAN](#) SERPINB5 protein (66)
197.[BAB18615](#) Heat shock cognate protein 54 (64)
198.[Q5S007_HUMAN](#) Leucine-rich repeat kinase 2 (64)
199.[S55024](#) nebulin, skeletal muscle (63)
200.[Q9ULL0_HUMAN](#) KIAA1210 protein (Fragment) (62)
201.[AAB31910](#) IgM autoantibody heavy chain; heavy chain; anti-GPIIb (62)
202.[CAB46473](#) IMMUNOGLOBULIN LIGHT CHAIN VARIABLE REGION (FRAGMENT) (61)
203.[Q9UL88_HUMAN](#) Myosin-reactive immunoglobulin heavy chain variable region (Fragment) (61)
204.[AAQ05553](#) Ig heavy chain variable region, VH3 family (60)
205.[BAC01921](#) Immunoglobulin heavy chain VHDJ region (Fragment) (60)
206.[FZFB](#) fibrinogen fragment double-d, chain B (60)
207.[Q86UQ4_HUMAN](#) ABC A13 (59)

- 208.[AAQ05543](#) Ig heavy chain variable region, VH3 family (59)
 209.[Q96ST0_HUMAN](#) Hypothetical protein FLJ14658 (57)
 210.[CAE45480](#) Immunoglobulin heavy chain variable region (56)
 211.[EZRI_HUMAN](#) Ezrin (p81) (Cytovillin) (Villin 2) (56)
 212.[AAB34564](#) Type 1 keratin K16 (56)
 213.[CAC17313](#) Sequence 1 from Patent WO0066730 precursor (Fragment) (56)
 214.[Q8WZ53_HUMAN](#) Novex-3 Titin Isoform (55)
 215.[CAF14727](#) Unnamed protein product (55)
 216.[Q5TBT2_HUMAN](#) Bullous pemphigoid antigen 1, 230/240kDa (55)
 217.[AAP35912](#) golgi autoantigen, golgi subfamily a, 2 (54)
 218.[FGHUB](#) fibrinogen beta chain precursor [validated] (53)
 219.[T03454](#) ALR protein (53)
 220.[1FZAB](#) fibrinogen fragment d, chain B (52)
 221.[AAQ05521](#) Ig heavy chain variable region, VH3 family (52)
 222.[NBHU](#) apolipoprotein H precursor [validated] (52)
 223.[Q5T9P7_HUMAN](#) PRP18 pre-mRNA processing factor 18 homolog (Yeast)(PRPF18) (Fragment) 52
 224.[Q14467_HUMAN](#) KIAA0068 protein (Fragment) (52)
 225.[AAD30784](#) IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION (FRAGMENT) (51)
 226.[Q7Z7D6_HUMAN](#) Bromodomain PHD finger transcription factor (51)
 227.[BPA1_HUMAN](#) Bullous pemphigoid antigen 1 isoforms 1/2/3/4/5/8 (230 kDa bullous pemphigoid antigen) (BPA) (51)
 228.[BAB13468](#) KIAA1642 protein (50)
 229.[Q6NZA0_HUMAN](#) HIST1H2BA protein (50)
 230.[1GSN](#) glutathione reductase (EC 1.6.4.2) (gsh 1030) sulfenic acid group in cea63, cea234, cea284 cea423 (50)
 231.[Q86U12_HUMAN](#) Full-length cDNA clone CS0CAP007YF18 of Thymus (49)
 232.[HHHU84](#) heat shock protein 90-beta [validated] (49)
 233.[CAC36022](#) OTTHUMP00000031430 protein (49)
 234.[Q14491_HUMAN](#) Hemoglobin gamma-G (Fragment) (49)
 235.[Q5SQI3_HUMAN](#) Calmodulin-like 5 (48)
 236.[O15021_HUMAN](#) KIAA0303 protein (Fragment) (47)
 237.[AAM75821](#) Immunoglobulin heavy chain variable region (Fragment) (47)
 238.[T00050](#) hypothetical protein KIAA0400 (47)
 239.[Q5T2M3_HUMAN](#) OTTHUMP00000017872 (Fragment) (47)
 240.[CAA65055](#) IMMUNOGLOBULIN HEAVY CHAIN (FRAGMENT) (47)
 241.[AAC17470](#) alpha actinin:
 242.[M3HUPM](#) Ig heavy chain V-III region (Pom) (tentative sequence) (47)
 243.[Q8N9Z3_HUMAN](#) Hypothetical protein FLJ36025 (47)
 244.[AAC96152](#) T-CELL RECEPTOR BETA CHAIN (FRAGMENT) (47)
 245.[AAB86742](#) Possible J 56 gene segment (Fragment) (47)
 246.[Q5T8W5_HUMAN](#) Novel PH domain-containing protein (47)
 247.[DMD_HUMAN](#) Dystrophin (47)
 248.[Q5QFI2_HUMAN](#) SRF-dependent transcription regulation associated protein

(47)

- 249.[AAP69525](#) protein kinase, DNA-activated, catalytic polypeptide (47)
250.[Q5VU43_HUMAN](#) Phosphodiesterase 4D interacting protein (Myomegalin) (47)
251.[NFASC_HUMAN](#) Neurofascin precursor (46)
252.[Q9UPV0_HUMAN](#) KIAA1052 protein (Fragment) (46)
253.[AAA17933](#) IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION (FRAGMENT) (46)
254.[PC2205](#) interferon-alpha LCA-2b binding subtype (fragment) (46)
255.[A57099](#) DNA-activated protein kinase, catalytic subunit (46)
256.[AAF65317](#) Beta V spectrin (46)
257.[AAQ05496](#) Ig heavy chain variable region, VH3 family (46)
258.[AAP23228](#) Antibody heavy chain (Fragment) (46)
259.[ADHUA](#) fructose-bisphosphate aldolase (EC 4.1.2.13) A [validated] (46)
260.[Q6S362_HUMAN](#) Usher syndrome 2A isoform B (Autosomal recessive, mild) (46)
261.[CAF15132](#) Unnamed protein product (45)
262.[Q7LG11_HUMAN](#) KIAA1758 protein (Fragment)(45)
263.[CAD44428](#) Sequence 16 from Patent WO02055701 (45)
264.[Q5T154_HUMAN](#) Peroxiredoxin 1 (45)
265.[Q5SZ57_HUMAN](#) OTTHUMP00000040139 (Utrophin) (Homologous to dystrophin) (45)
266.[B27725](#) small proline-rich protein spr2 (45)
267.[AAC50434](#) Trans-golgi p230 (45)
268.[A35715](#) fodrin alpha chain (44)
269.[VBHU](#) transthyretin precursor [validated] (44)
270.[CAA18150](#) HS454M7 NID (44)
271.[Q9ULM0_HUMAN](#) KIAA1200 protein (Fragment) (44)
272.[Q9UJU1_HUMAN](#) Cytovillin 2 (Fragment) (44)
273.[A46127](#) radixin (44)
274.[AAD29949](#) Myosin heavy chain IIb (44)
275.[CAB51722](#) IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION (FRAGMENT) (43)
276.[Q96M50_HUMAN](#) Hypothetical protein FLJ32825 (43)
277.[Q5VTR3_HUMAN](#) Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A (43)
278.[AAH00729](#) A-kinse anchor protein 1, isoform 1 precursor (43)
279.[Q9H3U1_HUMAN](#) SMAP-1b (Smooth muscle cell associated protein-1) (43)
280.[POP1_HUMAN](#) Ribonucleases P/MRP protein subunit POP1 (EC 3.1.26.5) (hPOP1) (43)
281.[BAA11498](#) KIAA0181 protein (43)
282.[Q7Z3D7_HUMAN](#) Hypothetical protein DKFZp686E2459 (43)
283.[AAK27700](#) Immunoglobulin heavy chain variable region (Fragment) (43)
284.[ZN294_HUMAN](#) Zinc finger protein 294 (43)
285.[Q5T4S7_HUMAN](#) Retinoblastoma-associated factor 600 (RBAF600) (43)
286.[ENO1_HUMAN](#) Beta enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Muscle-specific enolase) (MSE) (42)
287.[Q96RW7_HUMAN](#) Hemicentin (42)

- 288.[A54849](#) collagen alpha 1(VII) chain precursor (42)
 289.[1QABA](#) transthyretin, chain A (42)
 290.[Q8TC04_HUMAN](#) Keratin 23, isoform a (42)
 291.[Q5T1H6_HUMAN](#) OTTHUMP00000060196 protein (42)
 292.[LUHU](#) annexin I (42)
 293.[AAA52935](#) HUMAN IG REARRANGED GAMMA-CHAIN mRNA V-REGION, PARTIAL CDS (FRAGMENT) (42)
 294.[BAB40659](#) Membrane glycoprotein LIG-1 (42)
 295.[AAQ05705](#) Ig heavy chain variable region, VH3 family (42)
 296.[AAA18808](#) IMMUNOGLOBULIN HEAVY-CHAIN SUBGROUP VIII V-D-J REGION (FRAGMENT) (42)
 297.[AAO45432](#) Immunoglobulin heavy chain variable region (42)
 298.[Q8WX70_HUMAN](#) BA165P4.2 (Centrosomal protein 1) (41)
 299.[AAC96148](#) T-CELL RECEPTOR BETA CHAIN (FRAGMENT)
 300.[MCON](#) calmodulin – salmon (41)
 301.[1DH5L](#) immunoglobulin v1 kappa domain (41)
 302.[AAH20257](#) Neurofibromin 2, isoform 1 (41)
 303.[Q6AI24_HUMAN](#) Hypothetical protein DKFZp686F19113 (41)
 304.[CAA07619](#) lysine-ketoglutarate reductase /saccharopine dehydrogenase (41)
 305.[AAD50326](#) Truncated RAD50 protein (41)
 306.[Q5TF21_HUMAN](#) OTTHUMP00000017175 protein (41)
 307.[CAF00512](#) Unnamed protein product (40)
 308.[LPHUB](#) apolipoprotein B-100 precursor (40)
 309.[AAF98175](#) ATP-binding cassette transporter 1 (40)
 310.[CAE99963](#) Unnamed protein product (40)
 311.[AAF21617](#) Ig heavy chain (fragment) (40)
 312.[Q5THJ4_HUMAN](#) Vacuolar protein sorting 13D (Yeast) (OTTHUMP00000044487) (40)
 313.[Q6PID4_HUMAN](#) PTK2B protein tyrosine kinase 2 beta, isoform a (40)
 314.[CAF00248](#) AX886110 NID (40)
 315.[Q8WXQ6_HUMAN](#) Protein kinase A anchoring protein Ht31 (40)
 316.[AAC50431](#) Dystrobrevin-epsilon (40)
 317.[5VW21_HUMAN](#) Microtubule-actin crosslinking factor 1 (40)
 318.[CAI19320](#) ARFGEF2 (40)
 319.[AAL76042](#) partitioning-defective 3 protein splice variant a (40)
 320.[AAH04246](#) MutS homolog 6 (39)
 321.[Q8WXQ5_HUMAN](#) Serine/threonine protein kinase kkialre-like 1 (39)
 322.[Q7Z3R8_HUMAN](#) DNA-directed RNA polymerase (EC 2.7.7.6) (39)
 323.[CAD20680](#) Sequence 1 from Patent WO196561 (39)
 324.[CAD54734](#) Extracellular matrix protein (39)
 325.[AAF86276](#) ABCA1 (39)
 326.[LMO7_HUMAN](#) LIM domain only protein 7 (LOMP) (F-box only protein 20) (39)
 327.[Q5MJ67_HUMAN](#) CMYA3 (39)
 328.[CABL1_HUMAN](#) CDK5 and ABL1 enzyme substrate 1 (Interactor with CDK3 1) (Ik3-1) (39)
 329.[AAD05492](#) Kinesin superfamily motor KIF4 (39)

- 330.[Q8IXT1_HUMAN](#) Hypothetical protein FLJ25416 (39)
331.[Q5VZK9_HUMAN](#) OTTHUMP0000039401 protein (39)
332.[Q6IQ38_HUMAN](#) USP36 protein (39)
333.[Q6TV07_HUMAN](#) Breast cancer-associated antigen SGA-72M (39)
334.[CAF00143](#) Unnamed protein product (39)
335.[CAF17088](#) Unnamed protein product (39)
336.[CAF01896](#) Unnamed protein product (39)
337.[AAC95297](#) PITSLRE protein kinase beta SV2 isoform (39)
338.[Q6RUV3_HUMAN](#) Brain leucine zipper protein (Fragment) (38)
339.[BCHUCF](#) calgranulin A [validated] (38)
340.[TLN2_HUMAN](#) Talin 2 (38)
341.[Q76E58_HUMAN](#) Epiplakin (38)
342.[Q86TC5_HUMAN](#) Hypothetical protein DKFZp451J094 (38)
343.[AAR03497](#) KIAA1985 protein (38)
344.[A59188](#) ATP-binding cassette transporter ABC3 (38)
345.[CAD13085](#) Sequence 5 from Patent WO0174851 (38)
346.[AAH47793](#) TRNA splicing endonuclease 54 homolog (38)
347.[Q5T6T8_HUMAN](#) Novel protein (Fragment) (38)
348.[CAC88605](#) Sequence 19 from Patent WO0166748(38)
349.[AAG13956](#) (N6-adenosine) methyltransferase (38)
350.[SERA_HUMAN](#) D-3-phosphoglycerate dehydrogenase (EC 1.1.1.95) (3-PGDH) (37)
351.[CAF18161](#) Unnamed protein product (37)
352.[Q6ZWH5_HUMAN](#) Hypothetical protein FLJ41066 (37)
353.[Q8WTT2_HUMAN](#) AD24 (Chromosome 10 open reading frame 117) (37)
354.[Q9NXI4_HUMAN](#) Hypothetical protein FLJ20227 (37)
355.[Q5VW03_HUMAN](#) OTTHUMP0000063495 protein (37)
356.[Q92616_HUMAN](#) KIAA0219 protein (Fragment) (37)
357.[1A5Y](#) protein tyrosine phosphatase 1b catalytic domain, residues 1-321 (EC 3.1.3.48) (fragments) (37)
358.[S12712](#) small proline-rich protein spr2-1 (37)
359.[AAD42883](#) NY-REN-62 antigen (37)
360.[O95412_HUMAN](#) Beta globin (Fragment) (37)
361.[Q6IE36_HUMAN](#) Ovostatin-2 (Fragment) (36)
362.[Q8NCD3_HUMAN](#) Hypothetical protein FLJ90328 (Fetal liver expressing gene 1) (36)
363.[Q8IZC6_HUMAN](#) Collagen XXVII proalpha 1 chain precursor (OTTHUMP00000021970) (36)
364.[CAE99485](#) Unnamed protein product (36)
365.[CAC26777](#) Sequence 3 from Patent WO0100669 (36)
366.[Q5VV79_HUMAN](#) SNF2 histone linker PHD RING helicase (36)
367.[A57352](#) GPI-anchored protein p137 precursor (36)
368.[BAA83821](#) Actin binding protein ABP620 (36)
369.[CAI13370](#) RP11-155N3.2 (36)
370.[Q6NSI7_HUMAN](#) SLC12A6 protein (Fragment) (36)
371.[Q8WYB5_HUMAN](#) Histone acetyltransferase MOZ2 (36)
372.[Q8NE76_HUMAN](#) Hypothetical protein FLJ10786 (36)
373.[Q6ZVP5_HUMAN](#) Hypothetical protein FLJ42255 (36)

- 374.[Q8WVA6_HUMAN](#) FLJ20244 protein (35)
375.[AAL37885](#) Immunoglobulin kappa light chain variable region (Fragment) (35)
376.[CAA70844](#) HSPIBF1 NID (35)
377.[Q5VUN8_HUMAN](#) Ribosomal protein S6 kinase, 52kDa, polypeptide 1 (35)
378.[Q5VT25_HUMAN](#) CDC42 binding protein kinase alpha (DMPK-like) (35)
379.[AAD43326](#) adaptor-related protein complex AP-4 epsilon subunit (35)
380.[S71752](#) giant protein p619 (35)
381.[Q8N6K3_HUMAN](#) SNX25 protein (35)
382.[S14458](#) laminin alpha-1 chain precursor (35)
383.[Q9BUH5_HUMAN](#) Transgelin 2 (35)
384.[CAD69689](#) Sequence 1323 from Patent EP1270724 (35)
385.[BAB32435](#) WDC146 (35)
386.[Q86WF0_HUMAN](#) Sarcoma antigen NY-SAR-48 (Fragment) (35)
387.[Q5VST9_HUMAN](#) OTTHUMP00000061415 (Obscurin, cytoskeletal calmodulin and titin- interacting RhoGEF) (35)
388.[NBHUH](#) complement factor H precursor, long splice form [validated] (34)
389.[T00262](#) hypothetical protein KIAA0602 (fragment) (34)
390.[R5RT27](#) ribosomal protein L27, cytosolic [validated] – rat (34)
391.[Q96CE9_HUMAN](#) Hematopoietically expressed homeobox (34)
392.[Q6ZN75_HUMAN](#) Hypothetical protein FLJ16363 (34)
393.[CO4A6_HUMAN](#) Collagen alpha 6(IV) chain precursor (34)
394.[AAH14094](#) Regulator of G-protein signaling 14 (34)
395.[AAH36013](#) Mitogen activated protein kinase kinase kinase kinase 5 (34)

7.3 TAP-SUMO2 CELLS UNINFECTED (minus TAP cells uninfected data)

Accession number, name of protein, overall Mascot score and % peptide coverage

Gel chunks 1, 3, 7 and 14 (see figure 4.2)

- 1.[AAC08313](#) General transcription factor 2-I, 2593 (52%)
- 2.[AAH04978](#) Tripartite motif-containing 28 protein, 1814 (41%)
- 3.[JC5300](#) Ran GTPase activator 1, 1318 (43%)
- 4.[I65237](#) ubiquitin / ribosomal protein L40, cytosolic [validated] – rat, 792 (64%)
- 5.[Q6DSU6_HUMAN](#) GTF2I repeat domain containing protein 1, 709 (25%)
- 6.[AAA36787](#) Ubiquitin precursor, 657 (27%)
- 7.[BAA09487](#) KIAA0129 protein, 614 (22%)
- 8.[AAC00056](#) Hsp27 ERE-TATA-binding protein, 450 (21%)
- 9.[AAC18697](#) Scaffold attachment factor B, 450 (23%)
- 10.[AAH00036](#) Small ubiquitin-like modifier protein 3, 380 (45%)
- 11.[KR131_HUMAN](#) Keratin-associated protein 13-1 (High sulfur keratin-associated protein 13.1), 341 (51%)
- 12.[JC4760](#) SMT3 protein, 341 (57%)
- 13.[AAD23914](#) ubiquitin-like protein activating enzyme; sentrin activating enzyme, 333 (16%)
- 14.[ROC_HUMAN](#) Heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNP C1 / hnRNP C2), 321 (27%)
- 15.[T00034](#) SART-1 protein, 320 (17%)
- 16.[AAK57515](#) HBV pX associated protein 8 large isoform, 311 (17%)
- 17.[Q969M9_HUMAN](#) BRD8 protein, 301 (17%)
- 18.[CAD69652](#) Sequence 1245 from Patent EP1270724, 292 (10%)
- 19.[AAD17258](#) Trasncriptional intermediary factor 1 alpha; TIF1 alpha, 245 (14%)
- 20.[A40493](#) DNA topoisomerase (ATP-hydrolyzing) (EC 5.99.1.3) alpha , 239 (8%)
- 21.[Q96GX7_HUMAN](#) Selenium binding protein 1 (SELENBP1 protein) (OTTHUMP00000059969), 238 (16%)
- 22.[AAG43114](#) My001 protein, 199 (20%)
- 23.[Q5T5X7_HUMAN](#) OTTHUMP00000040500 protein, 190 (7%)
- 24.[IA5R](#) sumo-1, 188 (29%)
- 25.[Q6P4R8_HUMAN](#) NFRKB protein, 185 (6%)
- 26.[Q5TG72_HUMAN](#) Tripartite motif-containing 33, 180 (3%)
- 27.[I38344](#) titin, cardiac muscle [validated], 169 (2%)
- 28.[AAB63585](#) Transcription intermediary factor 1, 166 (11%)
- 29.[HHU27](#) heat shock protein 27, 156 (17%)
- 30.[Q6Y9L9_HUMAN](#) Desmoglein 4, 152 (3%)
- 31.[CAC14658](#) BA332A4.3 protein (Fragment), 142 (17%)
- 32.[Q5T3N1_HUMAN](#) Annexin A1 (Fragment), 139 (17%)
- 33.[G01872](#) selenium-binding protein, 139 (16%)
- 34.[FNBP3_HUMAN](#) Formin-binding protein 3 (Huntingtin yeast partner A) (Huntingtin- interacting protein HYPA/FBP11), 138 (9%)

- 35.[AAP15181](#) forkhead winged/helix transcription factor mutant 2, 134 (8%)
- 36.[CAA17879](#) novel protein similar to heterogeneous nuclear ribonucleoprotein C (C1\|C2) (HNRPC), 134 (16%)
- 37.[2GALB](#) galectin-7, chain B, 130 (25%)
- 38.[BAC05016](#) CDNA FLJ40040 fis, clone SYNOV2000426, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 129 (19%)
- 39.[BAC05009](#) CDNA FLJ40031 fis, clone STOMA2009253, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 122 (18%)
- 40.[BAB71560](#) CDNA FLJ25025 fis, clone CBL01928, highly similar to Ig gamma immunoglobulin heavy chain, 120 (17%)
- 41.[AAH78671](#) IGHG1 protein, 120 (18%)
- 42.[Q96E67_HUMAN](#) ACTB protein, 119 (17%)
- 43.[FABPA_HUMAN](#) Fatty acid-binding protein, adipocyte (AFABP) (Adipocyte lipid-binding protein) (ALBP) (A-FABP), 117 (32%)
- 44.[Q6A163_HUMAN](#) Ka35 protein, 115 (11%)
- 45.[CAA48197](#) DNA topoisomerase II, 114 (8%)
- 46.[Q5VUQ5_HUMAN](#) OTTHUMP00000059860 protein, 108 (9%)
- 47.[AAH63625](#) KRTAP2-4 protein, 107 (45%)
- 48.[Q5T2C6_HUMAN](#) AT rich interactive domain 4B (RBP1-like) (Fragment), 106 (5%)
- 49.[AAH47687](#) BCLAF1 protein, 106 (14%)
- 50.[Q8TC63_HUMAN](#) IGHG4 protein, 105 (15%)
- 51.[I58383](#) retinoblastoma binding protein 1, splice form I, 104 (5%)
- 52.[AAG00910](#) Recombinant IgG2 heavy chain (Fragment), 104 (14%)
- 53.[I54251](#) translation elongation factor eEF-1 alpha (fragment), 101 (24%)
- 54.[BAA11481](#) KIAA0164 protein, 99 (7%)
- 55.[EFHU1](#) translation elongation factor eEF-1 alpha-1 chain, 97 (14%)
- 56.[I38346](#) elastic titin (fragment), 97 (2%)
- 57.[CAD29721](#) High sulfur keratin associated protein 11.1, 95 (15%)
- 58.[Q6U276_HUMAN](#) Krev interaction trapped 1 variant, 92 (15%)
- 59.[KPYM_HUMAN](#) Pyruvate kinase, isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscle isozyme) (Cytosolic thyroid), 89 (17%)
- 60.[AAN16399](#) Thymine DNA glycosylase, 89 (8%)
- 61.[AAR32531](#) Immunoglobulin heavy chain variable region, 88 (23%)
- 62.[AAS85969](#) Immunoglobulin heavy chain, 88 (27%)
- 63.[Q5VWP1_HUMAN](#) Ryanodine receptor 2 (Cardiac), 86 (3%)
- 64.[Q9UEQ5_HUMAN](#) S164 (Fragment), 85 (6%)
- 65.[SYNE2_HUMAN](#) Nesprin 2 (Nuclear envelope spectrin repeat protein 2) (Syne-2) (Synaptic nuclear envelope protein), 84 (3%)
- 66.[Q7Z600_HUMAN](#) Apolipoprotein B (Including Ag(X) antigen), 84 (3%)
- 67.[CAC22372](#) Sequence 1 from Patent WO0075319, 84 (14%)
- 68.[CAC17371](#) Sequence 1 from Patent WO0066619, 82 (10%)
- 69.[Q9NR99_HUMAN](#) Adlican, 81 (3%)
- 70.[AAB41132](#) Microtubule-associated protein 1a, 81 (4%)
- 71.[Q5VYL3_HUMAN](#) Asp (Abnormal spindle)-like, microcephaly associated (Drosophila), 81 (2%)
- 72.[CAD48779](#) Sequence 23 from Patent EP1229047, 80 (6%)

- 73.[AAR32500](#) Immunoglobulin heavy chain variable region, 80 (24%)
 74.[S69131](#) Ig heavy chain (DOT) (fragment), 80 (13%)
 75.[BAA82099](#) Anti-Entamoeba histolytica immunoglobulin gamma heavy chain (Fragment), 78 (26%)
 76.[1PRXB](#) horf6 mutant C91S, chain B (fragments), 78 (13%)
 77.[AAD56724](#) acinusL, 78 (6%)
 78.[Q6ECI4_HUMAN](#) Zinc finger protein 470, 77 (12%)
 79.[PCLO_HUMAN](#) Piccolo protein (Aczonin), 77 (3%)
 80.[AAB25835](#) retinoblastoma binding protein 1 isoform III, 77 (2%)
 81.[AAM77722](#) Midasin, 77 (3%)
 82.[Q8NFQ0_HUMAN](#) Beige-like protein, 76 (3%)
 83.[ZCWC3_HUMAN](#) Zinc finger CW-type coiled-coil domain protein 3, 75 (4%)
 84.[AAS85999](#) Immunoglobulin heavy chain, 75 (25%)
 85.[Q8TCU4_HUMAN](#) ALMS1 protein, 74 (3%)
 86.[Q9BV61_HUMAN](#) TRAP1 protein (Fragment), 73 (5%)
 87.[Q8TDJ6_HUMAN](#) Rabconnectin, 73 (4%)
 88.[AAD02105](#) MKP-1 like protein tyrosine phosphatase, 72 (24%)
 89.[CAA04798](#) Ryanodine receptor 3, 72 (2%)
 90.[S42516](#) PML protein, splice form 1, 72 (3%)
 91.[Q8TD57_HUMAN](#) Axonemal heavy chain dynein type 3, 71 (3%)
 92.[NCOR1_HUMAN](#) Nuclear receptor corepressor 1 (N-CoR1) (N-CoR)71 (3%)
 93.[AAD51444](#) Methyl-CpG binding protein splice variant 3, 70 (10%)
 94.[BAA23795](#) Brain ryanodine receptor, 68 (2%)
 95.[Q6IEH8_HUMAN](#) Transcriptional regulator, 68 (3%)
 96.[Q8WY20_HUMAN](#) Centrosome-associated protein 350, 68 (3%)
 97.[AAD20946](#) Silencing mediator of retinoic acid and thyroid hormone receptor alpha, 68 (4%)
 98.[Q8WXX0_HUMAN](#) Ciliary dynein heavy chain 7, 68 (4%)
 99.[Q5VU48_HUMAN](#) Phosphodiesterase 4D interacting protein (Myomegalin),
 100.[MAPA_HUMAN](#) Microtubule-associated protein 1A (MAP 1A) (Proliferation-related protein p80) [Contains: MAP1 ligh, 68 (2%)
 101.[CAB35584](#) CALM2 P4 PROTEIN, 67 (17%)
 102.[Q7Z5C1_HUMAN](#) Glycoprotein receptor gp330/megalin, 66 (1%)
 103.[AT2B4_HUMAN](#) Plasma membrane calcium-transporting ATPase 4 (EC 3.6.3.8) (PMCA4) (Plasma membrane calcium pump is, 66 (9%)
 104.[AAC14667](#) Hsp27 ERE-TATA-binding protein, 65 (28%)
 105.[Q96L96_HUMAN](#) Muscle alpha-kinase, 65 (6%)
 106.[Q5TBJ7_HUMAN](#) OTTHUMP00000018803 protein, 65 (3%)
 107.[Q5VUM2_HUMAN](#) OTTHUMP00000040303 (Laminin, alpha 2) (Merosin, congenital muscular dystrophy), 65 (3%)
 108.[Q96Q15_HUMAN](#) Phosphatidylinositol 3-kinase-related protein kinase, 64 (3%)
 109.[Q5TBT3_HUMAN](#) OTTHUMP00000018069 (Fragment), 64 (17%)
 110.[S28261](#) centromere protein E , 64 (5%)
 111.[O95996_HUMAN](#) APCL protein, 64 (6%)

- 112.[AAD50325](#) RAD50-2 protein, 64 (6%)
 113.[BAA20770](#) KIAA0311 protein, 63 (5%)
 114.[Q7Z2L3_HUMAN](#) KIAA1749 protein, 63 (5%)
 115.[A42184](#) nuclear mitotic apparatus protein NuMA , 62 (6%)
 116.[ALU2_HUMAN](#) Alu subfamily SB sequence contamination warning entry, 61 (13%)
 117.[AAK00229](#) Myosin VI, 61 (4%)
 118.[AAR04774](#) Kinesin family member 21A, 61 (6%)
 119.[BAA25517](#) KIAA0591 protein, 61 (3%)
 120.[1A31A](#) topoisomerase i core domain and c-terminal domain (EC 5.99.1.2), chain A (fragments), 61 (13%)
 121.[SPTN4_HUMAN](#) Spectrin beta chain, brain 3 (Spectrin, non-erythroid beta chain 3) (Beta-IV spectrin), 60 (5%)
 122.[AAS45545](#) Ankyrin repeat-containing factor-2, 60 (4%)
 123.[AAC05330](#) Cell adhesion kinase beta, 60 (6%)
 124.[Q5VU45_HUMAN](#) Phosphodiesterase 4D interacting protein (Myomegalin), 60 (7%)
 125.[ABCA1_HUMAN](#) ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cass, 60 (5%)
 126.[CAI22960](#) Novel protein KIAA0117, 60 (10%)
 127.[AAR25662](#) Ankyrin repeat-containing protein, 60 (4%)
 128.[Q6VU68_HUMAN](#) Laminin alpha 3 splice variant b1, 59 (1%)
 129.[Q9H3T8_HUMAN](#) MOP-3, 59 (4%)
 130.[Q695L1_HUMAN](#) Striated muscle preferentially expressed protein (Fragment), 59 (3%)
 131.[A41137](#) heat shock transcription factor 1, 59 (6%)
 132.[Q9UPW7_HUMAN](#) KIAA1033 protein (Fragment), 59 (9%)
 133.[CAC20413](#) beta-myosin heavy chain, 59 (6%)
 134.[Q9UQB7_HUMAN](#) Huntingtin, 59 (3%)
 135.[AAH06536](#) Symplekin, 58 (11%)
 136.[Q8IWQ9_HUMAN](#) Helicase-like protein, 58 (5%)
 137.[S21054](#) DNA-directed RNA polymerase (EC 2.7.7.6) II largest chain , 58 (4%)
 138.[H2AQ_HUMAN](#) Histone H2A.q (H2A/q) (H2A-GL101), 58 (16%)
 139.[BAA23698](#) KIAA0402 protein, 57 (4%)
 140.[AAC83555](#) neuronal double zinc finger protein, 57 (2%)
 141.[ADHUC](#) fructose-bisphosphate aldolase (EC 4.1.2.13) C, 57 (5%)
 142.[Q6DC95_HUMAN](#) Interferon regulatory factor 2 binding protein 1, 57 (10%)
 143.[S21086](#) anion exchange protein 2, 57 (6%)
 144.[S37431](#) ankyrin 2, neuronal long splice form, 57 (3%)
 145.[DYH5_HUMAN](#) Ciliary dynein heavy chain 5 (Axonemal beta dynein heavy chain 5) (HL1), 56 (2%)
 146.[AAS85961](#) Immunoglobulin heavy chain, 56 (12%)
 147.[AAD22973](#) silencing mediator of retinoic acid and thyroid hormone receptor extended isoform, 56 (3%)
 148.[Q96QV0_HUMAN](#) PI-3-kinase-related kinase SMG-1, 56 (3%)
 149.[AAR02562](#) Immunoglobulin heavy chain variable region, 56 (12%)

- 150.[AAG42473](#) Spectrin beta IV, 55 (5%)
 151.[Q6DKQ9_HUMAN](#) Cell division cycle 2-like 5 (Cholinesterase-related cell division controller), 55 (4%)
 152.[Q5W0E7_HUMAN](#) Neurobeachin, 55 (2%)
 153.[CAB45385](#) Trithorax homologue 2, 55 (5%)
 154.[AAA70227](#) IMMUNOGLOBULIN GAMMA-1 CHAIN (FRAGMENT), 55 (13%)
 155.[A44265](#) trithorax homolog HTX, version 2, 54 (3%)
 156.[AAR08265](#) BCL-6 corepressor long isoform, 54 (2%)
 157.[CAC18956](#) Sequence 8 from Patent WO0068386, 54 (7%)
 158.[A59404](#) plectin [imported], 54 (3%)
 159.[A55144](#) autotaxin precursor, 54 (6%)
 160.[Q5VW21_HUMAN](#) Microtubule-actin crosslinking factor 1, 53 (2%)
 161.[PARP1_HUMAN](#) Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+)) ADP-ribosyltransferase-1 (Po, 53 (5%))
 162.[CAC10131](#) Sequence 26 from Patent DE19908423 (Fragment), 53 (5%)
 163.[CAA75436](#) Cxorf5 (71-7A) protein, 53 (5%)
 164.[PLEA6_HUMAN](#) Pleckstrin homology domain-containing protein family A member 6 (Phosphoinositol 3-phosphate-bindin, 53 (6%))
 165.[Q8IVL1_HUMAN](#) Steerin2 protein, 52 (5%)
 166.[CAD32046](#) Sequence 1 from Patent WO0218544 (Fragment).- synthetic construct, 52 (4%)
 167.[BAA20562](#) PKU-beta, 52 (7%)
 168.[PZRN3_HUMAN](#) PDZ domain containing RING finger protein 3 (Ligand of Numb-protein X 3) (Semaphorin cytoplasmic do, 52 (7%))
 169.[Q5T9S5_HUMAN](#) Sarcoma antigen NY-SAR-41 (NY-SAR-41), 52 (8%)
 170.[AAR32501](#) Immunoglobulin heavy chain variable region, 52 (28%)
 171.[Q6P7T4_HUMAN](#) IQCE protein (Fragment), 52 (14%)
 172.[AAD01550](#) RAB-R protein, 51 (3%)
 173.[Q8IWY8_HUMAN](#) KOX31-like zinc finger protein, 51 (6%)
 174.[Q7KYL3_HUMAN](#) Protein kinase ATR, 51 (6%)
 175.[AAL62061](#) Bullous pemphigoid antigen 1 eA, 51 (2%)
 176.[G59434](#) KIAA0411 protein [imported] , 51 (7%)
 177.[AAC50893](#) FUSE binding protein 3, 51 (9%)
 178.[ISHUT1](#) DNA topoisomerase (EC 5.99.1.2), 51 (8%)
 179.[Q9NZA0_HUMAN](#) FBP-interacting repressor (Fuse-binding protein-interacting repressor, isoform b), 50 (5%)
 180.[SJHUA](#) spectrin alpha chain , 50 (3%)
 181.[Q8IWB6_HUMAN](#) TEX14 protein, 50 (5%)
 182.[HSCH2F](#) histone H2A.F, embryonic – chicken, 50 (11%)
 183.[A35881](#) histone H2A.Z, 49 (11%)
 184.[Q9H1Q1_HUMAN](#) BA145E8.1 (KIAA1074), 49 (2%)
 185.[AAC25051](#) Advillin; p92, 49 (8%)
 186.[TSHUP1](#) thrombospondin 1 precursor, 49 (5%)
 187.[Q5VVL9_HUMAN](#) Protein tyrosine phosphatase, receptor type, D, 49 (5%)
 188.[O60498_HUMAN](#) VAV-like protein (Fragment), 49 (13%)

- 189.[AAL01416](#) importin 9, 49 (7%)
 190.[MYH11_HUMAN](#) Myosin heavy chain, smooth muscle isoform (SMMHC), 49 (5%)
 191.[AAB87381](#) CHD1, 49 (3%)
 192.[TCF20_HUMAN](#) Transcription factor 20 (Stromelysin 1 PDGF-responsive element-binding protein) (SPRE-binding protein) (Nuclear factor SPBP) (AR1), 49 (3%)
 193.[AAB51685](#) Wnt10B, 48 (7%)
 194.[AAR32489](#) Immunoglobulin heavy chain variable region, 48 (14%)
 195.[DOCK2_HUMAN](#) Dicator of cytokinesis protein 2, 48 (4%)
 196.[A30802](#) Ca2+-transporting ATPase (EC 3.6.3.8) 2, plasma membrane, 48 (7%)
 197.[Q8IVV0_HUMAN](#) RABGAP1L protein (Expressed in hematopoietic cells, heart, liver) (HLL), 48 (8%)
 198.[Q86UP3_HUMAN](#) Zinc finger homeodomain 4 protein, 48 (3%)
 199.[AAD42072](#) Oxygen-regulated protein 1, 48 (4%)
 200.[AAG28523](#) Adenocarcinoma protein ART1, 48 (1%)
 201.[CAC43729](#) Sequence 1 from Patent WO0142294, 47 (1%)
 202.[Q9P2H7_HUMAN](#) KIAA1370 protein (Fragment), 47 (3%)
 203.[AAD00186](#) Envoplakin, 47 (4%)
 204.[Q9P2D7_HUMAN](#) KIAA1410 protein (Fragment), 47 (2%)
 205.[Q86YS8_HUMAN](#) BRD4-NUT fusion oncoprotein, 47 (3%)
 206.[BAB33332](#) KIAA1662 protein, 47 (4%)
 207.[AAC41758](#) Nucleoporin, 47 (3%)
 208.[CAC34694](#) Sequence 1 from Patent WO0114414, 47 (1%)
 209.[AJHUPR](#) phosphoribosylamine-glycine ligase (EC 6.3.4.13), 47 (7%)
 210.[AAR02567](#) Immunoglobulin heavy chain variable region, 46 (18%)
 211.[STRC_HUMAN](#) Stereocilin precursor, 46 (3%)
 212.[CAA55632](#) Endosomal protein, 46 (4%)
 213.[Q5T791_HUMAN](#) OTTHUMP00000016860 (Fragment), 46 (5%)
 214.[CHTOG_HUMAN](#) CH-TOG protein (Colonic and hepatic tumor over-expressed protein) (Ch- TOG protein), 46 (5%)
 215.[AAH30221](#) ABI gene family, member 3 (NESH)binding protein, 46 (5%)
 216.[AAR02549](#) Immunoglobulin heavy chain variable region, 46 (12%)
 217.[CAD70100](#) Sequence 1369 from Patent EP1270724 (Fragment), 46 (12%)
 218.[AAL26987](#) Bromodomain-containing 4, 46 (3%)
 219.[CAC88675](#) Sequence 7 from Patent WO0166747, 46 (2%)
 220.[ALU4_HUMAN](#) Alu subfamily SB2 sequence contamination warning entry, 46 (15%)
 221.[AAK19279](#) L-periaxin, 46 (4%)
 222.[AAB25318](#) ANTI-TETANUS ANTIBODY HEAVY CHAIN VARIABLE REGION (FRAGMENT), 46 (23%)
 223.[Q9UCF5_HUMAN](#) Interleukin 2 (Fragment), 46 (34%)
 224.[Q7Z406_HUMAN](#) Myosin heavy chain, 46 (4%)
 225.[AAA52880](#) IMMUNOGLOBULIN HEAVY CHAIN PRECURSOR (FRAGMENT), 46 (21%)

- 226.[S22393](#) Ca2+-transporting ATPase (EC 3.6.3.8) 2, long splice form, 46 (4%)
- 227.[BAB18252](#) Anti TNF-alpha antibody heavy-chain Fab (Fragment), 46 (13%)
- 228.[AAH10381](#) Nuclear matrix protein p84, 46 (3%)
- 229.[Q5T670_HUMAN](#) MCM10 minichromosome maintenance deficient 10 (S. cerevisiae), 46 (4%)
- 230.[Q6IAM6_HUMAN](#) LAP3 protein, 45 (10%)
- 231.[Q5THZ5_HUMAN](#) OTTHUMP00000028561protein, 45 (4%)
- 232.[Q99736_HUMAN](#) HsGCN1 (Fragment), 45 (2%)
- 233.[AAD30389](#) GABA-B receptor R2, 45 (6%)
- 234.[AAR02498](#) Immunoglobulin heavy chain variable region, 45 (14%)
- 235.[Q9Y632_HUMAN](#) APC2 protein (Fragment), 45 (8%)
- 236.[A55575](#) ankyrin 3, long splice form , 45 (2%)
- 237.[Q6NXF1_HUMAN](#) VCY2IP1 protein (Fragment), 45 (3%)
- 238.[Q5VW08_HUMAN](#) OTTHUMP00000042245 protein, 45 (7%)
- 239.[Q7Z6E8_HUMAN](#) Retinoblastoma binding protein 6 isoform 2, 45 (3%)
- 240.[BAA76857](#) KIAA1014 protein, 45 (6%)
- 241.[CAC09161](#) Sequence 1 from Patent WO0043510, 45 (4%)
- 242.[LRP1B_HUMAN](#) Low-density lipoprotein receptor-related protein 1B precursor (Low- density lipoprotein receptor-re, 45 (2%)
- 243.[BAB17674](#) RFamide-related peptide precursor, 45 (11%)
- 244.[Q5R3M6_HUMAN](#) OTTHUMP00000028958 protein, 45 (3%)
- 245.[Q5VW20_HUMAN](#) OTTHUMP00000046304 (Microtubule-actin crosslinking factor 1), 45 (2%)
- 246.[8FABD](#) Ig gamma chain V-C region (Fab fragment HIL), chain D , 45 (12%)
- 247.[AAF61929](#) protocadherin Flamingo 1, 45 (2%)
- 248.[CAD33443](#) Sequence 170 from Patent WO0218424, 45 (2%)
- 249.[AAK14335](#) ABC transporter ABCA2, 45 (3%)
- 250.[AAA58669](#) HRX, 44 (2%)
- 251.[AAG30515](#) Immunoglobulin heavy chain (Fragment), 44 (17%)
- 252.[Q6PHY0_HUMAN](#) CABIN1 protein, 44 (2%)
- 253.[AAH68976](#) Phospholipase D1, phophatidlycholin-specific, 44 (5%)
- 254.[JC5712](#) adrenoleukodystrophy related protein, 44 (6%)
- 255.[Q5VZB7_HUMAN](#) Calcium channel, voltage-dependent, alpha 1E subunit, 44 (2%)
- 256.[AAS85977](#) Immunoglobulin heavy chain, 44 (13%)
- 257.[TPIS_HUMAN](#) Triosephosphate isomerase (EC 5.3.1.1) (TIM) (Triose-phosphate isomerase), 44 (6%)
- 258.[ALU8_HUMAN](#) Alu subfamily SX sequence contamination warning entry, 44 (15%)
- 259.[MLL3_HUMAN](#) Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog (Histone- lysine N-methyltransferase, H3 lysine-4 specific MLL3) (EC 2.1.1.43) (Homologous to ALR protein), 44 (1%)
- 260.[Q5SYX5_HUMAN](#) Golgi autoantigen, golgin subfamily a, 2 (Fragment), 44 (6%)
- 261.[CGHU2B](#) collagen alpha 2(IV) chain precursor – human, 44 (6%)

- 262.[1GC1H](#) antibody 17b antigen-binding fragment, fab cd4-induced site on gp120, chain H, 44 (10%)
263.[AAS86025](#) Immunoglobulin heavy chain, 44 (18%)
264.[AAF15590](#) Immunoglobulin heavy chain Fd region (fragment), 44 (15%)
265.[Q6NX52_HUMAN](#) ARHGEF1 protein, 43 (6%)
266.[AAR02513](#) Immunoglobulin heavy chain variable region, 43 (15%)
267.[Q6PJG2_HUMAN](#) C14orf43 protein (Fragment), 43 (5%)
268.[Q6YI51_HUMAN](#) Ubiquitin ligase protein MIB1 (Mind Bomb homologue 1 (MIB1), 43 (6%)
269.[Q5T0X9_HUMAN](#) REV3-like, catalytic subunit of DNA polymerase zeta (Yeast), 43 (3%)
270.[Q00375_HUMAN](#) Putative p150, 43 (4%)
271.[CHD7_HUMAN](#) Chromodomain-helicase-DNA-binding protein 7 (CHD-7) (Fragment), 43 (4%)
272.[Q5Y190_HUMAN](#) Anchor protein, 43 (1%)
273.[AAC06147](#) RPOM, 43 (3%)
274.[AAK82968](#) gamma-tubulin complex component, 43 (2%)
275.[Q6NSK0_HUMAN](#) Serine/threonine kinase 10, 43 (2%)
276.[CAC38606](#) Sequence 229 from Patent WO0129221, 43 (3%)
277.[AAH17232](#) Cleavage and polyadenylation specific factor 1, 160kDa, 43 (3%)
278.[Q5T9Q5_HUMAN](#) Succinate-CoA ligase, ADP-forming, beta subunit (Fragment), 43 (14%)
279.[BAA05393](#) KIAA0043 protein, 43 (2%)
280.[Q5TCU6_HUMAN](#) OTTHUMP00000045364 protein, 43 (3%)
281.[DDX21_HUMAN](#) Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21), 43 (5%)
282.[T13163](#) Rab6 GTPase activating protein, GAPCenA, 43 (4%)
283.[H2AX_HUMAN](#) Histone H2A.x (H2a/x), 43 (11%)
284.[AAH06192](#) Forkhead box M1, isoform 3, 43 (4%)
285.[AAC72360](#) Chromosome-associated protein-E, 43 (3%)
286.[Q9Y4F4_HUMAN](#) KIAA0423 protein (Fragment), 43 (2%)
287.[AAK52750](#) Nuclear receptor transcription cofactor, 43 (3%)
288.[AAC50550](#) PATCHED, 43 (3%)
289.[Q289.9BYW2_HUMAN](#) Huntingtin interacting protein 1 (HSPC069 isoform a), 43 (3%)
290.[AAR32425](#) Immunoglobulin heavy chain variable region, 42 (21%)
291.[CAI16520](#) Karyopherin (importin) beta 3, 42 (7%)
292.[AAS85947](#) Immunoglobulin heavy chain, 42 (13%)
293.[AAR02512](#) Immunoglobulin heavy chain variable region, 42 (20%)
294.[AAR32423](#) Immunoglobulin heavy chain variable region, 42 (14%)
295.[Q5VZ08_HUMAN](#) OTTHUMP00000044920 protein, 42 (3%)
296.[AAF86613](#) Phospholipase C beta 1, 42 (5%)
297.[Q5W9G1_HUMAN](#) KIAA0216 splice variant 1 (Fragment), 42 (4%)
298.[PKD1_HUMAN](#) Polycystin 1 precursor (Autosomal dominant polycystic kidney disease protein 1), 42 (2%)
299.[Q5T714_HUMAN](#) OTTHUMP00000060031 protein, 42 (2%)

- 300.[BAA14374](#) CCG1 protein, 42 (3%)
 301.[Q5TH00_HUMAN](#) OTTHUMP0000031017
 (OTTHUMP0000031013), 42 (13%)
 302.[Q5TYR7_HUMAN](#) Hemicentin (FIBL-6), 42 (2%)
 303.[AAF15452](#) Immunoglobulin heavy chain variable region (fragment),
 42 (24%)
 304.[1AQKH](#) fab b7-15a2 fab immunoglobulin fab, chain H , 42 (13%)
 305.[AAC06259](#) mitotic checkpoint kinase Bub1, 42 (6%)
 306.[BAB18258](#) Anti HBs antibody heavy-chain Fab (Fragment), 41 (13%)
 307.[Q5T011_HUMAN](#) KIAA0467 protein, 41 (2%)
 308.[AAC51331](#) CREB-binding protein, 41 (3%)
 309.[Q7L576_HUMAN](#) Cytoplasmic FMR1 interacting protein 1, 41 (4%)
 310.[AAF04015](#) DNA cytosine-5 methyltransferase 3B, 41 (6%)
 311.[AAD27647](#) SH3P12 protein, 41 (8%)
 312.[AAH38585](#) tripartate motif protein 15, isoform alpha, 41 (6%)
 313.[Q8WXG9_HUMAN](#) Very large G protein-coupled receptor 1b, 41 (2%)
 314.[Q8IU68_HUMAN](#) EVIN2 (Large EVER2 protein) (Transmembrane
 channel-like protein 8), 41 (4%)
 315.[AAC39746](#) Immunoglobulin heavy chain variable region (Fragment),
 41 (17%)
 316.[Q5VWF1_HUMAN](#) Protein kinase, lysine deficient 2, 41 (4%)
 317.[AAF75772](#) Ubiquitin-conjugating BIR-domain enzyme, 41 (1%)
 318.[JC5525](#) 1 alpha,25-dihydroxyvitamin D3-inducible, 41 (17%)
 319.[Q70CQ4_HUMAN](#) Ubiquitin-specific proteinase 31, 41 (4%)
 320.[CAD48627](#) Sequence 1 from Patent WO0210391, 41 (3%)
 321.[Q5T924_HUMAN](#) Sorbin and SH3 domain containing 1, 41 (4%)
 322.[AAA82889](#) CENP-F kinetochore protein, 41 (2%)
 323.[Q6A165_HUMAN](#) HHa7 protein (Fragment), 41 (2%)
 324.[CAC16626](#) Sequence 5 from Patent WO0063381, 41 (1%)
 325.[Q9UPF6_HUMAN](#) CD3e-associated protein, 41 (10%)
 326.[AAC14573](#) serum-inducible kinase, 41 (5%)
 327.[B33481](#) interferon-induced viral resistance protein MxB , 41 (2%)
 328.[A45259](#) desmoyokin (fragments), 41 (4%)
 329.[AAR32509](#) Immunoglobulin heavy chain variable region, 41 (17%)
 330.[BAC80154](#) Immunoglobulin gamma heavy chain, 41 (14%)
 331.[ALU7_HUMAN](#) Alu subfamily SQ sequence contamination warning
 entry, 40 (13%)
 332.[Q8WWZ7_HUMAN](#) ATP-binding cassette A5, 40 (2%)
 333.[Q9ULE0_HUMAN](#) KIAA1280 protein (Fragment), 40 (5%)
 334.[AAS86021](#) Immunoglobulin heavy chain, 40 (11%)
 335.[Q6ZNJ1_HUMAN](#) FLJ00341 protein (Fragment), 40 (4%)
 336.[AAR02496](#) Immunoglobulin heavy chain variable region, 40 (21%)
 337.[AAR32421](#) Immunoglobulin heavy chain variable region, 40 (18%)
 338.[D56695](#) transducin-like enhancer-of-split homolog TLE-3, 40 (3%)
 339.[Q5TCZ0_HUMAN](#) OTTHUMP0000059186 protein, 40 (26%)
 340.[Q5VYC4_HUMAN](#) OTTHUMP0000017476 protein, 40 (4%)
 341.[A44159](#) spectrin beta-G chain, 40 (5%)
 342.[Q6FI78_HUMAN](#) LYAR protein, 40 (12%)

- 343.[CAD69623](#) Sequence 1187 from Patent EP1270724, 40 (21%)
344.[Q6PIY4_HUMAN](#) TIP120A protein (Fragment), 40 (4%)
345.[Q8NHY3_HUMAN](#) GAS2-related protein isoform beta, 40 (8%)
346.[CO1A1_HUMAN](#) Collagen alpha 1(I) chain precursor, 40 (7%)
347.[AAR88379](#) anti-HIV-1 gp120 immunoglobulin 412d heavy chain, 40 (14%)
348.[Q5T5C0_HUMAN](#) Syntaxin binding protein 5 (Tomosyn), 40 (6%)
349.[AAR32397](#) anti-HIV-1 gp120 immunoglobulin 412d heavy chain, 40 (19%)
350.[Q5TIG6_HUMAN](#) Myeloid/lymphoid or mixed-lineage leukemia (Trithorax homolog, Drosophila); translocated to, 4, 40 (2%)
351.[AAR32446](#) Immunoglobulin heavy chain variable region, 40 (13%)
352.[Q8TDM9_HUMAN](#) Amplified in breast cancer 1, 40 (4%)
353.[AAR02490](#) Immunoglobulin heavy chain variable region, 40 (12%)
354.[MTMR1_HUMAN](#) Myotubularin-related protein 1 (EC 3.1.3.-), 40 (7%)
355.[AAC51317](#) karyopherin beta 3, 40 (7%)
356.[I2C2_HUMAN](#) Eukaryotic translation initiation factor 2C 2 (eIF2C 2) (eIF-2C 2), 40 (7%)
357.[Q8IWI9_HUMAN](#) MGA protein (Fragment), 40 (6%)
358.[CAA01501](#) RYANODINE RECEPTOR PROTEIN, 40 (1%)
359.[NFL_HUMAN](#) Neurofilament triplet L protein (68 kDa neurofilament protein) (Neurofilament light polypeptide), 40 (9%)
360.[BAA96056](#) KIAA1532 protein, 40 (8%)
361.[BAA07652](#) KIAA0002 protein, 40 (9%)
362.[WNK2_HUMAN](#) Serine/threonine-protein kinase WNK2 (EC 2.7.1.37) (Protein kinase with no lysine 2), 40 (2%)
363.[AAD09135](#) Trip230, 40 (3%)
364.[AAH64360](#) F-box and leucine-rich repeat protein 11, 40 (2%)
365.[CAI21723](#) testis-specific kinase 2, 40 (8%)
366.[CAC15498](#) RP4-591C20.6, 40 (5%)
367.[AAD13352](#) phosphatidylinositol 4-kinase, 40 (4%)
368.[O95785_HUMAN](#) Homolog of Mus musculus wizL protein (Fragment), 40 (6%)
369.[EGHU](#) epidermal growth factor precursor [validated], 40 (4%)
370.[Q86YW9_HUMAN](#) TRALPUSH, 39 (3%)
371.[S32436](#) collagen alpha 2(IX) chain (fragment), 39 (11%)
372.[NNTM_HUMAN](#) NAD(P) transhydrogenase, mitochondrial precursor (EC 1.6.1.2) (Pyridine nucleotide transhydrogenase), 39 (4%)
373.[Q5SU58_HUMAN](#) OTTHUMP00000044921 protein, 39 (5%)
374.[CAA67057](#) TCR JUNCTIONAL SEQUENCE (FRAGMENT), 39 (17%)
375.[AAF69004](#) Ciliary dynein heavy chain 9, 39 (3%)
376.[Q5VT62_HUMAN](#) Tripartite motif-containing 46, 39 (12%)
377.[Q8WXI7_HUMAN](#) Ovarian cancer related tumor marker CA125, 39 (<1%)
378.[Q5THR3_HUMAN](#) OTTHUMP00000028872 protein, 39 (3%)
379.[Q9Y4I0_HUMAN](#) Zinc-finger helicase, 39 (2%)
380.[Q96SE4_HUMAN](#) DNA polymerase theta, 39 (4%)

- 381.[Q6P991_HUMAN](#) Protein phosphatase 1D, 39 (4%)
 382.[Q8IWX1_HUMAN](#) 3'-5' RNA exonuclease, 39 (7%)
 383.[CAA93625](#) ALL-1 protein, 39 (3%)
 384.[AAC05370](#) membrane associated guanylate kinase 2, 39 (5%)
 385.[ALU3_HUMAN](#) Alu subfamily SB1 sequence contamination warning entry, 39 (16%)
 386.[Q9Y2H9_HUMAN](#) KIAA0973 protein (Fragment), 39 (4%)
 387.[AAB61453](#) Neuroendocrine-dlg, 39 (5%)
 388.[S69211](#) serine/threonine-specific protein kinase (EC 2.7.1.-), Rho-associated , 39 (4%)
 389.[Q5T2Q6_HUMAN](#) Novel protein (Fragment), 39 (3%)
 390.[CAD70045](#) Sequence 2055 from Patent EP1270724, 39 (7%)
 391.[Q6IC83_HUMAN](#) DJ90G24.6 protein (Novel protein), 39 (5%)
 392.[CXA3_HUMAN](#) Gap junction alpha-3 protein (Connexin 46) (Cx46), 39 (6%)
 393.[Q9UPP5_HUMAN](#) KIAA1107 protein (Fragment), 38 (2%)
 394.[Q6FI71_HUMAN](#) DKFZP434K1421 protein, 38 (7%)
 395.[A40201](#) artifact-warning sequence (translated ALU class A), 38 (8%)
 396.[Q66GS8_HUMAN](#) Centrosome protein Cep290, 38 (2%)
 397.[AAK01925](#) CGI-201 protein, type II, 38 (5%)
 398.[AAK85155](#) Kinesin superfamily protein 1B, 38 (3%)
 399.[Q8NDZ1_HUMAN](#) KIAA1737 protein, 38 (10%)
 400.[Q9NRN1_HUMAN](#) Ancient conserved domain protein 4, 38 (10%)
 401.[BAB47488](#) KIAA1859 protein, 38 (7%)
 402.[Q5TH93_HUMAN](#) Leucine zipper protein 1, 38 (4%)
 403.[SYHUQT](#) multifunctional aminoacyl-tRNA synthetase, 38 (5%)
 404.[BAA86433](#) KIAA1119 protein, 38 (3%)
 405.[AAC07988](#) Centrosomal Nek2-associated protein 1, 38 (3%)
 406.[DOT1L_HUMAN](#) Histone-lysine N-methyltransferase, H3 lysine-79 specific (EC 2.1.1.43) (Histone H3-K79 methyltransferase) (H3-K79-HMTase) (DOT1-like protein), 38 (3%)
 407.[BAA09488](#) KIAA0139 protein, 38 (5%)
 408.[Q6V1P9_HUMAN](#) Cadherin protein, 38 (3%)
 409.[A34581](#) oxysterol-binding protein, 38 (6%)
 410.[Q9NYZ5_HUMAN](#) Calcium channel alpha1E subunit, delta19 delta40 delta46 splice variant (Fragment), 38 (2%)
 411.[A41275](#) DNA ligase (ATP) (EC 6.5.1.1) I, 38 (5%)
 412.[BAA23661](#) GCP170, 38 (5%)
 413.[O95001_HUMAN](#) WUGSC:H_267D11.3 protein (Fragment), 38 (3%)
 414.[S32920](#) cell wall-associated protein precursor wapA [similarity] - Bacillus subtilis, 38 (4%)
 415.[BAA82612](#) Neural adhesion molecule NB-3, 38 (2%)
 416.[Q5W0B2_HUMAN](#) OTTHUMP00000016826 protein, 38 (2%)
 417.[Q709C8_HUMAN](#) VPS13C-2A protein, 38 (3%)
 418.[AAH19257](#) Zinc finger, CW type with coiled-coil domain 1, 38 (3%)
 419.[Q6IQ23_HUMAN](#) Pleckstrin homology domain containing, family A member 2, 37 (4%)
 420.[Q5VU13_HUMAN](#) Novel protein, 37 (5%)

- 421.[AAG60033](#) tRNA-guanine transglycosylase, 37 (5%)
 422.[AAA51995](#) Antigen CD9, 37 (9%)
 423.[AAF21944](#) Integrin alpha 10 subunit, 37 (3%)
 424.[CAC39142](#) GD: RRBP1, 37 (5%)
 425.[DVHU3](#) multidrug resistance protein 3, 37 (6%)
 426.[Q7LC09_HUMAN](#) STE20-like kinase (Fragment), 37 (6%)
 427.[CAA04154](#) spinocerebellar ataxia 7, 37 (7%)
 428.[Q5T583_HUMAN](#) Filaggrin, 37 (1%)
 429.[Q9UHA8_HUMAN](#) Splicing coactivator subunit SRm300, 37 (4%)
 430.[Q86X47_HUMAN](#) Neural cell adhesion molecule 1, 37 (3%)
 431.[AAH06551](#) Lamin B2, 37 (5%)
 432.[AAL38980](#) WD repeat protein Gemin5, 37 (3%)
 433.[JN0607](#) alpha-catenin 1, 37 (4%)
 434.[T43483](#) translation initiation factor IF-2 homolog [similarity] (fragment), 37 (3%)
 435.[CAA60685](#) Homologue of Drosophila Fat protein, 37 (2%)
 436.[BAA95972](#) KIAA1448 protein, 37 (4%)
 437.[Q6AZ91_HUMAN](#) RAB6B protein, 37 (14%)
 438.[CAD10507](#) voltage-gated sodium channel, 37 (1%)
 439.[BAA82444](#) SET-binding protein (SEB), 37 (4%)
 440.[Q5XG74_HUMAN](#) SEC31L1 protein, 37 (2%)
 441.[Q5SZZ1_HUMAN](#) OTTHUMP00000039688 (Testis expressed sequence 27), 37 (12%)
 442.[FBX38_HUMAN](#) F-box only protein 38 (Modulator of KLF7 activity homolog) (MoKA), 36 (3%)
 443.[Q5VYL4_HUMAN](#) Asp (Abnormal spindle)-like, microcephaly associated (Drosophila), 36 (3%)
 444.[AAB87382](#) CHD2, 36 (2%)
 445.[BAA04750](#) Protein tyrosin phosphatase type 1, 36 (3%)
 446.[CAD34838](#) Sequence 146 from Patent WO0222660, 36 (5%)
 447.[CAD91029](#) Sequence 1 from Patent WO0220619 (Fragment), 36 (21%)
 448.[Q96Q06_HUMAN](#) KIAA1881 protein (Fragment), 36 (10%)
 449.[RP1L1_HUMAN](#) Retinitis pigmentosa 1-like 1 protein, 36 (4%)
 450.[Q43469_HUMAN](#) Natural killer cell receptor KIR3DS1 variant (KIR3DS1), 36 (17%)
 451.[CAC88303](#) Sequence 3 from Patent WO0164871, 36 (2%)
 452.[AAD15903](#) sec7 domain family member, 36 (3%)
 453.[AAD43749](#) Protocadherin beta 1, 36 (2%)
 454.[Q5SQC5_HUMAN](#) Calcium channel, voltage-dependent, L type, alpha 1B subunit, 36 (2%)
 455.[Q5SQC6_HUMAN](#) Calcium channel, voltage-dependent, L type, alpha 1B subunit, 36 (2%)
 456.[Q13590_HUMAN](#) Homo sapiens Cri-du-chat region (Fragment), 36 (24%)
 457.[Q8IV76_HUMAN](#) PAS domain containing 1, 36 (4%)
 458.[Q9ULC9_HUMAN](#) KIAA1291 protein (Fragment), 36 (6%)
 459.[BAA25465](#) KIAA0539 protein , 36 (1%)

- 460.[Q9H3T7_HUMAN](#) MOP-4, 36 (6%)
 461.[Q6ICE0_HUMAN](#) CSF2RB protein, 36 (2%)
 462.[AAC50214](#) Pro-a2(XI), 36 (5%)
 463.[Q5VXT8_HUMAN](#) Nephronophthisis 4 (OTTHUMP00000044213), 36 (4%)
 464.[I38240](#) transcription factor SOX4, 36 (8%)
 465.[Q7Z4N1_HUMAN](#) Melastatin 1 splicing variant, 36 (4%)
 466.[NU205_HUMAN](#) Nuclear pore complex protein Nup205 (Nucleoporin Nup205) (205 kDa nucleoporin), 36 (3%)
 467.[Q5VXI3_HUMAN](#) Myosin IIIA, 36 (4%)
 468.[Q9H245_HUMAN](#) C1orf28, 36 (17%)
 469.[Q8NF52_HUMAN](#) FLJ00343 protein (Fragment), 36 (3%)
 470.[Q9UIW2_HUMAN](#) NOV/plexin-A1 protein (Fragment), 36 (3%)
 471.[CAA70889](#) Miz-1 protein, 35 (6%)
 472.[BAA86546](#) KIAA1232 protein, 35 (9%)
 473.[Q5SYA9_HUMAN](#) OTTHUMP00000046126 (OTTHUMP00000021391), 35 (3%)
 474.[Q5T8D5_HUMAN](#) Novel protein kinase domain containing protein, 35 (3%)
 475.[BAA06801](#) Proteoglycan PG-M(V3), 35 (3%)
 476.[AAA59487](#) Laminin B1, 35 (4%)
 477.[Q6PD57_HUMAN](#) EPRS protein (Fragment), 35 (7%)
 478.[DAAM1_HUMAN](#) Disheveled associated activator of morphogenesis 1, 35 (6%)
 479.[B40016](#) matrin 3 (fragment), 35 (4%)
 480.[Q9UPN4_HUMAN](#) KIAA1118 protein (Fragment), 35 (4%)
 481.[CGHU1E](#) collagen alpha 1(XI) chain precursor, 35 (5%)
 482.[Q5TZA0_HUMAN](#) Ciliary rootlet coiled-coil, rootletin (Fragment), 35 (2%)
 483.[CAD23837](#) Sequence 1 from Patent WO0204599, 35 (5%)

Gel Chunks 2-15 (see figure 4.2)

1. [AAC08313](#) General transcription factor 2-I, 2820(59%),
2. [JC5300](#) Ran GTPase activator 1, 2234 (61%)
3. [AAH04978](#) Tripartate motif-containing 28 protein, 2057 (48%)
4. [AAD23914](#) Ubiquitin-like protein activating enzyme; sentrin activating enzyme, 660 (35%)
5. [ROC_HUMAN](#) Heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNP C1 / hnRNP C2), 604 (36%)
6. [AAH08364](#) HNRPC protein, 603 (37%)
7. [1C3TA](#) 1d8 ubiquitin mutant YES, 597 (94%)
8. [Q5T5X7_HUMAN](#) OTTHUMP00000040500 protein, 538 (20%)
9. [T00034](#) SART-1 protein, 476 (19%)
10. [AAH00036](#) Small ubiquitin-like modifier protein 3, 462 (54%)
11. [CAA25855](#) alpha-tubulin, 439 (31%)
12. [JC4760](#) SMT3 protein – human, 361 (61%)

13. [Q8N532_HUMAN](#) TUBA6 protein, 359 (32%)
14. [Q5T8B6_HUMAN](#) OTTHUMP00000059759 protein, 352 (22%)
15. [Q6DSU6_HUMAN](#) GTF2I repeat domain containing protein 1, 346 (15%)
16. [BAA09487](#) KIAA0138 protein, 338 (13%)
17. [AAH20946](#) Tubulin, beta polypeptide, 335 (26%)
18. [AAN16399](#) thymine-DNA glycosylase, 328 (20%)
19. [AAP15181](#) Forkhead winged/helix transcription factor mutant 2, 322 (15%)
20. [Q8WZB3_HUMAN](#) N2B-Titin Isoform, 318 (3%)
21. [AAC18697](#) Scaffold attachment factor B, 317 (20%)
22. [ATCHSM](#) actin gamma, smooth muscle – chicken, 269 (29 %)
23. [A29821](#) dnaK-type molecular chaperone HSPA5 precursor, 267 (18%)
24. [S64732](#) scaffold attachment factor B, 261 (20%)
25. [A41137](#) heat shock transcription factor 1, 261 (19%)
26. [Q8IWP6_HUMAN](#) Class IVb beta tubulin, 255 (16%)
27. [AAB41132](#) Microtubule associated protein 1a, 251 (12%)
28. [CAA17879](#) novel protein similar to heterogeneous nuclear ribonucleoprotein C (C1\|C2) (HNRPC), 250 (24%)
29. [KPYM_HUMAN](#) Pyruvate kinase, isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscle isozyme) (Cytosolic thyroid h, 238 (17%)
30. [Q9BRZ9_HUMAN](#) IRF2BP1 protein (Fragment), 234 (20%)
31. [VIME_HUMAN](#) Vimentin, 216 (20%)
32. [JC5517](#) Gu/RNA helicase II binding protein, 200 (7%)
33. [Q6EKJ0_HUMAN](#) Transcription factor GTF2IRD2 beta, 198 (10%)
34. [MAPA_HUMAN](#) Microtubule-associated protein 1A (MAP 1A) (Proliferation-related protein p80) [Contains: MAP1 ligh, 198 (6%)
35. [Q6EKI9_HUMAN](#) General transcription factor II i repeat domain 2, 197 (10%)
36. [UBHU5B](#) tubulin beta chain, 194 (14%)
37. [AAG28523](#) Adenocarcinoma antigen ART1, 194 (14%)
38. [Q86U45_HUMAN](#) Full-length cDNA clone CS0DA009YK08 of Neuroblastoma of Homo sapiens, 193 (32%)
39. [LAP2B_HUMAN](#) Lamina-associated polypeptide 2, isoforms beta/gamma (Thymopoietin, isoforms beta/gamma) (TP beta/g, 189 (20%)
40. [1A5R](#) sumo-1, 169 (34%)
41. [Q9UPF6_HUMAN](#) CD3e-associated protein, 166 (9%)
42. [CC1_HUMAN](#) Nuclear protein Hcc-1 (HSPC316) (Proliferation associated cytokine- inducible protein CIP29), 145 (14%)
43. [CAA69400](#) Mesenchyme Fork Head-1, 144 (5%)
44. [Q5RI17_HUMAN](#) Heterogeneous nuclear ribonucleoprotein U (Scaffold attachment factor A) (Fragment), 141 (7%)
45. [AAB60654](#) Neuronal nitric oxide synthase, 140 (7%)
46. [Q6S382_HUMAN](#) Plectin 2, 139 (7%)
47. [AAB41497](#) beta-tubulin, 139 (12%)
48. [AAC19382](#) Scaffold attachment factor A, 137 (6%)
49. [AAR32531](#) Immunoglobulin heavy chain variable region, 135 (23%)
50. [Q96D94_HUMAN](#) SIAHBP1 protein, 133 (24%)
51. [Q6P094_HUMAN](#) LOC391257 protein, 133 (26%)

52. [AAD14062](#) 90 kDa heat shock protein beta HSP90 beta, 132 (61%)
 53. [AAD11466](#) Heat shock protein, 132 (8%)
 54. [PWHUA](#) H+-transporting two-sector ATPase (EC 3.6.3.14) alpha chain precursor, 131 (16%)
 55. [A42184](#) nuclear mitotic apparatus protein NuMA , 130 (8%)
 56. [S69131](#) Ig heavy chain (DOT) (fragment), 129 (13%)
 57. [A59404](#) plectin [imported], 128 (6%)
 58. [AAR32405](#) Immunoglobulin heavy chain variable region, 128 (22%)
 59. [FNBP3_HUMAN](#) Formin-binding protein 3 (Huntingtin yeast partner A) (Huntingtin- interacting protein HYPA/FBP11), 125 (8%)
 60. [AAR02539](#) Immunoglobulin heavy chain variable region, 124 (26%)
 61. [CAI12817](#) FKHL18 protein, 123 (11%)
 62. gamma heavy chain (Fragment), 123 (15%)
 63. [AAR02561](#) Immunoglobulin heavy chain variable region, 121 (24%)
 64. [Q9BV61_HUMAN](#) TRAP1 protein (Fragment), 121 (5%)
 65. [CAD69623](#) Sequence 1187 from Patent EP1270724, 121 (21%)
 66. [LAP2A_HUMAN](#) Lamina-associated polypeptide 2 isoform alpha, (Thymopoietin isoform alpha) (TP alpha) (Thymopoietin, 120 (14%)
 67. [AAR32527](#) Immunoglobulin heavy chain variable region, 119 (23%)
 68. [Q5VYL3_HUMAN](#) Asp (Abnormal spindle)-like, microcephaly associated (Drosophila), 119 (4%)
 69. [Q7Z7P5_HUMAN](#) IGHG1 protein, 118 (12%)
 70. [Q9UIE9_HUMAN](#) WUGSC:H_DJ0687K01.2 protein, 117 (8%)
 71. [AAH14258](#) Similar to immunoglobulin heavy constant gamma 3 (G3m marker), 117 (14%)
 72. [CAC49990](#) Sequence 22 from Patent WO0146261, 115 (11%)
 73. [Q8TD57_HUMAN](#) Axonemal heavy chain dynein type 3, 115 (4%)
 74. [CAC60121](#) Axonemal beta heavy chain dynein type 11, 113 (4%)
 75. [BAC05013](#) CDNA FLJ40037 fis, clone SYNOV2000279, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 113 (13%)
 76. [BAC05017](#) CDNA FLJ40041 fis, clone SYNOV2000700, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 113 (13%)
 77. [CAC49992](#) Sequence 52 from Patent WO0146261, 112 (10%)
 78. [CAC22488](#) Sequence 24 from Patent WO0077179, 112 (8%)
 79. [Q5VUQ5_HUMAN](#) OTTHUMP00000059860 protein, 111 (6%)
 80. [BAB71560](#) CDNA FLJ25025 fis, clone CBL01928, highly similar to Ig gamma immunoglobulin heavy chain, 111 (12%)
 81. [AAH73789](#) IGHG1 protein, 109 (15%)
 82. [AAO17821](#) Anti-rabies SO57 immunoglobulin heavy chain, 109 (12%)
 83. [B35098](#) MHC class III histocompatibility antigen HLA-B-associated protein 2 [imported], 109 (6%)
 84. [Q8WXI7_HUMAN](#) Ovarian cancer related tumor marker CA125, 109 (1%)
 85. [AAL62061](#) Bullous pemphigoid antigen 1 eA, 108 (3%)
 86. [BAC05016](#) CDNA FLJ40040 fis, clone SYNOV2000426, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 107 (14%)
 87. [Q14214_HUMAN](#) Nebulin (Fragment), 107 (7%)
 88. [Q9NR99_HUMAN](#) Adlican, 107 (3%)

89. [S33124](#) tpr protein – human, 105 (6%)
 90. [AAD01550](#) RAB-R protein, 104 (3%)
 91. [Q695C7_HUMAN](#) Paracingulin, 104 (3%)
 92. [AAA58669](#) HRX protein, 104 (2%)
 93. [Q99968_HUMAN](#) Tpr, 104 (5%)
 94. [AAF69004](#) Ciliary dynein heavy chain 9, 101 (4%)
 95. [S72269](#) ryanodine receptor isoform 2, cardiac muscle, 100 (4%)
 96. [Q8TBA3_HUMAN](#) TKT protein (Fragment), 100 (15%)
 97. [DYH5_HUMAN](#) Ciliary dynein heavy chain 5 (Axonemal beta dynein heavy chain 5) (HL1), 98 (3%)
 98. [VEHULA](#) lamin A, 97 (12%)
 99. [JC7555](#) C14orf4 protein, 97 (3%)
 100. [AAB04636](#) Apolipoprotein B-100 precursor, 97 (4%)
 101. [AAC36704](#) Protein inhibitor of activated STAT protein PIASx-alpha, 95 (6%)
 102. [Q6P9C6_HUMAN](#) AT rich interactive domain 3A (BRIGHT-like) protein, 95 (10%)
 103. [Q5W9G1_HUMAN](#) KIAA0216 splice variant 1 (Fragment), 94 (9%)
 104. [A56624](#) histone H2B.2, 94 (28%)
 105. [Q64I72_HUMAN](#) Death domain-containing protein p84N5 short isoform, 93 (6%)
 106. [CAC17315](#) Sequence 5 from Patent WO0066730 precursor, 93 (3%)
 107. [AAK85155](#) Kinesin superfamily protein 1B, 93 (11%)
 108. [Q7Z2L4_HUMAN](#) KIAA0445 protein (Fragment), 92 (8%)
 109. [AAD17258](#) transcriptional intermediary factor 1 alpha; TIF1 alpha, 91 (5%)
 110. [Q7KZS0_HUMAN](#) Ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast), 91 (22%)
 111. [Q6IEH8_HUMAN](#) Transcriptional regulator, 90 (5%)
 112. [Q6NWQ4_HUMAN](#) HIST1H2BM protein (Fragment), 89 (28%)
 113. [Q5SQ29_HUMAN](#) HLA-B associated transcript 2, 88 (5%)
 114. [BAA74927](#) KIAA0904 protein, 88 (4%)
 115. [CAA28420](#) apo-B100 precursor, 88 (3%)
 116. [Q96Q89_HUMAN](#) Mitotic kinesin-related protein, 87 (4%)
 117. [T08621](#) centrosome associated protein CEP250, 87 (5%)
 118. [Q7Z6E8_HUMAN](#) Retinoblastoma binding protein 6 isoform 2, 86 (5%)
 119. [CAC38606](#) Sequence 229 from Patent WO0129221, 86 (12%)
 120. [A40201](#) artifact-warning sequence (translated ALU class A), 85 (21%)
 121. [Q5VT06_HUMAN](#) OTTHUMP00000060646 (Novel protein), 85 (5%)
 122. [CAC20413](#) beta-myosin heavy chain, 84 (4%)
 123. [CAA48197](#) DNA topoisomerase II, 84 (9%)
 124. [AAK49332](#) Kinesin superfamily protein KIF1B, 84 (5%)
 125. [MBD1_HUMAN](#) Methyl-CpG binding protein 1 (Methyl-CpG binding domain protein 1) (Protein containing methyl-CpG-b, 83 (9%)
 126. [CAA01501](#) RYANODINE RECEPTOR PROTEIN, 83 (3%)
 127. [S22695](#) restin, 83 (9%)

128. [Q7KYL3_HUMAN](#) Protein kinase ATR, 83 (4%)
 129. [G02311](#) FREAC-8 (fragment), 83 (13%)
 130. [TCF20_HUMAN](#) Transcription factor 20 (Stromelysin 1 PDGF-responsive element-binding protein) (SPRE-binding protein), 83 (4%)
 131. [Q9P216_HUMAN](#) KIAA1512 protein (Fragment), 83 (7%)
 132. [AAK52750](#) Nuclear receptor transcription cofactor, 82 (4%)
 133. [Q5Y190_HUMAN](#) Anchor protein, 82 (2%)
 134. [CGHU1E](#) collagen alpha 1(XI) chain precursor, 81 (6%)
 135. [A59266](#) unconventional myosin-15, 80 (3%)
 136. [O75872_HUMAN](#) Rab3-GAP regulatory domain, 79 (7%)
 137. [AA95972](#) KIAA1448 protein, 79 (10%)
 138. [CAC07498](#) Sequence 1 from Patent WO9961607, 79 (7%)
 139. [AAL33548](#) NUANCE, 79 (3%)
 140. [AAM77722](#) Midasin, 79 (4%)
 141. [BAA07652](#) KIAA0002 protein, 79 (15%)
 142. [Q9Y2F3_HUMAN](#) KIAA0944 protein (Fragment), 79 (4%)
 143. [Q709C8_HUMAN](#) VPS13C-2A protein, 79 (2%)
 144. [Q5VUM2_HUMAN](#) OTTHUMP0000040303 (Laminin, alpha 2) (Merosin, congenital muscular dystrophy), 78 (3%)
 145. [CAI17184](#) MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D), 78 (4%)
 146. [Q96JB1_HUMAN](#) Axonemal dynein heavy chain 8, 78 (2%)
 147. [ALU7_HUMAN](#) Alu subfamily SQ sequence contamination warning entry, 78 (23%)
 148. [Q5W189_HUMAN](#) OTTHUMP0000028696 protein, 77 (4%)
 149. [AAD04629](#) PCAF-associated factor 400, 77 (3%)
 150. [CAE75579](#) Subtilase, 77 (5%)
 151. [AAA92354](#) A-kinase anchor protein, 77 (3%)
 152. [CAC50170](#) Sequence 7 from Patent WO0146231, 77 (5%)
 153. [Q8TDJ6_HUMAN](#) Rabconnectin, 77 (4%)
 154. [AAB63585](#) transcription intermediary factor 1, 76 (7%)
 155. [G59434](#) KIAA0411 protein [imported], 76 (6%)
 156. [AAH38596](#) CHD4 protein, 76 (6%)
 157. [Q5VTS4_HUMAN](#) OTTHUMP0000016553 protein, 76 (6%)
 158. [A44265](#) trithorax homolog HTX, version 2, 76 (2%)
 159. [1AO6A](#) serum albumin, chain A – human, 76 (7%)
 160. [AAB03679](#) Myosin VIIA, 76 (4%)
 161. [ALU1_HUMAN](#) Alu subfamily J sequence contamination warning entry, 75 (22%)
 162. [HRX_HUMAN](#) Zinc finger protein HRX (ALL-1) (Trithorax-like protein), 75 (2%)
 163. [BAA23698](#) KIAA0402 protein, 75 (5%)
 164. [Q5THK1_HUMAN](#) Novel protein, 75 (4%)
 165. [Q9UQ35_HUMAN](#) RNA binding protein, 75 (3%)
 166. [Q5VVM7_HUMAN](#) Centromere protein F, 350\400ka (Mitosin), 75 (5%)
 167. [Q5VYL4_HUMAN](#) Asp (Abnormal spindle)-like, microcephaly associated (Drosophila), 75 (7%)

168. [AAD20946](#) silencing mediator of retinoic acid and thyroid hormone receptor alpha, 74 (5%)
169. [AAD00186](#) Envoplakin, 74 (3%)
170. [AAR25662](#) Ankyrin repeat-containing protein, 74 (5%)
171. [AAM69365](#) Eukaryotic translation initiation factor 4GI, 74 (4%)
172. [Q76FK4_HUMAN](#) Nucleolar protein 8, 73 (6%)
173. [S09036](#) dnaK-type molecular chaperone HSPA6, 73 (11%)
174. [MMHUMH](#) laminin alpha-2 chain (fragment), 73 (5%)
175. [CAC22372](#) Sequence 1 from Patent WO0075319, 73 (10%)
176. [AAG01174](#) Smarce 1-related protein, 73 (19%)
177. [Q9Y2I5_HUMAN](#) KIAA0979 protein (Fragment), 73 (8%)
178. [Q5T9S5_HUMAN](#) Sarcoma antigen NY-SAR-41 (NY-SAR-41), 73 (7%)
179. [Q6VMB5_HUMAN](#) DNA polymerase theta, 73 (4%)
180. [CAA31492](#) Myosin heavy chain, 72 (10%)
181. [Q7Z561_HUMAN](#) Acetyl-CoA carboxylase 1, 72 (6%)
182. [Q5VZL5_HUMAN](#) Zinc finger protein 262 (OTTHUMP00000046188), 72 (5%)
183. [BAA78718](#) centrosome-and golgi-localized PKN-associated protein, 72 (3%)
184. [CAC22378](#) Sequence 13 from Patent WO0075319, 72 (10%)
185. [Q8TCU4_HUMAN](#) ALMS1 protein, 72 (3%)
186. [S42516](#) PML protein, splice form 1, 71 (4%)
187. [Q7L576_HUMAN](#) Cytoplasmic FMR1 interacting protein 1, 71 (7%)
188. [AAC25051](#) Advillin; p92, 71 (5%)
189. [Q9UIG2_HUMAN](#) Bromodomain PHD finger transcription factor, 71 (4%)
190. [Q86VJ1_HUMAN](#) E3 ligase for inhibin receptor, 71 (3%)
191. [O43314_HUMAN](#) KIAA0433 protein (Fragment), 71 (5%)
192. [PZRN3_HUMAN](#) PDZ domain containing RING finger protein 3 (Ligand of Numb-protein X 3) (Semaphorin cytoplasmic do, 71 (10%)
193. [Q92954_HUMAN](#) Megakaryocyte stimulating factor, 71 (4%)
194. [CAD48779](#) Sequence 23 from Patent EP1229047, 70 (4%)
195. [AAK29205](#) zinc finger protein 291, 70 (06%)
196. [O95714_HUMAN](#) HERC2 protein, 70 (3%)
197. [A55575](#) ankyrin 3, long splice form , 70 (3%)
198. [CAB45385](#) trithorax homologue 2, 70 (3%)
199. [A56539](#) giantin, 70 (4%)
200. [A45259](#) desmoyokin (fragments), 70 (6%)
201. [BAA25517](#) KIAA0591 protein. 70 (4%)
202. [CAA09762](#) DNA topoisomerase (ATP-hydrolysing); topoisomerase II alpha, 70 (6%)
203. [Q96Q15_HUMAN](#) Phosphatidylinositol 3-kinase-related protein kinase, 70 (3%)
204. [O95996_HUMAN](#) APCL protein, 70 (6%)
205. [BAA22378](#) Myosin phosphatase target subunit 1, 69 (6%)
206. [KIHUPL](#) pyruvate kinase (EC 2.7.1.40), hepatic splice form L, 69 (4%)

207. [CAC88303](#) Sequence 3 from Patent WO0164871, 69 (5%)
 208. [SKIV2_HUMAN](#) Helicase SKI2W (Helicase-like protein) (HLP), 69 (3%)
 209. [Q6XDA8_HUMAN](#) Acetyl-CoA carboxylase alpha, 69 (6%)
 210. [Q9Y5L9_HUMAN](#) Transcriptional activator SRCAP, 69 (4%)
 211. [Q9H3T8_HUMAN](#) MOP-3, 69 (3%)
 212. [AAH30221](#) ABI gene family, member 3 (NESH) binding protein, 68 (5%)
 213. [Q9UQ39_HUMAN](#) RNA binding protein (Fragment), 68 (7%)
 214. [O75592_HUMAN](#) Protein associated with Myc, 68 (2%)
 215. [ITSN2_HUMAN](#) Intersectin 2 (SH3 domain-containing protein 1B) (SH3P18) (SH3P18-like WASP associated protein), 68 (4%)
 216. [AAD10838](#) kendrin, 68 (5%)
 217. [Q69GU6_HUMAN](#) Transcription factor GTF2IRD2 beta isoform 1, 68 (15%)
 218. [Q5VZ08_HUMAN](#) OTTHUMP00000044920 protein, 68 (6%)
 219. [Q5TG72_HUMAN](#) Tripartite motif-containing 33, 67 (4%)
 220. [A61231](#) myosin heavy chain nonmuscle form A , 67 (5%)
 221. [AAR25661](#) Ankyrin repeat-containing protein, 67 (4%)
 222. [JC6148](#) subtilisin-like proprotein convertase (EC 3.4.21.-) homolog, 67 (8%)
 223. [CAC19642](#) Nebulette, 67 (11%)
 224. [Q5TCU6_HUMAN](#) OTTHUMP00000045364 protein, 67 (3%)
 225. [Q5VXR7_HUMAN](#) OTTHUMP00000059760 protein, 66 (4%)
 226. [AAB40700](#) putative DNA dependant ATPase and helicase, 66 (6%)
 227. [Q86UP3_HUMAN](#) Zinc finger homeodomain 4 protein, 66 (3%)
 228. [CAA62346](#) axonal transporter of synaptic vesicles, 66 (6%)
 229. [AAR32423](#) Immunoglobulin heavy chain variable region, 66 (20%)
 230. [CAI22960](#) Novel protein KIAA0117, 66 (12%)
 231. [AAL39080](#) transcription repressor p66, 66 (8%)
 232. [Q5THR3_HUMAN](#) OTTHUMP00000028872 protein, 66 (7%)
 233. [CAA60685](#) Homologue of Drosophila Fat protein, 66 (3%)
 234. [Q9P2D7_HUMAN](#) KIAA1410 protein, 66 (3%)
 235. [AAB49973](#) p97, 65 (10%)
 236. [BAA13200](#) KIAA0209 protein, 65 (4%)
 237. [A33370](#) H+-transporting two-sector ATPase (EC 3.6.3.14) beta chain precursor, mitochondrial, 65 (9%)
 238. [Q7Z5C1_HUMAN](#) Glycoprotein receptor gp330/megalin, 65 (2%)
 239. [Q8IZN0_HUMAN](#) KIAA0377 splice variant 4, 65 (6%)
 240. [ALU5_HUMAN](#) Alu subfamily SC sequence contamination warning entry, 65 (17%)
 241. [ALU8_HUMAN](#) Alu subfamily SX sequence contamination warning entry, 64 (20%)
 242. [Q6IPT9_HUMAN](#) Eukaryotic translation elongation factor 1 alpha 1, 64 (8%)
 243. [EFHUA2](#) translation elongation factor eEF-1 alpha-2 chain, 64 (9%)
 244. [AAH11684](#) GATA zinc finger domain containing 2A, 64 (9%)

245. [S65824](#) reverse transcriptase homolog - human transposon L1.1, 63 (7%)
246. [Q6PIY4_HUMAN](#) TIP120A protein (Fragment), 63 (6%)
247. [Q5THQ1_HUMAN](#) OTTHUMP0000028520 protein, 63 (5%)
248. [AAQ88893](#) Low temperature essential protein, 63 (6%)
249. [Q9UHA8_HUMAN](#) Splicing coactivator subunit SRm300, 63 (3%)
250. [PCLO_HUMAN](#) Piccolo protein (Aczonin), 63 (3%)
251. [Q6DKQ9_HUMAN](#) Cell division cycle 2-like 5 (Cholinesterase-related cell division controller), 63 (5%)
252. [CGHU6C](#) collagen alpha 1(II) chain precursor [validated], 63 (10%)
253. [AAD51793](#) GAG-PRO-POL-ENV PROTEIN, 63 (3%)
254. [Q5SYA9_HUMAN](#) OTTHUMP0000046126 (OTTHUMP0000021391), 63 (4%)
255. [Q86U86_HUMAN](#) Polybromo-1D, 63 (7%)
256. [AAK00229](#) Myosin VI, 63 (4%)
257. [BAA89209](#) Bromodomain adjacent to zinc finger domain 1A, 63 (5%)
258. [Q86T11_HUMAN](#) Full-length cDNA clone CS0DH002YN05 of T cells (Jurkat cell line) of Homo sapiens (human) (Fragment), 62 (9%)
259. [Q14333_HUMAN](#) Facioscapulohumeral muscular dystrophy, 62 (7%)
260. [ALU6_HUMAN](#) Alu subfamily SP sequence contamination warning entry, 62 (22%)
261. [Q96L96_HUMAN](#) Muscle alpha-kinase, 62 (5%)
262. [AAC50893](#) FUSE binding protein 3, 62 (14%)
263. [CAA34683](#) COL2A1, 62 (12%)
264. [AAB87381](#) CHD1, 62 (6%)
265. [ISHUT1](#) DNA topoisomerase (EC 5.99.1.2), 62 (9%)
266. [AAC14667](#) Hsp27 ERE-TATA-binding protein, 62 (28%)
267. [Q96DZ0_HUMAN](#) GDAP2 protein (Ganglioside induced differentiation associated protein 2), 61 (9%)
268. [DNJCD_HUMAN](#) DnaJ homolog subfamily C member 13, 61 (6%)
269. [AAK19738](#) channel-kinase 1, 61 (7%)
270. [Q8NF44_HUMAN](#) FLJ00354 protein (Fragment), 61 (7%)
271. [Q6PIC8_HUMAN](#) AQR protein (Fragment), 61 (7%)
272. [LRP4_HUMAN](#) Low-density lipoprotein receptor-related protein 4 precursor (Multiple epidermal growth factor-like, 61 (2%)
273. [Q5T9J7_HUMAN](#) Spastic ataxia of Charlevoix-Saguenay (Sacsin), 61 (2%)
274. [AAS85961](#) Immunoglobulin heavy chain, 61 (12%)
275. [CGHU1B](#) collagen alpha 4(IV) chain precursor, 61 (6%)
276. [Q9GZR2_HUMAN](#) XPMC2H (XPMC2 protein), 61 (22%)
277. [SPTN4_HUMAN](#) Spectrin beta chain, brain 3 (Spectrin, non-erythroid beta chain 3) (Beta-IV spectrin), 61 (6%)
278. [IHJOA](#) heat-shock 70kd protein 42kd atpase n-terminal fragment (EC 3.6.1.3), 61 (10%)
279. [Q5T2U8_HUMAN](#) OTTHUMP0000059294 protein, 61 (4%)
280. [S22610](#) DNA (cytosine-5-) -methyltransferase (EC 2.1.1.37), 61 (6%)

281. [Q6U276 HUMAN](#) Krev interaction trapped 1 variant, 60 (6%)
 282. [I54251](#) translation elongation factor eEF-1 alpha (fragment), 60 (13%)
 283. [EGHU](#) epidermal growth factor precursor [validated], 60 (4%)
 284. [Q5THM9 HUMAN](#) OTTHUMP0000040219 protein, 60 (6%)
 285. [CAD70044](#) Sequence 2053 from Patent EP1270724 (Fragment), 60 (18%)
 286. [AAD29948](#) Myosin heavy chain, 60 (5%)
 287. [MLL3 HUMAN](#) Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog (Histone- lysine N-methyltransferase, H3 lysine-4 specific MLL3) (EC 2.1.1.43) (Homologous to ALR protein), 60 (2%)
 288. [SMRC1 HUMAN](#) SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily C member 1 (SWI/, 60 (8%)
 289. [BAC80154](#) Immunoglobulin heavy chain, 60 (21%)
 290. [AAA19604](#) Similar to Drosophila splicing regulator, suppressor-of white-apricot: Swiss-Prot, 60 (6%)
 291. [Q9UL54 HUMAN](#) Prostate derived STE20-like kinase PSK, 60 (6%)
 292. [AAH43399](#) Ets variant 6 gene, 60 (10%)
 293. [Q9HCD1 HUMAN](#) KIAA1641 protein (Fragment), 60 (7%)
 294. [Q5VU48 HUMAN](#) Phosphodiesterase 4D interacting protein (Myomegalin), 60 (5%)
 295. [ZN646 HUMAN](#) Zinc finger protein 646, 60 (4%)
 296. [A57570](#) Bloom's syndrome related protein BLM, 59 (7%)
 297. [AAC72361](#) Chromosome-associated protein-C, 59 (9%)
 298. [AAD00702](#) Myosin heavy chain 12, 59 (6%)
 299. [A59252](#) myosin heavy chain, nonmuscle, form IIB, 59 (3%)
 300. [AAF36817](#) Guanine nucleotide exchange factor, 59 (3%)
 301. [Q60FE7 HUMAN](#) Filamin B, 59 (3%)
 302. [Q5VWI4 HUMAN](#) Nebulin-related anchoring protein, 59 (8%)
 303. [SIN3A HUMAN](#) Paired amphipathic helix protein Sin3a, 59 (6%)
 304. [Q5T4F9 HUMAN](#) OTTHUMP0000044259 (SET and MYND domain containing 3), 59 (15%)
 305. [Q5TCY3 HUMAN](#) OTTHUMP0000016423 protein, 59 (3%)
 306. [G02093](#) glycerol-3-phosphate dehydrogenase (EC 1.1.99.5), mitochondrial precursor, 59 (9%)
 307. [Q9Y474 HUMAN](#) DNA-binding protein, 59 (6%)
 308. [Q5SW99 HUMAN](#) OTTHUMP0000060857 protein, 59 (6%)
 309. [Q8IV76 HUMAN](#) PAS domain containing 1, 59 (3%)
 310. [AAR02485](#) Immunoglobulin heavy chain variable region, 59 (23%)
 311. [Q9P2D9 HUMAN](#) KIAA1408 protein (Fragment), 59 (5%)
 312. [A44125](#) high density lipoprotein-binding protein, 110K, 58 (8%)
 313. [Q5R3M6 HUMAN](#) OTTHUMP0000028958 protein, 58 (4%)
 314. [I38928](#) acetyl-CoA carboxylase (EC 6.4.1.2), hepatic, 58 (6%)
 315. [CAD10507](#) Voltage-gated sodium channel, 58 (4%)
 316. [BAA22957](#) KIAA0288 protein, 58 (7%)
 317. [AAD56402](#) Cyclophilin-related protein, 58 (6%)
 318. [Q95785 HUMAN](#) Homolog of Mus musculus wizL protein (Fragment), 58 (5%)

319. [CHD7_HUMAN](#) Chromodomain-helicase-DNA-binding protein 7 (CHD-7) (Fragment), 58 (4%)
320. [AAK07692](#) NREBP, 58 (3%)
321. [Q5TH00_HUMAN](#) OTTHUMP00000031017 (OTTHUMP00000031013) protein, 58 (3%)
322. [CAD89409](#) Immunoglobulin gamma heavy chain variable region (Fragment), 58 (14%)
323. [CAA09361](#) Hyperion protein, 58 (3%)
324. [AAR32505](#) Immunoglobulin heavy chain variable region, 58 (13%)
325. [Q96E87_HUMAN](#) KIAA0690 protein, 58 (5%)
326. [Q5TBJ7_HUMAN](#) OTTHUMP00000018803 protein, 58 (4%)
327. [Q8IVL1_HUMAN](#) Steerin2 protein, 58 (3%)
328. [AAD29951](#) Myosin heavy chain IIx/d, 58 (4%)
329. [JQ0129](#) 86K heat shock protein IV (fragment), 58 (9%)
330. [Q8WXG9_HUMAN](#) Very large G protein-coupled receptor 1b, 58 (1%)
331. [HSHUA1](#) histone H2A.1, 58 (16%)
332. [CAC60396](#) Sequence 77 from Patent WO0157209, 57 (6%)
333. [Q6ZNJ1_HUMAN](#) FLJ00341 protein (Fragment), 57 (4%)
334. [AAC50218](#) Myosin-VIIa, 57 (7%)
335. [Q69G40_HUMAN](#) Transcription factor GTF2IRD2 isoform 2, 57 (20%)
336. [RBM28_HUMAN](#) RNA-binding protein 28 (RNA binding motif protein 28), 57 (9%)
337. [B40016](#) matrin 3 (fragment), 57 (9%)
338. [A23767](#) myosin heavy chain, fast skeletal muscle (fragment), 57 (10%)
339. [GP125_HUMAN](#) Probable G protein-coupled receptor 125 precursor (UNQ556/PRO1113), 57 (4%)
340. [AAO18156](#) NALP5, 57 (5%)
341. [AAB41533](#) Lysosomal traffic regulator, 57 (6%)
342. [ALU2_HUMAN](#) Alu subfamily SB sequence contamination warning entry, 57 (15%)
343. [BAA86596](#) KIAA1282 protein, 57 (6%)
344. [Q68GC2_HUMAN](#) SIN3B long isoform, 57 (7%)
345. [AAD43749](#) Protocadherin beta, 57 (4%)
346. [S32920](#) cell wall-associated protein precursor wapA [similarity] - Bacillus subtilis, 57 (4%)
347. [AAC09299](#) hooook2 protein, 57 (8%)
348. [CAC25053](#) Sequence 3 from Patent WO0100849, 57 (5%)
349. [AAD50325](#) RAD50-2 protein, 57 (6%)
350. [Q5W0B2_HUMAN](#) OTTHUMP00000016826 protein, 56 (3%)
351. [Q5T0X9_HUMAN](#) REV3-like, catalytic subunit of DNA polymerase zeta (Yeast), 56 (3%)
352. [AAF24858](#) Nebulette, 56 (11%)
353. [S12788](#) transcription factor NF-IL6, 56 (10%)
354. [AAL85487](#) Zinc finger 298, 56 (6%)
355. [A39579](#) c-myc promoter-binding protein MBP-1, 56 (17%)
356. [AAR02490](#) Immunoglobulin heavy chain variable region, 56 (14%)
357. [Q00375_HUMAN](#) Putative p150, 56 (7%)

358. [CAC22310](#) LAMA5, 56 (2%)
 359. [AAN46668](#) G-protein coupled receptor GPR112, 56 (2%)
 360. [Q8WWR6_HUMAN](#) Beta 1,6-GlcNAc-transferase, 56 (6%)
 361. [Q5T1B0_HUMAN](#) Novel protein (OTTHUMP00000060640), 56 (9%)
 362. [Q8N4P9_HUMAN](#) PAXIP1L protein, 56 (13%)
 363. [AAH53903](#) SLIT-ROBO GTPase-activating protein 1, 56 (6%)
 364. [PUR4_HUMAN](#) Phosphoribosylformylglycinamide synthase (EC 6.3.5.3) (FGAM synthase) (FGAMS) (Formylglycinamide, 56 (3%))
 365. [T43483](#) translation initiation factor IF-2 homolog [similarity] (fragment), 56 (3%)
 366. [AAC96010](#) Chaperone containing t-complex polypeptide 1, delta subunit; CCT-delta, 56 (7%)
 367. [ALU3_HUMAN](#) Alu subfamily SB1 sequence contamination warning entry, 56 (18%)
 368. [JC5839](#) GTBP-N protein, 56 (6%)
 369. [DAHUAI](#) arachidonate 5-lipoxygenase (EC 1.13.11.34) [validated], 56 (10%)
 370. [Q5VW08_HUMAN](#) OTTHUMP0000042245 protein, 55 (9%)
 371. [I38110](#) 1-phosphatidylinositol 3-kinase (EC 2.7.1.137) alpha isoform, 55 (9%)
 372. [AAR32513](#) Immunoglobulin heavy chain variable region, 55 (13%)
 373. [AAR32515](#) Immunoglobulin heavy chain variable region, 55 (14%)
 374. [BAA20562](#) PKU-beta, 55 (7%)
 375. [IQGA2_HUMAN](#) Ras GTPase-activating-like protein IQGAP2, 55 (7%)
 376. [Q9Y2J4_HUMAN](#) Angiomotin like 2 (Fragment), 55 (7%)
 377. [Q6PRD1_HUMAN](#) GPR158-like 1 receptor, 55 (4%)
 378. [BAB67794](#) KIAA1901 protein, 55 (6%)
 379. [S57342](#) endopeptidase La homolog (EC 3.4.21.-) precursor, mitochondrial (version 1), 55 (4%)
 380. [A25773](#) dnaK-type molecular chaperone (fragment), 55 (17%)
 381. [Q5VVL9_HUMAN](#) Protein tyrosine phosphatase, receptor type, D, 55 (4%)
 382. [AAG09702](#) Zinc finger protein ZNF287, 55 (10%)
 383. [CAI14530](#) excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome)), 55 (5%)
 384. [AAF64304](#) Bcl2 associated transcription factor short form, 55 (7%)
 385. [AAC99959](#) DNA-directed RNA polymerase I, largest subunit, 55 (4%)
 386. [AAR02499](#) Immunoglobulin heavy chain variable region, 55 (13%)
 387. [AAR02507](#) Immunoglobulin heavy chain variable region, 55 (18%)
 388. [FLNC_HUMAN](#) Filamin C (Gamma-filamin) (Filamin 2) (Protein FLNC) (Actin-binding like protein) (ABP-L) (ABP-280-, 55 (6%)
 389. [Q5VYL1_HUMAN](#) OTTHUMP0000060079 protein, 55 (4%)
 390. [AAC06147](#) RPOM, 55 (6%)
 391. [CAA55632](#) Endosomal protein, 55 (5%)
 392. [AAR02562](#) Immunoglobulin heavy chain variable region, 54 (12%)

393. [Q9UE80_HUMAN](#) Signaling inositol polyphosphate phosphatase SHIP II
394. [AAA65018](#) Chondroitin sulfate proteoflycan versican V0 splice-variant precursor peptide, 54 (1%)
395. [Q7Z406_HUMAN](#) Myosin heavy chain, 54 (3%)
396. [NCOR1_HUMAN](#) Nuclear receptor corepressor 1 (N-CoR1) (N-CoR), 54 (4%)
397. [Q86Y04_HUMAN](#) MAP4 protein (Fragment), 54 (13%)
398. [CGHU2B](#) collagen alpha 2(IV) chain precursor, 54 (5%)
399. [AAR32448](#) Immunoglobulin heavy chain variable region, 54 (14%)
400. [Q8TDA3_HUMAN](#) Guanine nucleotide exchange factor GEF-H1 (Rho/rac guanine nucleotide exchange factor (GEF) 2), 54 (8%)
401. [AAA52420](#) Coagulation factor VIII, 54 (3%)
402. [S28261](#) centromere protein E, 54 (6%)
403. [Q5XJ05_HUMAN](#) ZNF638 protein, 54 (4%)
404. [CAC38608](#) Sequence 233 from Patent WO0129221, 54 (17%)
405. [Q86YH7_HUMAN](#) HDAC4 protein, 54 (8%)
406. [A43932](#) mucin 2 precursor, intestinal (fragments), 53 (3%)
407. [Q99736_HUMAN](#) HsGCN1 (Fragment), 53 (3%)
408. [O00362_HUMAN](#) Putative p150, 53 (7%)
409. [Q9Y4F4_HUMAN](#) KIAA0423 protein (Fragment), 53 (3%)
410. [BAA35073](#) Protein kinase, 53 (4%)
411. [CAI19008](#) OTTHUMP00000039037, 53 (7%)
412. [AAR02549](#) Immunoglobulin heavy chain variable region, 53 (12%)
413. [AAF15452](#) Immunoglobulin heavy chain variable region (fragment), 53 (33%)
414. [Q5VYK1_HUMAN](#) Collagen, type XII, alpha 1 (OTTHUMP00000039322), 53 (2%)
415. [Q5T1R5_HUMAN](#) Immunodeficiency virus type I enhancer binding protein 3, 53 (3%)
416. [AAF31262](#) Saccin, 53 (3%)
417. [AAL83937](#) DLG5, 53 (4%)
418. [AAQ94075](#) Truncated Krev interaction trapped 1 variant 1681 delta T, 53 (8%)
419. [Q8WWZ7_HUMAN](#) ATP-binding cassette A5, 53 (5%)
420. [FBX38_HUMAN](#) F-box only protein 38 (Modulator of KLF7 activity homolog) (MoKA), 53 (3%)
421. [CAC09161](#) Sequence 1 from Patent WO0043510, 53 (4%)
422. [AAA52898](#) IMMUNOGLOBULIN HEAVY CHAIN (FRAGMENT), 53 (18%)
423. [Q6PK08_HUMAN](#) NOP5/NOP58 protein (Fragment), 53 (9%)
424. [AAC50567](#) PTPsigma, 53 (5%)
425. [A42566](#) omega-conotoxin-sensitive N-type calcium channel alpha 1B-1 subunit (alternatively spliced), 53 (4%)
426. [Q7Z5J5_HUMAN](#) SNF2 histone linker PHD RING helicase, 53 (6%)
427. [JC2131](#) metabotropic glutamate receptor 5 B, 53 (6%)
428. [O96028_HUMAN](#) Putative WHSC1 protein (MMSET type II) (TRX5 protein), 53 (5%)

429. [Q5W0E7_HUMAN](#) Neurobeachin, 53 (2%)
 430. [AAF21616](#) Ig heavy chain (fragment), 53 (19%)
 431. [Q9UCF5_HUMAN](#) Interleukin 2 (Fragment), 52 (39%)
 432. [AAR32537](#) Immunoglobulin heavy chain variable region, 52 (14%)
 433. [Q5W041_HUMAN](#) Novel protein, 52 (7%)
 434. [CAI15935](#) protein phosphatase 1, regulatory (inhibitor) subunit 12B, 52 (8%)
 435. [BAB33332](#) KIAA1662 protein, 52 (4%)
 436. [Q6PJ56_HUMAN](#) TRDD3 protein, 52 (10%)
 437. [Q9H1S7_HUMAN](#) BA56H7.1.2 (Nebulette protein (NEBL, actin-binding Z-disc protein), 52 (14%)
 438. [A47008](#) transcription activator NF-IL6 beta, 52 (11%)
 439. [Q8IWV6_HUMAN](#) Tumor-associated microtubule-associated protein, 52 (9%)
 440. [BAA13403](#) KIAA0274 protein, 52 (8%)
 441. [AAF75772](#) Ubiquitin-conjugating BIR-domain enzyme APOLLON, 52 (2%)
 442. [Q8WVW8_HUMAN](#) COPG2 protein, 52 (14%)
 443. [S01991](#) transforming protein B-myb, 52 (7%)
 444. [Q86WG6_HUMAN](#) Transforming acidic coiled coil 2, long isoform, 52 (3%)
 445. [Q66GS8_HUMAN](#) Centrosome protein Cep290, 52 (4%)
 446. [AAH04274](#) H2A histone family, member V, isoform 1, 52 (19%)
 447. [Q70CQ2_HUMAN](#) Ubiquitin-specific proteinase 34, 52 (2%)
 448. [BAC85687](#) cytochrome b – Macaca fascicularis, 52 (6%)
 449. [BAA23795](#) Brain ryanodine receptor, 52 (3%)
 450. [S41121](#) acetyl-CoA carboxylase (EC 6.4.1.2), 52 (4%)
 451. [AAR32444](#) Immunoglobulin heavy chain variable region, 52 (18%)
 452. [Q9BWP7_HUMAN](#) Promyelocytic leukemia protein, isoform 11, 52 (2%)
 453. [BAA13198](#) KIAA0207 protein, 51 (8%)
 454. [AAG48558](#) LPS responsive and Beige-like anchor protein LBRA, 51 (3%)
 455. [CHTOG_HUMAN](#) CH-TOG protein (Colonic and hepatic tumor over-expressed protein) (Ch- TOG protein), 51 (4%)
 456. [Q14754_HUMAN](#) ORFII, 8%)
 457. [Q5TBS9_HUMAN](#) OTTHUMP00000040391 protein, 51 (4%)
 458. [CAC12756](#) dJ885L7.9.1 (Death associated transcription factor 1 (contains KIAA0333), isoform 1), 51 (9%)
 459. [S67527](#) protein kinase (EC 2.7.1.-) PRK2, 51 (5%)
 460. [Q5TH93_HUMAN](#) Leucine zipper protein 1, 51 (6%)
 461. [A27816](#) phosphoglycerate kinase (EC 2.7.2.3) 2, 51 (15%)
 462. [AAS85951](#) Immunoglobulin heavy chain, 51 (15%)
 463. [AAR02512](#) Immunoglobulin heavy chain variable region, 51 (20%)
 464. [ITA3_HUMAN](#) Integrin alpha-3 precursor (Galactoprotein B3) (GAPB3) (VLA-3 alpha chain) (CD49c) (FRP-2), 51 (6%)
 465. [AAF78783](#) Myosin 5c, Myosin Vc, 51 (4%)

466. [BAA02185](#) eIF-4 gamma, 51 (4%)
 467. [Q8WWS6_HUMAN](#) DJ537K23.3 (KIAA0266 protein) (Fragment), 51 (5%)
 468. [AAB87383](#) CHD3, 51 (4%)
 469. [ZCWC3_HUMAN](#) Zinc finger CW-type coiled-coil domain protein 3, 51 (7%)
 470. [IDFBH](#) Ig gamma chain V-C region (Fab fragment 3D6), chain H, 51 (12%)
 471. [AAC51107](#) Telomerase-associated protein TP-1, 51 (3%)
 472. [Q5T5R0_HUMAN](#) Protein tyrosine phosphatase, receptor type, C, 51 (4%)
 473. [Q9C099_HUMAN](#) KIAA1764 protein (Fragment), 51 (3%)
 474. [Q5W0J4_HUMAN](#) UPF2 regulator of nonsense transcripts homolog (Yeast), 51 (7%)
 475. [A28372](#) insulin-like growth factor 2 receptor precursor, 50 (2%)
 476. [PTPRS_HUMAN](#) Receptor-type tyrosine-protein phosphatase S precursor (EC 3.1.3.48) (R-PTP-S) (Protein-tyrosine phosphatase sigma) (R-PTP-sigma), 50 (5%)
 477. [CAC94915](#) Cyclin B3, 50 (4%)
 478. [AAC34245](#) Trio, 50 (3%)
 479. [AAK58565](#) Ubiquitin specific protease, 50 (7%)
 480. [NFM_HUMAN](#) Neurofilament triplet M protein (160 kDa neurofilament protein) (Neurofilament medium polypeptide), 50 (6%)
 481. [AAK16812](#) Cullin CUL4B, 50 (8%)
 482. [Q9UKZ4_HUMAN](#) Tenascin-M1, 50 (2%)
 483. [Q68G51_HUMAN](#) DDX42 protein (Fragment), 50 (5%)
 484. [AAC05243](#) Putative ATPase, 50 (5%)
 485. [Q695L1_HUMAN](#) Striated muscle preferentially expressed protein (Fragment), 50 (3%)
 486. [Q5SR01_HUMAN](#) OTTHUMP00000060747 protein, 50 (4%)
 487. [CE135_HUMAN](#) Centrosomal protein of 135 kDa (Cep135 protein), 50 (7%)
 488. [Q7Z478_HUMAN](#) DEAH (Asp-Glu-Ala-His) box polypeptide 29, 50 (5%)
 489. [Q8IVL0_HUMAN](#) Steerin3 protein, 50 (4%)
 490. [Q8IZK1_HUMAN](#) Tripin, 50 (6%)
 491. [Q70CQ4_HUMAN](#) Ubiquitin-specific proteinase 31, 50 (3%)
 492. [AAC51144](#) ATP-binding cassette transporter, 49 (3%)
 493. [Q5VXX7_HUMAN](#) Chromodomain helicase DNA binding protein 1-like, 49 (7%)
 494. [CAE55971](#) CaMK I Kinases regulated by Ca2+/CaM, 49 (11%)
 495. [SDG1_HUMAN](#) Serologically defined colon cancer antigen 1 (Antigen NY-CO-1), 49 (6%)
 496. [AAC24863](#) Gx protein, 49 (7%)
 497. [CAB61345](#) Integrin beta 4, 49 (2%)
 498. [Q5SZK8_HUMAN](#) OTTHUMP00000018288 (Fragment), 49 (3%)
 499. [CAC39142](#) GD: RRPB1, 49 (3%)
 500. [AAH11451](#) Alanyl-tRNA synthetase, 49 (8%)

501. [BAB84894](#) FLJ00139 protein, 49 (7%)
502. [A30789](#) creatine kinase (EC 2.7.3.2) precursor, mitochondrial, 49 (13%)
503. [D40201](#) artifact-warning sequence (translated ALU class D), 49 (15%)
504. [PLXB1_HUMAN](#) Plexin B1 precursor (Semaphorin receptor SEP), 49 (4%)
505. [Q7Z7G9_HUMAN](#) Insulin-like growth factor 2 receptor, 49 (2%)
506. [Q6YI51_HUMAN](#) Ubiquitin ligase protein MIB1 (Mind bomb homolog 1), 49 (6%)
507. [Q5SNV9_HUMAN](#) Novel protein (Fragment), 49 (6%)
508. [CAA04798](#) Ryanodine receptor 3, 49 (2%)
509. [AAR02544](#) Immunoglobulin heavy chain variable region, 49 (17%)
510. [Q9BZQ7_HUMAN](#) C1orf26, 49 (8%)
511. [BAB21799](#) KIAA1708 protein, 49 (3%)
512. [AAR32459](#) Immunoglobulin heavy chain variable region, 49 (11%)
513. [JC2460](#) pyruvate carboxylase (EC 6.4.1.1) precursor, 49 (4%)
514. [BAA25471](#) KIAA0545 protein, 49 (2%)
515. [AAF03094](#) Tousled-like kinase 1, 49 (7%)
516. [Q8NHY3_HUMAN](#) GAS2-related protein isoform beta, 49 (10%)
517. [AAA17374](#) Human homologue of E. coli mutL gene product, Swiss-Prot accession number P23367, 49 (8%)
518. [BAA13379](#) Similar to S. cerevisiae SEC7 protein, 49 (3%)
519. [Q5M775_HUMAN](#) NSP5beta3beta, 49 (5%)
520. [AAR02513](#) Immunoglobulin heavy chain variable region, 49 (15%)
521. [AAB31500](#) Pyruvate carboxylase; pyruvate:carbon dioxide ligase, 48 (5%)
522. [AAS86021](#) Immunoglobulin heavy chain, 48 (11%)
523. [NU205_HUMAN](#) Nuclear pore complex protein Nup205 (Nucleoporin Nup205) (205 kDa nucleoporin), 48 (5%)
524. [Q8TF72_HUMAN](#) Shroom-related protein, 48 (5%)
525. [CING_HUMAN](#) Cingulin, 48 (6%)
526. [Q5W9G0_HUMAN](#) KIAA0638 splice variant 2 (Fragment), 48 (9%)
527. [CAI16671](#) OTTHUMP00000018074, 48 (7%)
528. [CAC70714](#) Myosin heavy chain, 48 (2%)
529. [I38155](#) DNA-binding regulatory factor X5, 48 (13%)
530. [Q7Z6Q7_HUMAN](#) DJ470B24.1.3 (Myeloid/lymphoid or mixed-lineage leukemia (Trithorax (Drosophila) homolog); transloc, 48 (4%)
531. [Q6P987_HUMAN](#) Topoisomerase I binding, arginine-serine-rich, 48 (4%)
532. [Q8IXU4_HUMAN](#) RUN and TBC1 domain containing 1, 48 (4%)
533. [O15090_HUMAN](#) KIAA0390 protein (Fragment), 48 (4%)
534. [Q6IPN1_HUMAN](#) APG16L protein (Fragment), 48 (8%)
535. [AAC18034](#) Colon cancer antigen NY-CO-45, 48 (8%)
536. [AAF04726](#) collagen type XI alpha-a isoform B, 48 (6%)
537. [AAF17217](#) Matrin 3, 48 (7%)
538. [Q6VU68_HUMAN](#) Laminin alpha 3 splice variant b1, 48 (2%)

539. [Q5T2B1_HUMAN](#) ATP-binding cassette sub-family C (CFTR\MRP) member 2, 48 (4%)
540. [Q8IXF4_HUMAN](#) Fork head-related protein like B, 48 (3%)
541. [AAK14062](#) Nucleosomal binding protein 1, 48 (13%)
542. [AAC12728](#) ETS related protein-growth factor receptor tyrosine kinase fusion proteins (Fragment), 48 (10%)
543. [O95001_HUMAN](#) WUGSC:H_267D11.3 protein (Fragment), 48 (3%)
544. [Q96QH0_HUMAN](#) WDR9 protein, form A, 48 (5%)
545. [NFL_HUMAN](#) Neurofilament triplet L protein (68 kDa neurofilament protein) (Neurofilament light polypeptide), 48 (11%)
546. [Q6PJY8_HUMAN](#) TBC1D1 protein (Fragment), 48 (7%)
547. [Q5TFG6_HUMAN](#) OTTHUMP0000028769 protein, 48 (6%)
548. [CAD61491](#) Sequence 141 from Patent WO02083898, 48 (27%)
549. [Q5QPB6_HUMAN](#) OTTHUMP0000031267 protein, 48 (5%)
550. [Q6KE87_HUMAN](#) Acetyl-CoA carboxylase 2 (EC 6.4.1.2), 48 (3%)
551. [Q5M774_HUMAN](#) NSP5beta3alpha, 48 (7%)
552. [Q96EB4_HUMAN](#) Interleukin 11, 48 (13%)
553. [CAC34694](#) Sequence 1 from Patent WO0114414, 48 (3%)
554. [AAD51797](#) Gag-Pro-Pol protein, 48 (3%)
555. [BAA74875](#) KIAA0852 protein, 48 (4%)
556. [CAC69573](#) Sequence 1 from Patent WO0160985, 48 (12%)
557. [AAA52880](#) IMMUNOGLOBULIN HEAVY CHAIN PRECURSOR (FRAGMENT), 47 (17%)
558. [Q5SNV6_HUMAN](#) Novel protein (Fragment), 47 (11%)
559. [CAE55978](#) CaMK II KIN1/SNF1/Nim1 family, 47 (12%)
560. [BAC06833](#) HECT domain protein LASU1, 47 (3%)
561. [MYH8_HUMAN](#) Myosin heavy chain, skeletal muscle, perinatal (MyHC-perinatal), 47 (3%)
562. [Q5TIG6_HUMAN](#) Myeloid/lymphoid or mixed-lineage leukemia (Trithorax homolog, Drosophila)\; translocated to, 4, 47 (3%)
563. [Q5VYB2_HUMAN](#) OTTHUMP0000039211 (OTTHUMP0000015997), 47 (4%)
564. [Q9Y4D8_HUMAN](#) KIAA0614 protein (Fragment), 47 (2%)
565. [CAD58287](#) Sequence 1 from Patent WO02072826 precursor, 47 (28%)
566. [Q5VSR3_HUMAN](#) OTTHUMP0000044555 protein, 47 (10%)
567. [AAS85940](#) Immunoglobulin heavy chain, 47 (19%)
568. [RRP5_HUMAN](#) RRP5 protein homolog (Programmed cell death protein 11), 47 (4%)
569. [DATF1_HUMAN](#) Death associated transcription factor 1, 47 (9%)
570. [Q96JI4_HUMAN](#) KIAA1843 protein (Fragment), 47 (3%)
571. [AAC37520](#) MLL-AF4 DER(11) FUSION PROTEIN, 47 (2%)
572. [CU005_HUMAN](#) Protein C21orf5, 47 (3%)
573. [AAC39740](#) Immunoglobulin heavy chain variable region (Fragment), 47 (13%)
574. [DDX21_HUMAN](#) Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21), 47 (8%)
575. [BAA89210](#) bromodomain adjacent to zinc finger domain 1B, 47 (4%)

576. [CAD69337](#) Sequence 609 from Patent EP1270724, 47 (6%)
 577. [AAG09279](#) Cytoplasmic protein, 47 (6%)
 578. [Q8TC46_HUMAN](#) RBMY2FP protein, 47 (23%)
 579. [Q7Z7A1_HUMAN](#) CENTRIOLIN, 47 (4%)
 580. [AAH09372](#) MBD3 protein, 47 (15%)
 581. [Q6P7T4_HUMAN](#) IQCE protein (Fragment), 47 (12%)
 582. [AAK82958](#) pecanex-like protein 1, 47 (3%)
 583. [A47500](#) Ig mu chain switch region binding protein 2, 47 (7%)
 584. [S68142](#) probable transcription factor SMAP, 47 (6%)
 585. [BAA05384](#) type 2 inositol, 1,4,5-trisphosphate receptor, 47 (3%)
 586. [Q5T0F8_HUMAN](#) Novel protein, 47 (7%)
 587. [AAS86039](#) Immunoglobulin heavy chain, 47 (13%)
 588. [Q9UPP2_HUMAN](#) KIAA1110 protein (Fragment), 46 (5%)
 589. [AAR32397](#) Immunoglobulin heavy chain variable region, 46 (12%)
 590. [TB182_HUMAN](#) 182 kDa tankyrase 1-binding protein, 46 (6%)
 591. [AAH01661](#) ATP-binding cassette, sub-family F, member 2, isoform a, 46 (5%)
 592. [T43481](#) probable mucin DKFZp434C196.1 (fragment), 46 (13%)
 593. [AAD13352](#) Phosphatidylinositol 4-kinase 230, 46 (3%)
 594. [A49873](#) inositol 1,4,5-triphosphate receptor, type 3, 46 (3%)
 595. [Q7Z2L5_HUMAN](#) KIAA0580 protein (Fragment), 46 (3%)
 596. [Q5VTF9_HUMAN](#) MAP/microtubule affinity-regulating kinase, 46 (10%)
 597. [AAR02484](#) Immunoglobulin heavy chain variable region, 46 (15%)
 598. [AAO22172](#) Immunoglobulin heavy chain variable and constant region (Fragment), 46 (10%)
 599. [Q5VTB3_HUMAN](#) Component of oligomeric golgi complex 6 (Fragment), 46 (4%)
 600. [Q8IZC9_HUMAN](#) Cardiac sodium channel alpha subunit Nav1.5, 46 (3%)
 601. [S21054](#) DNA-directed RNA polymerase (EC 2.7.7.6) II largest chain, 46 (3%)
 602. [AAF05686](#) Anti-phospholipid immunoglobulin heavy chain VH-D-J region (fragment), 46 (15%)
 603. [Q8IVG5_HUMAN](#) KIAA2005 protein (Fragment), 46 (3%)
 604. [Q5VZ62_HUMAN](#) Multiple PDZ domain protein, 46 (3%)
 605. [SM1L2_HUMAN](#) Structural maintenance of chromosome 1-like 2 protein (SMC1beta protein), 46 (6%)
 606. [AAS85942](#) Immunoglobulin heavy chain, 46 (13%)
 607. [AAR32434](#) Immunoglobulin heavy chain variable region, 46 (17%)
 608. [AAS86004](#) Immunoglobulin heavy chain, 46 (15%)
 609. [Q5SY9_HUMAN](#) OTTHUMP00000020987 (OTTHUMP00000044319), 46 (9%)
 610. [Q6RI45_HUMAN](#) Bromodomain and WD repeat domain containing 3 variant BRWD3-A, 46 (4%)
 611. [AAC51166](#) translation repressor NAT1, 46 (8%)
 612. [A33183](#) microtubule-associated protein 4, 46 (8%)
 613. [Q6ZT07_HUMAN](#) Hypothetical protein FLJ45076, 46 (5%)

614. [CAC38829](#) OTT-MAL, 46 (4%)
615. [ALU4_HUMAN](#) Alu subfamily SB2 sequence contamination warning entry, 46 (13%)
616. [AAH68012](#) ATXN2L protein, 46 (7%)
617. [AAF65516](#) voltage-gated potassium channel Kv4.1, 46 (8%)
618. [Q60293_HUMAN](#) KIAA0546 protein (Fragment), 46 (3%)
619. [E59431](#) phosphoinositide-binding protein [imported], 46 (3%)
620. [Q9BXX2_HUMAN](#) Breast cancer antigen NY-BR-1.1 (Fragment), 46 (6%)
621. [AAF80245](#) filamin 2, 46 (7%)
622. [AAH39174](#) Vinculin, isoform VCL, 46 (10%)
623. [AAS85993](#) Immunoglobulin heavy chain, 45 (14%)
624. [Q8IWZ2_HUMAN](#) Multiple ankyrin repeats single KH domain protein isoform 2, 45 (2%)
625. [BAB21832](#) KIAA1741 protein, 45 (7%)
626. [Q7Z460_HUMAN](#) Multiple asters 1, 45 (6%)
627. [JC5263](#) transmembrane tyrosine phosphatase-like protein, ICAAR, 45 (7%)
628. [AAR32484](#) Immunoglobulin heavy chain variable region, 45 (17%)
629. [Q5T655_HUMAN](#) Novel protein, 45 (4%)
630. [Q8TEN7_HUMAN](#) FLJ00156 protein (Fragment), 45 (4%)
631. [CAI22429](#) KIAA1404 protein, 45 (4%)
632. [SYTL2_HUMAN](#) Synaptotagmin-like protein 2 (Exophilin 4), 45 (4%)
633. [AAR32416](#) Immunoglobulin heavy chain variable region, 45 (20%)
634. [CT160_HUMAN](#) Protein C20orf160, 45 (8%)
635. [Q8WV45_HUMAN](#) KIAA1217 protein, 45 (8%)
636. [Q9UKW0_HUMAN](#) T-cell lymphoma invasion and metastasis 2, 45 (5%)
637. [BAA02794](#) KIAA0004 protein, 45 (9%)
638. [AAA98443](#) TIAM1 protein, 45 (5%)
639. [HELC1_HUMAN](#) Activating signal cointegrator 1 complex subunit 3 (EC 3.6.1.-) (ASC-1 complex subunit p200) (Trip4), 45 (5%)
640. [Q5T476_HUMAN](#) OTTHUMP00000021504 (Fragment), 45 (1%)
641. [Q6IPA9_HUMAN](#) CLCN2 protein, 45 (7%)
642. [BAP31_HUMAN](#) B-cell receptor-associated protein 31 (BCR-associated protein Bap31) (p28 Bap31) (CDM protein) (6C6), 45 (15%)
643. [CAA75436](#) Cxorf5 (71-7A) protein, 45 (7%)
644. [AAS86020](#) Immunoglobulin heavy chain, 45 (16%)
645. [Q9UPP3_HUMAN](#) KIAA1109 protein (Fragment), 45 (3%)
646. [Q6P5V6_HUMAN](#) SNX5 protein (Fragment), 45 (10%)
647. [BAA12177](#) huMCM2, 45 (7%)
648. [Q86UW7_HUMAN](#) Ca2+-dependent activator protein for secretion 2, 45 (4%)
649. [Q8TBI9_HUMAN](#) Alpha isoform of regulatory subunit B56, protein phosphatase 2A, 45 (12%)
650. [CAD88683](#) Immunoglobulin heavy chain (Fragment), 45 (21%)
651. [I38614](#) helicase II, 45 (6%)
652. [Q5TFD7_HUMAN](#) OTTHUMP00000016784 protein, 45 (3%)

653. [Q9Y2K2 HUMAN](#) KIAA0999 protein (Fragment), 45 (4%)
654. [CAC88675](#) Sequence 7 from Patent WO0166747, 45 (2%)
655. [Q96JN2 HUMAN](#) KIAA1793 protein (Fragment), 45 (5%)
656. [AAA35913](#) Guanine nucleotide exchange factor, 45 (3%)
657. [CAC70712](#) Myosin heavy chain, 45 (2%)
658. [BAA76839](#) KIAA0995 protein, 45 (7%)
659. [Q9P0K0 HUMAN](#) Putative zinc finger protein, 44 (6%)
660. [Q8IUS3 HUMAN](#) NADPH oxidase activator 1, 44 (8%)
661. [Q5VW04 HUMAN](#) OTTHUMP00000063496 protein, 44 (3%)
662. [Q6PK59 HUMAN](#) C14orf43 protein (Fragment), 44 (8%)
663. [BAA03406](#) LTG19 protein, 44 (9%)
664. [BAB13393](#) KIAA1567 protein, 44 (8%)
665. [JH0628](#) caldesmon , 44 (8%)
666. [Q7Z3Z9 HUMAN](#) L1 cell adhesion molecule (Fragment), 44 (4%)
667. [POK5 HUMAN](#) HERV-K_19p13.11 provirus ancestral Pol protein
(HERV-K113 Pol protein) [Includes: Reverse transcriptase (RT) (EC
2.7.7.49); Ribonuclease H (EC 3.1.26.4) (RNase H); Integrase (IN)], 44
(5%)
668. [BAA92636](#) G protein alpha subunit i class, 44 (3%)
669. [AAB25318](#) ANTI-TETANUS ANTIBODY HEAVY CHAIN
VARIABLE REGION (FRAGMENT), 44 (17%)
670. [Q6P1M3 HUMAN](#) LLGL2 protein, 44 (4%)
671. [O15050 HUMAN](#) KIAA0342 protein (Fragment), 44 (3%)
672. [Q6P2Q9 HUMAN](#) U5 snRNP-specific protein, 44 (2%)
673. [S02004](#) phospholipase C (EC 3.1.4.3), phosphoinositol-specific, 44
(5%)
674. [S10812](#) phosphatidylserine-binding phosphoprotein (fragments), 44
(45%)
675. [Q5RGR9 HUMAN](#) Novel protein similar to cell recognition molecule
CASPR3 (CASPR3), 44 (5%)
676. [Q8NE12 HUMAN](#) Suppression of tumorigenicity 5, isoform 1, 44 (7%)
677. [Q9UNY1 HUMAN](#) JAW1-related protein MRVI1B short isoform, 44
(7%)
678. [A46054](#) GTP-binding protein ARD 1 , 44 (11%)
679. [DHX34 HUMAN](#) Probable ATP-dependent helicase DHX34 (DEAH-
box protein 34), 44 (11%)
680. [BAA32700](#) beta-spectrin III, 44 (5%)
681. [Q7L3D5 HUMAN](#) KIAA0841 protein (Fragment), 44 (4%)
682. [CAI17216](#) tetracopeptide repeat domain 13, 44 (5%)
683. [MMHUB2](#) laminin gamma-1 chain precursor , 44 (4%)
684. [AAM46640](#) Immunoglobulin heavy chain (Fragment, 44 (10%)
685. [AAC05330](#) Cell adhesion kinase beta, 44 (5%)
686. [AAA52897](#) IMMUNOGLOBULIN HEAVY CHAIN PRECURSOR
(FRAGMENT), 44 (18%)
687. [Q6NXF6 HUMAN](#) GPR protein, 44 (6%)
688. [AAB39753](#) Phosphoinositide 3'-hydroxykinase p110-alpha subunit,
44 (8%)

689. [IQCB1_HUMAN](#) IQ calmodulin-binding motif containing protein 1, 44 (9%)
690. [Q7Z6T2_HUMAN](#) DJ139D8.5.3 (Zinc finger transcription regulating protein TReP-132 (RAPA-1), variant 3), 43 (6%)
691. [Q5SQC9_HUMAN](#) OTTHUMP00000063563 protein, 43 (3%)
692. [S53869](#) laminin beta-2 chain precursor (version 2), 43 (2%)
693. [AAC39790](#) SP100-HMG nuclear autoantigen, 43 (3%)
694. [CAC28482](#) Sequence 4 from Patent WO0104326 (Fragment), 43 (8%)
695. [Q86Y92_HUMAN](#) Similar to KIAA0922 protein (Fragment), 43 (5%)
696. [Q6NVY5_HUMAN](#) GPR89 protein, 43 (8%)
697. [ZFP62_HUMAN](#) Zinc finger protein 62 homolog (Zfp-62), 43 (9%)
698. [Q9HCM1_HUMAN](#) KIAA1551 protein (Fragment), 43 (3%)
699. [BAA05392](#) KIAA0042 protein, 43 (6%)
700. [Q9NS89_HUMAN](#) Alpha1A-voltage-dependent calcium channel (Fragment), 43 (4%)
701. [CAD69830](#) Sequence 1613 from Patent EP1270724, 43 (18%)
702. [Q709C6_HUMAN](#) VPS13B-2A protein, 43 (2%)
703. [Q5VUU2_HUMAN](#) Leimodin 1 (Smooth muscle), 43 97%)
704. [Q8TDM9_HUMAN](#) Amplified in breast cancer 1, 43 (6%)
705. [Q5VZB8_HUMAN](#) OTTHUMP00000042486 protein, 43 (3%)
706. [Q6P3W4_HUMAN](#) Diacylglycerol kinase, theta, 43 (3%)
707. [RP1L1_HUMAN](#) Retinitis pigmentosa 1-like 1 protein, 43 (3%)
708. [AAL62062](#) Bullous pemphigoid antigen 1 eB, 43 (2%)
709. [Q9BR62_HUMAN](#) LLGL2 protein, 43 (11%)
710. [CAA66942](#) Ubiquitin hydroxylase, 43 (1%)
711. [Q5TI99_HUMAN](#) OTTHUMP00000060449 protein, 43 (1%)
712. [AAA36421](#) Protocadherin 43, 43 (7%)
713. [CAD01139](#) Putative TRP cation channel, 43 (4%)
714. [Q5U006_HUMAN](#) Cyclin-dependent kinase 9 (CDC2-related kinase), 43 (11%)
715. [GOGA3_HUMAN](#) Golgi autoantigen, golgin subfamily A member 3 (Golgin-160) (Golgi complex-associated protein of 170 kDa) (GCP170), 43 (1%)
716. [Q5T8A7_HUMAN](#) OTTHUMP00000064652 protein, 43 (4%)
717. [PDE6A_HUMAN](#) Rod cGMP-specific 3',5'-cyclic phosphodiesterase alpha-subunit (EC 3.1.4.17) (GMP-PDE alpha) (PDE V-B1), 43 (8%)
718. [Q8IVF2_HUMAN](#) KIAA2019 protein (Fragment), 43 (3%)
719. [AAC03241](#) Homeobox protein, 43 (9%)
720. [Q5VYM8_HUMAN](#) Unc-13 homolog B (C. elegans), 43 (3%)
721. [CAI12371](#) Rho GTPase activating protein 12, 43 (7%)
722. [Q7Z5W9_HUMAN](#) TCOF1 protein (Fragment), 43 (6%)
723. [CAD69990](#) Sequence 1941 from Patent EP1270724, 43 (11%)
724. [Q8TF46_HUMAN](#) KIAA1955 protein (Fragment), 43 (4%)
725. [Q76N46_HUMAN](#) KIAA0432 protein (Fragment), 43 (7%)
726. [Q8N607_HUMAN](#) Bruno-like 6, RNA binding protein, 43 (9%)
727. [S37431](#) ankyrin 2, neuronal long splice form, 42 (2%)
728. [AAB58382](#) Acetyl-coA carboxylase, 42 (2%)

729. [CAE55946](#) Fatty acid CoA ligase-like AMP-binding enzyme, 42 (3%)
 730. [Q6ECI4_HUMAN](#) Zinc finger protein 470, 42 (6%)
 731. [AAL93149](#) Golgin-160, 42 (1%)
 732. [Q6FI78_HUMAN](#) LYAR protein, 42 (8%)
 733. [T44500](#) fls485 protein [imported] , 42 (9%)
 734. [B55282](#) neurofibromatosis-related protein NF1, 42 (4%)
 735. [Q5VUP7_HUMAN](#) Leukemia-associated protein with a CXXC domain, 42 (2%)
 736. [AAL34497](#) SON DNA binding protein isoform A, 42 93%)
 737. [Q7Z7B6_HUMAN](#) Putative sulfhydryl oxidase precursor, 42 (8%)
 738. [Q96JI7_HUMAN](#) KIAA1840 protein (Fragment), 42 (2%)
 739. [BAA25465](#) KIAA0539 protein, 42 (5%)
 740. [AAC50059](#) ALL-1 fusion partner from chromosome 6, 42 (4%)
 741. [AAH00322](#) Mitotic spindle coiled-coil related protein, 42 (6%)
 742. [CAC15059](#) Putative protein kinase, 42 (5%)
 743. [AAL60502](#) Connector enhancer of KSR2A, 42 (5%)
 744. [BSN_HUMAN](#) Bassoon protein (Zinc-finger protein 231), 42 (5%)
 745. [EMR1_HUMAN](#) EGF-like module containing mucin-like hormone receptor-like 1 precursor (Cell surface glycoprotein), 42 (4%)
 746. [I54378](#) gene X104 protein, 42 (4%)
 747. [ECT2_HUMAN](#) ECT2 protein (Epithelial cell transforming sequence 2 oncogene), 42 (4%)
 748. [CAC16626](#) Sequence 5 from Patent WO0063381, 42 (2%)
 749. [Q6UWZ6_HUMAN](#) Disulfide isomerase, 42 (5%)
 750. [B28096](#) line-1 protein ORF2, 42 (4%)
 751. [Q5VUY9_HUMAN](#) Neuron navigator 1, 42 (5%)
 752. [AAG45223](#) Epsin 3, 42 (7%)
 753. [Q9ULE0_HUMAN](#) KIAA1280 protein (Fragment), 42 (4%)
 754. [GNHUL1](#) retrovirus-related reverse transcriptase pseudogene , 42 (6%)
 755. [CAD22864](#) Sequence 4 from Patent WO0194390, 42 (4%)
 756. [Q5VYQ4_HUMAN](#) OTTHUMP00000021403 (OTTHUMP00000046122), 42 (5%)
 757. [A46419](#) trophoblast-endothelial-activated lymphocyte surface protein 721P , 42 (10%)
 758. [BAB13459](#) KIAA1633 protein, 42 (3%)
 759. [Q5T8W7_HUMAN](#) Espin, 42 (12%)
 760. [AAD42072](#) Oxygen related protein 1, 42 (3%)
 761. [Q5SNV8_HUMAN](#) Novel protein, 42 (8%)
 762. [AAA03354](#) Microtubule-associated protein 2, 42 (4%)
 763. [Q9UEH3_HUMAN](#) Cytochrome P-450 2C (Fragment), 42 (15%)
 764. [Q5VY70_HUMAN](#) OTTHUMP00000003454 protein, 42 (10%)
 765. [CGHU3A](#) collagen alpha 3(VI) chain precursor [validated], 42 (3%)
 766. [Q9BWC2_HUMAN](#) Glyceroneophosphate O-acyltransferase, 42 (11%)
 767. [Q86X36_HUMAN](#) DHX8 protein, 42 (6%)
 768. [S42167](#) 190K protein, 42 (7%)
 769. [Q6Q377_HUMAN](#) Melanin-concentrating hormone receptor 2, 42 (6%)

770. [AIP1_HUMAN](#) Atrophin-1 interacting protein 1 (Atrophin-1 interacting protein A) (Membrane associated guanylate, 42 (4%))
771. [Q8N5U2_HUMAN](#) ATPase, H+/K+ transporting, nongastric, alpha polypeptide, 42 (7%)
772. [AAC39935](#) Alpha enolase like 1, 42 (17%)
773. [Q5S053_HUMAN](#) Secretory pathway calcium ATPase 2, 42 (6%)
774. [AAC78790](#) Zonadhesin, 41 (1%)
775. [Q5T795_HUMAN](#) MDN1, midasin homolog (Yeast) (Fragment), 41 (4%)
776. [Q9C0G6_HUMAN](#) KIAA1697 protein (Fragment), 41 (2%)
777. [Q8IWV8_HUMAN](#) Ubiquitin ligase E3 alpha-II (OTTHUMP00000039768), 41 (4%)
778. [AAC72089](#) PITSLRE protein kinase beta SV12, 41 (30%)
779. [Q5T1V6_HUMAN](#) Novel protein, 41 (7%)
780. [Q6UVW7_HUMAN](#) F-box and leucine-rich repeat protein 13 transcript variant 2, 41 (6%)
781. [Q92815_HUMAN](#) Cytoplasmic dynein 2 heavy chain (Fragment), 41 (9%)
782. [RBHUAP](#) adenomatous polyposis coli protein, 41 (5%)
783. [Q9H4G2_HUMAN](#) Novel protein (Fragment), 41 (3%)
784. [KNTC1_HUMAN](#) Kinetochore-associated protein 1 (Rough deal homolog) (hRod) (HsROD) (Rod), 41 (3%)
785. [AAC72360](#) Chromosome-associated protein-E, 41 (7%)
786. [Q5VVN3_HUMAN](#) Serine/arginine repetitive matrix 1, 41 (8%)
787. [A55178](#) neurotrophin receptor trkC precursor, 41 (6%)
788. [AAD04160](#) RNA binding motif protein 6, 41 (5%)
789. [CAC43730](#) Sequence 7 from Patent WO0142294, 41 (2%)
790. [AAC51833](#) Ubiquitin specific protease 9, 41 (<1%)
791. [Q9C0A8_HUMAN](#) KIAA1755 protein (Fragment), 41 (6%)
792. [A49651](#) replication factor C large subunit, 41 (7%)
793. [AAM49063](#) Plexin D1, 41 (3%)
794. [Q5XLT4_HUMAN](#) Breast and ovarian cancer susceptibility protein (Fragment), 41 (4%)
795. [Q9C0D6_HUMAN](#) KIAA1727 protein (Fragment), 41 (6%)
796. [CK5P2_HUMAN](#) CDK5 regulatory subunit associated protein 2 (CDK5 activator-binding protein C48), 41 (3%)
797. [Q70Z35_HUMAN](#) P-Rex2 protein, 41 (5%)
798. [BAA92616](#) KIAA1378 protein, 41 (5%)
799. [Q6DHZ8_HUMAN](#) Activity-dependent neuroprotector, 41 (6%)
800. [Q9P2N9_HUMAN](#) KIAA1307 protein (Fragment), 41 (3%)
801. [AAB38240](#) Huntington's disease protein, 41 (3%)
802. [MYH11_HUMAN](#) Myosin heavy chain, smooth muscle isoform (SMMHC), 41 (5%)
803. [Q9Y485_HUMAN](#) X-like 1 protein, 41 (5%)
804. [CAA33387](#) Alpha-1 (III) collagen, 41 (11%)
805. [CAA61132](#) SEX protein, 41 (3%)
806. [AAH08825](#) DEAH (Asp-Glu-Ala-His) box polypeptide 16, 41 (6%)
807. [P78409_HUMAN](#) Butyrophilin, 41 (6%)

808. [BAB84925](#) FLJ00170 protein, 41 (5%)
809. [Q5XKH0_HUMAN](#) MGC42174 protein, 41 (7%)
810. [Q9UP21_HUMAN](#) Heparan N-deacetylase/N-sulfotransferase 3, 41 (5%)
811. [Q8IVF9_HUMAN](#) KIAA2012 protein (Fragment), 41 (10%)
812. [Q7Z401_HUMAN](#) C-MYC promoter-binding protein IRLB, 40 (4%)
813. [BAB15795](#) Polyprotein, 40 (6%)
814. [CAC15498](#) RP4-591C20.6, 40 (7%)
815. [Q5VU37_HUMAN](#) Formin 2, 40 (5%)
816. [Q8WYK1_HUMAN](#) Caspr5, 40 (5%)
817. [Q5T0F6_HUMAN](#) MAD, mothers against decapentaplegic homolog (Drosophila) interacting protein, receptor activation, 40 (4%)
818. [Q9NY74_HUMAN](#) ETAA16 protein, 40 (5%)
819. [Q6P4R7_HUMAN](#) Sterol regulatory element binding transcription factor 1, isoform a, 40 (5%)
820. [Q5T5N2_HUMAN](#) OTTHUMP00000043243 protein, 40 (5%)
821. [JN0607](#) alpha-catenin 1, 40 (8%)
822. [JC2542](#) alpha-2(E)-catenin, 40 (10%)
823. [POK12_HUMAN](#) HERV-K_1q22 provirus ancestral Pol protein (HERV-K102 Pol protein) (HERV-K(III) Pol protein) [Includes: Reverse transcriptase (RT) (EC 2.7.7.49); Ribonuclease H (EC 3.1.26.4) (RNase H); Integrase (IN)], 40 (4%)
824. [Q6PD62_HUMAN](#) SH2 domain binding protein 1, 40 (5%)
825. [Q7L8R2_HUMAN](#) DJ691N24.1.1 (KIAA0980 protein, isoform 1) (Fragment), 40 (3%)
826. [CAD69495](#) Sequence 931 from Patent EP1270724, 40 (3%)
827. [BAA86576](#) KIAA1262 protein, 40 (3%)
828. [Q5T3W8_HUMAN](#) Interferon, gamma-inducible protein 16, 40 (7%)
829. [Q5W5T7_HUMAN](#) Long transient receptor potential channel 3, 40 (3%)
830. [Q8IUL8_HUMAN](#) Cartilage intermediate layer protein-like protein CLIP-2, 40 (5%)
831. [G02257](#) NAD(P) transhydrogenase (B-specific) (EC 1.6.1.1) precursor, mitochondrial, 40 (4%)
832. [T09474](#) forkhead protein FREAC-2, 40 (9%)
833. [FSCN1_HUMAN](#) Fascin (Singed-like protein) (55 kDa actin bundling protein) (p55), 40 (10%)
834. [Q8TEN3_HUMAN](#) FLJ00160 protein (Fragment), 40 (3%)
835. [Q6H969_HUMAN](#) Myomesin 1, 40 (6%)
836. [Q9HBG5_HUMAN](#) WDR10p-L, 40 (5%)
837. [S59136](#) estradiol 17beta-dehydrogenase (EC 1.1.1.62) type 4, 40 (5%)
838. [Q5T3F8_HUMAN](#) OTTHUMP00000039890 protein, 40 (3%)
839. [CAD42444](#) Sequence 1 from Patent WO0226817, 40 (4%)
840. [Q5TBA0_HUMAN](#) Novel protein similar to Drosophila CG11486 protein, variant 1 (Fragment), 40 (8%)
841. [Q5VZ18_HUMAN](#) Novel protein (LOC126669), 40 (7%)

842. [SLC2B_HUMAN](#) Slp homolog lacking C2 domains-b (Exophilin 5), 40 (4%)
843. [Q5VY62_HUMAN](#) DEAH (Asp-Glu-Ala-His) box polypeptide 9 (OTTHUMP00000060702), 40 (5%)
844. [I37183](#) gene APXL protein , 40 (4%)
845. [Q6IBT3_HUMAN](#) CCT7 protein, 40 (8%)
846. [1BF5A](#) stat-1, chain A (fragments), 40 (8%)
847. [Q96Q74_HUMAN](#) Mitochondrial ribosomal protein L7/L12 (Fragment), 40 (54%)
848. [AAF66048](#) Immunoglobulin kappa light chain variable region (fragment), 40 (11%)
849. [Q43585_HUMAN](#) CD2 binding protein 1 short form, 40 (9%)
850. [CGHUL7](#) collagen alpha 1(III) chain precursor, 40 (10%)
851. [Q5VXU9_HUMAN](#) OTTHUMP00000021923 protein, 40 (2%)
852. [Q96BX6_HUMAN](#) KIAA0252 protein, 40 (10%)
853. [Q5VTC7_HUMAN](#) Hexokinase domain containing 1, 40 (5%)
854. [T50839](#) U4/U6 small nuclear ribonucleoprotein hPrp3 [imported], 40 (6%)
855. [URA6_HUMAN](#) Unknown protein from 2D-PAGE of red blood cells (Spot 17) (Fragment), 40 (100%)
856. [Q9UK88_HUMAN](#) Genethonin 3 (Fragment), 40 (7%)
857. [AAB60689](#) beta-adrenergic receptor kinase, 40 (12%)
858. [Q8IVM0_HUMAN](#) C3orf6 protein (Chromosome 3 open reading frame 6), 40 (7%)
859. [Q5VV01_HUMAN](#) OTTHUMP00000044924 protein, 40 (5%)
860. [BAA20783](#) KIAA0325 protein, 40 (1%)
861. [AAC41758](#) Nucleoporin, 40 (2%)
862. [AAC50213](#) Pro-a2(XI), 40 (7%)
863. [Q5T2F6_HUMAN](#) Neurofascin (NFASC), 39 (7%)
864. [Q5T0G1_HUMAN](#) Novel protein (Fragment), 39 (8%)
865. [PH0268](#) epidermal autoantigen 450K (clone pE450-C/D)(fragment), 39 (11%)
866. [Q5TDR4_HUMAN](#) OTTHUMP00000031418 protein, 39 (6%)
867. [JC7988](#) uridine phosphorylase (EC 2.4.2.3)-2, 39 (17%)
868. [CAC69377](#) Sequence 1 from Patent WO0144473, 39 (3%)
869. [Q5T4W7_HUMAN](#) Artemin, 39 (14%)
870. [Q96IF6_HUMAN](#) FBF1 protein (Fragment), 39 (6%)
871. [BAA09762](#) KIAA0109 protein, 39 (5%)
872. [Q86VN8_HUMAN](#) Phospholipase C, delta 1, 39 (7%)
873. [AAF60188](#) Toll-like receptor 7, 39 (3%)
874. [I38240](#) transcription factor SOX4, 39 (5%)
875. [AAC51202](#) Armadillo repeat protein, 39 (3%)
876. [Q5T924_HUMAN](#) Sorbin and SH3 domain containing 1, 39 (4%)
877. [AAN40840](#) TBP-associated factor RNA polymerase 1-like, 39 (5%)
878. [Q6ISK3_HUMAN](#) Ubiquitin associated and SH3 domain containing, A, long form, 39 (5%)
879. [AAB93981](#) Pro-alpha 2(I) collagen, 39 (9%)
880. [A57640](#) retinoblastoma binding protein RBQ-1, 39 (3%)

881. [Q6PI49_HUMAN](#) LOC389008 protein (Fragment), 39 (11%)
882. [S45344](#) TUP1 like enhancer, 39 (5%)
883. [Q5T2T1_HUMAN](#) Novel protein, 39 (7%)
884. [BAA14323](#) Collagen alpha 1(V) chain precursor, 39 (7%)
885. [JH0564](#) calcium channel alpha-1D chain, 39 (3%)
886. [Q8IZA4_HUMAN](#) ELYS transcription factor-like protein TMBS62, 39 (2%)
887. [AAH68976](#) Phospholipase D1, phophatidylcholine-specific, 39 (5%)
888. [Q86YW9_HUMAN](#) TRALPUSH, 39 (5%)
889. [AAA18904](#) Microtubule associated protein 1B, 39 (5%)
890. [AAA72125](#) Voltage-operated calcium channel, alpha-1 subunit, 39 (3%)
891. [Q9ULE4_HUMAN](#) KIAA1276 protein (Fragment), 39 (4%)
892. [T02214](#) ubiquitous TPR motif isoform Y, 39 (4%)
893. [Q8TF30_HUMAN](#) KIAA1971 protein (Fragment), 39 (4%)
894. [Q5T3H8_HUMAN](#) OTTHUMP00000060347 protein, 39 (5%)
895. [AAD45121](#) somatostatin receptor interactin protein splice variant, 39 (4%)
896. [1B3OB](#) inosine monophosphate dehydrogenase 2 (EC 1.1.1.205), chain B (fragments), 39 (5%)
897. [Q5VZP5_HUMAN](#) OTTHUMP00000060403 protein, 39 (6%)
898. [AAH05022](#) sorting nexin 9, 39 (4%)
899. [AAD43761](#) protocadherin beta 7, 39 (3%)
900. [CAA71610](#) Ankyrin-like protein, 39 (6%)
901. [AAC23915](#) ATP-binding cassette transporter, 39 (2%)
902. [Q6ICE0_HUMAN](#) CSF2RB protein, 39 (4%)
903. [G01627](#) androgen receptor 1 , 39 (10%)
904. [TCPZ_HUMAN](#) T-complex protein 1, zeta subunit (TCP-1-zeta) (CCT-zeta) (CCT-zeta-1) (Tcp20) (HTR3) (Acute morphine dependence related protein 2), 39 (8%)
905. [TLK2_HUMAN](#) Serine/threonine-protein kinase tousled-like 2 (EC 2.7.1.37) (Tousled- like kinase 2) (PKU-alpha), 39 (5%)
906. [Q9NZS4_HUMAN](#) GR AF-1 specific protein phosphatase (Fragment), 39 (5%)
907. [CAC09149](#) Sequence 1 from Patent DE19856882, 39 (6%)
908. [O94943_HUMAN](#) KIAA0863 protein (Fragment), 39 (5%)
909. [Q8TD84_HUMAN](#) Down syndrome cell adhesion molecule 2, 39 (3%)
910. [Q9UPW7_HUMAN](#) KIAA1033 protein (Fragment), 39 (4%)
911. [Q8IVJ2_HUMAN](#) ABCA5 transporter, 39 (8%)
912. [Q8N4G8_HUMAN](#) KIAA1683 protein, 39 (10%)
913. [PCF11_HUMAN](#) Pre-mRNA cleavage complex II protein Pcf11 (Fragment), 39 (6%)
914. [Q9NRV0_HUMAN](#) X 004 protein, 39 (9%)
915. [AAK08131](#) Hephaestin, 39 (3%)
916. [A56236](#) probable RNA helicase 1, 39 (6%)
917. [ISAV](#) annexin v mutant P13, P87, P119, P163, AND P248 SUBSTITUTED WITH thioproline (prs) (fragments), 39 (5%)
918. [Q7Z5R8_HUMAN](#) Deleted in liver cancer 1, isoform 1, 38 (3%)

919. [AAH37964](#) DEAH (Asp-Glu-Ala-His) box polypeptide 37, 38(5%)
 920. [Q8N0X2_HUMAN](#) PF20 variant 1a (Sperm-associated WD repeat protein), 38 (11%)
 921. [Q9ULA1_HUMAN](#) R31155_1, 38 (6%)
 922. [Q86TU7_HUMAN](#) Full-length cDNA clone CS0DI069YD09 of Placenta of Homo sapiens, 38 (5%)
 923. [Q5VX33_HUMAN](#) ATP-binding cassette, sub-family A (ABC1), member 1, 38 (4%)
 924. [Q9ULE3_HUMAN](#) KIAA1277 protein (Fragment), 38 (2%)
 925. [AAF19816](#) Dynein intermediate chain DNAI1, 38 (6%)
 926. [Q92799_HUMAN](#) Mitochondrial trifunctional protein beta subunit (Fragment), 38 (8%)
 927. [AAV31784](#) B-cell CLL/lymphoma 11B/T-cell receptor delta constant region fusion protein, 38 (9%)
 928. [Q8IVE8_HUMAN](#) KIAA2023 protein (Fragment), 38 (4%)
 929. [AAF88143](#) Hyd protein, 38 (2%)
 930. [Q5UAW9_HUMAN](#) G protein-coupled receptor 157, 38 (3%)
 931. [AAH06395](#) Cell division cycle 25B, isoform 3, 38 (9%)
 932. [Q5TG08_HUMAN](#) OTTHUMP00000028720 protein, 38 (7%)
 933. [Q8TC05_HUMAN](#) Nuclear protein double minute 1, 38 (9%)
 934. [Q7Z5V7_HUMAN](#) Serine arginine-rich pre-mRNA splicing factor SR-A1, 38 (4%)
 935. [A54600](#) 1-phosphatidylinositol 3-kinase (EC 2.7.1.137) 110K chain beta isoform , 38 (9%)
 936. [Q5T791_HUMAN](#) OTTHUMP00000016860 (Fragment), 38 (4%)
 937. [Q7Z437_HUMAN](#) Putative NFkB activating protein, 38 (6%)
 938. [CAD48663](#) Sequence 44 from Patent WO0220569, 38 (2%)
 939. [Q5TB80_HUMAN](#) Chromosome 6 open reading frame 84, 38 (3%)
 940. [BAD16733](#) Fibrillin 3, 38 (3%)
 941. [BAB13469](#) KIAA1643 protein, 38 (7%)
 942. [BAB85807](#) Polycystin-1L1, 38 (2%)
 943. [Q8IXX8_HUMAN](#) ProSAPiP1 protein, 38 (11%)
 944. [Q5SR00_HUMAN](#) Novel protein (Fragment), 38 (6%)
 945. [WNK2_HUMAN](#) Serine/threonine-protein kinase WNK2 (EC 2.7.1.37) (Protein kinase with no lysine 2) (Protein kinase, lysine-deficient 2) (P/OKcl.13), 38 (3%)
 946. [AAP97269](#) CDV protein, 38 (5%)
 947. [AAG03000](#) Vitronectin receptor alpha polypeptide, 38 (5%)
 948. [Q6P3U2_HUMAN](#) BRD2 protein, 38 (7%)
 949. [AAC51331](#) CREB binding protein, 38 (3%)
 950. [Q8IZX7_HUMAN](#) JNK-associated leucine-zipper protein, 38 (3%)
 951. [AAG33700](#) tensin, 38 (3%)
 952. [CAC33877](#) KIAA1404 protein, 38 (3%)
 953. [Q86WX7_HUMAN](#) PRF1 protein (Fragment), 38 (3%)
 954. [ACSL6_HUMAN](#) Long-chain-fatty-acid--CoA ligase 6 (EC 6.2.1.3) (Long-chain acyl-CoA synthetase 6) (LACS 6), 38 (4%)
 955. [Q8WWW1_HUMAN](#) Smoothelin-B3, 38 (7%)

956. [U520_HUMAN](#) U5 small nuclear ribonucleoprotein 200 kDa helicase (EC 3.6.1.-) (U5 snRNP-specific 200 kDa protein) (U5-200KD) (Activating signal cointegrator 1 complex subunit 3-like 1), 38 (3%)
957. [Q5TA69_HUMAN](#) Nucleoporin 133kDa, 38 (3%)
958. [Q9BSS5_HUMAN](#) CLPTM1 protein (Cleft lip and palate associated transmembrane protein 1), 38 (4%)
959. [Q9P2H0_HUMAN](#) KIAA1377 protein (Fragment), 38 (7%)
960. [S45142](#) translation initiation factor eIF-4A2 homolog, 38 (9%)
961. [Q8NFY8_HUMAN](#) Neuroblastoma-amplified protein, 38 (4%)
962. [JC7359](#) splicing factor, Ssf-1, 38 (5%)
963. [Q5SZH6_HUMAN](#) Novel protein, 38 (12%)
964. [A38219](#) GAP-associated tyrosine phosphoprotein p62, 38 (12%)
965. [AAC34210](#) tuberin, 38 (3%)
966. [UN13C_HUMAN](#) Unc-13 homolog C (Munc13-3) (Fragment), 38 (4%)
967. [Q6PCB7_HUMAN](#) Solute carrier family 27 (Fatty acid transporter), member 1, 38 (7%)
968. [JC7388](#) M83 protein
969. [I38902](#) retinoblastoma binding protein RIZ, 38 (4%)
970. [Q86WF7_HUMAN](#) Sarcoma antigen NY-SAR-16 (Fragment), 38 (6%)
971. [AAN45861](#) Scavenger receptor type F, 37 (5%)
972. [Q9UNS1_HUMAN](#) Timeless homolog, 37 (6%)
973. [Q5QPI6_HUMAN](#) OTTHUMP00000030478 (Fragment), 37 (8%)
974. [AAA93229](#) ADP-ribosylation factor, 37 (14%)
975. [Q5T5U3_HUMAN](#) Rho GTPase activating protein 21, 37 (4%)
976. [Q5VU18_HUMAN](#) Nasopharyngeal epithelium specific protein 1 (NESG1), 37 (12%)
977. [Q5R206_HUMAN](#) Carbamoylphosphate synthetase I, 37 (2%)
978. [Q8N2U5_HUMAN](#) SYMPK protein, 37 (6%)
979. [Q5VTE4_HUMAN](#) OTTHUMP00000021897 (OTTHUMP00000063891), 37 (1%)
980. [Q8WX93_HUMAN](#) Myoneurin, 37 (5%)
981. [Q9BSJ7_HUMAN](#) CPSF6 protein, 37 (8%)
982. [Q969N1_HUMAN](#) Poly(A) polymerase gamma (EC 2.7.7.19) (Neopoly(A) polymerase), 37 (9%)
983. [Q7Z6K9_HUMAN](#) C22orf9 protein, 37 (16%)
984. [O15129_HUMAN](#) Breast and ovarian cancer susceptibility protein splice variant, 37 (4%)
985. [Q5T361_HUMAN](#) OTTHUMP00000064771 protein, 37 (8%)
986. [Q6IQ43_HUMAN](#) PTPN9 protein, 37 (9%)
987. [Q8TED9_HUMAN](#) FLJ00258 protein (Fragment), 37 (6%)
988. [A38197](#) protein kinase (EC 2.7.1.37) cdc2-like, 37 (11%)
989. [AAC05370](#) Membrane associated guanylate kinase 2, 37 (2%)
990. [Q86WG2_HUMAN](#) ARM protein, 37 (8%)
991. [CAI15569](#) Novel protein, 37 (12%)
992. [Q8NEK9_HUMAN](#) Chromosome 1 open reading frame 26, 37 (8%)
993. [Q9NRH2_HUMAN](#) HSNFRK protein, 37 (6%)
994. [Q9BWX1_HUMAN](#) NYD-SP6 (PHD finger protein 7, isoform 1), 37 (8%)

995. [Q5TCY1_HUMAN](#) OTTHUMP00000016425 (Fragment), 37 (7%)
 996. [Q6P4I8_HUMAN](#) IGHD protein, 37 (5%)
 997. [Q5Q9G7_HUMAN](#) Polymerase kappa isoform 2, 37 (8%)
 998. [Q8IZC6_HUMAN](#) Collagen XXVII proalpha 1 chain precursor
 (OTTHUMP00000021970), 37 (6%)
 999. [BAB15783](#) FLJ00098 protein, 37 (5%)
 1000. [Q75T46_HUMAN](#) SNAP-25-interacting protein, 37 (5%)
 1001. [CAI23322](#) ENPP3 protein, 37 (5%)
 1002. [Q96DI7_HUMAN](#) U5 snRNP-specific 40 kDa protein (HPrp8-binding)
 (HPRP8BP), 37 911%
 1003. [AAC51105](#) Human reelin, 37 (1%)
 1004. [Q14654_HUMAN](#) Insulin receptor substrate 4 (DA24A23.2), 37 (3%)
 1005. [Q9HCE3_HUMAN](#) KIAA1629 protein (Fragment), 37 (7%)
 1006. [AAF21944](#) Integrin alpha 10 subunit, 37 (2%)
 1007. [Q5T4T6_HUMAN](#) Novel protein similar to synaptonemal complex
 protein 2 (SYCP2), 37 (6%)
 1008. [Q5RGS1_HUMAN](#) Novel protein similar to cell recognition molecule
 CASPR3 (CASPR3) (Fragment), 37 (4%)
 1009. [AAB49993](#) N-methyl-D-aspartate receptor 2B subunit precursor, 37
 (5%)
 1010. [Q96S80_HUMAN](#) Transcription factor RFX4, 37 (10%)
 1011. [Q6IBF4_HUMAN](#) HMGCS2 protein, 37 (8%)
 1012. [JN0599](#) DNA-binding protein PO-GA , 37 (8%)
 1013. [Q94958_HUMAN](#) KIAA0882 protein (Fragment), 37 (5%)
 1014. [DMN_HUMAN](#) Desmuslin, 37 (5%)
 1015. [RASL1_HUMAN](#) RasGAP-activating-like protein 1, 37 (6%)
 1016. [Q7Z3Y0_HUMAN](#) Catenin, alpha 2, 37 (6%)
 1017. [KBTB3_HUMAN](#) Kelch repeat and BTB domain containing protein 3
 (BTB and kelch domain containing protein 3), 37 94%
 1018. [Q71U35_HUMAN](#) Transcription enhancer factor-5, 37 (12%)
 1019. [Q9UQ28_HUMAN](#) KIAA0722 protein (Fragment), 37 96%
 1020. [JC5785](#) ATP-dependent RNA helicase (EC 3.6.-.-), 37 (7%)
 1021. [Q6NUN1_HUMAN](#) Chromosome 13 open reading frame 11, 37 (5%)
 1022. [Q9UL68_HUMAN](#) Myelin transcription factor 1-like, 37 (6%)
 1023. [A57187](#) bumetanide-sensitive Na-K-Cl cotransporter, 37 (2%)
 1024. [CAI13886](#) N-acetylneuraminc acid synthase (sialic acid synthase), 37
 (15%)
 1025. [Q8IV81_HUMAN](#) Similar to splicing factor, arginine-serine-rich 8
 (Suppressor-of- white-apricot homolog, Drosophila) (Fragment), 37 (5%)
 1026. [Q86YL4_HUMAN](#) INM02, 36 (9%)
 1027. [CAC38936](#) Sequence 11 from Patent WO0131014, 36 (5%)
 1028. [Q6NXF1_HUMAN](#) VCY2IP1 protein (Fragment), 36 (4%)
 1029. [A35363](#) synapsin I splice form a, 36 (12%)
 1030. [ARHG4_HUMAN](#) Rho guanine nucleotide exchange factor 4 (APC-
 stimulated guanine nucleotide exchange factor) (Asef), 36 (9%)
 1031. [COG5_HUMAN](#) Conserved oligomeric Golgi complex component 5
 (13S Golgi transport complex 90 kDa subunit) (GTC-90) (Golgi transport
 complex 1), 36 (10%)

1032. [Q6P0M2 HUMAN](#) Translocase of outer mitochondrial membrane 70 homolog A, 36 (4%)
1033. [Q94836 HUMAN](#) KIAA0731 protein (Fragment), 36 (5%)
1034. [CAE48436](#) p162XLC helicase, 36 (2%)
1035. [Q5VSH6 HUMAN](#) Melanoma antigen, family D, 1, 36 (3%)
1036. [BAA11495](#) KIAA0178 protein, 36 (6%)
1037. [Q8TF02 HUMAN](#) P250R, 36 (6%)
1038. [Q6UW90 HUMAN](#) Sialyltransferase HI, 36 (6%)
1039. [Q6ZNL6 HUMAN](#) FLJ00274 protein (Fragment), 36 (4%)
1040. [Q5VUX1 HUMAN](#) OTTHUMP00000017386
(OTTHUMP00000040156), 36 (6%)
1041. [AAL57039](#) DNA cytosine methyltransferase 3 alpha, 36 (9%)
1042. [Q8TC96 HUMAN](#) AE2 protein, 36 (7%)
1043. [Q8WWV6 HUMAN](#) Fc alpha/mu receptor, 36 (5%)
1044. [Q76I76 HUMAN](#) Slingshot-2L, 36 (1%)
1045. [S69211](#) serine/threonine-specific protein kinase (EC 2.7.1.-), Rho-associated, 36 (7%)
1046. [CAD10523](#) Sequence 1 from Patent WO0177323, 36 (5%)
1047. [ZCC3 HUMAN](#) Zinc finger CCCH type domain containing protein 3, 36 (9%)
1048. [CAA44373](#) Human basement membrane heparan sulphate proteoglycan core protein, 36 (2%)
1049. [DAPK1 HUMAN](#) Death-associated protein kinase 1 (EC 2.7.1.37) (DAP kinase 1), 36 (5%)
1050. [S27257](#) methionine adenosyltransferase (EC 2.5.1.6) 2 alpha chain, 36 (5%)
1051. [Q9UPN4 HUMAN](#) KIAA1118 protein (Fragment), 36 (7%)
1052. [Q9HCD2 HUMAN](#) KIAA1640 protein (Fragment), 36 (5%)
1053. [Q9BTD3 HUMAN](#) Hole protein, 36 (9%)
1054. [Q9NS19 HUMAN](#) HHM protein (Cyclin D-type binding-protein 1) (CCNDBP1 protein), 36 (15%)
1055. [Q96B88 HUMAN](#) Zinc finger protein 37a (OTTHUMP00000046353), 36 (4%)

Gel chunks 10-13 (see figure 4.2)

1. [BAA23486](#) Polyubiquitin, 539 (12%)
2. [Q96H31 HUMAN](#) UBC protein (Fragment), 488 (13%)
3. [Q6FGD4 HUMAN](#) SMT3H1 protein, 411 (48%)
4. [Q96JJ2 HUMAN](#) KIAA1835 protein (Fragment), 358 (17%)
5. [HNRPC HUMAN](#) Heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNP C1 / hnRNP C2), 197 (18%)
6. [Q7KZS0 HUMAN](#) Ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast), 150 (30%)
7. [Q6PKD2 HUMAN](#) LOC343069 protein (Fragment), 86 (18%)
8. [Q6PK98 HUMAN](#) H2A histone family, member V, isoform 1 (Fragment), 64 (14%)

9. [Q59GV8_HUMAN](#) H2A histone family, member V isoform 1 variant (Fragment), 61 (20%)
10. [CAB35584](#) CALM2 P4 PROTEIN, 50 (16%)
11. [SYNE2_HUMAN](#) Nesprin 2 (Nuclear envelope spectrin repeat protein 2) (Syne-2) (Synaptic nuclear envelope protein 2) (Nucleus and actin connecting element protein) (NUANCE protein), 46 (<1%)
12. [Q5T911_HUMAN](#) OTTHUMP00000018392 (Fragment), 47 (1%)
13. [Q9HCF8_HUMAN](#) KIAA1614 protein (Fragment), 41 (2%)
14. [Q8WXI7_HUMAN](#) Ovarian cancer related tumor marker CA125, 41 (<1%)
15. [SYTL2_HUMAN](#) Synaptotagmin-like protein 2 (Exophilin 4), 40 (1%)
16. [Q9UHA8_HUMAN](#) Splicing coactivator subunit SRm300, 39 (1%)
17. [Q6P158_HUMAN](#) DHX57 protein (Fragment), 39 (2%)
18. [Q5H9A2_HUMAN](#) Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae), 39 (1%)
19. [Q7L3D5_HUMAN](#) KIAA0841 protein (Fragment), 38 (3%)
20. [Q5T9M9_HUMAN](#) M-phase phosphoprotein 1, 38 (2%)
21. [Q5TBT1_HUMAN](#) OTTHUMP00000040015 protein, 38 (1%)
22. [Q9P2D7_HUMAN](#) KIAA1410 protein (Fragment), 38 (1%)
23. [Q9UQ39_HUMAN](#) RNA binding protein (Fragment), 38 (2%)
24. [AAH30018](#) RNA binding motif protein, Y-linked, family 1, member F, 37 (5%)
25. [MMHUMH](#) laminin alpha-2 chain (fragment), 37 (1%)
26. [CNHUB](#) chromogranin B precursor [validated] , 36 (2%)
27. [CAC20413](#) Beta myosin heavy chain, 36 (2%)
28. [Q6P4C7_HUMAN](#) KRT8 protein (Fragment), 36 (6%)

**7.4 TAP-SUMO2 CELLS INFECTED WITH ADENOVIRUS TYPE 5
AT A M.O.I OF 10 FOR 24 HOURS (minus TAP cells uninfected data)**

Accession number, name of protein, overall Mascot score and % peptide coverage

Gel chunks 1-9 (see figure 4.6)

- 1.[AAC08313](#) General transcription factor 2-I, 2498 (61%)
- 2.[JC5300](#) Ran GTPase activator 1 – human, 2361 (63%)
- 3.[AAH04978](#) Tripartate motif-containing 28 protein (TRIM 28), 1372 (41%)
- 4.[Q5T5X7_HUMAN](#) OTTHUMP00000040500, 1051 (33%)
- 5.[AAD23914](#) Ubiquitin-like protein activating enzyme; sentrin activating enzyme, 732 (40%)
- 6.[AAF42836](#) Endoplasmic reticulum luminal Ca^{2+} binding protein grp78; BiP, 698 (64%)
- 7.[I65237](#) ubiquitin / ribosomal protein L40, cytosolic [validated] – rat, 686 (64%)
- 8.[BAA09860](#) Polyubiquitin, 657 (17%)
- 9.[Q5T8B6_HUMAN](#) OTTHUMP00000059759, 607 (33%)
- 10.[1C3TA](#) 1d8 ubiquitin mutant YES , 568 (94%)
- 11.[ROC_HUMAN](#) Heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNP C1 / hnRNP C2), 489 (33%)
- 12.[Q6DC95_HUMAN](#) Interferon regulatory factor 2 binding protein 1, 448 (29%)
- 13.[A27077](#) dnaK-type molecular chaperone , 419 (27%)
- 15.[Q6DSU6_HUMAN](#) GTF2I repeat domain containing protein 1, 413 (18%)
- 16.[AAF19786](#) General transcription factor 3, 412 (21%)
- 17.[Q5SP17_HUMAN](#) Heat shock 70kDa protein 1A, 381 (19%)
- 18.[AAC18697](#) Scaffold attachment factor B, 356 (17%)
- 19.[AAC00056](#) Hsp27-ERE-TATA binding protein, 342 (16%)
- 20.[BAA09487](#) KIAA0138 protein, 341 (14%)
- 21.[JC7555](#) C14orf4 protein – human, 334 (14%)
- 22.[A41137](#) heat shock transcription factor 1, 322 (16%)
- 23.[AAH00036](#) Small ubiquitin-like modifier protein 3, 307 (45%)
- 24.[S64732](#) scaffold attachment factor B 303 (17%)
- 25.[HHHU86](#) heat shock protein 90-alpha , 299 (10%)
- 26.[S52863](#) DNA-binding protein R kappa B, 294 (14%)
- 27.[HHHU27](#) heat shock protein 27 , 274 (40%)
- 28.[Q969M9_HUMAN](#) BRD8 protein, 274 (8%)
- 29.[CAA98022](#) Plakophilin, 273 (24%)
- 30.[Q96B34_HUMAN](#) Beta actin, 266 (34%)
- 31.[A25873](#) tubulin alpha chain (version 2) , 268 (39%)

- 32.[AAN16399](#) Thymine-DNA glycosylase, 246 (12%)
33.[KPYM_HUMAN](#) Pyruvate kinase, isozymes M1/M2 (EC 2.7.1.40)
(Pyruvate kinase muscle isozyme) (Cytosolic thyroid), 241 (23%)
34.[VIME_HUMAN](#) Vimentin, 239 (33%)
35.[JC4760](#) SMT3 protein , 232 (57%)
36.[T00034](#) SART-1 protein , 225 (17%)
37.[AAH43399](#) Ets variant gene 6, 225 (16%)
38.[1A5R](#) sumo-1, 214 (48%)
39.[Q8IWR2_HUMAN](#) Class II beta tubulin isotype, 213 (33%)
40.[Q8IWP6_HUMAN](#) Class IVb beta tubulin, 213 (31%)
41.[Q9UPF6_HUMAN](#) CD3e-associated protein, 203 (16%)
42.[Q5T8M7_HUMAN](#) Actin, alpha 1, skeletal muscle, 194 (21%)
43.[Q8IWI9_HUMAN](#) MGA protein (Fragment), 185 (9%)
44.[Q5TG72_HUMAN](#) Tripartite motif-containing 33, 183 (7%)
45.[ZCWC3_HUMAN](#) Zinc finger CW-type coiled-coil domain protein 3,
182 (14%)
46.[Q5RI17_HUMAN](#) Heterogeneous nuclear ribonucleoprotein U
(Scaffold attachment factor A) (Fragment), 180 (7%)
47.[HCC1_HUMAN](#) Nuclear protein Hcc-1 (HSPC316) (Proliferation
associated cytokine- inducible protein CIP29), 178 (13%)
48.[AAG28523](#) Adenocarcinoma antigen ART1, 163 (11%)
49.[UBHU5B](#) tubulin beta chain , 162 (28%)
50.[Q6EEV6_HUMAN](#) Small ubiquitin-like protein 4
(OTTHUMP00000040124), 161 (35%)
51.[A57198](#) splicing factor, arginine-serine-rich 7, 160 (28%)
52.[AAN60442](#) Nesprin-1, 154 (3%)
53.[LAP2B_HUMAN](#) Lamina-associated polypeptide 2, isoforms
beta/gamma (Thymopoietin, isoforms beta/gamma) (TP beta/g, 152 (13%)
54.[S09036](#) dnaK-type molecular chaperone HSPA6, 151 (8%)
55.[Q6EKI9_HUMAN](#) General transcription factor II i repeat domain 2, 150
(8%)
56.[Q9NZA0_HUMAN](#) FBP-interacting repressor (Fuse-binding protein-
interacting repressor, isoform b), 149 (19%)
57.[4PGTA](#) glutathione s-transferase (EC 2.5.1.18) mutant VAL 104
VARIANT naturally occurring variants of hgst, 148 (29%)
58.[CAI23317](#) Arginase, liver, 147 (28%)
59.[EFHU1](#) translation elongation factor eEF-1 alpha-1 chain, 146
(14%)
60.[CAA48197](#) DNA topoisomerase II, 144 (7%)
61.[A43700](#) BN51 protein, 144 (14%)
62.[Q719H9_HUMAN](#) Potassium channel tetramerization domain-
containing 1 (KCTD1 protein), 136 (30%)
63.[Q8IV76_HUMAN](#) PAS domain containing 1, 135 (8%)
64.[AAH10578](#) Threonyl-tRNA synthetase, 133 (6%)

- 65.Q7KZS0_HUMAN Ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast), 129 (3%)
- 66.A39579 c-myc promoter-binding protein MBP-1, 128 (19%)
- 67.Q5VY62_HUMAN DEAH (Asp-Glu-Ala-His) box polypeptide 9 (OTTHUMP00000060702), 125 (5%)
- 68.A47363 RNA helicase A, 120 (5%)
- 69.AAD10197 DNA recombination and repair protein, 119 (7%)
- 70.CAA09762 DNA topoisomerase (ATP-hydrolysing); topoisomerase II alpha, 118 (7%)
- 71.Q5VZL5_HUMAN Zinc finger protein 262 (OTTHUMP00000046188), 116 (4%)
- 72.Q5SYZ0_HUMAN Chaperonin containing TCP1, subunit 3 (Gamma), 115 (17%)
- 73.AAB63585 Transcription intermediary factor 1, 114 (8%)
- 74.AAA61281 Vimentin, 109 (34%)
- 75.Q5VT06_HUMAN OTTHUMP00000060646 (Novel protein), 109 (4%)
- 76.Q695C7_HUMAN Paracingulin, 107 (6%)
- 77.Q9H552_HUMAN OTTHUMP00000021786, 105 (5%)
- 78.AAH75846 IGHG1 protein, 101 (13%)
- 79.Q9BV61_HUMAN TRAP1 protein (Fragment), 101 (7%)
- 80.AAK57515 HBV pX protein 8 large isoform, 98 (6%)
- 81.Q5SQX9_HUMAN Novel protein similar to beta-tubulin 4Q (TUBB4Q) (LOC253936) (Fragment), 97 (15%)
- 82.CAA28420 Apo-B100 precursor, 97 (4%)
- 83.AAH75842 IGHG1 protein, 97 (13%)
- 84.Q5T9J7_HUMAN Spastic ataxia of Charlevoix-Saguenay (Sacsin), 97 (2%)
- 85.CAC14658 BA332A4.3 protein (Fragment), 96 (17%)
- 86.CAC49990 Sequence 22 from Patent WO0146261, 96 (10%)
- 87.AAH78670 IGHG1 protein, 95 (14%)
- 88.BAC04226 CDNA FLJ36487 fis, clone THYMU2017844, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 94 (13%)
- 89.BAC05203 CDNA FLJ40631 fis, clone THYMU2014777, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 93 (14%)
- 90.FNBP3_HUMAN Formin-binding protein 3 (Huntingtin yeast partner A) (Huntingtin- interacting protein HYPA/FBP11) , 92 (8%)
- 91.BAC05012 CDNA FLJ40035 fis, clone SYNOV2000173, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 90 (13%)
- 92.AAP15181 Forkhead winged/helix transcription factor mutant 2, 90 (13%)
- 93.VEHULA lamin A , 89 (9%)
- 94.JC5517 Gu/RNA helicase II binding protein, 87 (5%)
- 95.JC1087 RNA helicase, ATP-dependent, 87 (9%)
- 96.Q6PK08_HUMAN NOP5/NOP58 protein (Fragment), 86 (13%)

- 97.Q9BYF8_HUMAN SCCA1b, 85 (20%)
98-AAH15030 ZBED1 protein, 85 (7%)
99.A42184 nuclear mitotic apparatus protein NuMA, 85 (7%)
100.TVHUJB transforming protein jun-B, 84 (13%)
101.AAL33548 NUANCE (actin-binding protein associated with nuclear envelope), 84 (4%)
102.AAK48938 PIAS-NY protein, 82 (16%)
103.SJHUA spectrin alpha chain, 81 (4%)
104.Q8TD57_HUMAN Axonemal heavy chain dynein type 3, 79 (3%)
105.AAL33798 Nesprin-1 beta, 77 (3%)
106.AAG00912 Recombinant IgG4 heavy chain (Fragment), 77 (8%)
107.RL18_HUMAN 60S ribosomal protein L18, 76 (13%)
108.AAG13404 Topoisomerase II alpha-3, 75 (9%)
109.AAB41497 beta-tubulin, 75 (15%)
110.Q6FGQ8_HUMAN NFIL3 protein (Fragment), 73 (10%)
111.Q7Z406_HUMAN Myosin heavy chain, 73 (5%)
112.Q8WXI7_HUMAN Ovarian cancer related tumor marker CA125, 72 (1%)
113.Q9NPL4_HUMAN Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Fragment), 72 (24%)
114.AAB41132 Microtubule-associated protein 1a, 72 (6%)
115.TCPQ_HUMAN T-complex protein 1, theta subunit (TCP-1-theta) (CCT-theta), 71 (8%)
116.Q6PFW1_HUMAN KIAA0377 gene product, 71 (3%)
117.TVHUJN transcription factor AP-1, 70 (4%)
118.Q96AA2_HUMAN Obscurin, 70 (3%)
119.AAG01174 smarce 1-related protein, 70 (11%)
120.Q99968_HUMAN Tpr, 69 (3%)
121.AAC41758 Nucleoporin, 67 (1%)
122.Q8IXV2_HUMAN ATP5A1 protein (Fragment), 67 (9%)
123.Q7Z791_HUMAN TKT protein, 66 (12%)
124.AAH10381 Nuclear matrix protein p84, 66 (9%)
125.Q5TEM5_HUMAN Myosin VI, 65 (6%)
126.Q5THR3_HUMAN OTTHUMP00000028872, 65 (4%)
127.BAA78718 Centrosome- and golgi-localized PKN-associated protein (CG-NAP), 64 (3%)
128.Q7L576_HUMAN Cytoplasmic FMR1 interacting protein 1, 64 (4%)
129.Q5VUM2_HUMAN OTTHUMP00000040303 (Laminin, alpha 2) (Merosin, congenital muscular dystrophy), 64 (4%)
130.Q6P158_HUMAN DHX57 protein (Fragment), 63 (4%)
131.LAP2A_HUMAN Lamina-associated polypeptide 2 isoform alpha (Thymopoietin isoform alpha) (TP alpha) (Thymopoietin), 63 (4%)
132.Q96Q15_HUMAN Phosphatidylinositol 3-kinase-related protein kinase, 62 (3%)

- 133.Q5T7P4_HUMAN Involucrin, 62 (8%)
134.CAD23331 Sequence 1 from Patent WO200863, 62 (8%)
135.A48133 pre-mRNA splicing SRp75, 62 (7%)
136.GCC2_HUMAN GRIP and coiled-coil domain-containing protein 2
(Golgi coiled coil protein GCC185) (CTCL tumor ant), 61 (3%)
137.Q16716_HUMAN Pyruvate kinase PK-L isoenzyme, 61 (5%)
138.AAD00186 Envoplakin, 61 (4%)
139.CAD48779 Sequence 23 from Patent EP1229047, 61 (5%)
140.D40201 artifact-warning sequence (translated ALU class D), 61
(22%)
141.Q5W9F9_HUMAN KIAA0216 splice variant 2 (Fragment), 61 (4%)
142.Q5VWP1_HUMAN Ryanodine receptor 2 (Cardiac), 61 (2%)
143.Q8NF44_HUMAN FLJ00354 protein (Fragment), 60 (5%)
144.BAC77401 Putative MAPK activating protein, 60 (9%)
145.A33370 H+-transporting two-sector ATPase (EC 3.6.3.14) beta
chain precursor, mitochondrial, 59 (13%)
146.BAC15608 FELE-2, 59 (3%)
147.HSHUA1 histone H2A.1, 59 (21%)
148.Q6PJT4_HUMAN MSN protein (Fragment), 59 (9%)
149.Q6PIK4_HUMAN DIX domain containing 1, 59 (6%)
150.AAC07988 centrosomal Nek2—associated protein 1, 59 (5%)
151.A43358 macrophage capping protein, 58 (3%)
152.PDRN4_HUMAN PDZ domain containing RING finger protein 4
(Ligand of Numb-protein X 4) (SEMACAP3-like protein), 58 (4%)
153.PCNT2_HUMAN Pericentrin 2 (Pericentrin B) (Kendrin), 58 (2%)
154.BAA75062 apg-2, 58 (5%)
155.AAD21786 Similar to clagizzarin; similar to PID:g3115349, 57
(38%)
156.MBD1_HUMAN Methyl-CpG binding protein 1 (Methyl-CpG
binding domain protein 1) (Protein containing methyl-CpG-b), 57 (8%)
157.BAA05384 Type 2 inositol 1,4,5-trisphosphate receptor, 57 (4%)
158.Q9UPP3_HUMAN KIAA1109 protein (Fragment), 57 (6%)
159.AAC50662 Monocytic leukemia zinc finger protein, 57 (3%)
160.Q5T9M9_HUMAN M-phase phosphoprotein 1, 56 (3%)
161.S43692 transcription factor erm , 56 (8%)
162.Q5T2F1_HUMAN Neurofascin (NFASC) (Fragment), 57 (5%)
163.BAA13192 KIAA0201 protein, 56 (7%)
164.Q6ZML1_HUMAN FLJ00298 protein (Fragment), 56 (5%)
165.Q9P2D7_HUMAN KIAA1410 protein (Fragment), 56 (2%)
166.TPIS_HUMAN Triosephosphate isomerase (EC 5.3.1.1) (TIM)
(Triose-phosphate isomerase), 55 (6%)
167.Q8TEP3_HUMAN FLJ00150 protein (Fragment), 55 (3%)
168.CAD19369 Sequence 5 from Patent WO0181417, 55 (12%)
169.CAA02940 SEQUENCE 2 FROM PATENT WO9527070, 55 (5%)

- 170.Q5W0B2_HUMAN OTTHUMP0000016826, 55 (4%)
171.Q6PFW2_HUMAN ARHGEF11 protein (OTTHUMP0000060353), 54 (5%)
172.Q6FG89_HUMAN G22P1 protein, 54 (8%)
173.CAC22372 Sequence 1 from Patent WO0075319, 54 (8%)
174.Q5VZ08_HUMAN OTTHUMP0000044920, 54 (5%)
175.Q5VUA4_HUMAN OTTHUMP0000039830 (Zinc finger protein 318), 54 (3%)
176.AAH39612 MYO18A protein, 54 (3%)
177.O95996_HUMAN APCL protein, 53 (3%)
178.Q6IPN1_HUMAN APG16L protein (Fragment), 53 (7%)
179.Q6UXW4_HUMAN SSGL9393, 53 (11%)
180.Q6P9C6_HUMAN AT rich interactive domain 3A (BRIGHT-like) protein, 53 (9%)
181.PKD1_HUMAN Polycystin 1 precursor (Autosomal dominant polycystic kidney disease protein 1), 53 (2%)
182.CAD48781 Sequence 27 from Patent EP1229047, 53 (7%)
183.BAA86576 KIAA1262 protein, 53 (3%)
184.AAF87083 Tropomyosin isoform, 53 (29%)
185.Q9Y2J4_HUMAN Angiomotin like 2 (Fragment), 53 (6%)
186.EF1G_HUMAN Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) (PRO1608), 53 (7%)
187.Q9UPS0_HUMAN KIAA1082 protein (Fragment), 53 (3%)
188.CAC70712 Myosin heavy chain, 52 (6%)
189.Q6Q759_HUMAN PF6 (OTTHUMP0000059528), 52 (2%)
190.A46160 interferon alpha-induced transcription activator ISGF-3, 113K chain , 52 (5%)
191.TCPW_HUMAN T-complex protein 1, zeta-2 subunit (TCP-1-zeta-2) (CCT-zeta-2) (TCP- 1-zeta-like) (CCT-zeta-like) (Testis-specific Tcp20) (Testis-specific protein TSA303), 52 (5%)
192.CAC70714 Myosin heavy chain, 52 (6%)
193.CAI13123 PRP18 pre-mRNA processing factor 18 homolog, 52 (10%)
194.ITB4_HUMAN Integrin beta-4 precursor (GP150) (CD104 antigen), 52 (4%)
195.AAC51833 Ubiquitin specific protease 9, 52 (2%)
196.AAM19206 S100 calcium binding protein A14, 52 (10%)
197.Q9BYW2_HUMAN Huntingtin interacting protein 1 (HSPC069 isoform a), 51 (2%)
198.CSMSA peptidylprolyl isomerase (EC 5.2.1.8) A – mouse, 51 (21%)
199.Q5TZA2_HUMAN Ciliary rootlet coiled-coil, rootletin (OTTHUMP0000044629), 50 (4%)
200.Q5R206_HUMAN Carbamoylphosphate synthetase I, 50 (4%)

- 201.Q6R954_HUMAN Polymerase delta interacting protein 46, 50 (7%)
202.T08796 tropomyosin (fragment), 50 (18%)
203.AAH50321 TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, 50 (4%)
204.SFRS1_HUMAN Splicing factor, arginine-serine-rich 1 (pre-mRNA splicing factor SF2, P33 subunit) (Alternative sp), 50 (7%)
205.CAC69813 Sequence 6 from Patent WO0162928, 50 (5%)
206.Q8N4P9_HUMAN PAXIP1L protein, 50 (5%)
207.Q5W093_HUMAN OTTHUMP00000059187 (Programmed cell death 11), 50 (2%)
208.CAC22488 Sequence 24 from Patent WO0077179, 50 (4%)
209.Q99736_HUMAN HsGCN1 (Fragment), 50 (4%)
210.AAK52750 nuclear receptor transcription cofactor, 49 (1%)
211.AAF24980 voltage-gated sodium channel alpha subunit, alternate splice variant SCN12A-s, 49 (4%)
212.Q5TBA9_HUMAN Chromosome 13 open reading frame 14, 49 (3%)
213.Q9H4G2_HUMAN Novel protein (Fragment), 49 (3%)
214.AAD00702 Myosin heavy chain 12, 49 (5%)
215.Q69GU6_HUMAN Transcription factor GTF2IRD2 beta isoform 1, 49 (18%)
216.DOCK2_HUMAN Dediator of cytokinesis protein 2, 49 (1%)
217.Q7Z519_HUMAN Maid, 49 (7%)
218.NCOR1_HUMAN Nuclear receptor corepressor 1 (N-CoR1) (N-CoR), 49 (3%)
219.Q9UQB7_HUMAN Huntingtin, 49 (3%)
220.Q9P2N5_HUMAN KIAA1311 protein (Fragment), 48 (3%)
221.AAC96010 chaperonin containing t-complex polypeptide 1, delta subunit; CCT-delta, 48 (13%)
222.Q8NFX2_HUMAN PI-3-kinase ATX, 48 (2%)
223.Q6P189_HUMAN Minor histocompatibility antigen HA-1, 48 (2%)
224.Q96SE4_HUMAN DNA polymerase theta, 48 (3%)
225.AAK40350 DNA-dependant protein kinase, 48 (12%)
226.AAR02550 Immunoglobulin heavy chain variable region, 48 (25%)
227.AAD29948 Myosin heavy chain, 48 (4%)
228.Q6PJ32_HUMAN FLJ11806 protein (Fragment), 48 (5%)
229.Q5VUA6_HUMAN OTTHUMP00000046844 (InaD-like protein) (INADL), 48 (5%)
230.T08599 probable transcription factor CA150, 48 (2%)
231.AAM77722 Midasin, 48 (2%)
232.A24199 tropomyosin NM, skeletal muscle, 48 (22%)
233.JQ1348 carbamoyl-phosphate synthase (ammonia) (EC 6.3.4.16) precursor, 48 (4%)
234.Q7Z5Y2_HUMAN Rho-interacting protein 3, 47 (7%)
235.Q5VXD5_HUMAN Ankyrin 3, node of Ranvier (Ankyrin G), 47 (1%)

- 236.[AAC43042](#) trio isoform, 47 (4%)
237.[1B3OB](#) inosine monophosphate dehydrogenase 2 (EC 1.1.1.205), chain B (fragments), 47 (5%)
238.[CAA99729](#) RAD50 homologue hsRAD50, 47 (5%)
239.[BAC80154](#) Immunoglobulin gamma heavy chain, 47 (18%)
240.[Q5TCZ0_HUMAN](#) OTTHUMP00000059186, 46 (14%)
241.[Q6I9V9_HUMAN](#) LOC63929 protein, 46 (11%)
242.[Q9NR99_HUMAN](#) Adlican, 46 (6%)
243.[A59404](#) plectin [imported] , 46 (2%)
244.[CD2L2_HUMAN](#) PITSLRE serine/threonine-protein kinase CDC2L2 (EC 2.7.1.37) (Galactosyltransferase associated protein, 46 (5%)
245.[A59266](#) unconventional myosin-15, 46 (2%)
246.[CAB61345](#) Integrin beta 4, 46 (4%)
247.[Q5SYX5_HUMAN](#) Golgi autoantigen, golgin subfamily a, 2 (Fragment), 46 (3%)
248.[AAR02531](#) Immunoglobulin heavy chain variable region, 46 (24%)
249.[G59434](#) KIAA0411 protein [imported] , 46 (3%)
250.[Q5SQI3_HUMAN](#) Calmodulin-like 5, 46 (7%)
251.[T09551](#) ribosomal protein L4, 46 (8%)
252.[AAF78783](#) Myosin Vc, 46 (6%)
253.[2FB4H](#) Ig heavy chain V region (Fab fragment KOL), chain H , 45 (18%)
254.[Q9BS48_HUMAN](#) HSF2 protein (Hypothetical protein), 45 (21%)
255.[BAA20783](#) KIAA0325 protein, 45 (2%)
256.[Q9HCD1_HUMAN](#) KIAA1641 protein (Fragment), 45 (4%)
257.[Q96BI2_HUMAN](#) ARL6IP4 protein, 45 (14%)
258.[CAC34694](#) Sequence 1 from Patent WO0114414, 45 (4%)
259.[CAC38606](#) Sequence 229 from Patent WO0129221, 45 (3%)
260.[Q86VH0_HUMAN](#) KIF27C, 45 (5%)
261.[Q5VVU8_HUMAN](#) OTTHUMP00000059324 (Transcription termination factor, RNA polymerase II), 45 (6%)
262.[Q5VWI3_HUMAN](#) Nebulin-related anchoring protein, 45 (8%)
263.[Q8WXV8_HUMAN](#) Alpha 1 chain-like collagen COLA1L precursor (Collagen, type XXI, alpha 1), 44 (11%)
264.[O95001_HUMAN](#) WUGSC:H_267D11.3 protein (Fragment), 44 (4%)
265.[Q76L81_HUMAN](#) Chimeric MOZ-ASXH2 fusion protein, 44 (3%)
266.[A47212](#) transcription factor TMF, TATA element modulatory factor, 44 (5%)
267.[Q86VH1_HUMAN](#) KIF27B, 44 (5%)
268.[Q9H281_HUMAN](#) Serologically defined breast cancer antigen NY-BR-49 (Fragment), 44 (16%)
269.[Q6IC06_HUMAN](#) RFPL1 protein, 44 (7%)
270.[Q5T7X7_HUMAN](#) OTTHUMP00000018794 (OTTHUMP00000040880) (Fragment), 44 (5%)

- 271.[Q66GS8_HUMAN](#) Centrosome protein Cep290, 44 (5%)
272.[AAR02539](#) Immunoglobulin heavy chain variable region, 44 (15%)
273.[Q96PH3_HUMAN](#) Proliferation potential-related protein, 44 (3%)
274.[AAR32467](#) Immunoglobulin heavy chain variable region, 44 (29%)
275.[Q7KZ68_HUMAN](#) Putative M phase phosphoprotein (MPP1 (Fragment), 44 (8%)
276.[Q6PIC7_HUMAN](#) FLJ14503 protein (Fragment), 44 (6%)
277.[Q9ULU8_HUMAN](#) KIAA1121 protein (Fragment), 44 (6%)
278.[PIAS3_HUMAN](#) Protein inhibitor of activated STAT protein 3, 44 (3%)
279.[CAI13459](#) Novel protein, 44 (17%)
280.[BAA78533](#) Protein inhibitor of activated STAT3, 44 (3%)
281.[Q5T9S5_HUMAN](#) Sarcoma antigen NY-SAR-41 (NY-SAR-41), 44 (5%)
282.[1P1GA](#) macrophage migration inhibitory factor mutant P1G, chain A, 43 (17%)
283.[BAA22586](#) SCP-1, 43 (9%)
284.[BAB21832](#) KIAA1741 protein, 43 (4%)
285.[Q5VYQ4_HUMAN](#) OTTHUMP00000021403 (OTTHUMP00000046122), 43 (5%)
286.[Q8TB9_HUMAN](#) RIC3 protein, 43 (13%)
287.[BAA02054](#) Nucleotide pyrophosphatase, 43 (6%)
288.[Q9NZE6_HUMAN](#) BM-010, 43 (14%)
289.[AAR32484](#) Immunoglobulin heavy chain variable region, 43 (27%)
290.[A57169](#) [heparan sulfate]-glucosamine N-sulfotransferase (EC 2.8.2.8) , 43 (3%)
291.[Q8NEQ7_HUMAN](#) Interferon-inducible IFI 16, 43 (2%)
292.[BAA20836](#) KIAA0382 protein, 43 (5%)
293.[1FVEB](#) Fab fragment of humanized antibody 4d5, version 7, chain B, 43 (20%)
294.[AAR02524](#) Immunoglobulin heavy chain variable region, 43 (29%)
295.[Q5VVL9_HUMAN](#) Protein tyrosine phosphatase, receptor type, D, 43 (5%)
296.[AAC52012](#) SET-DOMAIN PROTEIN AND MARINER TRANSPOSON HSMAR1 TRANSPOSASE FUSION PROTEIN, 43 (7%)
297.[AAR00504](#) Immunoglobulin heavy chain, VH region, 43 (17%)
298.[AAK19936](#) IgG1 immunoglobulin heavy chain variable region (Fragment), 43 (22%)
299.[ROCK2_HUMAN](#) Rho-associated protein kinase 2 (EC 2.7.1.37) (Rho-associated, coiled- coil containing protein kinase, 42 (5%)
300.[MITF_HUMAN](#) Microphthalmia-associated transcription factor, 42 (3%)
301.[AAL62061](#) Bullous pemphigoid antigen 1 eA, 42 (2%)
302.[Q76D35_HUMAN](#) Nop132, 42 (4%)

- 303.[Q6IBF4_HUMAN](#) HMGCS2 protein, 42 (5%)
304.[AAD50325](#) RAD50-2 protein, 42 (5%)
305.[AAA58669](#) HRX, 42 (1%)
306.[BAA97670](#) Immunoglobulin gamma heavy chain (Fragment)
(OTTHUMP00000021970), 42 (15%)
307.[O60307_HUMAN](#) KIAA0561 protein (Fragment), 42 (3%)
308.[Q6K0P6_HUMAN](#) Interferon-inducible protein X beta 2 isoform
(IFIX), 42 (12%)
309.[Q5VZN0_HUMAN](#) OTTHUMP00000044227, 42 (9%)
310.[AAR32419](#) Immunoglobulin heavy chain variable region, 41 (28%)
311.[AAO22169](#) Immunoglobulin heavy chain variable and constant
region (Fragment), 41 (24%)
312.[A40201](#) artifact-warning sequence (translated ALU class A), 41
(12%)
313.[Q5TYR7_HUMAN](#) Hemicentin (FIBL-6), 41 (1%)
314.[Q6YMW8_HUMAN](#) Rac-GTP binding protein-like protein, 41 (8%)
315.[Q96I57_HUMAN](#) MYH7B protein (Fragment), 41 (6%)
316.[AAA52805](#) OMM protein (Ig gamma 3) heavy chain, 41 (7%)
317.[BAA31614](#) KIAA0639 protein, 41 (4%)
318.[AAL05571](#) SERPINB12, 41 (4%)
319.[1GC1H](#) antibody 17b antigen-binding fragment, fab cd4-induced
site on gp120, chain H, 41 (16%)
320.[Q6Y1F2_HUMAN](#) FHOS, 41 (2%)
321.[Q5T749_HUMAN](#) Novel protein, 41 (7%)
322.[BAA95526](#) ANKRD3, 41 (6%)
323.[Q8TB65_HUMAN](#) Cytochrome c oxidase subunit Va, (COX5A
protein), 41 (910%)
324.[AAF07045](#) A-kinase anchoring protein 220, 41 (3%)
325.[Q5T714_HUMAN](#) OTTHUMP00000060031, 41 (3%)
326.[S21424](#) nestin, 41 (3%)
327.[I38510](#) neuronal kinesin heavy chain, 41 (8%)
328.[CAC07506](#) Sequence 1 from Patent WO9960116, 41 (2%)
329.[DDX21_HUMAN](#) Nucleolar RNA helicase II (Nucleolar RNA
helicase Gu) (RH II/Gu) (DEAD-box protein 21), 41 (5%)
330.[Q5T655_HUMAN](#) Novel protein, 41 (5%)
331.[AAC05330](#) Cell adhesion kinase beta, 40 (4%)
332.[AAR32529](#) Immunoglobulin heavy chain variable region, 40 (25%)
333.[AAC72089](#) PITSLRE protein kinase beta SV12 isoform, 40 (113%)
334.[CAA55632](#) Endosomal protein, 40 (3%)
335.[FLNC_HUMAN](#) Filamin C (Gamma-filamin) (Filamin 2) (Protein
FLNc) (Actin-binding like protein) (ABP-L) (ABP-280), 40 (4%)
336.[MYH6_HUMAN](#) Myosin heavy chain, cardiac muscle alpha isoform
(MyHC-alpha), 40 (3%)
337.[AAR32426](#) Immunoglobulin heavy chain variable region, 40 (16%)

- 338.Q6KE87_HUMAN Acetyl-CoA carboxylase 2 (EC 6.4.1.2), 40 (1%)
339.AAP13528 rho/rac-interactin citron kinase, 40 (2%)
340.I39152 zinc finger protein, 40 (12%)
341.PEPL_HUMAN Periplakin (195 kDa cornified envelope precursor protein) (190 kDa paraneoplastic pemphigus antigen), 40 (2%)
342.ZN292_HUMAN Zinc finger protein 292, 40 (5%)
343.MATN4_HUMAN Matrilin-4 precursor, 40 (7%)
344.AAF03782 Traf2 and NCK interacting kinase, splice variant 1, 40 (5%)
345.Q6PHY0_HUMAN CABIN1 protein, 40 (1%)
346.1SAV annexin v mutant P13, P87, P119, P163, AND P248 SUBSTITUTED WITH thioproline (prs) - human (fragment), 40 (8%)
347.Q9Y5L9_HUMAN Transcriptional activator SRCAP, 40 (2%)
348.Q8IYF3_HUMAN Testis expressed sequence 11, isoform 2, 40 (7%)
349.AAH53903 SLIT-ROBO Rho GTPase-activating protein 1, 40 (4%)
350.AAF75772 Ubiquitin-conjugating BIR-domain enzyme APOLLON, 40 (1%)
351.AAB87381 CHD1, 40 (2%)
352.Q7Z5J5_HUMAN SNF2 histone linker PHD RING helicase, 39 (2%)
353.BAB15795 FLJ00115 protein, 39 (8%)
354.Q5T527_HUMAN Small proline-rich protein 1A, 39 (34%)
355.Q96PY5_HUMAN KIAA1902 protein (Fragment), 39 (3%)
356.DDX18_HUMAN ATP-dependent RNA helicase DDX18 (EC 3.6.1.-) (DEAD-box protein 18) (Myc-regulated DEAD-box protein) (MrDb), 39 (6%)
357.AAC39790 SP100-HMG nuclear autoantigen, 39 (2%)
358.AAF36817 Guanine nucleotide exchange factor, 39 (3%)
359.Q9UDT6_HUMAN KIAA0291; similar rodent cytoplasmic linker protein CLIP-115 and restin, 39 (4%)
360.BAA08260 Phosphodiesterase I alpha, 39 (5%)
361.Q8N4H8_HUMAN Smoothelin, isoform b, 39 (4%)
362.AAD20946 Silencing mediator of retinoic acid and thyroid hormone receptor alpha, 39 (3%)
363.Q6H969_HUMAN Myomesin 1, 39 (4%)
364.AAH25393 PSMA8 protein, 39 (15%)
365.CAC20413 beta-myosin heavy chain, 39 (5%)
366.I38155 DNA-binding regulatory factor X5 – human, 39 (12%)
367.AAC51331 CREB-binding protein, 39 (3%)
369.Q5TFG6_HUMAN OTTHUMP00000028769, 39 (4%)
370.CAC60121 Axonemal beta heavy chain dynein type 11, 39 (2%)
371.ABCA1_HUMAN ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cassette 1) (ABC-1) (Cholesterol efflux regulatory protein), 39 (2%)
372.AAF98175 ATP-binding cassette transporter 1, 39 (3%)

- 373.[Q5TIG6_HUMAN](#) Myeloid\lymphoid or mixed-lineage leukemia (Trithorax homolog, Drosophila)\; translocated to, 4, 38 (4%)
- 374.[CHSS3_HUMAN](#) Chondroitin sulfate synthase 3 (EC 2.4.1.175) (Glucuronosyl-N- acetylgalactosaminyl-proteoglycan 4, 38 (11%)
- 375.[TVHUFF](#) protein-tyrosine kinase (EC 2.7.1.112) fes/fps , 38 (5%)
- 376.[CAA04747](#) IMMUNOGLOBULIN IGG2 (FRAGMENT), 38 (34%)
- 377.[PDE6A_HUMAN](#) Rod cGMP-specific 3',5'-cyclic phosphodiesterase alpha-subunit (EC 3.1.4.17) (GMP-PDE alpha) (PDE V-B1), 38 (4%)
- 378.[DYH5_HUMAN](#) Ciliary dynein heavy chain 5 (Axonemal beta dynein heavy chain 5) (HL1), 38 (2%)
- 379.[AAH06536](#) Sympleki, 38 (1%)
- 380.[A61231](#) myosin heavy chain nonmuscle form A , 38 (2%)
- 381.[CAD61482](#) Sequence 132 from Patent WO02083898, 38 (20%)
- 382.[Q5ZL2_HUMAN](#) OTTHUMP00000040396, 38 (5%)
- 383.[BAA20770](#) KIAA0311 protein, 38 (4%)
- 384.[CAD33317](#) Sequence 44 from Patent WO0218424, 38 (3%)
- 385.[Q9H430_HUMAN](#) DJ756N5.1.1 (Continues in Em:AL133324 as dJ1161H23.3) (Fragment), 38 (4%)
- 386.[Q5TE18_HUMAN](#) OTTHUMP00000030379, 38 (2%)
- 387.[AAD29857](#) NAD+ ADP-ribosyltransferase 2, 38 (6%)
- 388.[SPTN4_HUMAN](#) Spectrin beta chain, brain 3 (Spectrin, non-erythroid beta chain 3) (Beta-IV spectrin), 38 (6%)
- 390.[Q6NZ98_HUMAN](#) HIST1H2BA protein (Fragment), 38 (26%)
- 391.[BAA82981](#) KIAA1029 protein, 38 (2%)
- 392.[A54971](#) protein-tyrosine-phosphatase (EC 3.1.3.48) PTPN13, nonreceptor type 13, splice form 1 [validated] , 38 (4%)
- 393.[Q5VXF6_HUMAN](#) Dediator of cyto-kinesis 1 (Fragment), 38 (3%)
- 394.[A43346](#) 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase (EC 3.1.4.11) beta-2, 38 (6%)
- 395.[CAA93625](#) All-1 protein, 38 (2%)
- 396.[BAA07536](#) PK-120 precursor, 38 (7%)
- 397.[A32611](#) beta-galactosidase (EC 3.2.1.23) precursor, 38 (6%)
- 398.[CAD33289](#) Sequence 16 from Patent WO0218424, 38 (20%)
- 399.[Q5VYL3_HUMAN](#) Asp (Abnormal spindle)-like, microcephaly associated (Drosophila), 38 (2%)
- 400.[Q86XK4_HUMAN](#) PLCL3 protein (Fragment), 37 (4%)
- 401.[CAC94536](#) Sequence 1 from Patent WO0170982, 37 (68%)
- 402.[MYH3_HUMAN](#) Myosin heavy chain, fast skeletal muscle, embryonic (Muscle embryonic myosin heavy chain) (SMHCE), 37 (4%)
- 403.[CAA67057](#) TCR JUNCTIONAL SEQUENCE (FRAGMENT), 37 (21%)
- 404.[Q9Y6X6_HUMAN](#) KIAA0865 protein (Fragment), 37 (3%)
- 405.[AAL85487](#) Zinc finger 298, 37 (5%)
- 406.[GAK_HUMAN](#) Cyclin G-associated kinase (EC 2.7.1.-), 37 (5%)

- 407.[Q9P2N0_HUMAN](#) KIAA1316 protein (Fragment), 37 (3%)
408.[CAD10594](#) Sequence 2 from Patent WO0162977, 37 (2%)
409.[Q5SY9_HUMAN](#) OTTHUMP00000020987
(OTTHUMP00000044319), 37 (5%)
410.[Q9ULM3_HUMAN](#) KIAA1197 protein (Fragment), 37 (3%)
411.[A45259](#) desmoyokin (fragments), 37 (6%)
412.[AAG16639](#) Helicase SMARCAD1, 37 (7%)
413.[Q8N137_HUMAN](#) LYST-interacting protein LIP8 (Hypothetical
protein DKFZp434I1519), 37 (4%)
414.[AAF80245](#) Filamin 2, 37 (5%)
415.[S45340](#) FKBP-rapamycin-associated protein (FRAP) , 37 (3%)
416.[Q9UPU7_HUMAN](#) KIAA1055 protein (Fragment), 37 (4%)
417.[B28096](#) line-1 protein ORF2, 37 (3%)
418.[Q6UWF7_HUMAN](#) Brush border, 37 (2%)
419.[AAD51797](#) Gag-Pro-Pol protein, 37 (3%)
420.[Q6IQ54_HUMAN](#) PTK7 protein tyrosine kinase 7, isoform a, 37 (4%)
421.[Q5VT08_HUMAN](#) OTTHUMP00000046616, 37 (1%)
422.[Q5VT94_HUMAN](#) Growth hormone inducible transmembrane protein,
37 (9%)
423.[CO1A2_HUMAN](#) Collagen alpha 2(I) chain precursor, 37 (4%)
424.[Q8TD98_HUMAN](#) MLL/AF4 fusion protein (Fragment), 37 (9%)
425.[S31975](#) 14-3-3 protein epsilon, renal – mouse, 37 (12%)
426.[Q68GC2_HUMAN](#) SIN3B long isoform, 37 (5%)
427.[S12788](#) transcription factor NF-IL6, 37 (10%)
428.[CAB65786](#) DJ269M15.1 (similar to peptidylprolyl isomerase
(cyclophilin)), 37 (15%)
429.[AAA52897](#) IMMUNOGLOBULIN HEAVY CHAIN PRECURSOR
(FRAGMENT), 37 (13%)
430.[AAD04629](#) PCAF-associated factor 400, 36 (3%)
431.[S69211](#) serine/threonine-specific protein kinase (EC 2.7.1.-), Rho-
associated, 36 (7%)
432.[A55575](#) ankyrin 3, long splice form , 36 91%)
433.[QFHUH](#) neurofilament triplet H protein, 36 (7%)
434.[Q96P05_HUMAN](#) Transient receptor potential channel 4 epsilon splice
variant (Transient receptor potential cation), 36 (6%)
435.[E973183](#) TETRANECTIN, 36 (6%)
436.[Q5U5L5_HUMAN](#) Jumonji, AT rich interactive domain 2 protein
(OTTHUMP00000016058), 36 (5%)
437.[Q9H1S7_HUMAN](#) BA56H7.1.2 (Nebulette protein (NEBL, actin-
binding Z-disc protein)), 36 (8%)
438.[AAH07557](#) F-box and leucine-rich repeat protein 20
439.[AAC51202](#) Armadillo repeat protein, 36 (1%)
440.[Q9UPP6_HUMAN](#) KIAA1106 protein (Fragment), 36 (3%)
441.[AAD28285](#) Bile salt export pump, 36 (7%)

- 442.[SIN3A_HUMAN](#) Paired amphipathic helix protein Sin3a, 36 (1%)
443.[CAE55985](#) PTX XI Eph/Elk/Eck orphan receptor family, 36 (3%)
444.[G3P1_HUMAN](#) Glyceraldehyde-3-phosphate dehydrogenase, muscle (EC 1.2.1.12) (GAPDH), 36 (11%)
445.[Q7Z4G5_HUMAN](#) K-Cl cotransporter KCC3a-X2M isoform, 36 (2%)
446.[Q9NS87_HUMAN](#) Kinesin-like protein 2, 36 (5%)
447.[AAN10118](#) cAMP-specific phosphodiesterase PDE4D7, 36 (5%)
448.[Q6P9G1_HUMAN](#) DHX57 protein, 36 (6%)
449.[Q6WRI0_HUMAN](#) Bone specific CMF608, 36 (3%)
450.[Q5T791_HUMAN](#) OTTHUMP00000016860 (Fragment), 35 (2%)
451.[AAA52025](#) creatine kinase M, 35 (10%)
452.[BAA82612](#) Neural adhesion molecule NB-3, 35 (5%)
453.[DDX3_HUMAN](#) DEAD-box protein 3 (Helicase-like protein 2) (HLP2) (DEAD-box, X isoform), 35 (2%)
454.[CAD32046](#) Sequence 1 from Patent WO0218544 (Fragment).- synthetic construct, 35 (3%)
455.[AAG33700](#) Tensin, 35 (4%)
456.[BAA11423](#) Multifunctional protein CAD, 35 (4%)
457.[O43598_HUMAN](#) RCL (OTTHUMP00000016424) (Putative c-Myc-responsive, isoform 1), 35 (14%)
458.[CAD43233](#) Chondroitin synthase 2, 35 (13%)
459.[Q8TES3_HUMAN](#) FLJ00120 protein (Fragment), 35 (5%)
460.[BAB17674](#) RFamide-related peptide precursor, 35 (5%)
461.[Q8TC68_HUMAN](#) MGC40042 protein (Fragment), 35 (7%)
462.[A30789](#) creatine kinase (EC 2.7.3.2) precursor, mitochondrial, 35 (6%)
463.[Q96E39_HUMAN](#) Kynurenine aminotransferase III (Novel protein similar to RNA binding motif protein, X-linked) (RBM), 35 (9%)
464.[Q8IVF4_HUMAN](#) KIAA2017 protein (Fragment), 35 (5%)
465.[I67630](#) protein-tyrosine-phosphatase (EC 3.1.3.48) PTPN13, nonreceptor type 13, splice form 3, 35 (4%)
466.[Q5VW08_HUMAN](#) OTTHUMP00000042245, 35 (5%)
467.[Q96HU2_HUMAN](#) SRPR protein, 35 (10%)
468.[Q9Y4D8_HUMAN](#) KIAA0614 protein (Fragment), 35 (2%)
469.[Q5QPB6_HUMAN](#) OTTHUMP00000031267, 35 91%)
470.[Q5TG00_HUMAN](#) OTTHUMP00000039711 (OTTHUMP00000017871), 35 (2%)
471.[O00370_HUMAN](#) Putative p150, 35 (2%)
472.[AAH21908](#) Zinc finger, DHHC domain containing 1, 35 (7%)
473.[Q6IEH8_HUMAN](#) Transcriptional regulator, 35 (2%)
474.[CAD69464](#) Sequence 867 from Patent EP1270724, 35 91%)
475.[AAK29402](#) Nucleolar protein GU2, 35 (10%)
476.[CHTOG_HUMAN](#) CH-TOG protein (Colonic and hepatic tumor over-expressed protein) (Ch- TOG protein), 35 92%)

- 477.Q5TAP6_HUMAN KIAA0266 protein, 35 (4%)
 478.Q8NET9_HUMAN Vanilloid receptor like 3 protein splice variant b, 35 (6%)
 479.AAH41700 RABEP1 protein, 35 (2%)
 480.SM1L2_HUMAN Structural maintenance of chromosome 1-like 2 protein (SMC1beta protein), 35 (5%)
 481.AAS85957 Immunoglobulin heavy chain, 35 (15%)
 482.I78879 retinoblastoma binding protein 2, 35 (3%)
 483.Q14828_HUMAN MG44 protein (Fragment), 35 (8%)
 484.CAA65265 Rox protein 35 (4%)
 485.Q9C0D5_HUMAN KIAA1728 protein (Fragment), 34 (4%)
 486.AAH07082 SMARCE 1 protein, 34 (3%)
 487.Q8WU85_HUMAN RBM4 protein (Hypothetical protein DKFZp547K0918), 34 (16%)
 488.Q5TCU3_HUMAN Tropomyosin 2 (Beta), 34 (17%)
 489.ALU3_HUMAN Alu subfamily SB1 sequence contamination warning entry, 34 (15%)
 490.Q5TGI4_HUMAN SAM domain containing 1, 34 (23%)
 491.MCHUNB calmodulin-related protein NB-1, 34 (10%)
 492.Q8WUG8_HUMAN MTB protein, 34 (5%)
 493.Q9C0D4_HUMAN KIAA1729 protein (Fragment), 34 (2%)
 494.AAH37964 DEAH (Asp-Glu-Ala-His) box polypeptide 37, 34 (5%)
 495.BAA92573 KIAA1335 protein, 34 (2%)
 496.E59435 myosin IXA [imported] , 34 (2%)
 497.Q5VZN1_HUMAN Novel DnaJ domain-containing protein (Fragment), 34 (10%)
 498.MLL3_HUMAN Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog (Histone- lysine N-methyltransferase, H3 lysine-4 specific MLL3) (EC 2.1.1.43) (Homologous to ALR protein), 34 (1%)
 499.Q8IW75_HUMAN Serine (Or cysteine) proteinase inhibitor, clade A (Alpha-1 antiproteinase, antitrypsin), member 12, 34 (9%)
 500.AAH40945 PCMTD2 protein, 34 (14%)
 501.Q9P2B8_HUMAN KIAA1429 protein (Fragment), 34 (3%)

Gel chunks 10-18 (see figure 4.6)

- 1.Q96JJ2_HUMAN KIAA1835 protein (Fragment), 709 (31%)
- 2.BAA09860 Polyubiquitin, 578 (12%)
- 3.Q96H31_HUMAN UBC protein (Fragment), 485 (13%)
- 4.1C3TA 1d8 ubiquitin mutant YES , 452 (94%)
- 5.AAH00036 Small ubiquitin-like modifier protein 3, 380 (48%)
- 6.Q96B34_HUMAN Beta actin, 340 (31%)
- 7.A57198 splicing factor, arginine-serine-rich 7 , 232 (28%)

- 8.[ROC_HUMAN](#) Heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNP C1 / hnRNP C2), 207 (25%)
- 9.[Q8IWP6_HUMAN](#) Class IVb beta tubulin, 206 (29%)
- 10.[Q719H9_HUMAN](#) Potassium channel tetramerization domain-containing 1 (KCTD1 protein), 200 (30%)
- 11.[R3HUS3](#) ribosomal protein S3, cytosolic , 192 (32%)
- 12.[H2BE_HUMAN](#) Histone H2B.e (H2B/e), 192 (54%)
- 13.[Q7KZS0_HUMAN](#) Ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast), 179 (30%)
- 14.[DEHULH](#) L-lactate dehydrogenase (EC 1.1.1.27) chain H, 179 (23%)
- 15.[Q6EEV6_HUMAN](#) Small ubiquitin-like protein 4 (OTTHUMP00000040124), 176 (26%)
- 16.[SFRS1_HUMAN](#) Splicing factor, arginine/serine-rich 1 (pre-mRNA splicing factor SF2, P33 subunit) (Alternative sp), 172 (25%)
- 17.[UBH5B](#) tubulin beta chain , 167 (21%)
- 18.[DEHULM](#) L-lactate dehydrogenase (EC 1.1.1.27) chain M, 164 (18%)
- 19.[ENOA_HUMAN](#) Alpha enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Non- neural enolase) (NNE) (Enolase), 163 (30%)
- 20.[Q8IWR2_HUMAN](#) Class II beta tubulin isotype, 147 (19%)
- 21.[A33370](#) H+-transporting two-sector ATPase (EC 3.6.3.14) beta chain precursor, mitochondrial, 142 (13%)
- 22.[R5RTL8](#) ribosomal protein L8, cytosolic [validated] – rat, 119 (13%)
- 23.[S00985](#) translation initiation factor eIF-4A II – mouse, 119 (10%)
- 24.[FIMS4A](#) translation initiation factor eIF-4A I – mouse, 118 (10%)
- 25.[Q8N5Z7_HUMAN](#) Ribosomal protein L6, 106 (15%)
- 26.[R5HU7A](#) ribosomal protein L7a, cytosolic, 106 (24%)
- 27.[AAK58567](#) RBMX, 105 (19%)
- 28.[Q5VWK1_HUMAN](#) Voltage-dependent anion channel 2, 98 (13%)
- 29.[MMHUP3](#) voltage-dependent anion channel 1 [validated] , 97 (28%)
- 30.[KIHUG](#) phosphoglycerate kinase (EC 2.7.2.3) , 96 (23%)
- 31.[RL18_HUMAN](#) 60S ribosomal protein L18, 96 (25%)
- 32.[BAA77335](#) IDN3, 94 (3%)
- 33.[AAA60288](#) Ribosomal protein S6, 93 (26%)
- 34.[T03829](#) transcription factor TFII-I , 92 (6%)
- 35.[CAI14804](#) FUS interacting protein (serine-arginine rich), 91 (17%)
- 36.[AAB41497](#) Beta-tubulin, 90 (12%)
- 37.[Q96E39_HUMAN](#) Kynurenine aminotransferase III (Novel protein similar to RNA binding motif protein, X-linked) (RBM), 87 (18%)
- 38.[PWHUA](#) H+-transporting two-sector ATPase (EC 3.6.3.14) alpha chain precursor , 87 (6%)
- 39.[AAA60287](#) Ribosomal protein S6, 85 (26%)

- 40.[G3P1_HUMAN](#) Glyceraldehyde-3-phosphate dehydrogenase, muscle (EC 1.2.1.12) (GAPDH), 84 (11%)
- 41.[S08228](#) ribosomal protein S2, cytosolic (fragment), 80 (15%)
- 42.[Q5SWL5_HUMAN](#) Eukaryotic translation elongation factor 1 alpha 1, 77 (11%)
- 43.[R5HU7](#) ribosomal protein L7, cytosolic , 76 (18%)
- 44.[JE0241](#) Ig kappa chain Am37 precursor , 75 (23%)
- 45.[R3RT8](#) ribosomal protein S8, cytosolic [validated] – rat, 72 (11%)
- 46.[Q6NZ55_HUMAN](#) Ribosomal protein L13, 71 (17%)
- 47.[AAH75846](#) proteasome activator subunit 1, 70 (10%)
- 48.[AAH04978](#) Tripartate motif-containing 28 protein, 69 (3%)
- 49.[AAD23914](#) ubiquitin-like activating enzyme; sentrin activating enzyme, 69 (8%)
- 50.[Q5VUM2_HUMAN](#) OTTHUMP00000040303 (Laminin, alpha 2) (Merosin, congenital muscular dystrophy), 68 (3%)
- 51.[BAC01761](#) Immunoglobulin kappa light chain VLJ region (Fragment), 67 (18%)
- 52.[BAC05014](#) CDNA FLJ40038 fis, clone SYNOV2000297, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 66 (12%)
- 53.[EFHUI1](#) translation elongation factor eEF-1 alpha-1 chain , 66 (10%)
- 54.[Q5T8U3_HUMAN](#) Ribosomal protein L7a (Fragment), 65 (23%)
- 55.[1IBRA](#) ran, chain A, 63 (30%)
- 56.[Q9P2D7_HUMAN](#) KIAA1410 protein (Fragment), 63 (2%)
- 57.[DEHULC](#) L-lactate dehydrogenase (EC 1.1.1.27) chain X , 62 (9%)
- 58.[Q5TBT0_HUMAN](#) Bullous pemphigoid antigen 1, 230\240kDa, 61 (2%)
- 59.[AAA02914](#) IGG PRECURSOR, 60 (10%)
- 60.[CAC49990](#) Sequence 22 from Patent WO0146261, 60 (9%)
- 61.[EFHUA2](#) translation elongation factor eEF-1 alpha-2 chain, 60 (6%)
- 62.[BPAEA_HUMAN](#) Bullous pemphigoid antigen 1, isoforms 6/9/10 (Trabeculin-beta) (Bullous pemphigoid antigen) (BPA) , 59 (2%)
- 63.[Q5TZA2_HUMAN](#) Ciliary rootlet coiled-coil, rootletin (OTTHUMP00000044629), 58 (4%)
- 64.[G59434](#) KIAA0411 protein [imported] , 58 (2%)
- 65.[BAC05012](#) CDNA FLJ40035 fis, clone SYNOV2000173, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 58 (10%)
- 66.[CAA73095](#) Golgi-associated microtubule-binding protein, 58 (6%)
- 67.[AAH14667](#) Similar to immunoglobulin heavy constant gamma 3 (G3m marker), 58 (9%)
- 68.[A45259](#) desmoyokin (fragments), 57 (4%)
- 69.[AAH73789](#) IGHG1 protein, 57 (14%)
- 70.[CAD42369](#) Sequence 11 from Patent WO0214368, 55 (17%)
- 71.[CAI12385](#) Kinesin family member 21B, 54 (4%)

- 72.Q5VT06_HUMAN OTTHUMP00000060646 (Novel protein), 54 (3%)
 73.AAB41132 Microtubule-associated protein 1a, 54 (4%)
 74.CAC70714 Myosin heavy chain, 53 (4%)
 75.AAD00186 Envoplakin, 53 (4%)
 76.BAB71634 CDNA FLJ25308 fis, clone SYN00939, highly similar to Ig gamma immunoglobulin heavy chain, 53 (7%)
 77.Q8TD57_HUMAN Axonemal heavy chain dynein type 3, 53 (3%)
 78.AAR32538 Immunoglobulin heavy chain variable region, 53 (23%)
 79.CU005_HUMAN Protein C21orf5, 53 (3%)
 80.HSHUA1 histone H2A.1, 52 (18%)
 81.AAR32470 Immunoglobulin heavy chain variable region, 52 (23%)
 82.CAI16903 Antigen identified by monoclonal antibody Ki-67, 52 (4%)
 83.AAH50321 TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, 52 (3%)
 84.MLL3_HUMAN Myeloid/lymphoid or mixed-lineage leukemia protein 3 homolog (Histone- lysine N-methyltransferase, 51 (1%)
 85.Q5VXD5_HUMAN Ankyrin 3, node of Ranvier (Ankyrin G), 51 (2%)
 86.PGK2_HUMAN Phosphoglycerate kinase, testis specific (EC 2.7.2.3), 51 (5%)
 87.PCLO_HUMAN Piccolo protein (Aczonin), 51 (1%)
 88.Q6P158_HUMAN DHX57 protein (Fragment), 51 (2%)
 89.CAD69623 Sequence 1187 from Patent EP1270724, 51 (19%)
 90.AAR02489 Immunoglobulin heavy chain variable region, 51 (21%)
 91.KAD2_HUMAN Adenylate kinase isoenzyme 2, mitochondrial (EC 2.7.4.3) (ATP-AMP transphosphorylase), 51 (18%)
 92.CAD34981 Sequence 289 from Patent WO0222660, 50 (5%)
 93.Q7L576_HUMAN Cytoplasmic FMR1 interacting protein 1, 50 (5%)
 94.JC5423 2-hydroxyacylsphingosine 1-beta-galactosyltransferase (EC 2.4.1.45), 50 (7%)
 95.Q5VW08_HUMAN OTTHUMP00000042245, 50 (3%)
 96.Q5T7K0_HUMAN OTTHUMP00000040616, 50 (6%)
 97.Q86YW0_HUMAN PLC-zeta, 49 (5%)
 98.AAR32440 Immunoglobulin heavy chain variable region, 49 (33%)
 99.AAS86025 Immunoglobulin heavy chain, 49 (21%)
 100.SJHUA spectrin alpha chain , 48 (3%)
 101.CAC70712 Myosin heavy chain, 48 (4%)
 102.CAC08835 Sequence 36 from Patent WO0006605, 48 (6%)
 103.AAC02903 eIF4GII, 48 (5%)
 104.Q5T583_HUMAN Filaggrin, 48 (2%)
 105.Q96L96_HUMAN Muscle alpha-kinase, 48 (2%)
 106.A56236 probable RNA helicase 1, 48 (9%)
 107.BAA37168 IgG heavy chain (Fragment), 48 (16%)
 108.CAD33443 Sequence 170 from Patent WO0218424, 47 (2%)

- 109.Q5T7Y7_HUMAN DKFZP547E1010 protein, 47 (40%)
110.AAC51833 Ubiquitin specific protease 9, 47 (1%)
111-AAF03881 IgG1 heavy chain (fragment), 47 (16%)
112.Q8IWV7_HUMAN Ubiquitin ligase E3 alpha-I, 47 (2%)
113.Q8WXI7_HUMAN Ovarian cancer related tumor marker CA125, 46 (<1%)
114.AAS86039 Immunoglobulin heavy chain, 46 (21%)
115.MCPH1_HUMAN Microcephalin, 46 (6%)
116.AAH14789 UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-like 2, 46 (4%)
117.A42184 nuclear mitotic apparatus protein NuMA , 46 (3%)
118.Q96JP6_HUMAN Guanine nucleotide exchange factor Lbc, 46 (1%)
119.CAA28420 apo-B100 precursor, 46 (1%)
120.Q5RGR9_HUMAN Novel protein similar to cell recognition molecule CASPR3 (CASPR3), 45 (3%)
121.AAL33548 NUANCE, 45 (2%)
122-AAF75772 ubiquitin-conjugating BIR-domain enzyme APOLLON, 45 (1%)
123.PDZK3_HUMAN PDZ domain containing protein 3 (PDZ domain containing protein 2) (Activated in prostate cancer pro, 44 (2%)
124.PLXB1_HUMAN Plexin B1 precursor (Semaphorin receptor SEP), 44 (3%)
125.I37360 phosphopyruvate hydratase (EC 4.2.1.11), lung , 44 (9%)
126.Q5SPY5_HUMAN OTTHUMP00000064733, 44 (1%)
127.AAR88375 anti-HIV-1 gp120 immunoglobulin 23e heavy chain, 44 (18%)
128.I38869 transcription factor NFX1 [imported] , 44 (4%)
129.AAR25661 ankyrin repeat-containing protein, 43 (2%)
130.1A5R sumo-1, 43 (33%)
131.A59404 plectin [imported], 43 (3%)
132.AAS85951 Immunoglobulin heavy chain, 43 (26%)
133.AAR32396 Immunoglobulin heavy chain variable region, 43 (25%)
134.AAR32529 Immunoglobulin heavy chain variable region, 43 (25%)
135.Q5VW20_HUMAN OTTHUMP00000046304 (Microtubule-actin crosslinking factor 1), 42 (2%)
136.AAR04774 Kinesin family member 21A, 42 (2%)
137.AAS85961 Immunoglobulin heavy chain, 42 (28%)
138.Q6RAS0_HUMAN Microcephalin (Fragment), 42 (9%)
139.E977534 H.SAPIENS PROTEIN FRAGMENT (PR14-5.3.3.1), 42 (2%)
140.ENOG_HUMAN Gamma enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Neural enolase) (Neuron-specific enolase, 42 (9%)
141.Q5THM4_HUMAN Chromosome 6 open reading frame 71, 41 (6%)
142.AAA19595 PITSLRE isoform PBETA2241 (6%)

- 143.Q5T9J7_HUMAN Spastic ataxia of Charlevoix-Saguenay (Sacsin), 41 (2%)
- 144.CAI19680 DKFZP547E1010 protein, 41 (23%)
- 145.AAR02560 Immunoglobulin heavy chain, 41 (25%)
- 146.AAR32446 Immunoglobulin heavy chain variable region, 41 (30%)
- 147.AAA58669 HRX, 41 (2%)
- 148.Q5SVH7_HUMAN Neuron navigator 1, 41 (4%)
- 149.R3HDM_HUMAN R3H domain protein 1, 41 (5%)
- 150.Q5THJ0_HUMAN PR domain containing 2, with ZNF domain (Fragment), 41 (5%)
- 151.AAB07496 leptin receptor, 41 (3%)
- 152.CAD33317 Sequence 44 from Patent WO0218424, 41 (4%)
- 153.BAA96000 KIAA1476 protein, 41 (1%)
- 154.Q8NGB0_HUMAN Seven transmembrane helix receptor
- 155.Q5R3M6_HUMAN OTTHUMP00000028958, 40 (3%)
- 156.Q5SYA9_HUMAN OTTHUMP00000046126
(OTTHUMP00000021391), 40 (2%)
- 157.AAC51202 armadillo repeat protein, 40 (3%)
- 158.Q8WY53_HUMAN PAD, 40 (6%)
- 159.Q5T791_HUMAN OTTHUMP00000016860 (Fragment), 40 (1%)
- 160.AAA85638 TFIIIC alpha subunit, 40 (3%)
- 161.AAF21616 Ig heavy chain (fragment), 40 (17%)
- 162.CAE11569 Sequence 9 from Patent WO03048202, 39 (1%)
- 163.S02392 alpha-2-macroglobulin receptor precursor, 39 (1%)
- 164.SYNE2_HUMAN Nesprin 2 (Nuclear envelope spectrin repeat protein 2) (Synaptic nuclear envelope protein , 39 (1%)
- 165.Q5VUP7_HUMAN Leukemia-associated protein with a CXXC domain, 39 (2%)
- 166.CAA69325 Mosaic protein LR11, 39 (2%)
- 167.Q6P4G7_HUMAN TRA@ protein, 39 (13%)
- 168.POK5_HUMAN HERV-K_19p13.11 provirus ancestral Pol protein (HERV-K113 Pol protein) [Includes: Reverse transcript, 39 (5%)
- 169.PSA7L_HUMAN Proteasome subunit alpha type 7-like (EC 3.4.25.1), 39 (6%)
- 170.Q6NSK0_HUMAN Serine/threonine kinase 10, 38 (<1%)
- 171.TP2B_HUMAN DNA topoisomerase II, beta isozyme (EC 5.99.1.3), 38 (4%)
- 172.Q5VZN6_HUMAN OTTHUMP00000018523
(OTTHUMP00000040824), 38 (1%)
- 173.PCNT2_HUMAN Pericentrin 2 (Pericentrin B) (Kendrin), 38 (2%)
- 174.AAF05687 Anti-phospholipid immunoglobulin heavy chain VH-D-J-C region (fragment), 38 (18%)
- 175.ALU3_HUMAN Alu subfamily SB1 sequence contamination warning entry, 38 (16%)

- 176.Q8WWS6_HUMAN DJ537K23.3 (KIAA0266 protein) (Fragment), 38 (3%)
177.Q5THR3_HUMAN OTTHUMP00000028872, 37 (3%)
178.Q5SY9_HUMAN OTTHUMP00000020987
(OTTHUMP00000044319), 37 (6%)
179.CAD01139 putative TRP cation channel, 37 (2%)
180.AAC51825 3-phosphoinositide dependent protein kinase-1, 37 (2%)
181.BAA20778 KIAA0320 protein, 37 (2%)
182.Q6GPH5_HUMAN Eukaryotic translation initiation factor 4B, 37 (5%)
183.AAL39000 Macrophin 1 isoform 2, 37 (1%)
184.Q5STU9_HUMAN HLA-B associated transcript 1, 37 (6%)
185.AAP13528 rho/rac-interacting citron kinase, 37 (3%)
186.Q9NZA0_HUMAN FBP-interacting repressor (Fuse-binding protein-interacting repressor, isoform b), 37 (4%)
187.AAG43364 MAK-related kinase, 37 (9%)
188.AAK19738 channel-kinase 1, 37 (3%)
189.Q5THQ1_HUMAN OTTHUMP00000028520, 37 (2%)
190.Q6PFW1_HUMAN KIAA0377 gene product, 37 (3%)
191.JC2444 ribosomal protein L24, cytosolic [validated] – rat, 37 (8%)
192.AAH03413 Nucleolar protein family A, member 1, 37 (13%)
193.AAC26019 alpha-tectorin, 37 (2%)
194.YLPM1_HUMAN YLP motif containing protein 1 (Nuclear protein ZAP3) (ZAP113), 37 (3%)
195.Q5TYR7_HUMAN Hemicentin (FIBL-6), 37 (1%)
196.Q13544_HUMAN Signaling inositol polyphosphate 5 phosphatase SIP-110, 37 (10%)
197.SYTL2_HUMAN Synaptotagmin-like protein 2 (Exophilin 4), 36 (2%)
198.AAD51793 GAG-PRO-POL-ENV PROTEIN, 36 (1%)
199.A35938 profilaggrin (fragments), 36 (1%)
200.CAA32530 Eosinophil preperoxidase (AA-127 to 575), 36 (6%)
201.Q7LAX7_HUMAN CKII beta binding protein 2, 36 (16%)
202.JC7687 nucleolar protein p40/Epstein-Barr virus nuclear antigen 1 binding protein 2 , 36 (3%)
203.CAC20419 a disintegrin-like and metalloprotease (replosin type), 36 (2%)
204.Q5T1U7_HUMAN OTTHUMP00000021972 (Fragment), 36 (7%)
205.A59266 unconventional myosin-15, 36 (<1%)
206.Q8NG37_HUMAN Microtubule affinity-regulating kinase-like1, 36 (5%)
207.BAA05384 Type 2 inositol 1,4,5-trisphosphate receptor, 36 (2%)
208.AAB38530 Plasma membrane calcium ATPase isoform 3x/b, 36 (4%)
209.RABE2_HUMAN Rab GTPase binding effector protein 2 (Rabaptin-

- 5beta), 35 (8%)
- 210.[DOC10_HUMAN](#) Dediator of cytokinesis protein 10 (Protein zizimin 3), 35 (2%)
- 211.[AAD29948](#) Myosin heavy chain, 35 (2%)
- 212.[Q9UPS0_HUMAN](#) KIAA1082 protein (Fragment), 35 (3%)
- 213.[T08711](#) gamma-adaptin homolog DKFZp564D066.1 , 35 (4%)
- 214.[POK20_HUMAN](#) HERV-K_3q27.3 provirus ancestral Pol protein [Includes: Reverse transcriptase (RT) (EC 2.7.7.49); R, 35 (4%)
- 215.[AAH45551](#) TSEN54 protein, 35 (4%)
- 216.[Q8NHY3_HUMAN](#) GAS2-related protein isoform beta, 35 (3%)
- 217.[Q5SQZ8_HUMAN](#) Thyroid hormone receptor interactor 8, 35 (3%)
- 218.[AAC51191](#) Skeletal muscle ryanodine receptor, 35 (1%)
- 219.[AAH09304](#) DDX27 protein, 35 (3%)
- 220.[AAA51758](#) apolipoprotein B-100, 35 (1%)
- 221.[CGHU2E](#) collagen alpha 2(XI) chain precursor (fragment), 35 (4%)
- 222.[O43149_HUMAN](#) KIAA0399 protein (Fragment), 34 (3%)
- 223.[AAR32522](#) Immunoglobulin heavy chain variable region, 34 (25%)
- 224.[AAR25662](#) ankyrin repeat-containing protein, 34 (2%)
- 225.[AAB04947](#) Inositol 1,4,5-trisphosphate receptor type 1, 34 (1%)
- 226.[D56695](#) transducin-like enhancer-of-split homolog TLE-3, 34 (3%)
- 227.[BAA20783](#) inositol 1,4,5-trisphosphate receptor type 1, 34 (1%)
- 228.[CAC38563](#) Sequence 143 from Patent WO0129221, 34 (9%)
- 229.[CAC33149](#) Sequence 54 from Patent WO0100826, 34 (4%)
- 230.[CGHU3A](#) collagen alpha 3(VI) chain precursor [validated] , 34 (1%)
- 231.[Q96CV2_HUMAN](#) ACCN2 protein (Fragment), 34 (4%)
- 232.[AAH00528](#) Arginyl-tRNA synthetase, 34 (7%)
- 233.[ALU2_HUMAN](#) Alu subfamily SB sequence contamination warning entry, 34 (10%)
- 234.[AAB08431](#) hGrb1Rbeta/hGrb10, 34 (3%)

7.5 PROTEINS IDENTIFIED IN ADENOVIRUS INFECTED TAP-SUMO2 CELLS BUT NOT IN UNINFECTED CELLS (minus TAP cells uninfected data)

Accession number, name of protein, overall Mascot score and % peptide coverage

Gel chunks 1-9

1. **AAF42836** Endoplasmic reticulum luminal Ca²⁺ binding protein grp78; BiP, 698 (64%)
2. **BAA09860** Polyubiquitin, 657 (17%)
3. **A27077** dnaK-type molecular chaperone , 419 (27%)
4. **AAF19786** General transcription factor 3, 412 (21%)
5. **Q5SP17_HUMAN** Heat shock 70kDa protein 1A, 381 (19%)
6. **HHU86** heat shock protein 90-alpha , 299 (10%)
7. **S52863** DNA-binding protein R kappa B, 294 (14%)
8. **CAA98022** Plakophilin, 273 (24%)
9. **Q96B34_HUMAN** Beta actin, 266 (34%)
10. **A25873** tubulin alpha chain (version 2) , 268 (39%)
11. **Q8IWR2_HUMAN** Class II beta tubulin isotype, 213 (33%)
12. **Q5T8M7_HUMAN** Actin, alpha 1, skeletal muscle, 194 (21%)
13. **HCC1_HUMAN** Nuclear protein Hcc-1 (HSPC316) (Proliferation associated cytokine- inducible protein CIP29), 178 (13%)
14. **Q6EEV6_HUMAN** Small ubiquitin-like protein 4 (OTTHUMP00000040124), 161 (35%)
15. **A57198** splicing factor, arginine-serine-rich 7, 160 (28%)
16. **AAN60442** Nesprin-1, 154 (3%)
17. **Q9NZA0_HUMAN** FBP-interacting repressor (Fuse-binding protein-interacting repressor, isoform b), 149 (19%)
18. **4PGTA** glutathione s-transferase (EC 2.5.1.18) mutant VAL 104 VARIANT naturally occurring variants of hgst, 148 (29%)
19. **CAI23317** Arginase, liver, 147 (28%)
20. **A43700** BN51 protein, 144 (14%)
21. **Q719H9_HUMAN** Potassium channel tetramerization domain-containing 1 (KCTD1 protein), 136 (30%)
22. **AAH10578** Threonyl-tRNA synthetase, 133 (6%)
23. **A47363** RNA helicase A, 120 (5%)
24. **AAD10197** DNA recombination and repair protein, 119 (7%)
25. **Q5SZY0_HUMAN** Chaperonin containing TCP1, subunit 3 (Gamma), 115 (17%)
26. **AAA61281** Vimentin, 109 (34%)
27. **Q9H552_HUMAN** OTTHUMP00000021786, 105 (5%)
28. **AAH75846** IGHG1 protein, 101 (13%)
29. **Q5SQX9_HUMAN** Novel protein similar to beta-tubulin 4Q

- (TUBB4Q) (LOC253936) (Fragment), 97 (15%)
30. [AAH75842](#) IGHG1 protein, 97 (13%)
31. [AAH78670](#) IGHG1 protein, 95 (14%)
32. [BAC04226](#) CDNA FLJ36487 fis, clone THYMU2017844, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 94 (13%)
33. [BAC05203](#) CDNA FLJ40631 fis, clone THYMU2014777, highly similar to Homo sapiens immunoglobulin lambda heavy chain, 93 (14%)
34. [BAC05012](#) CDNA FLJ40035 fis, clone SYNOV2000173, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 90 (13%)
35. [JC1087](#) RNA helicase, ATP-dependent, 87 (9%)
36. [Q9BYF8_HUMAN](#) SCCA1b, 85 (20%)
37. [AAH15030](#) ZBED1 protein, 85 (7%)
38. [TVHUJB](#) transforming protein jun-B, 84 (13%)
39. [AAK48938](#) PIAS-NY protein, 82 (16%)
40. [AAL33798](#) Nesprin-1 beta, 77 (3%)
41. [AAG00912](#) Recombinant IgG4 heavy chain (Fragment), 77 (8%)
42. [RL18_HUMAN](#) 60S ribosomal protein L18, 76 (13%)
43. [AAG13404](#) Topoisomerase II alpha-3, 75 (9%)
44. [Q6FGQ8_HUMAN](#) NFIL3 protein (Fragment), 73 (10%)
45. [Q9NPL4_HUMAN](#) Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Fragment), 72 (24%)
46. [TCPQ_HUMAN](#) T-complex protein 1, theta subunit (TCP-1-theta) (CCT-theta), 71 (8%)
47. [Q6PFW1_HUMAN](#) KIAA0377 gene product, 71 (3%)
48. [TVHUJN](#) transcription factor AP-1, 70 (4%)
49. [Q96AA2_HUMAN](#) Obscurin, 70 (3%)
50. [Q8IXV2_HUMAN](#) ATP5A1 protein (Fragment), 67 (9%)
51. [Q7Z791_HUMAN](#) TKT protein, 66 (12%)
52. [Q5TEM5_HUMAN](#) Myosin VI, 65 (6%)
53. [Q5T7P4_HUMAN](#) Involucrin, 62 (8%)
54. [CAD23331](#) Sequence 1 from Patent WO0200863, 62 (8%)
55. [A48133](#) pre-mRNA splicing SRp75, 62 (7%)
56. [GCC2_HUMAN](#) GRIP and coiled-coil domain-containing protein 2 (Golgi coiled coil protein GCC185) (CTCL tumor ant), 61 (3%)
57. [Q16716_HUMAN](#) Pyruvate kinase PK-L isoenzyme, 61 (5%)
58. [Q5W9F9_HUMAN](#) KIAA0216 splice variant 2 (Fragment), 61 (4%)
59. [BAC77401](#) Putative MAPK activating protein, 60 (9%)
60. [BAC15608](#) FELE-2, 59 (3%)
61. [Q6PJ4_HUMAN](#) MSN protein (Fragment), 59 (9%)
62. [Q6PIK4_HUMAN](#) DIX domain containing 1, 59 (6%)
63. [A43358](#) macrophage capping protein, 58 (3%)

64. [PDRN4_HUMAN](#) PDZ domain containing RING finger protein 4
 (Ligand of Numb-protein X 4) (SEMACAP3-like protein), 58 (4%)
 65. [PCNT2_HUMAN](#) Pericentrin 2 (Pericentrin B) (Kendrin), 58 (2%)
 66. [BAA75062](#) apg-2, 58 (5%)
 67. [AAD21786](#) Similar to clagizzarin; similar to PID:g3115349, 57
 (38%)
 68. [AAC50662](#) Monocytic leukemia zinc finger protein, 57 (3%)
 69. [S43692](#) transcription factor erm , 56 (8%)
 70. [Q5T2F1_HUMAN](#) Neurofascin (NFASC) (Fragment), 57 (5%)
 71. [BAA13192](#) KIAA0201 protein, 56 (7%)
 72. [Q6ZML1_HUMAN](#) FLJ00298 protein (Fragment), 56 (5%)
 73. [Q8TEP3_HUMAN](#) FLJ00150 protein (Fragment), 55 (3%)
 74. [CAD19369](#) Sequence 5 from Patent WO0181417, 55 (12%)
 75. [CAA02940](#) SEQUENCE 2 FROM PATENT WO9527070, 55
 (5%)
 76. [Q6PFW2_HUMAN](#) ARHGEF11 protein
 (OTTHUMP00000060353), 54 (5%)
 77. [Q6FG89_HUMAN](#) G22P1 protein, 54 (8%)
 78. [Q5VUA4_HUMAN](#) OTTHUMP00000039830 (Zinc finger protein
 318), 54 (3%)
 79. [AAH39612](#) MYO18A protein, 54 (3%)
 80. [Q6UXW4_HUMAN](#) SSGL9393, 53 (11%)
 81. [CAD48781](#) Sequence 27 from Patent EP1229047, 53 (7%)
 82. [AAF87083](#) Tropomyosin isoform, 53 (29%)
 83. [EF1G_HUMAN](#) Elongation factor 1-gamma (EF-1-gamma) (eEF-
 1B gamma) (PRO1608), 53 (7%)
 84. [Q9UPS0_HUMAN](#) KIAA1082 protein (Fragment), 53 3%)
 85. [Q6Q759_HUMAN](#) PF6 (OTTHUMP00000059528), 52 (2%)
 86. [A46160](#) interferon alpha-induced transcription activator ISGF-3,
 113K chain , 52 (5%)
 87. [TCPW_HUMAN](#) T-complex protein 1, zeta-2 subunit (TCP-1-zeta-
 2) (CCT-zeta-2) (TCP- 1-zeta-like) (CCT-zeta-like) (Testis-specific
 Tcp20) (Testis-specific protein TSA303), 52 (5%)
 88. [CAI13123](#) PRP18 pre-mRNA processing factor 18 homolog, 52
 (10%)
 89. [ITB4_HUMAN](#) Integrin beta-4 precursor (GP150) (CD104
 antigen), 52 (4%)
 90. [AAM19206](#) S100 calcium binding protein A14, 52 (10%)
 91. [Q9BYW2_HUMAN](#) Huntington interacting protein 1 (HSPC069
 isoform a), 51 (2%)
 92. [CSMSA](#) peptidylprolyl isomerase (EC 5.2.1.8) A – mouse, 51
 (21%)
 93. [Q5TZA2_HUMAN](#) Ciliary rootlet coiled-coil, rootletin
 (OTTHUMP00000044629), 50 (4%)

94. [Q6R954_HUMAN](#) Polymerase delta interacting protein 46, 50 (7%)
95. [T08796](#) tropomyosin (fragment), 50 (18%)
96. [AAH50321](#) TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, 50 (4%)
97. [SFRS1_HUMAN](#) Splicing factor, arginine/serine-rich 1 (pre-mRNA splicing factor SF2, P33 subunit) (Alternative sp), 50 (7%)
98. [CAC69813](#) Sequence 6 from Patent WO0162928, 50 (5%)
99. [Q5W093_HUMAN](#) OTTHUMP0000059187 (Programmed cell death 11), 50 (2%)
100. [AAF24980](#) voltage-gated sodium channel alpha subunit, alternate splice variant SCN12A-s, 49 (4%)
101. [Q5TBA9_HUMAN](#) Chromosome 13 open reading frame 14, 49 (3%)
102. [Q7Z519_HUMAN](#) Maid, 49 (7%)
103. [Q9P2N5_HUMAN](#) KIAA1311 protein (Fragment), 48 (3%)
104. [Q8NFX2_HUMAN](#) PI-3-kinase ATX, 48 (2%)
105. [Q6P189_HUMAN](#) Minor histocompatibility antigen HA-1, 48 (2%)
106. [AAK40350](#) DNA-dependant protein kinase, 48 (12%)
107. [AAR02550](#) Immunoglobulin heavy chain variable region, 48 (25%)
108. [Q6PJ32_HUMAN](#) FLJ11806 protein (Fragment), 48 (5%)
109. [Q5VUA6_HUMAN](#) OTTHUMP0000046844 (InaD-like protein) (INADL), 48 (5%)
110. [T08599](#) probable transcription factor CA150, 48 (2%)
111. [A24199](#) tropomyosin NM, skeletal muscle, 48 (22%)
112. [JQ1348](#) carbamoyl-phosphate synthase (ammonia) (EC 6.3.4.16) precursor, 48 (4%)
113. [Q7Z5Y2_HUMAN](#) Rho-interacting protein 3, 47 (7%)
114. [Q5VXD5_HUMAN](#) Ankyrin 3, node of Ranvier (Ankyrin G), 47 (1%)
115. [AAC43042](#) trio isoform, 47 (4%)
116. [CAA99729](#) RAD50 homologue hsRAD50, 47 (5%)
117. [Q6I9V9_HUMAN](#) LOC63929 protein, 46 (11%)
118. [CD2L2_HUMAN](#) PITSLRE serine/threonine-protein kinase CDC2L2 (EC 2.7.1.37) (Galactosyltransferase associated protein, 46 (5%)
119. [AAR02531](#) Immunoglobulin heavy chain variable region, 46 (24%)
120. [Q5SQI3_HUMAN](#) Calmodulin-like 5, 46 (7%)
121. [T09551](#) ribosomal protein L4, 46 (8%)
122. [2FB4H](#) Ig heavy chain V region (Fab fragment KOL), chain H , 45 (18%)
123. [Q9BS48_HUMAN](#) HSF2 protein (Hypothetical protein), 45 (21%)
124. [Q96BI2_HUMAN](#) ARL6IP4 protein, 45 (14%)

- 125.[Q86VH0_HUMAN](#) KIF27C, 45 (5%)
126.[Q5VVU8_HUMAN](#) OTTHUMP00000059324 (Transcription termination factor, RNA polymerase II), 45 (6%)
127.[Q5VWI3_HUMAN](#) Nebulin-related anchoring protein, 45 (8%)
128.[Q8WXV8_HUMAN](#) Alpha 1 chain-like collagen COLA1L precursor (Collagen, type XXI, alpha 1), 44 (11%)
129.[Q76L81_HUMAN](#) Chimeric MOZ-ASXH2 fusion protein, 44 (3%)
130.[A47212](#) transcription factor TMF, TATA element modulatory factor, 44 (5%)
131.[Q86VH1_HUMAN](#) KIF27B, 44 (5%)
132.[Q9H281_HUMAN](#) Serologically defined breast cancer antigen NY-BR-49 (Fragment), 44 (16%)
133.[Q6IC06_HUMAN](#) RFPL1 protein, 44 (7%)
134.[Q96PH3_HUMAN](#) Proliferation potential-related protein, 44 (3%)
135.[AAR32467](#) Immunoglobulin heavy chain variable region, 44 (29%)
136.[Q7KZ68_HUMAN](#) Putative M phase phosphoprotein (MPP1) (Fragment), 44 (8%)
138.[Q6PIC7_HUMAN](#) FLJ14503 protein (Fragment), 44 (6%)
139.[Q9ULU8_HUMAN](#) KIAA1121 protein (Fragment), 44 (6%)
140.[PIAS3_HUMAN](#) Protein inhibitor of activated STAT protein 3, 44 (3%)
141.[CAI13459](#) Novel protein, 44 (17%)
142.[BAA78533](#) Protein inhibitor of activated STAT3, 44 (3%)
143.[1P1GA](#) macrophage migration inhibitory factor mutant P1G, chain A, 43 (17%)
144.[BAA22586](#) SCP-1, 43 (9%)
145.[Q8TBJ9_HUMAN](#) RIC3 protein, 43 (13%)
146.[BAA02054](#) Nucleotide pyrophosphatase, 43 (6%)
147.[Q9NZE6_HUMAN](#) BM-010, 43 (14%)
148.[A57169](#) [heparan sulfate]-glucosamine N-sulfotransferase (EC 2.8.2.8), 43 (3%)
149.[Q8NEQ7_HUMAN](#) Interferon-inducible IFI 16, 43 (2%)
150.[BAA20836](#) KIAA0382 protein, 43 (5%)
151.[1FVEB](#) Fab fragment of humanized antibody 4d5, version 7, chain B, 43 (20%)
152.[AAR02524](#) Immunoglobulin heavy chain variable region, 43 (29%)
153.[AAC52012](#) SET-DOMAIN PROTEIN AND MARINER TRANSPOSON HSMAR1 TRANSPOSASE FUSION PROTEIN, 43 (7%)
154.[AAR00504](#) Immunoglobulin heavy chain, VH region, 43 (17%)
155.[AAK19936](#) IgG1 immunoglobulin heavy chain variable region (Fragment), 43 (22%)

- 156.[ROCK2_HUMAN](#) Rho-associated protein kinase 2 (EC 2.7.1.37) (Rho-associated, coiled- coil containing protein kinase, 42 (5%))
157.[MITF_HUMAN](#) Microphthalmia-associated transcription factor, 42 (3%)
158.[Q76D35_HUMAN](#) Nop132, 42 (4%)
159.[BAA97670](#) Immunoglobulin gamma heavy chain (Fragment)
160.(OTTHUMP00000021970), 42 (15%)
161.[O60307_HUMAN](#) KIAA0561 protein (Fragment), 42 (3%)
162.[Q6K0P6_HUMAN](#) Interferon-inducible protein X beta 2 isoform (IFIX), 42 (12%)
163.[Q5VZN0_HUMAN](#) OTTHUMP00000044227, 42 (9%)
164.[AAR32419](#) Immunoglobulin heavy chain variable region, 41 (28%)
165.[AAO22169](#) Immunoglobulin heavy chain variable and constant region (Fragment), 41 (24%)
166.[Q6YMW8_HUMAN](#) Rac-GTP binding protein-like protein, 41 (8%)
167.[Q96I57_HUMAN](#) MYH7B protein (Fragment), 41 (6%)
168.[AAA52805](#) OMM protein (Ig gamma 3) heavy chain, 41 (7%)
169.[BAA31614](#) KIAA0639 protein, 41 (4%)
170.[AAL05571](#) SERPINB12, 41 (4%)
171.[Q6Y1F2_HUMAN](#) FHOS, 41 (2%)
172.[Q5T749_HUMAN](#) Novel protein, 41 (7%)
173.[BAA95526](#) ANKRD3, 41 (6%)
174.[Q8TB65_HUMAN](#) Cytochrome c oxidase subunit Va, (COX5A protein), 41 910%)
175.[AAF07045](#) A-kinase anchoring protein 220, 41 (3%)
176.[S21424](#) nestin, 41 (3%)
177.[I38510](#) neuronal kinesin heavy chain, 41 (8%)
178.[CAC07506](#) Sequence 1 from Patent WO9960116, 41 (2%)
179.[AAR32529](#) Immunoglobulin heavy chain variable region, 40 (25%)
180.[MYH6_HUMAN](#) Myosin heavy chain, cardiac muscle alpha isoform (MyHC-alpha), 40 (3%)
181.[AAR32426](#) Immunoglobulin heavy chain variable region, 40 (16%)
182.[AAP13528](#) rho/rac-interactin citron kinase, 40 (2%)
183.[I39152](#) zinc finger protein, 40 (12%)
184.[PEPL_HUMAN](#) Periplakin (195 kDa cornified envelope precursor protein) (190 kDa paraneoplastic pemphigus antigen), 40 (2%)
185.[ZN292_HUMAN](#) Zinc finger protein 292, 40 (5%)
186.[MATN4_HUMAN](#) Matrilin-4 precursor, 40 (7%)
187.[AAF03782](#) Traf2 and NCK interacting kinase, splice variant 1, 40 (5%)

- 188.[Q8IYF3_HUMAN](#) Testis expressed sequence 11, isoform 2, 40 (7%)
189.[Q5T527_HUMAN](#) Small proline-rich protein 1A, 39 (34%)
190.[Q96PY5_HUMAN](#) KIAA1902 protein (Fragment), 39 (3%)
191.[DDX18_HUMAN](#) ATP-dependent RNA helicase DDX18 (EC 3.6.1.-) (DEAD-box protein 18) (Myc-regulated DEAD-box protein) (MrDb), 39 (6%)
192.[Q9UDT6_HUMAN](#) KIAA0291; similar rodent cytoplasmic linker protein CLIP-115 and restin, 39 (4%)
193.[BAA08260](#) Phosphodiesterase I alpha, 39 (5%)
194.[Q8N4H8_HUMAN](#) Smoothelin, isoform b, 39 (4%)
195.[AAH25393](#) PSMA8 protein, 39 (15%)
196.[ABCA1_HUMAN](#) ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cassette 1) (ABC-1) (Cholesterol efflux regulatory protein), 39 (2%)
197.[AAF98175](#) ATP-binding cassette transporter 1, 39 (3%)
198.[CHSS3_HUMAN](#) Chondroitin sulfate synthase 3 (EC 2.4.1.175) (Glucuronosyl-N- acetylgalactosaminyl-proteoglycan 4, 38 (11%)
199.[TVHUFF](#) protein-tyrosine kinase (EC 2.7.1.112) fes/fps , 38 (5%)
200.[CAA04747](#) IMMUNOGLOBULIN IGG2 (FRAGMENT), 38 (34%)
201.[CAD61482](#) Sequence 132 from Patent WO02083898, 38 (20%)
202.[Q5S2L2_HUMAN](#) OTTHUMP00000040396, 38 (5%)
203.[CAD33317](#) Sequence 44 from Patent WO0218424, 38 (3%)
204.[Q9H430_HUMAN](#) DJ756N5.1.1 (Continues in Em:AL133324 as dJ1161H23.3) (Fragment), 38 (4%)
205.[Q5TE18_HUMAN](#) OTTHUMP00000030379, 38 (2%)
206.[AAD29857](#) NAD+ ADP-ribosyltransferase 2, 38 (6%)
207.[Q6NZ98_HUMAN](#) HIST1H2BA protein (Fragment), 38 (26%)
208.[BAA82981](#) KIAA1029 protein, 38 (2%)
209.[A54971](#) protein-tyrosine-phosphatase (EC 3.1.3.48) PTPN13, nonreceptor type 13, splice form 1 [validated] , 38 (4%)
210.[Q5VXF6_HUMAN](#) Dedicator of cyto-kinesis 1 (Fragment), 38 (3%)
211.[A43346](#) 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase (EC 3.1.4.11) beta-2, 38 (6%)
212.[BAA07536](#) PK-120 precursor, 38 (7%)
213.[A32611](#) beta-galactosidase (EC 3.2.1.23) precursor, 38 (6%)
214.[CAD33289](#) Sequence 16 from Patent WO0218424, 38 (20%)
215.[Q86XK4_HUMAN](#) PLCL3 protein (Fragment), 37 (4%)
216.[CAC94536](#) Sequence 1 from Patent WO0170982, 37 (68%)
217.[MYH3_HUMAN](#) Myosin heavy chain, fast skeletal muscle, embryonic (Muscle embryonic myosin heavy chain) (SMHCE), 37 (4%)

- 218.Q9Y6X6_HUMAN KIAA0865 protein (Fragment), 37 (3%)
 219.GAK_HUMAN Cyclin G-associated kinase (EC 2.7.1.-), 37 (5%)
 220.Q9P2N0_HUMAN KIAA1316 protein (Fragment), 37 (3%)
 221.CAD10594 Sequence 2 from Patent WO0162977, 37 (2%)
 222.Q9ULM3_HUMAN KIAA1197 protein (Fragment), 37 (3%)
 223.AAG16639 Helicase SMARCAD1, 37 (7%)
 224.Q8N137_HUMAN LYST-interacting protein LIP8 (Hypothetical protein DKFZp434I1519), 37 (4%)
 225.S45340 FKBP-rapamycin-associated protein (FRAP) , 37 (3%)
 226.Q9UPU7_HUMAN KIAA1055 protein (Fragment), 37 (4%)
 227.Q6UWF7_HUMAN Brush border, 37 (2%)
 228.Q6IQ54_HUMAN PTK7 protein tyrosine kinase 7, isoform a, 37 (4%)
 229.Q5VT08_HUMAN OTTHUMP0000046616, 37 (1%)
 230.Q5VT94_HUMAN Growth hormone inducible transmembrane protein, 37 (9%)
 231.CO1A2_HUMAN Collagen alpha 2(I) chain precursor, 37 (4%)
 232.Q8TD98_HUMAN MLL/AF4 fusion protein (Fragment), 37 (9%)
 233.S31975 14-3-3 protein epsilon, renal – mouse, 37 (12%)
 234.CAB65786 DJ269M15.1 (similar to peptidylprolyl isomerase (cyclophilin)), 37 (15%)
 235.QFHUH neurofilament triplet H protein, 36 (7%)
 236.Q96P05_HUMAN Transient receptor potential channel 4 epsilon splice variant (Transient receptor potential cation), 36 (6%)
 237.E973183 TETRANECTIN, 36 (6%)
 238.Q5U5L5_HUMAN Jumonji, AT rich interactive domain 2 protein (OTTHUMP0000016058), 36 (5%)
 239.AAH07557 F-box and leucine-rich repeat protein 20
 240.Q9UPP6_HUMAN KIAA1106 protein (Fragment), 36 (3%)
 241.AAD28285 Bile salt export pump, 36 (7%)
 242.CAE55985 PTX XI Eph/Elk/Eck orphan receptor family, 36 (3%)
 243.G3P1_HUMAN Glyceraldehyde-3-phosphate dehydrogenase, muscle (EC 1.2.1.12) (GAPDH), 36 (11%)
 244.Q7Z4G5_HUMAN K-Cl cotransporter KCC3a-X2M i
 245.AAN10118 cAMP-specific phosphodiesterase PDE4D7, 36 (5%)
 246.Q6P9G1_HUMAN DHX57 protein, 36 (6%)
 247.Q6WRI0_HUMAN Bone specific CMF608, 36 (3%)
 248.AAA52025 creatine kinase M, 35 (10%)
 249.DDX3_HUMAN DEAD-box protein 3 (Helicase-like protein 2) (HLP2) (DEAD-box, X isoform), 35 (2%)
 250.BAA11423 Multifunctional protein CAD, 35 (4%)
 251.O43598_HUMAN RCL (OTTHUMP0000016424) (Putative c-Myc-responsive, isoform 1), 35 (14%)

- 252.[CAD43233](#) Chondroitin synthase 2, 35 (13%)
253.[Q8TES3_HUMAN](#) FLJ00120 protein (Fragment), 35 (5%)
254.[Q8TC68_HUMAN](#) MGC40042 protein (Fragment), 35 (7%)
255.[Q96E39_HUMAN](#) Kynurenine aminotransferase III (Novel protein similar to RNA binding motif protein, X-linked) (RBM), 35 (9%)
256.[Q8IVF4_HUMAN](#) KIAA2017 protein (Fragment), 35 (5%)
257.[I67630](#) protein-tyrosine-phosphatase (EC 3.1.3.48) PTPN13, nonreceptor type 13, splice form 3, 35 (4%)
259.[Q96HU2_HUMAN](#) SRPR protein, 35 (10%)
260.[Q5TG00_HUMAN](#) OTTHUMP00000039711 (OTTHUMP0000017871), 35 (2%)
261.[O00370_HUMAN](#) Putative p150, 35 (2%)
262.[AAH21908](#) Zinc finger, DHHC domain containing 1, 35 (7%)
263.[CAD69464](#) Sequence 867 from Patent EP1270724, 35 91%)
264.[AAK29402](#) Nucleolar protein GU2, 35 (10%)
265.[Q5TAP6_HUMAN](#) KIAA0266 protein, 35 (4%)
266.[Q8NET9_HUMAN](#) Vanilloid receptor like 3 protein splice variant b, 35 (6%)
267.[AAH41700](#) RABEP1 protein, 35 (2%)
268.[AAS85957](#) Immunoglobulin heavy chain, 35 (15%)
269.[I78879](#) retinoblastoma binding protein 2, 35 (3%)
270.[Q14828_HUMAN](#) MG44 protein (Fragment), 35 (8%)
271.[CAA65265](#) Rox protein 35 (4%)
272.[Q9C0D5_HUMAN](#) KIAA1728 protein (Fragment), 34 (4%)
273.[AAH07082](#) SMARCE 1 protein, 34 (3%)
274.[Q8WU85_HUMAN](#) RBM4 protein (Hypothetical protein DKFZp547K0918), 34 (16%)
275.[Q5TCU3_HUMAN](#) Tropomyosin 2 (Beta), 34 (17%)
276.[Q5TGI4_HUMAN](#) SAM domain containing 1, 34 (23%)
277.[MCHUNB](#) calmodulin-related protein NB-1, 34 (10%)
278.[Q8WUG8_HUMAN](#) MTB protein, 34 (5%)
279.[Q9C0D4_HUMAN](#) KIAA1729 protein (Fragment), 34 (2%)
280.[BAA92573](#) KIAA1335 protein, 34 (2%)
281.[E59435](#) myosin IXA [imported] , 34 (2%)
282.[Q5VZN1_HUMAN](#) Novel DnaJ domain-containing protein (Fragment), 34 (10%)
283.[Q8IW75_HUMAN](#) Serine (Or cysteine) proteinase inhibitor, clade A (Alpha-1 antiproteinase, antitrypsin), member 12, 34 (9%)
284.[AAH40945](#) PCMTD2 protein, 34 (14%)
285.[Q9P2B8_HUMAN](#) KIAA1429 protein (Fragment), 34 (3%)

Gel chunks 10-18

1. **Q96B34_HUMAN** Beta actin, 340 (31%)
2. **A57198** splicing factor, arginine/serine-rich 7 , 232 (28%)
3. **Q719H9_HUMAN** Potassium channel tetramerization domain-containing 1 (KCTD1 protein), 200 (30%)
4. **R3HUS3** ribosomal protein S3, cytosolic , 192 (32%)
5. **H2BE_HUMAN** Histone H2B.e (H2B/e), 192 (54%)
6. **DEHULH** L-lactate dehydrogenase (EC 1.1.1.27) chain H, 179 (23%)
7. **Q6EEV6_HUMAN** Small ubiquitin-like protein 4 (OTTHUMP00000040124), 176 (26%)
8. **SFRS1_HUMAN** Splicing factor, arginine/serine-rich 1 (pre-mRNA splicing factor SF2, P33 subunit) (Alternative sp), 172 (25%)
9. **DEHULM** L-lactate dehydrogenase (EC 1.1.1.27) chain M, 164 (18%)
10. **ENOA_HUMAN** Alpha enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Non- neural enolase) (NNE) (Enolase), 163 (30%)
11. **Q8IWR2_HUMAN** Class II beta tubulin isotype, 147 (19%)
12. **R5RTL8** ribosomal protein L8, cytosolic [validated] – rat, 119 (13%)
13. **S00985** translation initiation factor eIF-4A II – mouse, 119 (10%)
14. **FIMS4A** translation initiation factor eIF-4A I – mouse, 118 (10%)
15. **Q8N5Z7_HUMAN** Ribosomal protein L6, 106 (15%)
16. **R5HU7A** ribosomal protein L7a, cytosolic, 106 (24%)
17. **AAK58567** RBMX, 105 (19%)
18. **Q5VWK1_HUMAN** Voltage-dependent anion channel 2, 98 (13%)
19. **MMHUP3** voltage-dependent anion channel 1 [validated] , 97 (28%)
20. **KIHUG** phosphoglycerate kinase (EC 2.7.2.3) , 96 (23%)
21. **RL18_HUMAN** 60S ribosomal protein L18, 96 (25%)
22. **BAA77335** IDN3, 94 (3%)
23. **AAA60288** Ribosomal protein S6, 93 (26%)
24. **T03829** transcription factor TFII-I , 92 (6%)
25. **CAI14804** FUS interacting protein (serine-arginine rich), 91 (17%)
26. **Q96E39_HUMAN** Kynurene aminotransferase III (Novel protein similar to RNA binding motif protein, X-linked) (RBM), 87 (18%)
27. **AAA60287** Ribosomal protein S6, 85 (26%)
28. **G3P1_HUMAN** Glyceraldehyde-3-phosphate dehydrogenase, muscle (EC 1.2.1.12) (GAPDH), 84 (11%)
29. **S08228** ribosomal protein S2, cytosolic (fragment), 80 (15%)

30. [Q5SWL5_HUMAN](#) Eukaryotic translation elongation factor 1 alpha 1, 77 (11%)
31. [R5HU7](#) ribosomal protein L7, cytosolic , 76 (18%)
32. [JE0241](#) Ig kappa chain Am37 precursor , 75 (23%)
33. [R3RT8](#) ribosomal protein S8, cytosolic [validated] – rat, 72 (11%)
34. [Q6NZ55_HUMAN](#) Ribosomal protein L13, 71 (17%)
35. [AAH75846](#) proteasome activator subunit 1, 70 (10%)
36. [BAC01761](#) Immunoglobulin kappa light chain VLJ region (Fragment), 67 (18%)
37. [BAC05014](#) CDNA FLJ40038 fis, clone SYNOV2000297, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 66 (12%)
38. [Q5T8U3_HUMAN](#) Ribosomal protein L7a (Fragment), 65 (23%)
39. [1IBRA](#) ran, chain A, 63 (30%)
40. [DEHULC](#) L-lactate dehydrogenase (EC 1.1.1.27) chain X , 62 (9%)
41. [Q5TBT0_HUMAN](#) Bullous pemphigoid antigen 1, 230\240kDa, 61 (2%)
42. [AAA02914](#) IGG PRECURSOR, 60 (10%)
43. [BPAEA_HUMAN](#) Bullous pemphigoid antigen 1, isoforms 6/9/10 (Trabeculin-beta) (Bullous pemphigoid antigen) (BPA) , 59 (2%)
44. [Q5TZA2_HUMAN](#) Ciliary rootlet coiled-coil, rootletin (OTTHUMP00000044629), 58 (4%)
45. [BAC05012](#) CDNA FLJ40035 fis, clone SYNOV2000173, highly similar to Human (hybridoma H210) anti-hepatitis A Ig, 58 (10%)
46. [CAA73095](#) Golgi-associated microtubule-binding protein, 58 (6%)
47. [AAH14667](#) Similar to immunoglobulin heavy constant gamma 3 (G3m marker), 58 (9%)
48. [CAD42369](#) Sequence 11 from Patent WO0214368, 55 (17%)
49. [CAI12385](#) Kinesin family member 21B, 54 (4%)
50. [BAB71634](#) CDNA FLJ25308 fis, clone SYN00939, highly similar to Ig gamma immunoglobulin heavy chain, 53 (7%)
51. [AAR32538](#) Immunoglobulin heavy chain variable region, 53 (23%)
52. [AAR32470](#) Immunoglobulin heavy chain variable region, 52 (23%)
53. [CAI16903](#) Antigen identified by monoclonal antibody Ki-67, 52 (4%)
54. [AAH50321](#) TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1, 52 (3%)
55. [Q5VXD5_HUMAN](#) Ankyrin 3, node of Ranvier (Ankyrin G), 51 (2%)
56. [PGK2_HUMAN](#) Phosphoglycerate kinase, testis specific (EC

- 2.7.2.3), 51 (5%)
57. [AAR02489](#) Immunoglobulin heavy chain variable region, 51 (21%)
58. [KAD2_HUMAN](#) Adenylate kinase isoenzyme 2, mitochondrial (EC 2.7.4.3) (ATP-AMP transphosphorylase), 51 (18%)
59. [CAD34981](#) Sequence 289 from Patent WO0222660, 50 (5%)
60. [JC5423](#) 2-hydroxyacylsphingosine 1-beta-galactosyltransferase (EC 2.4.1.45), 50 (7%)
61. [Q5T7K0_HUMAN](#) OTTHUMP00000040616, 50 (6%)
62. [Q86YW0_HUMAN](#) PLC-zeta, 49 (5%)
63. [AAR32440](#) Immunoglobulin heavy chain variable region, 49 (33%)
64. [CAC08835](#) Sequence 36 from Patent WO0006605, 48 (6%)
65. [AAC02903](#) eIF4GII, 48 (5%)
66. [BAA37168](#) IgG heavy chain (Fragment), 48 (16%)
67. [Q5T7Y7_HUMAN](#) DKFZP547E1010 protein, 47 (40%)
68. [AAF03881](#) IgG1 heavy chain (fragment), 47 (16%)
69. [Q8IWV7_HUMAN](#) Ubiquitin ligase E3 alpha-I, 47 (2%)
70. [MCPH1_HUMAN](#) Microcephalin, 46 (6%)
71. [AAH14789](#) UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-like 2, 46 (4%)
72. [Q96JP6_HUMAN](#) Guanine nucleotide exchange factor Lbc, 46 (1%)
73. [PDZK3_HUMAN](#) PDZ domain containing protein 3 (PDZ domain containing protein 2) (Activated in prostate cancer pro, 44 (2%)
74. [I37360](#) phosphopyruvate hydratase (EC 4.2.1.11), lung , 44 (9%)
75. [Q5SPY5_HUMAN](#) OTTHUMP00000064733, 44 (1%)
76. [AAR88375](#) anti-HIV-1 gp120 immunoglobulin 23e heavy chain, 44 (18%)
77. [I38869](#) transcription factor NFX1 [imported] , 44 (4%)
78. [AAR32396](#) Immunoglobulin heavy chain variable region, 43 (25%)
79. [AAR32529](#) Immunoglobulin heavy chain variable region, 43 (25%)
80. [Q6RAS0_HUMAN](#) Microcephalin (Fragment), 42 (9%)
81. [E977534](#) H.SAPIENS PROTEIN FRAGMENT (PR14-5.3.3.1), 42 (2%)
82. [ENOG_HUMAN](#) Gamma enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (Neural enolase) (Neuron-specific enolase, 42 (9%)
83. [Q5THM4_HUMAN](#) Chromosome 6 open reading frame 71, 41 (6%)
84. [AAA19595](#) PITSLRE isoform PBETA2241 (6%)
85. [CAI19680](#) DKFZP547E1010 protein, 41 (23%)
86. [AAR02560](#) Immunoglobulin heavy chain, 41 (25%)
87. [Q5SVH7_HUMAN](#) Neuron navigator 1, 41 (4%)

- 88.[R3HDM_HUMAN](#) R3H domain protein 1, 41 (5%)
89.[Q5THJ0_HUMAN](#) PR domain containing 2, with ZNF domain (Fragment), 41 (5%)
90.[AAB07496](#) leptin receptor, 41 (3%)
91.[CAD33317](#) Sequence 44 from Patent WO0218424, 41 (4%)
92.[BAA96000](#) KIAA1476 protein, 41 (1%)
93.[Q8NGB0_HUMAN](#) Seven transmembrane helix receptor
94.[Q8WY53_HUMAN](#) PAD, 40 (6%)
95.[AAA85638](#) TFIIIC alpha subunit, 40 (3%)
96.[CAE11569](#) Sequence 9 from Patent WO03048202, 39 (1%)
97.[S02392](#) alpha-2-macroglobulin receptor precursor, 39 (1%)
98.[CAA69325](#) Mosaic protein LR11, 39 (2%)
99.[Q6P4G7_HUMAN](#) TRA@ protein, 39 (13%)
100.[PSA7L_HUMAN](#) Proteasome subunit alpha type 7-like (EC 3.4.25.1), 39 (6%)
101.[TP2B_HUMAN](#) DNA topoisomerase II, beta isozyme (EC 5.99.1.3), 38 (4%)
102.[Q5VZN6_HUMAN](#) OTTHUMP00000018523 (OTTHUMP00000040824), 38 (1%)
103.[PCNT2_HUMAN](#) Pericentrin 2 (Pericentrin B) (Kendrin), 38 (2%)
104.[AAF05687](#) Anti-phospholipid immunoglobulin heavy chain VH-D-J-C region (fragment), 38 (18%)
105.[AAC51825](#) 3-phosphoinositide dependent protein kinase-1, 37 (2%)
106.[BAA20778](#) KIAA0320 protein, 37 (2%)
107.[Q6GPH5_HUMAN](#) Eukaryotic translation initiation factor 4B, 37 (5%)
108.[AAL39000](#) Macrophin 1 isoform 2, 37 (1%)
109.[Q5STU9_HUMAN](#) HLA-B associated transcript 1, 37 (6%)
110.[AAP13528](#) rho/rac-interacting citron kinase, 37 (3%)
111.[AAG43364](#) MAK-related kinase, 37 (9%)
112.[Q6PFW1_HUMAN](#) KIAA0377 gene product, 37 (3%)
113.[JC2444](#) ribosomal protein L24, cytosolic [validated] – rat, 37 (8%)
114.[AAH03413](#) Nucleolar protein family A, member 1, 37 (13%)
115.[AAC26019](#) alpha-tectorin, 37 (2%)
116.[YLPM1_HUMAN](#) YLP motif containing protein 1 (Nuclear protein ZAP3) (ZAP113), 37 (3%)
117.[Q13544_HUMAN](#) Signaling inositol polyphosphate 5 phosphatase SIP-110, 37 (10%)
118.[A35938](#) profilaggrin (fragments), 36 (1%)
119.[CAA32530](#) Eosinophil preperoxidase (AA-127 to 575), 36 (6%)
120.[Q7LAX7_HUMAN](#) CKII beta binding protein 2, 36 (16%)
121.[JC7687](#) nucleolar protein p40/Epstein-Barr virus nuclear

- antigen 1 binding protein 2 , 36 (3%)
- 122.[CAC20419](#) a disintegrin-like and metalloprotease (replosin type), 36 (2%)
- 123.[Q5T1U7_HUMAN](#) OTTHUMP00000021972 (Fragment), 36 (7%)
- 124.[Q8NG37_HUMAN](#) Microtubule affinity-regulating kinase-like1, 36 (5%)
- 125.[AAB38530](#) Plasma membrane calcium ATPase isoform 3x/b, 36 (4%)
- 126.[RABE2_HUMAN](#) Rab GTPase binding effector protein 2 (Rabaptin-5beta), 35 (8%)
- 128.[DOC10_HUMAN](#) Dedicator of cytokinesis protein 10 (Protein zizimin 3), 35 (2%)
- 129.[Q9UPS0_HUMAN](#) KIAA1082 protein (Fragment), 35 (3%)
- 130.[T08711](#) gamma-adaptin homolog DKFZp564D066.1 , 35 (4%)
- 131.[POK20_HUMAN](#) HERV-K_3q27.3 provirus ancestral Pol protein [Includes: Reverse transcriptase (RT) (EC 2.7.7.49); R, 35 (4%)
- 132.[AAH45551](#) TSEN54 protein, 35 (4%)
- 133.[Q5SQZ8_HUMAN](#) Thyroid hormone receptor interactor 8, 35 (3%)
- 134.[AAC51191](#) Skeletal muscle ryanodine receptor, 35 (1%)
- 135.[AAH09304](#) DDX27 protein, 35 (3%)
- 136.[AAA51758](#) apolipoprotein B-100, 35 (1%)
- 137.[CGHU2E](#) collagen alpha 2(XI) chain precursor (fragment), 35 (4%)
- 138.[O43149_HUMAN](#) KIAA0399 protein (Fragment), 34 (3%)
- 139.[AAR32522](#) Immunoglobulin heavy chain variable region, 34 (25%)
- 140.[AAB04947](#) Inositol 1,4,5-trisphosphate receptor type 1, 34 (1%)
- 141.[CAC38563](#) Sequence 143 from Patent WO0129221, 34 (9%)
- 142.[CAC33149](#) Sequence 54 from Patent WO0100826, 34 (4%)
- 143.[Q96CV2_HUMAN](#) ACCN2 protein (Fragment), 34 (4%)
- 144.[AAH00528](#) Arginyl-tRNA synthetase, 34 (7%)
- 145.[AAB08431](#) hGrbIRbeta/hGrb10, 34 (3%)

BIBLIOGRAPHY

- Adamson, A. L., Darr, D., Holley-Guthrie, E., Johnson, R. A., Mauser, A., Swenson, J., and Kenney, S. (2000). Epstein-Barr virus immediate-early proteins BZLF1 and BRLF1 activate the ATF2 transcription factor by increasing the levels of phosphorylated p38 and c-Jun N-terminal kinases. *J Virol* 74, 1224-1233.
- Adamson, A. L., and Kenney, S. (1999). The Epstein-Barr virus BZLF1 protein interacts physically and functionally with the histone acetylase CREB-binding protein. *J Virol* 73, 6551-6558.
- Ahn, J. H., and Hayward, G. S. (1997). The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. *J Virol* 71, 4599-4613.
- Ahn, J. H., Xu, Y., Jang, W. J., Matunis, M. J., and Hayward, G. S. (2001). Evaluation of interactions of human cytomegalovirus immediate-early IE2 regulatory protein with small ubiquitin-like modifiers and their conjugation enzyme Ubc9. *J Virol* 75, 3859-3872.
- Arnberg N., Pring-Akerblom P., Wadell G. (2002). Adenovirus type 37 uses sialic acid as a cellular receptor on Chang C cells . *J Virol* 76(17), 8834-8841.
- Ascoli, C. A., and Maul, G. G. (1991). Identification of a novel nuclear domain. *J Cell Biol* 112, 785-795.
- Avvakumov, N., Wheeler, R., D'Halluin, J. C., and Mymryk, J. S. (2002). Comparative sequence analysis of the largest E1A proteins of human and simian adenoviruses. *J Virol* 76, 7968-7975.

- Azuma, Y., Arnaoutov, A., Anan, T., and Dasso, M. (2005). PIASy mediates SUMO-2 conjugation of Topoisomerase-II on mitotic chromosomes. *Embo J* 24, 2172-2182.
- Azuma, Y., Tan, S. H., Cavenagh, M. M., Ainsztein, A. M., Saitoh, H., and Dasso, M. (2001). Expression and regulation of the mammalian SUMO-1 E1 enzyme. *Faseb J* 15, 1825-1827.
- Babiss, L. E., and Ginsberg, H. S. (1984). Adenovirus type 5 early region 1b gene product is required for efficient shutoff of host protein synthesis. *J Virol* 50, 202-212.
- Bailey, D., and O'Hare, P. (2004). Characterization of the localization and proteolytic activity of the SUMO-specific protease, SENP1. *J Biol Chem* 279, 692-703.
- Benko, M., Elo, P., Ursu, K., Ahne, W., LaPatra, S. E., Thomson, D., and Harrach, B. (2002). First molecular evidence for the existence of distinct fish and snake adenoviruses. *J Virol* 76, 10056-10059.
- Best, J. L., Ganiatsas, S., Agarwal, S., Changou, A., Salomoni, P., Shirihai, O., Meluh, P. B., Pandolfi, P. P., and Zon, L. I. (2002). SUMO-1 protease-1 regulates gene transcription through PML. *Mol Cell* 10, 843-855.
- Bischoff, J. R., Kirn, D. H., Williams, A., Heise, C., Horn, S., Muna, M., Ng, L., Nye, J. A., Sampson-Johannes, A., Fattaey, A., and McCormick, F. (1996). An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274, 373-376.
- Blanchette, P., Cheng, C. Y., Yan, Q., Ketner, G., Ornelles, D. A., Dobner, T., Conaway, R. C., Conaway, J. W., and Branton, P. E. (2004). Both BC-box motifs

of adenovirus protein E4orf6 are required to efficiently assemble an E3 ligase complex that degrades p53. *Mol Cell Biol* 24, 9619-9629.

Boddy, M. N., Howe, K., Etkin, L. D., Solomon, E., and Freemont, P. S. (1996). PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. *Oncogene* 13, 971-982.

Bohren, K. M., Nadkarni, V., Song, J. H., Gabbay, K. H., and Owerbach, D. (2004). A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. *J Biol Chem* 279, 27233-27238.

Bondesson, M., Ohman, K., Manervik, M., Fan, S., and Akusjarvi, G. (1996). Adenovirus E4 open reading frame 4 protein autoregulates E4 transcription by inhibiting E1A transactivation of the E4 promoter. *J Virol* 70, 3844-3851.

Bonne-Andrea, C., Santucci, S., Clertant, P., and Tillier, F. (1995). Bovine papillomavirus E1 protein binds specifically DNA polymerase alpha but not replication protein A. *J Virol* 69, 2341-2350.

Booher, R., and Beach, D. (1987). Interaction between cdc13+ and cdc2+ in the control of mitosis in fission yeast; dissociation of the G1 and G2 roles of the cdc2+ protein kinase. *Embo J* 6, 3441-3447.

Booher, R., and Beach, D. (1988). Involvement of cdc13+ in mitotic control in *Schizosaccharomyces pombe*: possible interaction of the gene product with microtubules. *Embo J* 7, 2321-2327.

- Booher, R. N., Alfa, C. E., Hyams, J. S., and Beach, D. H. (1989). The fission yeast cdc2/cdc13/suc1 protein kinase: regulation of catalytic activity and nuclear localization. *Cell* 58, 485-497.
- Bottger A., Bottger V., Sparks A., Liu W. L., Howard S. F., and Lane D. P. (1997) Design of a synthetic Mdm2-binding mini protein that activates the p53 response *in vivo*. *Curr Biol* 7, 860-869.
- Boulukos, K. E., and Ziff, E. B. (1993). Adenovirus 5 E1A proteins disrupt the neuronal phenotype and growth factor responsiveness of PC12 cells by a conserved region 1-dependent mechanism. *Oncogene* 8, 237-248.
- Boyer, J., Rohleider, K., and Ketner, G. (1999). Adenovirus E4 34k and E4 11k inhibit double strand break repair and are physically associated with the cellular DNA-dependent protein kinase. *Virology* 263, 307-312.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72, 248-254.
- Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998). Retinoblastoma protein recruits histone deacetylase to repress transcription. *Nature* 391, 597-601.
- Bridge, E., and Ketner, G. (1989). Redundant control of adenovirus late gene expression by early region 4. *J Virol* 63, 631-638.
- Brizuela, L., Draetta, G., and Beach, D. (1989). Activation of human CDC2 protein as a histone H1 kinase is associated with complex formation with the p62 subunit. *Proc Natl Acad Sci U S A* 86, 4362-4366.

- Cartwright, P., Muller, H., Wagener, C., Holm, K., and Helin, K. (1998). E2F-6: a novel member of the E2F family is an inhibitor of E2F-dependent transcription. *Oncogene* *17*, 611-623.
- Carvalho, T., Seeler, J. S., Ohman, K., Jordan, P., Pettersson, U., Akusjarvi, G., Carmo-Fonseca, M., and Dejean, A. (1995). Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. *J Cell Biol* *131*, 45-56.
- Cathomen, T., and Weitzman, M. D. (2000). A functional complex of adenovirus proteins E1B-55kDa and E4orf6 is necessary to modulate the expression level of p53 but not its transcriptional activity. *J Virol* *74*, 11407-11412.
- Cayrol, C., and Flemington, E. K. (1996). The Epstein-Barr virus bZIP transcription factor Zta causes G0/G1 cell cycle arrest through induction of cyclin-dependent kinase inhibitors. *Embo J* *15*, 2748-2759.
- Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y., and Evans, R. M. (1999). A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. *Cell* *96*, 393-403.
- Challberg, M. D., Desiderio, S. V., and Kelly, T. J., Jr. (1980). Adenovirus DNA replication in vitro: characterization of a protein covalently linked to nascent DNA strands. *Proc Natl Acad Sci U S A* *77*, 5105-5109.
- Challberg, M. D., and Kelly, T. J., Jr. (1981). Processing of the adenovirus terminal protein. *J Virol* *38*, 272-277.
- Challberg, M. D., and Rawlins, D. R. (1984). Template requirements for the initiation of adenovirus DNA replication. *Proc Natl Acad Sci U S A* *81*, 100-104.

- Chen, A., Mannen, H., and Li, S. S. (1998a). Characterization of mouse ubiquitin-like SMT3A and SMT3B cDNAs and gene/pseudogenes. *Biochem Mol Biol Int* 46, 1161-1174.
- Chen, P., Tian, J., Kovesdi, I., and Bruder, J. T. (1998b). Interaction of the adenovirus 14.7-kDa protein with FLICE inhibits Fas ligand-induced apoptosis. *J Biol Chem* 273, 5815-5820.
- Chen, P. H., Ornelles, D. A., and Shenk, T. (1993). The adenovirus L3 23-kilodalton proteinase cleaves the amino-terminal head domain from cytokeratin 18 and disrupts the cytokeratin network of HeLa cells. *J Virol* 67, 3507-3514.
- Chiocca, S., Kurzbauer, R., Schaffner, G., Baker, A., Mautner, V., and Cotten, M. (1996). The complete DNA sequence and genomic organization of the avian adenovirus CELO. *J Virol* 70, 2939-2949.
- Conger, K. L., Liu, J. S., Kuo, S. R., Chow, L. T., and Wang, T. S. (1999). Human papillomavirus DNA replication. Interactions between the viral E1 protein and two subunits of human dna polymerase alpha/primase. *J Biol Chem* 274, 2696-2705.
- Corbin-Lickfett, K. A., and Bridge, E. (2003). Adenovirus E4-34kDa requires active proteasomes to promote late gene expression. *Virology* 315, 234-244.
- Cress, W. D., and Nevins, J. R. (1994). Interacting domains of E2F1, DP1, and the adenovirus E4 protein. *J Virol* 68, 4213-4219.
- Davison, A. J., Benko, M., and Harrach, B. (2003). Genetic content and evolution of adenoviruses. *J Gen Virol* 84, 2895-2908.
- Davison, A. J., Wright, K. M., and Harrach, B. (2000). DNA sequence of frog adenovirus. *J Gen Virol* 81, 2431-2439.

- Davison, A. J. and Harrach B. (2002). Siadenovirus. Adenoviridae. (Berlin, Heidelberg, New York: Springer-Verlag).
- de Bruin, A., Maiti, B., Jakoi, L., Timmers, C., Buerki, R., and Leone, G. (2003). Identification and characterization of E2F7, a novel mammalian E2F family member capable of blocking cellular proliferation. *J Biol Chem* 278, 42041-42049.
- Dechechchi M. C., Tamanini A., Bonizzato A., and Cabrini G. (2000). Heparin sulphate glycosaminoglycans are involved in adenovirus type 5 and 2-host cell interactions. *Virol* 268, 382-390
- DeLeo, A. B., Jay, G., Appella, E., Dubois, G. C., Law, L. W., and Old, L. J. (1979). Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci U S A* 76, 2420-2424.
- Deryckere, F., Ebenau-Jehle, C., Wold, W. S., and Burgert, H. G. (1995). Tumor necrosis factor alpha increases expression of adenovirus E3 proteins. *Immunobiology* 193, 186-192.
- Desterro, J. M., Rodriguez, M. S., Kemp, G. D., and Hay, R. T. (1999). Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J Biol Chem* 274, 10618-10624.
- Di Stefano, L., Jensen, M. R., and Helin, K. (2003). E2F7, a novel E2F featuring DP-independent repression of a subset of E2F-regulated genes. *Embo J* 22, 6289-6298.
- Dix, I., and Leppard, K. N. (1995). Expression of adenovirus type 5 E4 Orf2 protein during lytic infection. *J Gen Virol* 76 (Pt 4), 1051-1055.

- Dobbelstein, M., Roth, J., Kimberly, W. T., Levine, A. J., and Shenk, T. (1997). Nuclear export of the E1B 55-kDa and E4 34-kDa adenoviral oncoproteins mediated by a rev-like signal sequence. *Embo J* 16, 4276-4284.
- Dohmen, R. J., Stappen, R., McGrath, J. P., Forrova, H., Kolarov, J., Goffeau, A., and Varshavsky, A. (1995). An essential yeast gene encoding a homolog of ubiquitin-activating enzyme. *J Biol Chem* 270, 18099-18109.
- Dorsman, J. C., Teunisse, A. F., Zantema, A., and van der Eb, A. J. (1997). The adenovirus 12 E1A proteins can bind directly to proteins of the p300 transcription co-activator family, including the CREB-binding protein CBP and p300. *J Gen Virol* 78 (Pt 2), 423-426.
- Dosch, T., Horn, F., Schneider, G., Kratzer, F., Dobner, T., Hauber, J., and Stauber, R. H. (2001). The adenovirus type 5 E1B-55K oncoprotein actively shuttles in virus-infected cells, whereas transport of E4orf6 is mediated by a CRM1-independent mechanism. *J Virol* 75, 5677-5683.
- Dulic, V., Lees, E., and Reed, S. I. (1992). Association of human cyclin E with a periodic G1-S phase protein kinase. *Science* 257, 1958-1961.
- Duprez, E., Saurin, A. J., Desterro, J. M., Lallemand-Breitenbach, V., Howe, K., Boddy, M. N., Solomon, E., de The, H., Hay, R. T., and Freemont, P. S. (1999). SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. *J Cell Sci* 112 (Pt 3), 381-393.
- Dyson, N. (1998). The regulation of E2F by pRB-family proteins. *Genes Dev* 12, 2245-2262.

- Eaton, E. M., and Sealy, L. (2003). Modification of CCAAT/enhancer-binding protein-beta by the small ubiquitin-like modifier (SUMO) family members, SUMO-2 and SUMO-3. *J Biol Chem* 278, 33416-33421.
- el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* 75, 817-825.
- Elledge, S. J., Winston, J., and Harper, J. W. (1996). A question of balance: the role of cyclin-kinase inhibitors in development and tumorigenesis. *Trends Cell Biol* 6, 388-392.
- Elsing, A., and Burgert, H. G. (1998). The adenovirus E3/10.4K-14.5K proteins down-modulate the apoptosis receptor Fas/Apo-1 by inducing its internalization. *Proc Natl Acad Sci U S A* 95, 10072-10077.
- Enders, J. F., Bell, J. A., Dingle, J. H., Francis, T., Jr., Hilleman, M. R., Huebner, R. J., and Payne, A. M. (1956). Adenoviruses: group name proposed for new respiratory-tract viruses. *Science* 124, 119-120.
- Endter, C., Kzhyshkowska, J., Stauber, R., and Dobner, T. (2001). SUMO-1 modification required for transformation by adenovirus type 5 early region 1B 55-kDa oncoprotein. *Proc Natl Acad Sci U S A* 98, 11312-11317.
- Ensinger, M. J., and Ginsberg, H. S. (1972). Selection and preliminary characterization of temperature-sensitive mutants of type 5 adenovirus. *J Virol* 10, 328-339.
- Faha, B., Ewen, M. E., Tsai, L. H., Livingston, D. M., and Harlow, E. (1992). Interaction between human cyclin A and adenovirus E1A-associated p107 protein. *Science* 255, 87-90.

Faha, B., Harlow, E., and Lees, E. (1993). The adenovirus E1A-associated kinase consists of cyclin E-p33cdk2 and cyclin A-p33cdk2. *J Virol* 67, 2456-2465.

Fallaux, F. J., Bout, A., van der Velde, I., van den Wollenberg, D. J., Hehir, K. M., Keegan, J., Auger, C., Cramer, S. J., van Ormondt, H., van der Eb, A. J., *et al.* (1998). New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Hum Gene Ther* 9, 1909-1917.

Fallaux, F. J., Kranenburg, O., Cramer, S. J., Houweling, A., Van Ormondt, H., Hoeben, R. C., and Van Der Eb, A. J. (1996). Characterization of 911: a new helper cell line for the titration and propagation of early region 1-deleted adenoviral vectors. *Hum Gene Ther* 7, 215-222.

Fang S., Jensen J. P., Ludwig R. L., Vousden K. H., and Weissman A. M. (2000). Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J Biol Chem* 275, 8945-8951.

Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F., and Whitehouse, C. M. (1989). Electrospray ionization for mass spectrometry of large biomolecules. *Science* 246, 64-71.

Fields, S., and Song, O. (1989). A novel genetic system to detect protein-protein interactions. *Nature* 340, 245-246.

Flores-Rozas, H., Kelman, Z., Dean, F. B., Pan, Z. Q., Harper, J. W., Elledge, S. J., O'Donnell, M., and Hurwitz, J. (1994). Cdk-interacting protein 1 directly binds with proliferating cell nuclear antigen and inhibits DNA replication catalyzed by the DNA polymerase delta holoenzyme. *Proc Natl Acad Sci U S A* 91, 8655-8659.

- Forsburg, S. L., and Nurse, P. (1991). Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Annu Rev Cell Biol* 7, 227-256.
- Foster C. J., and Lozano G. (2002). Loss of p19ARF enhances the defects in Mdm2 overexpression in the mammary gland. *Oncogene* 21, 3525-3531.
- Fueyo, J., Gomez-Manzano, C., Alemany, R., Lee, P. S., McDonnell, T. J., Mitlianga, P., Shi, Y. X., Levin, V. A., Yung, W. K., and Kyritsis, A. P. (2000). A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene* 19, 2-12.
- Gabler, S., Schutt, H., Groitl, P., Wolf, H., Shenk, T., and Dobner, T. (1998). E1B 55-kilodalton-associated protein: a cellular protein with RNA-binding activity implicated in nucleocytoplasmic transport of adenovirus and cellular mRNAs. *J Virol* 72, 7960-7971.
- Gaggar A., Shayakhmetov D. M., and Lieber A. (2003). CD46 is a cellular receptor for group B adenoviruses. *Nat Med* 9, 1408-1412.
- Gallimore, P. H., and Turnell, A. S. (2001). Adenovirus E1A: remodelling the host cell, a life or death experience. *Oncogene* 20, 7824-7835.
- Garnett, C. T., Erdman, D., Xu, W., and Gooding, L. R. (2002). Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *J Virol* 76, 10608-10616.
- Gaubatz, S., Wood, J. G., and Livingston, D. M. (1998). Unusual proliferation arrest and transcriptional control properties of a newly discovered E2F family member, E2F-6. *Proc Natl Acad Sci U S A* 95, 9190-9195.

Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A. M., Cruciat, C. M., *et al.* (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* *415*, 141-147.

Geisberg, J. V., Lee, W. S., Berk, A. J., and Ricciardi, R. P. (1994). The zinc finger region of the adenovirus E1A transactivating domain complexes with the TATA box binding protein. *Proc Natl Acad Sci U S A* *91*, 2488-2492.

Giordano, A., McCall, C., Whyte, P., and Franzia, B. R., Jr. (1991). Human cyclin A and the retinoblastoma protein interact with similar but distinguishable sequences in the adenovirus E1A gene product. *Oncogene* *6*, 481-485.

Girard, F., Strausfeld, U., Fernandez, A., and Lamb, N. J. (1991). Cyclin A is required for the onset of DNA replication in mammalian fibroblasts. *Cell* *67*, 1169-1179.

Girdwood, D., Bumpass, D., Vaughan, O. A., Thain, A., Anderson, L. A., Snowden, A. W., Garcia-Wilson, E., Perkins, N. D., and Hay, R. T. (2003). P300 transcriptional repression is mediated by SUMO modification. *Mol Cell* *11*, 1043-1054.

Gong, L., Millas, S., Maul, G. G., and Yeh, E. T. (2000). Differential regulation of sentrinized proteins by a novel sentrin-specific protease. *J Biol Chem* *275*, 3355-3359.

Gooding, L. R., Aquino, L., Duerksen-Hughes, P. J., Day, D., Horton, T. M., Ye, S. P., and Wold, W. S. (1991a). The E1B 19,000-molecular-weight protein of group C adenoviruses prevents tumor necrosis factor cytotoxicity of human cells but not of mouse cells. *J Virol* *65*, 3083-3094.

Gooding, L. R., Elmore, L. W., Tollefson, A. E., Brady, H. A., and Wold, W. S. (1988). A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. *Cell* 53, 341-346.

Gooding, L. R., Ranheim, T. S., Tollefson, A. E., Aquino, L., Duerksen-Hughes, P., Horton, T. M., and Wold, W. S. (1991b). The 10,400- and 14,500-dalton proteins encoded by region E3 of adenovirus function together to protect many but not all mouse cell lines against lysis by tumor necrosis factor. *J Virol* 65, 4114-4123.

Goodrum, F. D., Shenk, T., and Ornelles, D. A. (1996). Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. *J Virol* 70, 6323-6335.

Grable, M., and Hearing, P. (1992). cis and trans requirements for the selective packaging of adenovirus type 5 DNA. *J Virol* 66, 723-731.

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 36, 59-74.

Graumann, J., Dunipace, L. A., Seol, J. H., McDonald, W. H., Yates, J. R., 3rd, Wold, B. J., and Deshaies, R. J. (2004). Applicability of tandem affinity purification MudPIT to pathway proteomics in yeast. *Mol Cell Proteomics* 3, 226-237.

Gravel, A., Gosselin, J., and Flamand, L. (2002). Human Herpesvirus 6 immediate-early 1 protein is a sumoylated nuclear phosphoprotein colocalizing with promyelocytic leukemia protein-associated nuclear bodies. *J Biol Chem* 277, 19679-19687.

- Gregory, D. J., Garcia-Wilson, E., Poole, J. C., Snowden, A. W., Roninson, I. B., and Perkins, N. D. (2002). Induction of transcription through the p300 CRD1 motif by p21WAF1/CIP1 is core promoter specific and cyclin dependent kinase independent. *Cell Cycle* 1, 343-350.
- Gu, W., and Roeder, R. G. (1997). Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 90, 595-606.
- Gu, W., Shi, X. L., and Roeder, R. G. (1997). Synergistic activation of transcription by CBP and p53. *Nature* 387, 819-823.
- Gu, Y., Turck, C. W., and Morgan, D. O. (1993). Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. *Nature* 366, 707-710.
- Hagemeier, C., Walker, S. M., Sissons, P. J., and Sinclair, J. H. (1992). The 72K IE1 and 80K IE2 proteins of human cytomegalovirus independently trans-activate the c-fos, c-myc and hsp70 promoters via basal promoter elements. *J Gen Virol* 73 (Pt 9), 2385-2393.
- Hagmeyer, B. M., Angel, P., and van Dam, H. (1995). Modulation of AP-1/ATF transcription factor activity by the adenovirus-E1A oncogene products. *Bioessays* 17, 621-629.
- Halbert, D. N., Cutt, J. R., and Shenk, T. (1985). Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. *J Virol* 56, 250-257.
- Hammarskjold, M. L., and Winberg, G. (1980). Encapsidation of adenovirus 16 DNA is directed by a small DNA sequence at the left end of the genome. *Cell* 20, 787-795.

- Harada, J. N., Shevchenko, A., Pallas, D. C., and Berk, A. J. (2002). Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. *J Virol* 76, 9194-9206.
- Harbour, J. W., and Dean, D. C. (2000). The Rb/E2F pathway: expanding roles and emerging paradigms. *Genes Dev* 14, 2393-2409.
- Harbour, J. W., Luo, R. X., Dei Santi, A., Postigo, A. A., and Dean, D. C. (1999). Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. *Cell* 98, 859-869.
- Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75, 805-816.
- Hashimoto, S., Ishii, A., and Yonehara, S. (1991). The E1b oncogene of adenovirus confers cellular resistance to cytotoxicity of tumor necrosis factor and monoclonal anti-Fas antibody. *Int Immunol* 3, 343-351.
- Hasson, T. B., Ornelles, D. A., and Shenk, T. (1992). Adenovirus L1 52- and 55-kilodalton proteins are present within assembling virions and colocalize with nuclear structures distinct from replication centers. *J Virol* 66, 6133-6142.
- Hasson, T. B., Soloway, P. D., Ornelles, D. A., Doerfler, W., and Shenk, T. (1989). Adenovirus L1 52- and 55-kilodalton proteins are required for assembly of virions. *J Virol* 63, 3612-3621.
- Haupt Y., Maya R., Kazaz A. and Oren M. (1997). Mdm2 promotes the rapid degradation of p53. *Nature* 387, 296-299.
- Hay, J. G., Shapiro, N., Sauthoff, H., Heitner, S., Phupakdi, W., and Rom, W. N. (1999). Targeting the replication of adenoviral gene therapy vectors to lung cancer

cells: the importance of the adenoviral E1b-55kD gene. *Hum Gene Ther* 10, 579-590.

Hearing, P., Samulski, R. J., Wishart, W. L., and Shenk, T. (1987). Identification of a repeated sequence element required for efficient encapsidation of the adenovirus type 5 chromosome. *J Virol* 61, 2555-2558.

Heise, C., Hermiston, T., Johnson, L., Brooks, G., Sampson-Johannes, A., Williams, A., Hawkins, L., and Kirn, D. (2000). An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat Med* 6, 1134-1139.

Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D. D., and Kirn, D. H. (1997). ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nat Med* 3, 639-645.

Hermiston, T. W., Malone, C. L., and Stinski, M. F. (1990). Human cytomegalovirus immediate-early two protein region involved in negative regulation of the major immediate-early promoter. *J Virol* 64, 3532-3536.

Herrmann, C. H., Su, L. K., and Harlow, E. (1991). Adenovirus E1A is associated with a serine/threonine protein kinase. *J Virol* 65, 5848-5859.

Higashino, F., Pipas, J. M., and Shenk, T. (1998). Adenovirus E4orf6 oncoprotein modulates the function of the p53-related protein, p73. *Proc Natl Acad Sci U S A* 95, 15683-15687.

Hilleman, M. R., and Werner, J. H. (1954). Recovery of new agent from patients with acute respiratory illness. *Proc Soc Exp Biol Med* 85, 183-188.

- Ho, J. C., and Watts, F. Z. (2003). Characterization of SUMO-conjugating enzyme mutants in *Schizosaccharomyces pombe* identifies a dominant-negative allele that severely reduces SUMO conjugation. *Biochem J* 372, 97-104.
- Ho, Y., Gruhler, A., Heilbut, A., Bader, G. D., Moore, L., Adams, S. L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., *et al.* (2002). Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415, 180-183.
- Hobbs S. Jitrapakdee S. and Wallace J. C. (1998). Development of a bicistronic vector driven by the human polypeptide chain elongation factor 1alpha promoter for creation of stable mammalian cell lines that express high levels of recombinant proteins. *Biochem Biophys Res Commun* 252(2), 368-372.
- Hochstrasser, M. (2000). Biochemistry. All in the ubiquitin family. *Science* 289, 563-564.
- Hofmann, H., Floss, S., and Stamminger, T. (2000). Covalent modification of the transactivator protein IE2-p86 of human cytomegalovirus by conjugation to the ubiquitin-homologous proteins SUMO-1 and hSMT3b. *J Virol* 74, 2510-2524.
- Honda R., Tanaka H., and Yasuda H. (1997). Oncoprotein Mdm2 is a ubiquitin ligase E3 for human suppressor p53. *FEBS Lett* 420, 25-27.
- Honda R., and Yasuda H. (2000). Activity of Mdm2, a ubiquitin ligase, towards p53 or itself is dependent on the ring finger domain of the E3 ligase. *Oncogene* 19, 1473-1476.
- Horridge, J. J., and Leppard, K. N. (1998). RNA-binding activity of the E1B 55-kilodalton protein from human adenovirus type 5. *J Virol* 72, 9374-9379.

- Huang, M. M., and Hearing, P. (1989). Adenovirus early region 4 encodes two gene products with redundant effects in lytic infection. *J Virol* 63, 2605-2615.
- Hunter, T., and Pines, J. (1994). Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. *Cell* 79, 573-582.
- Hutton, F. G., Turnell, A. S., Gallimore, P. H., and Grand, R. J. (2000). Consequences of disruption of the interaction between p53 and the larger adenovirus early region 1B protein in adenovirus E1 transformed human cells. *Oncogene* 19, 452-462.
- Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S., Sameshima, M., Hase, A., Seto, Y., and Nagata, S. (1991). The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66, 233-243.
- Jackson, M. R., Nilsson, T., and Peterson, P. A. (1993). Retrieval of transmembrane proteins to the endoplasmic reticulum. *J Cell Biol* 121, 317-333.
- Jackson M. W., and Berberich S. J. (2000) Mdmx protects p53 from Mdm2-mediated degradation. *Mol Cell Biol* 20(3), 1001-1007.
- Javier, R. T. (1994). Adenovirus type 9 E4 open reading frame 1 encodes a transforming protein required for the production of mammary tumors in rats. *J Virol* 68, 3917-3924.
- Jimenez-Garcia, L. F., Green, S. R., Mathews, M. B., and Spector, D. L. (1993). Organization of the double-stranded RNA-activated protein kinase DAI and virus-associated VA RNAI in adenovirus-2-infected HeLa cells. *J Cell Sci* 106 (Pt 1), 11-22.
- Johnson, E. S. (2004). Protein modification by SUMO. *Annu Rev Biochem* 73, 355-382.

- Johnson, E. S., and Blobel, G. (1999). Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J Cell Biol* 147, 981-994.
- Johnson, E. S., Schwienhorst, I., Dohmen, R. J., and Blobel, G. (1997). The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *Embo J* 16, 5509-5519.
- Jones, D., Crowe, E., Stevens, T. A., and Candido, E. P. (2001). Functional and phylogenetic analysis of the ubiquitylation system in *Caenorhabditis elegans*: ubiquitin-conjugating enzymes, ubiquitin-activating enzymes, and ubiquitin-like proteins. *Genome Biol* 3(1), research 0002.1-0002.15.
- Jones S. N., Roe A. E., Donehower L. A., and Bradley A. (1995). Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* 378, 206-208.
- Kao, C. C., Yew, P. R., and Berk, A. J. (1990). Domains required for in vitro association between the cellular p53 and the adenovirus 2 E1B 55K proteins. *Virology* 179, 806-814.
- Karas, M., and Hillenkamp, F. (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal Chem* 60, 2299-2301.
- Keblusek, P., Dorsman, J. C., Teunisse, A. F., Teunissen, H., van der Eb, A. J., and Zantema, A. (1999). The adenoviral E1A oncoproteins interfere with the growth-inhibiting effect of the cdk-inhibitor p21(CIP1/WAF1). *J Gen Virol* 80 (Pt 2), 381-390.
- Kelly, C., Van Driel, R., and Wilkinson, G. W. (1995). Disruption of PML-associated nuclear bodies during human cytomegalovirus infection. *J Gen Virol* 76 (Pt 11), 2887-2893.

Kieff, R. a. (2001). Epstein-Barr virus and its replication (Philadelphia: Lippincott, Williams and Wilkins).

Kim, K. I., Baek, S. H., Jeon, Y. J., Nishimori, S., Suzuki, T., Uchida, S., Shimbara, N., Saitoh, H., Tanaka, K., and Chung, C. H. (2000). A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. *J Biol Chem* 275, 14102-14106.

Kimelman, D., Miller, J. S., Porter, D., and Roberts, B. E. (1985). E1a regions of the human adenoviruses and of the highly oncogenic simian adenovirus 7 are closely related. *J Virol* 53, 399-409.

Kleinberger, T., and Shenk, T. (1991). A protein kinase is present in a complex with adenovirus E1A proteins. *Proc Natl Acad Sci U S A* 88, 11143-11147.

Klucher, K. M., Sommer, M., Kadonaga, J. T., and Spector, D. H. (1993). In vivo and in vitro analysis of transcriptional activation mediated by the human cytomegalovirus major immediate-early proteins. *Mol Cell Biol* 13, 1238-1250.

Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M., and Roberts, J. M. (1991). Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. *Cell* 66, 1217-1228.

Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franzia, B. R., and Roberts, J. M. (1992). Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science* 257, 1689-1694.

Konig, C., Roth, J., and Dobbelstein, M. (1999). Adenovirus type 5 E4orf3 protein relieves p53 inhibition by E1B-55-kilodalton protein. *J Virol* 73, 2253-2262.

- Korioth, F., Maul, G. G., Plachter, B., Stamminger, T., and Frey, J. (1996). The nuclear domain 10 (ND10) is disrupted by the human cytomegalovirus gene product IE1. *Exp Cell Res* 229, 155-158.
- Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998). Transcription factor-specific requirements for coactivators and their acetyltransferase functions. *Science* 279, 703-707.
- Kovesdi, I., Reichel, R., and Nevins, J. R. (1986). Identification of a cellular transcription factor involved in E1A trans-activation. *Cell* 45, 219-228.
- Krajcsi, P., Dimitrov, T., Hermiston, T. W., Tollefson, A. E., Ranheim, T. S., Vande Pol, S. B., Stephenson, A. H., and Wold, W. S. (1996). The adenovirus E3-14.7K protein and the E3-10.4K/14.5K complex of proteins, which independently inhibit tumor necrosis factor (TNF)-induced apoptosis, also independently inhibit TNF-induced release of arachidonic acid. *J Virol* 70, 4904-4913.
- Kratzer, F., Rosorius, O., Heger, P., Hirschmann, N., Dobner, T., Hauber, J., and Stauber, R. H. (2000). The adenovirus type 5 E1B-55K oncoprotein is a highly active shuttle protein and shuttling is independent of E4orf6, p53 and Mdm2. *Oncogene* 19, 850-857.
- Kubbutat M. H. G., Jones S. N., and Vousden K. H. (1997). Regulation of p53 stability by p53. *Nature* 387, 299-303.
- Kung, A. L., Rebel, V. I., Bronson, R. T., Ch'ng, L. E., Sieff, C. A., Livingston, D. M., and Yao, T. P. (2000). Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev* 14, 272-277.

- Lane, D. P., and Crawford, L. V. (1979). T antigen is bound to a host protein in SV40-transformed cells. *Nature* *278*, 261-263.
- Laster, S. M., Wood, J. G., and Gooding, L. R. (1988). Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. *J Immunol* *141*, 2629-2634.
- Lee, G. W., Melchior, F., Matunis, M. J., Mahajan, R., Tian, Q., and Anderson, P. (1998). Modification of Ran GTPase-activating protein by the small ubiquitin-related modifier SUMO-1 requires Ubc9, an E2-type ubiquitin-conjugating enzyme homologue. *J Biol Chem* *273*, 6503-6507.
- Lees, E., Fahy, B., Dulic, V., Reed, S. I., and Harlow, E. (1992). Cyclin E/cdk2 and cyclin A/cdk2 kinases associate with p107 and E2F in a temporally distinct manner. *Genes Dev* *6*, 1874-1885.
- Leppard, K. N. (1997). E4 gene function in adenovirus, adenovirus vector and adeno-associated virus infections. *J Gen Virol* *78* (Pt 9), 2131-2138.
- Leppard, K. N., and Everett, R. D. (1999). The adenovirus type 5 E1b 55K and E4 Orf3 proteins associate in infected cells and affect ND10 components. *J Gen Virol* *80* (Pt 4), 997-1008.
- Lethbridge, K. J., Scott, G. E., and Leppard, K. N. (2003). Nuclear matrix localization and SUMO-1 modification of adenovirus type 5 E1b 55K protein are controlled by E4 Orf6 protein. *J Gen Virol* *84*, 259-268.
- Lew, D. J., Dulic, V., and Reed, S. I. (1991). Isolation of three novel human cyclins by rescue of G1 cyclin (Cln) function in yeast. *Cell* *66*, 1197-1206.
- Li, S. J., and Hochstrasser, M. (1999). A new protease required for cell-cycle progression in yeast. *Nature* *398*, 246-251.

- Li, S. J., and Hochstrasser, M. (2000). The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol Cell Biol* 20, 2367-2377.
- Li, Y., Kang, J., Friedman, J., Tarassishin, L., Ye, J., Kovalenko, A., Wallach, D., and Horwitz, M. S. (1999). Identification of a cell protein (FIP-3) as a modulator of NF-kappaB activity and as a target of an adenovirus inhibitor of tumor necrosis factor alpha-induced apoptosis. *Proc Natl Acad Sci U S A* 96, 1042-1047.
- Link, A. J., Eng, J., Schieltz, D. M., Carmack, E., Mize, G. J., Morris, D. R., Garvik, B. M., and Yates, J. R., 3rd (1999). Direct analysis of protein complexes using mass spectrometry. *Nat Biotechnol* 17, 676-682.
- Linzer, D. I., Maltzman, W., and Levine, A. J. (1979). The SV40 A gene product is required for the production of a 54,000 MW cellular tumor antigen. *Virology* 98, 308-318.
- Liu, L., Scolnick, D. M., Trievel, R. C., Zhang, H. B., Marmorstein, R., Halazonetis, T. D., and Berger, S. L. (1999). p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. *Mol Cell Biol* 19, 1202-1209.
- Liu, Y., Colosimo, A. L., Yang, X. J., and Liao, D. (2000). Adenovirus E1B 55-kilodalton oncoprotein inhibits p53 acetylation by PCAF. *Mol Cell Biol* 20, 5540-5553.
- Look, D. C., Roswit, W. T., Frick, A. G., Gris-Alevy, Y., Dickhaus, D. M., Walter, M. J., and Holtzman, M. J. (1998). Direct suppression of Stat1 function during adenoviral infection. *Immunity* 9, 871-880.

- Lowe, S. W., and Ruley, H. E. (1993). Stabilization of the p53 tumor suppressor is induced by adenovirus 5 E1A and accompanies apoptosis. *Genes Dev* 7, 535-545.
- Lundberg, A. S., and Weinberg, R. A. (1998). Functional inactivation of the retinoblastoma protein requires sequential modification by at least two distinct cyclin-cdk complexes. *Mol Cell Biol* 18, 753-761.
- Luo, Y., Hurwitz, J., and Massague, J. (1995). Cell-cycle inhibition by independent CDK and PCNA binding domains in p21Cip1. *Nature* 375, 159-161.
- Lutz, P., Rosa-Calatrava, M., and Kedinger, C. (1997). The product of the adenovirus intermediate gene IX is a transcriptional activator. *J Virol* 71, 5102-5109.
- Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997). A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 88, 97-107.
- Mahajan, R., Gerace, L., and Melchior, F. (1998). Molecular characterization of the SUMO-1 modification of RanGAP1 and its role in nuclear envelope association. *J Cell Biol* 140, 259-270.
- Malette, P., Yee, S. P., and Branton, P. E. (1983). Studies on the phosphorylation of the 58000 dalton early region 1B protein of human adenovirus type 5. *J Gen Virol* 64, 1069-1078.
- Malone, C. L., Vesole, D. H., and Stinski, M. F. (1990). Transactivation of a human cytomegalovirus early promoter by gene products from the immediate-early gene IE2 and augmentation by IE1: mutational analysis of the viral proteins. *J Virol* 64, 1498-1506.

- Mann, D. J., and Jones, N. C. (1996). E2F-1 but not E2F-4 can overcome p16-induced G1 cell-cycle arrest. *Curr Biol* 6, 474-483.
- Mannervik, M., Fan, S., Strom, A. C., Helin, K., and Akusjarvi, G. (1999). Adenovirus E4 open reading frame 4-induced dephosphorylation inhibits E1A activation of the E2 promoter and E2F-1-mediated transactivation independently of the retinoblastoma tumor suppressor protein. *Virology* 256, 313-321.
- Marcellus, R. C., Teodoro, J. G., Wu, T., Brough, D. E., Ketner, G., Shore, G. C., and Branton, P. E. (1996). Adenovirus type 5 early region 4 is responsible for E1A-induced p53-independent apoptosis. *J Virol* 70, 6207-6215.
- Masterson, P. J., Stanley, M. A., Lewis, A. P., and Romanos, M. A. (1998). A C-terminal helicase domain of the human papillomavirus E1 protein binds E2 and the DNA polymerase alpha-primase p68 subunit. *J Virol* 72, 7407-7419.
- Mathews, M. B., and Shenk, T. (1991). Adenovirus virus-associated RNA and translation control. *J Virol* 65, 5657-5662.
- Matsushime, H., Roussel, M. F., Ashmun, R. A., and Sherr, C. J. (1991). Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell* 65, 701-713.
- Matunis, M. J., Coutavas, E., and Blobel, G. (1996). A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Cell Biol* 135, 1457-1470.
- Matunis, M. J., Wu, J., and Blobel, G. (1998). SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. *J Cell Biol* 140, 499-509.

- Mazzarelli, J. M., Mengus, G., Davidson, I., and Ricciardi, R. P. (1997). The transactivation domain of adenovirus E1A interacts with the C terminus of human TAF(II)135. *J Virol* 71, 7978-7983.
- McCormack, A. L., Schieltz, D. M., Goode, B., Yang, S., Barnes, G., Drubin, D., and Yates, J. R., 3rd (1997). Direct analysis and identification of proteins in mixtures by LC/MS/MS and database searching at the low-femtomole level. *Anal Chem* 69, 767-776.
- McElroy, A. K., Dwarakanath, R. S., and Spector, D. H. (2000). Dysregulation of cyclin E gene expression in human cytomegalovirus-infected cells requires viral early gene expression and is associated with changes in the Rb-related protein p130. *J Virol* 74, 4192-4206.
- Melchior, F. (2000). SUMO--nonclassical ubiquitin. *Annu Rev Cell Dev Biol* 16, 591-626.
- Meluh, P. B., and Koshland, D. (1995). Evidence that the MIF2 gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Mol Biol Cell* 6, 793-807.
- Midgley C. A., Desterro J. M. P., Saville M. K., Howard S., Sparks A., Hay R. T., and Lane D. P. (2000). An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination *in vitro* and can activate p53 *in vivo*. *Oncogene* 19, 2312-2323.
- Minshull, J., Golsteyn, R., Hill, C. S., and Hunt, T. (1990). The A- and B-type cyclin associated cdc2 kinases in Xenopus turn on and off at different times in the cell cycle. *Embo J* 9, 2865-2875.

- Montes de Oca Luna R., Wagner D. S., and Lozano G. (1995). Rescue of early embryonic lethality in Mdm2-deficient mice by deletion of p53. *Nature* 378, 203-206.
- Moore, M., Horikoshi, N., and Shenk, T. (1996). Oncogenic potential of the adenovirus E4orf6 protein. *Proc Natl Acad Sci U S A* 93, 11295-11301.
- Moran (1994). Mammalian cell growth controls reflected through protein interactions with the adenovirus E1A gene products. *Seminars in Virology* 5, 327-340.
- Morkel, M., Wenkel, J., Bannister, A. J., Kouzarides, T., and Hagemeier, C. (1997). An E2F-like repressor of transcription. *Nature* 390, 567-568.
- Motokura, T., Bloom, T., Kim, H. G., Juppner, H., Ruderman, J. V., Kronenberg, H. M., and Arnold, A. (1991). A novel cyclin encoded by a bcl1-linked candidate oncogene. *Nature* 350, 512-515.
- Muller (1992). Adenovirus E4 Orf4 protein reduces phosphorylation of c-fos and E1A proteins while simultaneously reducing the level of AP-1. *Journal of Virology* 66, 5867-5878.
- Muller (1998). Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *Embo J* 17, 61-70.
- Muller, S., and Dejean, A. (1999). Viral immediate-early proteins abrogate the modification by SUMO-1 of PML and Sp100 proteins, correlating with nuclear body disruption. *J Virol* 73, 5137-5143.
- Mundle, S. D., and Saberwal, G. (2003). Evolving intricacies and implications of E2F1 regulation. *Faseb J* 17, 569-574.

- Mymryk, J. S., Shire, K., and Bayley, S. T. (1994). Induction of apoptosis by adenovirus type 5 E1A in rat cells requires a proliferation block. *Oncogene* 9, 1187-1193.
- Nasmyth, K., and Hunt, T. (1993). Cell cycle. Dams and sluices. *Nature* 366, 634-635.
- Nevels, M., Rubenwolf, S., Spruss, T., Wolf, H., and Dobner, T. (1997). The adenovirus E4orf6 protein can promote E1A/E1B-induced focus formation by interfering with p53 tumor suppressor function. *Proc Natl Acad Sci U S A* 94, 1206-1211.
- Nevels, M., Rubenwolf, S., Spruss, T., Wolf, H., and Dobner, T. (2000). Two distinct activities contribute to the oncogenic potential of the adenovirus type 5 E4orf6 protein. *J Virol* 74, 5168-5181.
- Nevels, M., Spruss, T., Wolf, H., and Dobner, T. (1999a). The adenovirus E4orf6 protein contributes to malignant transformation by antagonizing E1A-induced accumulation of the tumor suppressor protein p53. *Oncogene* 18, 9-17.
- Nevels, M., Tauber, B., Kremmer, E., Spruss, T., Wolf, H., and Dobner, T. (1999b). Transforming potential of the adenovirus type 5 E4orf3 protein. *J Virol* 73, 1591-1600.
- Nevels, M., Tauber, B., Spruss, T., Wolf, H., and Dobner, T. (2001). "Hit-and-run" transformation by adenovirus oncogenes. *J Virol* 75, 3089-3094.
- Nishida, T., Kaneko, F., Kitagawa, M., and Yasuda, H. (2001). Characterization of a novel mammalian SUMO-1/Smt3-specific isopeptidase, a homologue of rat axam, which is an axin-binding protein promoting beta-catenin degradation. *J Biol Chem* 276, 39060-39066.

- Nishida, T., Tanaka, H., and Yasuda, H. (2000). A novel mammalian Smt3-specific isopeptidase 1 (SMT3IP1) localized in the nucleolus at interphase. *Eur J Biochem* *267*, 6423-6427.
- Noda, A., Ning, Y., Venable, S. F., Pereira-Smith, O. M., and Smith, J. R. (1994). Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. *Exp Cell Res* *211*, 90-98.
- O'Connor, R. J., and Hearing, P. (2000). The E4-6/7 protein functionally compensates for the loss of E1A expression in adenovirus infection. *J Virol* *74*, 5819-5824.
- Obert, S., O'Connor, R. J., Schmid, S., and Hearing, P. (1994). The adenovirus E4-6/7 protein transactivates the E2 promoter by inducing dimerization of a heteromeric E2F complex. *Mol Cell Biol* *14*, 1333-1346.
- Ohman (1995). Effects of adenovirus early region 4 products on E1 transformation. *International Journal of Oncology* *6*, 663-668.
- Okura, T., Gong, L., Kamitani, T., Wada, T., Okura, I., Wei, C. F., Chang, H. M., and Yeh, E. T. (1996). Protection against Fas/APO-1- and tumor necrosis factor-mediated cell death by a novel protein, sentrin. *J Immunol* *157*, 4277-4281.
- Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics* *1*, 376-386.
- Pagano, M., Pepperkok, R., Verde, F., Ansorge, W., and Draetta, G. (1992). Cyclin A is required at two points in the human cell cycle. *Embo J* *11*, 961-971.

- Pajovic, S., Wong, E. L., Black, A. R., and Azizkhan, J. C. (1997). Identification of a viral kinase that phosphorylates specific E2Fs and pocket proteins. *Mol Cell Biol* 17, 6459-6464.
- Park, P., Copeland, W., Yang, L., Wang, T., Botchan, M. R., and Mohr, I. J. (1994). The cellular DNA polymerase alpha-primase is required for papillomavirus DNA replication and associates with the viral E1 helicase. *Proc Natl Acad Sci U S A* 91, 8700-8704.
- Pepper, D. S., and Zantema, A. (1993). Adenovirus-E1A proteins transform cells by sequestering regulatory proteins. *Mol Biol Rep* 17, 197-207.
- Perissi, V., Dasen, J. S., Kurokawa, R., Wang, Z., Korzus, E., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1999). Factor-specific modulation of CREB-binding protein acetyltransferase activity. *Proc Natl Acad Sci U S A* 96, 3652-3657.
- Perkins, N. D., Felzien, L. K., Betts, J. C., Leung, K., Beach, D. H., and Nabel, G. J. (1997). Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. *Science* 275, 523-527.
- Peters, G. (1994). Cell cycle. Stifled by inhibitions. *Nature* 371, 204-205.
- Philipson, L. (1984). The interplay between host and viral genes in adenovirus gene expression. *Klin Wochenschr* 62, 433-440.
- Pichler, A., Gast, A., Seeler, J. S., Dejean, A., and Melchior, F. (2002). The nucleoporin RanBP2 has SUMO1 E3 ligase activity. *Cell* 108, 109-120.
- Pierce, A. M., Schneider-Broussard, R., Philhower, J. L., and Johnson, D. G. (1998). Differential activities of E2F family members: unique functions in regulating transcription. *Mol Carcinog* 22, 190-198.

- Pilder, S., Logan, J., and Shenk, T. (1984). Deletion of the gene encoding the adenovirus 5 early region 1b 21,000-molecular-weight polypeptide leads to degradation of viral and host cell DNA. *J Virol* 52, 664-671.
- Pilder, S., Moore, M., Logan, J., and Shenk, T. (1986). The adenovirus E1B-55K transforming polypeptide modulates transport or cytoplasmic stabilization of viral and host cell mRNAs. *Mol Cell Biol* 6, 470-476.
- Pizzorno, M. C., O'Hare, P., Sha, L., LaFemina, R. L., and Hayward, G. S. (1988). trans-activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. *J Virol* 62, 1167-1179.
- Punga, T., and Akusjarvi, G. (2000). The adenovirus-2 E1B-55K protein interacts with a mSin3A/histone deacetylase 1 complex. *FEBS Lett* 476, 248-252.
- Putzer, B. M., Stiewe, T., Parssanedjad, K., Rega, S., and Esche, H. (2000). E1A is sufficient by itself to induce apoptosis independent of p53 and other adenoviral gene products. *Cell Death Differ* 7, 177-188.
- Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W. G., Conaway, R. C., Conaway, J. W., and Branton, P. E. (2001a). Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes Dev* 15, 3104-3117.
- Querido, E., Marcellus, R. C., Lai, A., Charbonneau, R., Teodoro, J. G., Ketner, G., and Branton, P. E. (1997a). Regulation of p53 levels by the E1B 55-kilodalton protein and E4orf6 in adenovirus-infected cells. *J Virol* 71, 3788-3798.
- Querido, E., Morrison, M. R., Chu-Pham-Dang, H., Thirlwell, S. W., Boivin, D., Branton, P. E., and Morisson, M. R. (2001b). Identification of three functions of

the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6-E1B55K complex. *J Virol* 75, 699-709.

Querido, E., Teodoro, J. G., and Branton, P. E. (1997b). Accumulation of p53 induced by the adenovirus E1A protein requires regions involved in the stimulation of DNA synthesis. *J Virol* 71, 3526-3533.

Rangasamy, D., and Wilson, V. G. (2000). Bovine papillomavirus E1 protein is sumoylated by the host cell Ubc9 protein. *J Biol Chem* 275, 30487-30495.

Rangasamy, D., Woytek, K., Khan, S. A., and Wilson, V. G. (2000). SUMO-1 modification of bovine papillomavirus E1 protein is required for intranuclear accumulation. *J Biol Chem* 275, 37999-38004.

Rao, L., Debbas, M., Sabbatini, P., Hockenberry, D., Korsmeyer, S., and White, E. (1992). The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. *Proc Natl Acad Sci U S A* 89, 7742-7746.

Reid, J. L., Bannister, A. J., Zegerman, P., Martinez-Balbas, M. A., and Kouzarides, T. (1998). E1A directly binds and regulates the P/CAF acetyltransferase. *Embo J* 17, 4469-4477.

Rekosh, D. M., Russell, W. C., Bellet, A. J., and Robinson, A. J. (1977). Identification of a protein linked to the ends of adenovirus DNA. *Cell* 11, 283-295.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* 17, 1030-1032.

Rodriguez, M. S., Dargemont, C., and Hay, R. T. (2001). SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *J Biol Chem* 276, 12654-12659.

- Roth, J., Konig, C., Wienzek, S., Weigel, S., Ristea, S., and Dobbelstein, M. (1998). Inactivation of p53 but not p73 by adenovirus type 5 E1B 55-kilodalton and E4 34-kilodalton oncoproteins. *J Virol* 72, 8510-8516.
- Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. G. (1953). Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc Exp Biol Med* 84, 570-573.
- Rubenwolf, S., Schutt, H., Nevels, M., Wolf, H., and Dobner, T. (1997). Structural analysis of the adenovirus type 5 E1B 55-kilodalton-E4orf6 protein complex. *J Virol* 71, 1115-1123.
- Russell, W. C., (2000). Update on adenovirus and its vectors. *J Gen Virol* 81, 2573-2604.
- Saitoh, H., and Hinchey, J. (2000). Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. *J Biol Chem* 275, 6252-6258.
- Sakaguchi, K., Herrera, J. E., Saito, S., Miki, T., Bustin, M., Vassilev, A., Anderson, C. W., and Appella, E. (1998). DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev* 12, 2831-2841.
- Sarisky, R. T., Gao, Z., Lieberman, P. M., Fixman, E. D., Hayward, G. S., and Hayward, S. D. (1996). A replication function associated with the activation domain of the Epstein-Barr virus Zta transactivator. *J Virol* 70, 8340-8347.
- Sarnow, P., Hearing, P., Anderson, C. W., Halbert, D. N., Shenk, T., and Levine, A. J. (1984). Adenovirus early region 1B 58,000-dalton tumor antigen is physically associated with an early region 4 25,000-dalton protein in productively infected cells. *J Virol* 49, 692-700.

- Sarnow, P., Sullivan, C. A., and Levine, A. J. (1982). A monoclonal antibody detecting the adenovirus type 5-E1b-58Kd tumor antigen: characterization of the E1b-58Kd tumor antigen in adenovirus-infected and -transformed cells. *Virology* *120*, 510-517.
- Schwienhorst, I., Johnson, E. S., and Dohmen, R. J. (2000). SUMO conjugation and deconjugation. *Mol Gen Genet* *263*, 771-786.
- Seufert, W., Futcher, B., and Jentsch, S. (1995). Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* *373*, 78-81.
- Shen, Y., Kitzes, G., Nye, J. A., Fattaey, A., and Hermiston, T. (2001). Analyses of single-amino-acid substitution mutants of adenovirus type 5 E1B-55K protein. *J Virol* *75*, 4297-4307.
- Shen, Y., Zhu, H., and Shenk, T. (1997). Human cytomegalovirus IE1 and IE2 proteins are mutagenic and mediate "hit-and-run" oncogenic transformation in cooperation with the adenovirus E1A proteins. *Proc Natl Acad Sci U S A* *94*, 3341-3345.
- Shen, Z., Pardington-Purtymun, P. E., Comeaux, J. C., Moyzis, R. K., and Chen, D. J. (1996). UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins. *Genomics* *36*, 271-279.
- Shenk, T. (1996). Adenoviridae: the viruses and their replication, 3rd edition edn (Philadelphia: Lippincott-Raven).
- Shenk, T., and Flint, J. (1991). Transcriptional and transforming activities of the adenovirus E1A proteins. *Adv Cancer Res* *57*, 47-85.
- Sherr, C. J. (1994). G1 phase progression: cycling on cue. *Cell* *79*, 551-555.
- Sherr, C. J. (1996). Cancer cell cycles. *Science* *274*, 1672-1677.

- Shim, J., Lee, H., Park, J., Kim, H., and Choi, E. J. (1996). A non-enzymatic p21 protein inhibitor of stress-activated protein kinases. *Nature* *381*, 804-806.
- Shisler, J., Yang, C., Walter, B., Ware, C. F., and Gooding, L. R. (1997). The adenovirus E3-10.4K/14.5K complex mediates loss of cell surface Fas (CD95) and resistance to Fas-induced apoptosis. *J Virol* *71*, 8299-8306.
- Short J. J., Vasu C., Holterman M. J., Curiel D. T., and Pereboev A. (2006). Members of adenovirus species B utilise CD80 and CD86 as cellular attachment receptors. *Virus Res* *122(1-2)*, 144-153.
- Snowden, A. W., Anderson, L. A., Webster, G. A., and Perkins, N. D. (2000). A novel transcriptional repression domain mediates p21(WAF1/CIP1) induction of p300 transactivation. *Mol Cell Biol* *20*, 2676-2686.
- Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M., and Kirschner, M. W. (1990). Cyclin activation of p34cdc2. *Cell* *63*, 1013-1024.
- Spengler, M. L., Kurapatwinski, K., Black, A. R., and Azizkhan-Clifford, J. (2002). SUMO-1 modification of human cytomegalovirus IE1/IE72. *J Virol* *76*, 2990-2996.
- Stanton, R., Fox, J. D., Caswell, R., Sherratt, E., and Wilkinson, G. W. (2002). Analysis of the human herpesvirus-6 immediate-early 1 protein. *J Gen Virol* *83*, 2811-2820.
- Steegenga, W. T., Riteco, N., Jochemsen, A. G., Fallaux, F. J., and Bos, J. L. (1998). The large E1B protein together with the E4orf6 protein target p53 for active degradation in adenovirus infected cells. *Oncogene* *16*, 349-357.
- Steen, H., and Mann, M. (2004). The ABC's (and XYZ's) of peptide sequencing. *Nat Rev Mol Cell Biol* *5*, 699-711.

- Stenberg, R. M., and Stinski, M. F. (1985). Autoregulation of the human cytomegalovirus major immediate-early gene. *J Virol* 56, 676-682.
- Stillman, B. W., Lewis, J. B., Chow, L. T., Mathews, M. B., and Smart, J. E. (1981). Identification of the gene and mRNA for the adenovirus terminal protein precursor. *Cell* 23, 497-508.
- Stracker, T. H., Carson, C. T., and Weitzman, M. D. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* 418, 348-352.
- Stracker, T. H., Lee, D. V., Carson, C. T., Araujo, F. D., Ornelles, D. A., and Weitzman, M. D. (2005). Serotype-specific reorganization of the Mre11 complex by adenoviral E4orf3 proteins. *J Virol* 79, 6664-6673.
- Straus (1984). The Adenoviruses (New York: Plenum).
- Sundquist, B., Everitt, E., Philipson, L., and Hoglund, S. (1973). Assembly of adenoviruses. *J Virol* 11, 449-459.
- Swanson, S. K., and Washburn, M. P. (2005). The continuing evolution of shotgun proteomics. *Drug Discov Today* 10, 719-725.
- Swenson, K. I., Farrell, K. M., and Ruderman, J. V. (1986). The clam embryo protein cyclin A induces entry into M phase and the resumption of meiosis in *Xenopus* oocytes. *Cell* 47, 861-870.
- Takemori, N., Cladaras, C., Bhat, B., Conley, A. J., and Wold, W. S. (1984). cyt gene of adenoviruses 2 and 5 is an oncogene for transforming function in early region E1B and encodes the E1B 19,000-molecular-weight polypeptide. *J Virol* 52, 793-805.

- Tatham, M. H., Jaffray, E., Vaughan, O. A., Desterro, J. M., Botting, C. H., Naismith, J. H., and Hay, R. T. (2001). Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J Biol Chem* **276**, 35368-35374.
- Taylor, D. L., Ho, J. C., Oliver, A., and Watts, F. Z. (2002). Cell-cycle-dependent localisation of Ulp1, a *Schizosaccharomyces pombe* Pmt3 (SUMO)-specific protease. *J Cell Sci* **115**, 1113-1122.
- Teodoro, J. G., and Branton, P. E. (1997a). Regulation of apoptosis by viral gene products. *J Virol* **71**, 1739-1746.
- Teodoro, J. G., and Branton, P. E. (1997b). Regulation of p53-dependent apoptosis, transcriptional repression, and cell transformation by phosphorylation of the 55-kilodalton E1B protein of human adenovirus type 5. *J Virol* **71**, 3620-3627.
- Teodoro, J. G., Halliday, T., Whalen, S. G., Takayesu, D., Graham, F. L., and Branton, P. E. (1994). Phosphorylation at the carboxy terminus of the 55-kilodalton adenovirus type 5 E1B protein regulates transforming activity. *J Virol* **68**, 776-786.
- Teodoro, J. G., Shore, G. C., and Branton, P. E. (1995). Adenovirus E1A proteins induce apoptosis by both p53-dependent and p53-independent mechanisms. *Oncogene* **11**, 467-474.
- Tibbetts, C. (1977). Physical organization of subgroup B human adenovirus genomes. *J Virol* **24**, 564-579.
- Tollefson, A. E., Hermiston, T. W., Lichtenstein, D. L., Colle, C. F., Tripp, R. A., Dimitrov, T., Toth, K., Wells, C. E., Doherty, P. C., and Wold, W. S. (1998).

Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. *Nature* 392, 726-730.

Tollefson, A. E., Ryerse, J. S., Scaria, A., Hermiston, T. W., and Wold, W. S. (1996a). The E3-11.6-kDa adenovirus death protein (ADP) is required for efficient cell death: characterization of cells infected with adp mutants. *Virology* 220, 152-162.

Tollefson, A. E., Scaria, A., Hermiston, T. W., Ryerse, J. S., Wold, L. J., and Wold, W. S. (1996b). The adenovirus death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. *J Virol* 70, 2296-2306.

Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997). The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* 387, 677-684.

Tribouley, C., Lutz, P., Staub, A., and Kedinger, C. (1994). The product of the adenovirus intermediate gene IVa2 is a transcriptional activator of the major late promoter. *J Virol* 68, 4450-4457.

Trimarchi, J. M., Fairchild, B., Verona, R., Moberg, K., Andon, N., and Lees, J. A. (1998). E2F-6, a member of the E2F family that can behave as a transcriptional repressor. *Proc Natl Acad Sci U S A* 95, 2850-2855.

Trimarchi, J. M., and Lees, J. A. (2002). Sibling rivalry in the E2F family. *Nat Rev Mol Cell Biol* 3, 11-20.

Tsai, L. H., Lees, E., Faha, B., Harlow, E., and Riabowol, K. (1993). The cdk2 kinase is required for the G1-to-S transition in mammalian cells. *Oncogene* 8, 1593-1602.

Virtanen, A., Gilardi, P., Naslund, A., LeMoullec, J. M., Pettersson, U., and Perricaudet, M. (1984). mRNAs from human adenovirus 2 early region 4. *J Virol* 51, 822-831.

Vollmer, C. M., Ribas, A., Butterfield, L. H., Dissette, V. B., Andrews, K. J., Eilber, F. C., Montejo, L. D., Chen, A. Y., Hu, B., Glaspy, J. A., *et al.* (1999). p53 selective and nonselective replication of an E1B-deleted adenovirus in hepatocellular carcinoma. *Cancer Res* 59, 4369-4374.

Waga, S., Hannon, G. J., Beach, D., and Stillman, B. (1994). The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* 369, 574-578.

Wang, D., Russell, J. L., and Johnson, D. G. (2000). E2F4 and E2F1 have similar proliferative properties but different apoptotic and oncogenic properties in vivo. *Mol Cell Biol* 20, 3417-3424.

Weiden, M. D., and Ginsberg, H. S. (1994). Deletion of the E4 region of the genome produces adenovirus DNA concatemers. *Proc Natl Acad Sci U S A* 91, 153-157.

Weigel, S., and Dobbelstein, M. (2000). The nuclear export signal within the E4orf6 protein of adenovirus type 5 supports virus replication and cytoplasmic accumulation of viral mRNA. *J Virol* 74, 764-772.

Weiss, R. S., Lee, S. S., Prasad, B. V., and Javier, R. T. (1997). Human adenovirus early region 4 open reading frame 1 genes encode growth-transforming proteins that may be distantly related to dUTP pyrophosphatase enzymes. *J Virol* 71, 1857-1870.

- Westendorf, J. M., Swenson, K. I., and Ruderman, J. V. (1989). The role of cyclin B in meiosis I. *J Cell Biol* 108, 1431-1444.
- Whalen, S. G., Marcellus, R. C., Whalen, A., Ahn, N. G., Ricciardi, R. P., and Branton, P. E. (1997). Phosphorylation within the transactivation domain of adenovirus E1A protein by mitogen-activated protein kinase regulates expression of early region 4. *J Virol* 71, 3545-3553.
- White, E., and Cipriani, R. (1989). Specific disruption of intermediate filaments and the nuclear lamina by the 19-kDa product of the adenovirus E1B oncogene. *Proc Natl Acad Sci U S A* 86, 9886-9890.
- White, E., and Cipriani, R. (1990). Role of adenovirus E1B proteins in transformation: altered organization of intermediate filaments in transformed cells that express the 19-kilodalton protein. *Mol Cell Biol* 10, 120-130.
- White, E., Cipriani, R., Sabbatini, P., and Denton, A. (1991). Adenovirus E1B 19-kilodalton protein overcomes the cytotoxicity of E1A proteins. *J Virol* 65, 2968-2978.
- White, E., Denton, A., and Stillman, B. (1988). Role of the adenovirus E1B 19,000-dalton tumor antigen in regulating early gene expression. *J Virol* 62, 3445-3454.
- White, E., Fahy, B., and Stillman, B. (1986). Regulation of adenovirus gene expression in human WI38 cells by an E1B-encoded tumor antigen. *Mol Cell Biol* 6, 3763-3773.
- White, E., Grodzicker, T., and Stillman, B. W. (1984). Mutations in the gene encoding the adenovirus early region 1B 19,000-molecular-weight tumor antigen cause the degradation of chromosomal DNA. *J Virol* 52, 410-419.

White, E., Sabbatini, P., Debbas, M., Wold, W. S., Kushner, D. I., and Gooding, L. R. (1992). The 19-kilodalton adenovirus E1B transforming protein inhibits programmed cell death and prevents cytolysis by tumor necrosis factor alpha. *Mol Cell Biol* 12, 2570-2580.

Wickham T. J., Mathias P., Cheresh D. A., and Nemerow G. R. (1993). Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalisation but not virus attachment. *Cell* 73, 309-319.

Wilson, V. G., West, M., Woytek, K., and Rangasamy, D. (2002). Papillomavirus E1 proteins: form, function, and features. *Virus Genes* 24, 275-290.

Wu, L., Timmers, C., Maiti, B., Saavedra, H. I., Sang, L., Chong, G. T., Nuckolls, F., Giangrande, P., Wright, F. A., Field, S. J., *et al.* (2001). The E2F1-3 transcription factors are essential for cellular proliferation. *Nature* 414, 457-462.

Xiong, Y., Connolly, T., Futcher, B., and Beach, D. (1991). Human D-type cyclin. *Cell* 65, 691-699.

Xu, Y., Ahn, J. H., Cheng, M., apRhys, C. M., Chiou, C. J., Zong, J., Matunis, M. J., and Hayward, G. S. (2001). Proteasome-independent disruption of PML oncogenic domains (PODs), but not covalent modification by SUMO-1, is required for human cytomegalovirus immediate-early protein IE1 to inhibit PML-mediated transcriptional repression. *J Virol* 75, 10683-10695.

Yang X., Yang E. M., Brugarolas J., Jacks T., and Baltimore D. (1998). Involvement of p53 and p21 in cellular defects and tumorigenesis in Atm-/- mice. *Mol Cell Biol* 18(7), 4385-4390.

- Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* 382, 319-324.
- Yao, T. P., Oh, S. P., Fuchs, M., Zhou, N. D., Ch'ng, L. E., Newsome, D., Bronson, R. T., Li, E., Livingston, D. M., and Eckner, R. (1998). Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* 93, 361-372.
- Yasugi, T., and Howley, P. M. (1996). Identification of the structural and functional human homolog of the yeast ubiquitin conjugating enzyme UBC9. *Nucleic Acids Res* 24, 2005-2010.
- Yee, S. P., Rowe, D. T., Tremblay, M. L., McDermott, M., and Branton, P. E. (1983). Identification of human adenovirus early region 1 products by using antisera against synthetic peptides corresponding to the predicted carboxy termini. *J Virol* 46, 1003-1013.
- Yew, P. R., Liu, X., and Berk, A. J. (1994). Adenovirus E1B oncoprotein tethers a transcriptional repression domain to p53. *Genes Dev* 8, 190-202.
- Zantema, A., Fransen, J. A., Davis-Olivier, A., Ramaekers, F. C., Vooijs, G. P., DeLeys, B., and Van der Eb, A. J. (1985). Localization of the E1B proteins of adenovirus 5 in transformed cells, as revealed by interaction with monoclonal antibodies. *Virology* 142, 44-58.
- Zhang, Q., Gutsch, D., and Kenney, S. (1994). Functional and physical interaction between p53 and BZLF1: implications for Epstein-Barr virus latency. *Mol Cell Biol* 14, 1929-1938.

- Zhang, W., and Arcos, R. (2005). Interaction of the adenovirus major core protein precursor, pVII, with the viral DNA packaging machinery. *Virology* 334, 194-202.
- Zhang, X., Turnell, A. S., Gorbea, C., Mymryk, J. S., Gallimore, P. H., and Grand, R. J. (2004). The targeting of the proteasomal regulatory subunit S2 by adenovirus E1A causes inhibition of proteasomal activity and increased p53 expression. *J Biol Chem* 279, 25122-25133.
- Zhao, L. Y., Colosimo, A. L., Liu, Y., Wan, Y., and Liao, D. (2003). Adenovirus E1B 55-kilodalton oncoprotein binds to Daxx and eliminates enhancement of p53-dependent transcription by Daxx. *J Virol* 77, 11809-11821.
- Zhu, H., Shen, Y., and Shenk, T. (1995). Human cytomegalovirus IE1 and IE2 proteins block apoptosis. *J Virol* 69, 7960-7970.
- Zindy, F., Lamas, E., Chenivesse, X., Sobczak, J., Wang, J., Fesquet, D., Henglein, B., and Brechot, C. (1992). Cyclin A is required in S phase in normal epithelial cells. *Biochem Biophys Res Commun* 182, 1144-1154.