Identification of TLS-interacting Proteins Using a Modified Yeast Two-Hybrid Screen

by

Gracjan Boguslaw Bozek

A Thesis submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree of

Master of Science

Department of Physiology
Faculty of Medicine
University of Manitoba
2005

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Identification of TLS-interacting Proteins Using a Modified Yeast Two-Hybrid Screen

BY

Gracjan Boguslaw Bozek

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Table of Contents

Some people think football is a matter of life and death. I don't like that attitude. I can assure them it is much more serious than that.

-Bill Shankly

Abstract

TLS (*FUS*, hnRNP P2) is found at the breakpoint region of chromosomal translocations in human liposarcoma with the *CHOP* gene t12:16 {q13:p11}, and in acute myeloid leukemia with the ERG1 gene t16:21 {p11:q22}. While it's clear that *TLS* translocation is a primary genetic determinant of these cancers, the function of TLS *in vivo* is relatively unknown. We have previously shown in knockout mice that *TLS* is essential for genome maintenance, the development and the proliferative response of lymphocytes, and viability. To begin to understand the function of wild-type TLS at the molecular level, we searched for TLS-interacting proteins that may provide insight into cellular processes that underlie the *TLS* knockout phenotype. Since the N-terminal region of TLS is a known potent transactivation domain, a modified cytoplasmic yeast two-hybrid system was used. Two-hybrid analysis of a rat pituitary cDNA expression library identified 15 TLS-interacting proteins using the Ras Recruitment System. cDNA for the TLS-interacting proteins include the *DNA J*, *My004* and *Hspca* stress response genes, the *MAN1* and *MIF* immune response genes, and the DNA damage response genes *SMARCAD1* and *YB-1*. Understanding the molecular mechanisms by which TLS interacts with these proteins will lead to a better understanding of TLS function, especially in regard to the *TLS* knockout mice and to *TLS*-associated cancer.

Acknowledgements

I would like to express my deep gratitude to my mentor and supervisor Dr. Geoff G. Hicks, who has, throughout the years, always greeted me with enthusiasm and a smile. His office door was always open to provide constant and unwavering motivation, insight, and guidance.

I would also like to thank my committee members, Drs. Jim. Davie, R. Daniel Gietz, and Sabina Mai for their assistance and encouragement. With an additional merit and appreciation to Dr. Gietz for his unending knowledge and expertise that greatly contributed to the yeast two-hybrid section of this project.

To the many past and present members of the Hicks' laboratory and the contemporaries within the department of Cell Biology, who are too numerous to mention, I would like to thank you all for your contributions: from stimulating discussions to technical expertise, for the humour and the occasional friendly competition. I would like to express my sincerest gratitude to Drs. Denis Bosc and Songyang Liu for their contributions of knowledge and expertise towards cDNA library propagation and data analysis portions of this project, respectively.

Regardless of the choices I make in my career or in life my family, my mom and my brother, have always supported me. I would like to thank them for their support, encouragement and strength. Their support has been my foundation, inspiration and motivation and it is to them that I would like to dedicate the following work.

List of Figures

List of Tables

1. Introduction

Cancer can be considered as a disease of uncontrolled cell proliferation. The process of transformation from a normal cell into a cancerous one typically occurs as the cumulative outcome of somatic mutations that result in anomalies in cellular regulatory processes (Weinberg, 1996). Consequently, understanding these abnormalities and the affected regulatory processes resulting in cancerous growth may allow for more complete comprehension of the mechanisms that control normal cell proliferation, differentiation and survival.

Neoplasms, literally "new growths", can develop from normal cells of virtually any tissue (Cooper et al., 1980). Accordingly there are a wide variety of tumors that differ in their origin, growth and prognosis. A simple way of distinguishing tumors is by allotting them into one of two main biological categories: benign and malignant (Liotta & Stetler-Stevenson, 1991). A benign tumor remains confined to its original location. Malignant tumors are both invasive and metastatic, that is they are capable of extravagation and eradication of adjacent normal tissues as well as spreading to distant organ sites through circulation.

Although cancer can generally be considered a collection of similar illnesses, each specific cancer is recognized and historically classified according to its origin. Most neoplasms are divided into three major groups: carcinomas, sarcomas, and leukemias or lymphomas. Carcinomas develop from epithelial cells of either endodermal or ectodermal origin. Tumors originating from mesodermal origin including circulating

cells of the lymph and blood systems, fibroblasts, muscle, and bone cells give rise to leukemias or lymphomas, and sarcomas, respectively.

Since more than one hundred different kinds of human cancers are recognized one might consider cancer as a diverse collection of similar yet different diseases. Almost every tissue and thus cell type can yield a malignancy, some even several types (Fearon & Vogelstein, 1990). However there are common characteristics in the development of all the different tumors that form the basis of our current understanding of carcinogenesis (Vogelstein & Kinzler, 1993). The basic processes include cellular hypertrophy, clonal growth, and aberrant inheritance through cell division (Weinberg, 1996).

A fundamental feature of cancer cells is uncontrolled cellular hypertrophy resulting in the formation of more tumor cells as tumor surveillance and apoptosis mechanisms fail (Weinberg, 1988). Many tumors have been found to arise from a single cell of origin (Hanahan & Weinberg, 2000). As tumor progression continues, neoplasms contain bursts of clonal expansions in which tumor cells represent the descendants of a previous progenitor cell that continues to acquire additional mutations. Thus, the abnormalities that result in cancerous growth are selectively inherited at the cellular level as the neoplastic phenotype is reliably inherited at subsequent cell divisions. This validates the hypothesis that genetic mutations are responsible for neoplastic transformation. The development of a tumor is a complex multi-step process in which cells progressively acquire the neoplastic phenotype through a series of alterations in between successive cell divisions (Fearon & Vogelstein, 1990). Fearon and Vogelstein

(1990) first proposed this multi-step theory, and although it deals with colorectal tumors, the genetic basis is applicable to most initiation and progression of tumors.

An overall "successful" development of a cancer cell requires aberrations in at least 5 to 7 genes for the development of a tumor (Hahn & Weinberg, 2002). This is consistent with the majority of cancers encountered in later stages of life, and that its predominance has increased drastically with the increased life span of human beings (Fearon & Vogelstein, 1990). Tumors arise from sequential mutations of growth-regulatory genes, particularly the inactivation of tumor suppressor genes, and deregulation of oncogenes. The consequences of such genetic mutations are due more to the number of mutations rather than the sequence in which they occur or accumulate.

Although the above is a summary of necessary formulae to achieve malignancy by a cell, it is consistent with Darwinian evolution (Cahill et al., 1999; Weinberg, 1989). Each mutational event is a barrier that a particular cell must breach in order to become successful at deregulating the normal growth constraints. Accordingly each change would be subjected to Darwinian selection to provide the altered cell the most advantageous value to a multitude of selective pressures. Thus, each cell can be viewed as an individual experiment in cellular evolution driven by genomic instability to progress toward an endpoint, that being malignancy (Bishop, 1985).

There are many events that can lead to genomic instability. The most obvious consequence of genomic instability is chromosomal translocations. Chromosomal translocations are present in most solid tumors of mesenchymal origins and up to 65% of acute leukemia (Look, 1997; Rabbitts, 1999). Translocations represent a region of

chromosomes where breakage and rejoining has occurred, in all likelihood creating two new chromosomal derivatives. These chromosomal derivatives contain novel combinations of genetic regions that may juxtapose two genes or regulatory elements onto a single continuous DNA molecule. Two main consequences can result from such chromosomal anomalies that posses altered, in structure or expression, genes or regulatory elements. First is a forced expression of a gene, e.g. an oncogene, and even though that gene is not altered in its structure proper, such an event leads to altered expression pattern either spatial or quantitative. The second involves an amalgamation between the coding regions of two genes, resulting in expression of a chimera protein (Look, 1997).

Chromosomal translocations are common in hematopoietic tumors and solid tumors of mesenchymal origin. Such chromosome aberrations frequently involve transcription factors, creating novel chimera proteins that retain their ability to bind DNA (Cleary, 1991; Look, 1997; Rabbitts, 1991; Rabbitts, 1994). *TLS* is one such gene that was discovered as a chimera protein in myxoid liposarcoma – a solid tumor within mesenchymal tissue of adipose origin (Crozat et al., 1993a).

In 1993, two separate groups isolated a translocation between chromosomes 12 and 16 in a solid tumor of myxoid liposarcoma (Crozat et al., 1993a; Rabbitts et al., 1993). Upon closer analysis of this chromosomal t12:16 aberration (Rabbitts et al.), they identified that two genes were fused together. This chimera gene consisted of *CHOP* on chromosome 12q13 and novel unnamed gene on chromosome 16p11, which they designated as *TLS* (for translocated in liposarcoma). A year later, two separate

laboratories isolated an acute myeloid leukemia tumor that contained a chromosomal translocation of t16:21 (Ichikawa et al., 1994; Panagopoulos et al., 1994; Prasad et al., 1994). The two groups identified that the same unnamed gene from chromosome 16p11, which they termed *FUS*, is fused to *ERG* on chromosome 21q22. Hereafter the gene from chromosome 16p11 designated as *TLS* or *FUS*, which is also known as *hnRNP P2*, will be referred to as *TLS* (Calvio et al., 1995).

The *TLS* gene contains 15 exons spanning 11 kb on human chromosome 16p11. The gene encodes an open reading frame of 1580 nucleotides, expressing a 526 amino acid protein of 78 kDa (see Figure 1). The TLS protein can be subdivided into two sub domains, the amino and carboxyl terminal. The amino terminal domain of *TLS* spans approximately 200 amino acids and is rich in hexapeptide repeats commonly associated with transactivation (TA) properties of a transcription factor (Yang et al., 2000), such as the TA domain of SP-1 (Courey & Tjian, 1988). This N-terminal domain is necessary for oncogenic potential of *TLS–CHOP* and *TLS–ERG* (Crozat et al., 1993a; Prasad et al., 1994). It has also been shown to exhibit potent TA activity when placed near a promoter (Bailly et al., 1994; Brown et al., 1995; Lessnick et al., 1995; May et al., 1993; Zinszner et al., 1994). The 3' end of the *TLS* gene contains an RNA recognition motif (RRM), a C2-C2 zinc finger, both of which are flanked by arg-gly-gly (RGG) rich regions (Aman et al., 1996). The zinc finger found within the TLS protein is homologous to those found in snRNP-associated protein 69KD and RBP56 while the RRM contains an 80 amino acid ribonucleoprotein consensus sequence (Crozat et al., 1993b; Hackl & Luhrmann, 1996; Morohoshi et al., 1996).

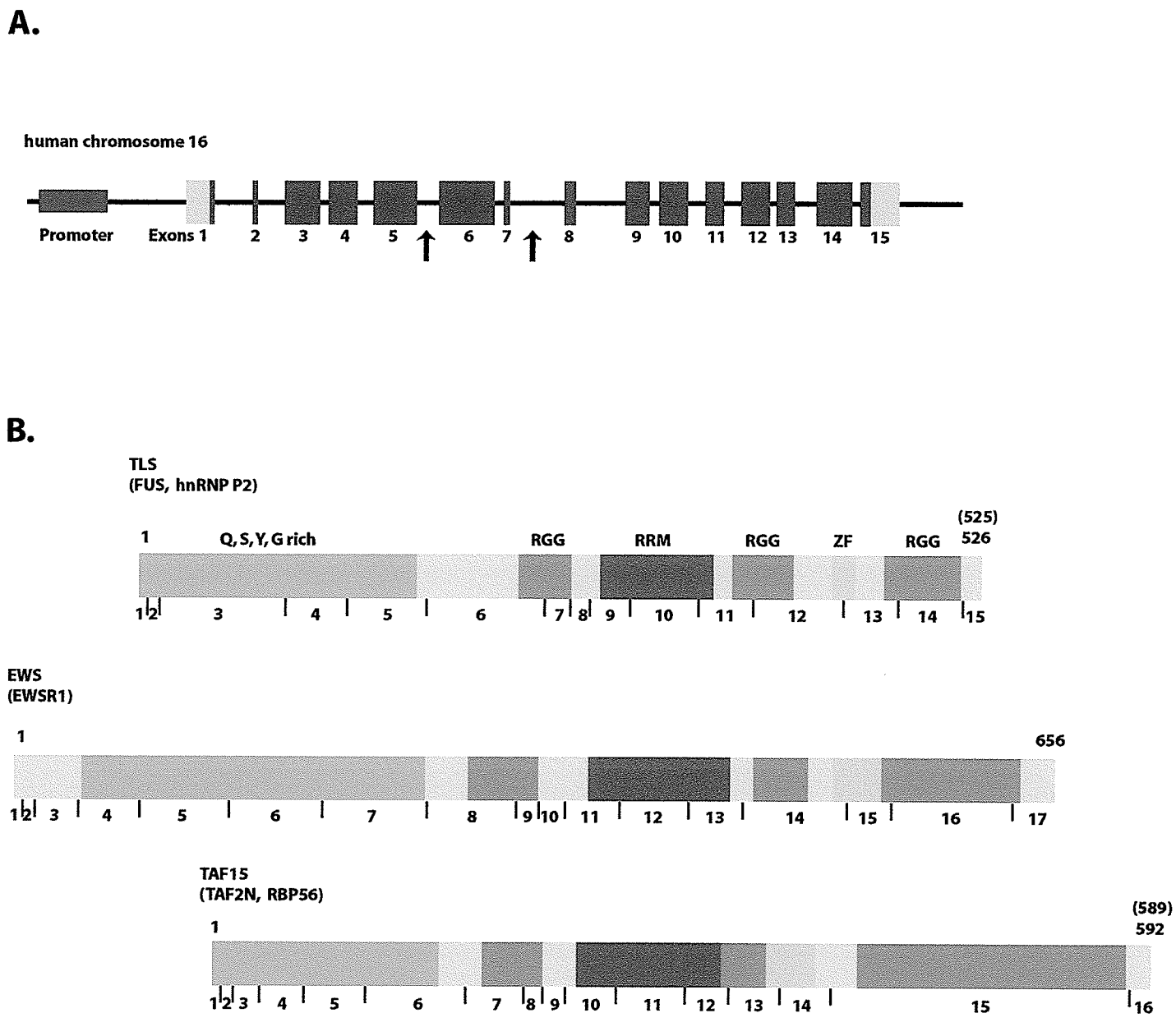


Figure 1. TLS structure and functional domains. **A.** Exon structure of TLS is shown to scale. Arrows indicate common sites of chromosomal transloactions in human neoplasms. **B.** TLS, EWS and Taf15 proteins proteins are shown with functional motifs highlighted. Q, S, Y, G rich, region enriched with gln, ser, tyr, gly amino acids; RGG, arg-gly-gly repeat; RRM, RNA.

The gene for *TLS* is part of a family of genes that includes *TLS*, *EWS* and *TAF15* (TET family). The *EWS* gene is involved in clear cell sarcomas of a tendons or aponeurosis, intra-abdominal desmoplastic small round cell tumors, myxoid chondrosarcomas or liposarcomas and, of course, the tumor it is named after Ewing's sarcoma (Ewing, 1972). *TAF15*, also known as *RBP56* or *TAF2N*, has been isolated in a single case from a myxoid chondrosarcoma (Morohoshi et al., 1996). All three genes are related through amino acid similarity of the expressed gene products and as the genetic determinants of related cancers, as outlined in Table 1. Both *TLS* and *EWS* genes are found translocated in three tumors with the same fusion partners of *CHOP, ERG* and *ATF1* genes causing the same or similar pathology. Transformation potential of chimera proteins of TLS–CHOP and TLS–ERG was confirmed in mouse cell (Ichikawa et al., 1999; Zinszner et al., 1994) and human hematopoietic cell assays (Pereira et al., 1998). Similar experiments performed with EWS–FLI and EWS–ERG chimera proteins, demonstrated the same transformation potential of the *EWS* gene product in hematopoietic cell lines as that of the *TLS* gene product (Ichikawa et al., 1999).

The protein encoded by the *TLS* gene is expressed at high levels in most tissues including cells of hematopoietic origins (Aman et al., 1996; Morohoshi et al., 1996). Since the TLS protein has been found to primarily localize to the nucleus and the fact that it has been independently co-purified with other hnRNPs suggests it might be involved in nucleo-cytoplasmic chaperoning of mRNA or pre-mRNA, or as part of the spliceosome (Calvio et al., 1995; Crozat et al., 1993a; Meissner et al., 2003; Zinszner et al., 1997b).

TLS might also function as a basal transcription factor due to its presence in RNA polymerase II transcription complexes

Table 1: *TLS* and TET family Translocations in Human Neoplasms

TET Gene	Location	Fused Gene	Location	Malignancy type	Reference
FUS (TLS, hnRNP P2)	(16p11.2)	*c/ebpξ*	12q13.1	Myxoid liposarcoma	(Crozat et al., 1993a)
		ERG	21q22.3	Acute myeloid leukemia	(Ichikawa et al., 1994)
		ATF1	12q13	Fibrous hystocytoma	(Waters et al., 2000)
			7q33-q34	Low-grade fibromyxoid sarcoma	(Storlazzi et al., 2003)
EWS (EWSR1)	(22q12)	*FEV*	2	EFT	(Peter et al., 1997)
		POU5F1	6p21.3	Undifferentiated sarcoma	(Yamaguchi et al., 2005)
		ETV1	7p22	EFT	(Jeon et al., 1995)
		FLI-1	11q24	EFT	(Delattre et al., 1992)
		E1AF	17q12	EFT	(Urano et al., 1996)
		ERG	21q22.3	ETF	(Sorensen et al., 1994)
		ZSG	1p36	Round cell sarcoma	(Mastrangelo et al., 2000)
		ATF1	12q13	MMSP	(Zucman et al., 1993)
		WT1	11p13	DSRCT	(Ladanyi & Gerald, 1994)
		c/ebpζ	12q13.1	Myxoid liposarcoma	(Panagopoulos et al., 1996)
		CSFM	9q22	Myxoid chondrosarcoma	(Gill et al., 1995)
TAF15 (TAF2N, RBP56)	(17q12)	*CSFM*	9q22	Myxoid chondrosarcoma	(Sjogren et al., 1999)

(Immanuel et al., 1995; Zinszner et al., 1994). Interestingly, the other members of the TET family have also been detected in transcription complexes with TAF2N persistently found to inhabit RNA polymerase II pre-initiation complexes, while EWS interacts with hsRPB7, a RNA polymerase II subunit (Bertolotti et al., 1996; Petermann et al., 1998). Another presumed function of the TLS protein is as a co-regulator of nuclear receptors, as the *TLS* gene product is found to interact with the receptors of steroid and thyroid hormones, retinoids and vitamin D (Powers et al., 1998). The overall amino acid sequence and nature of TLS as well as its interactions imply a role in coupled transcription and processing of RNA.

To further understand the function of the *TLS* gene a number of laboratories, including ours, developed transgenic mouse models. Our lab constructed a murine model in which the *TLS* gene has been knocked out through insertion mutagenesis via retrovirus integration into genomic DNA (Hicks et al., 2000). Mice that are homozygous for the mutation are not viable as they failed to suckle and died within a day of parturition. A closer analysis of these $tls^{-/-}$ mice revealed a number of hematopoietic deficiencies including decreased thymus size, a defect in proliferation response to specific stimuli in mature B-cells and an overall reduction in lymphopoiesis particularly of IgM^{+} B-lymphocytes. Examination of metaphase spreads, of cells harvested from $tls^{-/-}$ mice, revealed a wide variety of karyotypic anomalies, including aneuploidy, centromeric fusions, chromosomal breaks, and extrachromosomal elements (Hicks et al., 2000). The B-lymphocytes defects of $tls^{-/-}$ murine models resemble those of $abl^{-/-}$ mice (Hardin et al.,

1995). The products of *ABL* and *ATM* genes exert a significant role in repair of DNA damage and genomic stability (Baskaran et al., 1997; Shafman et al., 1997).

Other *tls* transgenic murine models have been developed that primarily focused on the fusion between *TLS* plus *CHOP* as an attempt to reconstruct tumor phenotype in mice. While mice generated by David Ron's group did not induce liposarcoma in their mice, they did detect that male mice homozygous for the chimera protein are sterile. Extracts from testes of those males revealed that they are incapable of pairing homologous chromosomes during meiosis. Overall, the chromosomes from that particular animal model system are more sensitive to ionizing radiation than their wild-type littermates (Kuroda et al., 2000). Sanchez-Garcia's group constructed similar mice, which did develop liposarcoma when the *TLS-CHOP* chimera is over-expressed in adipocyte tissues and that the amino-terminal domain of *tls* is necessary for neogenesis, as mice over-expressing CHOP only do not exhibit any clinical signs or symptoms of sarcoma (Perez-Losada et al., 2000; Perez-Mancera et al., 2002).

Perhaps the most important finding of the murine studies is the necessity of *TLS* for the maintenance of genomic stability. A separate study has provided a plausible reason for this global genomic instability exhibited by $tls^{-/-}$ mice. Lopez's group were able to show that viable TLS protein is essential for D-loop formation, an initiation complex in double-strand break repair of chromosomal DNA (Bertrand et al., 1999). In an DNA pairing assay, they demonstrated that TLS binds double-stranded DNA, and promotes annealing of complimentary single-stranded DNA, which led them to postulate

that gene product of *TLS* is involved in the maintenance of genomic integrity during cell division of somatic cells and those of meiotic origin (Baechtold et al., 1999).

The main focus of our laboratory is the identification of cellular processes TLS is involved in. Upon the determination of TLS function, a more complete understanding of the role TLS contributes to the maintenance of genomic integrity, and hence neogenesis will be possible. Yet, despite the wealth of knowledge on TLS structure, binding specificity, and transactivating properties, very little information is available on the actual mechanisms, function and their regulation through which TLS acts in a native cell is understood. Therefore this project will focus on the determination and identification of such interacting partners of TLS in order to better characterize the role of the wild type gene.

In all *TLS* translocations analyzed thus far, only the first 215 or 245 amino acids out of 526 of TLS are found within the chimera proteins of TLS-CHOP, TLS-ERG or TLS-ETV1. Therefore, we were interested in the identification of the function of this N-terminal domain through the determination of proteins this domain is capable to interact with (Crozat et al., 1993a; Panagopoulos et al., 1994). While a number of different strategies have been developed to perform experiments to identify interactions between proteins, a yeast two-hybrid method was chosen for this project. A similar approach was previously successful in the identification of the carboxy-terminal domain of TLS (aa 357 to 526) as having function in maturation of mRNA through interactions with three splicing proteins, Sc35 and two novel proteins named TASR 1 and 2 (Yang et al., 1998).

Hickstein's group utilized the classical yeast two-hybrid method as described by Fields and Song for their identification of genes capable of interaction with the C-terminal domain of the TLS protein (Yang et al., 1998). However, in this thesis project we will perform a search for interacting proteins for the amino-terminal domain of TLS through a modified yeast two-hybrid method, called the Ras Recruitment System (Aronheim & Karin, 2000).

Interactions between two proteins form the basis for a variety of biochemical reactions, and researchers have in recent years developed a number of such methods capable of identifying protein-protein interactions (Uetz et al., 2000). One such technique is a yeast two-hybrid screen. Fields and Song originally described the first yeast two-hybrid screen in 1989. Their method relies on the reconstitution of a transcriptional activator molecule in yeast through the interaction of two proteins being investigated (Fields & Song, 1989). In this technique, the protein under examination (bait protein) is fused to the DNA binding domain (BD) of Gal4p, a known *E. coli* transcription factor with its own transactivation properties. A second set of plasmid constructs containing the activation domain (AD) of the same Gal4 protein fused to a cDNA library of choice is created and forming, upon expression, a library-Gal4p AD chimera protein (prey protein). If an interaction of the bait-BD and library-AD fusion partners occurs, the Gal4p transcriptional activator is reconstituted. Therefore, allowing expression of reporter genes that are under the transcription control of upstream activator sequences specific for *Saccharomyces cerevisiae* Gal4p. Thus a yeast cell harboring the

two chimera proteins is conveyed an advantage and can be assayed for the presence of growth on nutritional selective media or through a color-based method.

Following the initial report by Stanley Fields in 1989, several modifications of the yeast two-hybrid system have been constructed in an attempt to broaden its usefulness or apply its concepts to specific cellular mechanisms. This has been achieved through the use of other transcription factors, such as LexA and VP-16. Also the engineering of a variety of different reporter strains of *S. cerevisiae* has allowed for the improvement in the reliability of experiments by attempts in decreasing the number of false positives (Young, 1998).

One particular modification of interest to the classical yeast two-hybrid approach is the Ras Recruitment System (RRS) (Aronheim et al., 1994). The RRS contains two novel alterations; first it relocates the actual interaction outside the nucleus into the cytoplasm, and secondly it employs an activation of a biochemical pathway. This system relies on the activation of the known oncoprotein, Ras, and the consequently the MAP kinase cascade. The cascade activation in *S. cerevisiae* allows for rapid screening as it changes the phenotype, for example by growth on galactose as a sole carbon source.

One of the plasmid constructs contains the desired cDNA library, which are expressed under a galactose responsive promoter with a myristoylation signal peptide fused to the 5' end of the library proteins. The signal peptide directs all library proteins to the inner leaflet of the plasma membrane, and thus effectively directing the protein interaction to the cytoplasm. The bait construct, besides possessing the protein of interest (or a portion of it), contains on open reading frame of the *H. sapiens SOS* gene upstream

of the cloning site. However, if the fusion SOS-bait protein contained the *SOS* gene in its entirety, the expressed chimera would auto-activate the Ras protein and the MAP kinase cascade even in the absence of galactose, thus providing a high number of false positives and making the entire screen very inefficient. Therefore, the expressed chimera protein contains the *SOS* gene located on the bait construct, which lacks the transmembrane domain and thus precludes it from activating the Ras protein autonomously. Hence, only in the presence of a library protein capable of interacting with the bait protein (or a portion of it) there will be activation of Ras. As such interaction between the bait and library proteins is necessary to localize the SOS protein to the membrane where it is capable of interacting with the Ras gene product. This leads to the induction of the MAP kinase pathway under the non-permissive temperature of 37°C and the presence of galactose (Aronheim, 2001).

In this project, the amino-terminal region of TLS was used as bait in the Ras Recruitment Screen to identify interacting proteins from a cDNA expression library.

2. Materials and Methods

2.1. Plasmids

Table 2: List of plasmids used throughout this project.

Plasmid Name	Vector Size (bp)	*E. coli* selection	*S. cerevisiae* selection	GenBank accession #	Source
pSOS	11,259	amp	leu2	AF102576	(Aronheim et al., 1994)
pYes2	5, 856	amp	ura3		(Aronheim et al., 1994)
pCR-2.1	3,929	kan/amp	---		Invitrogen
pCR-Blunt	3,512	kan	---		Invitrogen
pGBT9	5,524	amp	trp1	U07646	(Gietz et al., 1997)
pGAD424	6,659	amp	leu2	U07647	(Gietz et al., 1997)

2.2. cDNA libraries

Table 3: cDNA libraries used throughout these experiments.

Species	Organ	Vector	Source
H. sapiens	Adult brain	?	B. Triggs-Raine
R. norvetigus	Pituitary gland	pYes2	(Aronheim et al., 1994)

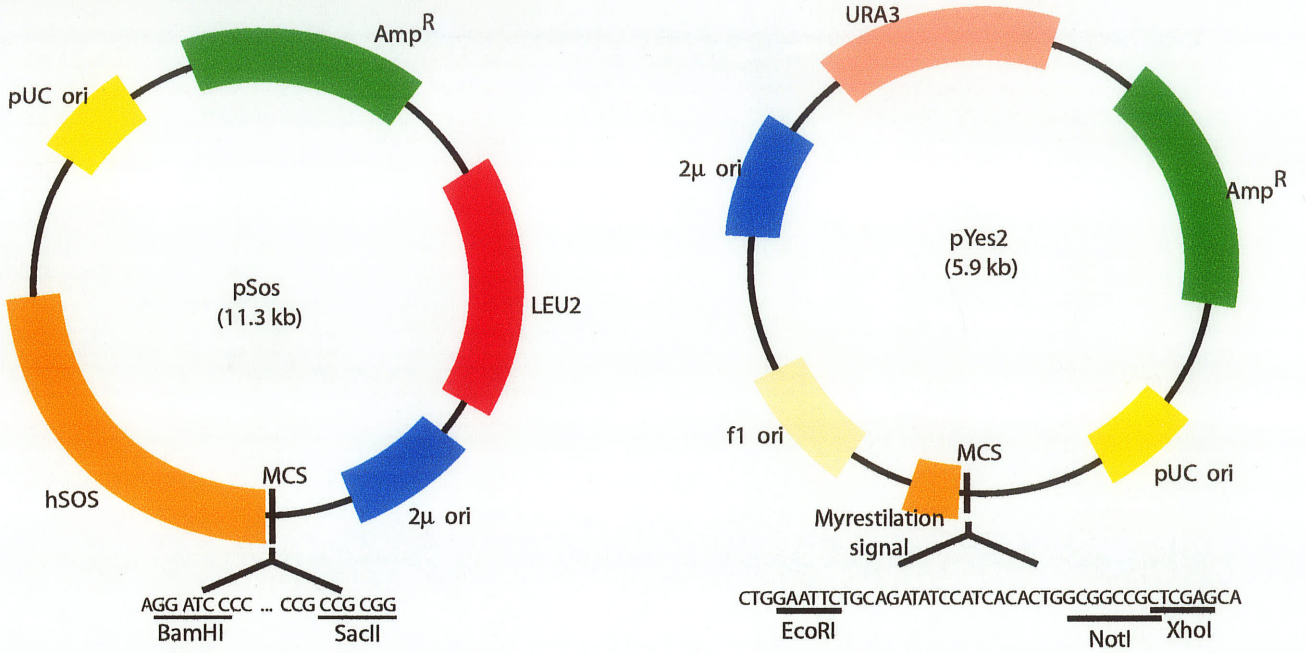
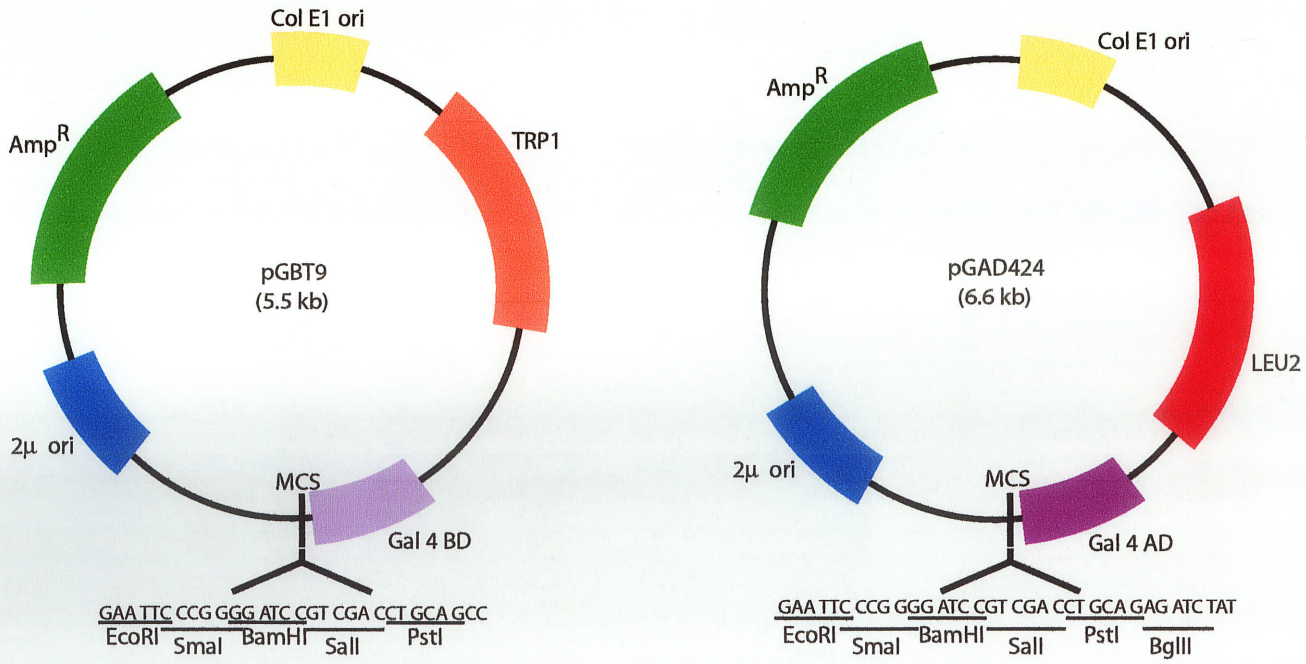


Figure 2. Maps of vectors used in the Ras Recruitment and yeast two-hybrid systems. Vector diagrams showing size, pertinent features and multiple cloning sites. For a detailed sequence of vectors see manufacturer (or genbank accession #). pSos: Stratagene (#AF102576), pGAD424 and pGBT9: both Clontech (#'s U07647 and U07646, respectively), pYes2: Invitrogen.



2.3. Bacterial Strains

Table 4: *E. coli* strains used in throughout these studies.

Cells	Source	Genotype
DH5α	Gibco	F⁻ ϕ80ΔlacZΔM15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hsdR17(r_k^-, m_k^+) phoA supE44 λ-thi-1 gyrA96 relA1
DH10B	Gibco	F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) 80ΔlacZΔM15ΔlacX74 deoR recA1 endA1 ara139D(ara, leu)7697 galU galK l⁻ rpsL nupG
TOP10F'	Invitrogen	F′ {lacI^q Tn10 (Tet^R)} mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (Str^R) endA1 nupG
INVαF'	Invitrogen	F′ endA1 recA1 hsdR17 (r_k^-, m_k^+) supE44 thi-1 gyrA96 relA1 ϕ80lacZΔM15 Δ(lacZYA –argF)U169 λ⁻
KC8	Clonetech	hsdR, leuB600, trpC9830, pyrF::Tn5, hisB463, lacDX74, strA, galU,K

2.4. Two-hybrid *S. cerevisiae* strains

Table 5: List of *S. cerevisiae* strains used during the yeast two-hybrid screening.

Strain	Genotype	Source
Cdc25H	MATα, ura3, lys2, leu2, trp1, his200, ade 101, cdc25-2, GAL+	Stratagene
KGY37	MATa ade2 gal4 gal80 his3-Δ200 trp1-Δ901 leu2 ::pUC18 ura3::GAL1-lacZ, lys2::UASG17 MERS(x3) GAL1-HIS3	R.D. Gietz

2.5. Antibodies

Table 6: List of antibodies utilized in experiments.

Name	Type	Host	Recognition sequence	Dilution	Source	Catalogue #
α-SOS	Mc	Mouse	SOS aa 1–145	1: 2,000	BD Biotech	610095
α-TLS	Pc	Rabbit	TLS aa 78–244	1:10,000	(Zinszner et al., 1997a)	N/A
gαm HRP	Pc	Goat	Mouse	1:10,000	Santa Cruz	2033
gαr HRP	Pc	Goat	Rabbit	1:10,000	Santa Cruz	2004

Mc = monoclonal; Pc = polyclonal

2.6. Determination of DNA concentration

DNA concentration was determined through the use of the following formula:

$$\text{DNA concentration} = (A_{260})\ (50\ \mu g/\mu l)\ (\text{dilution factor})$$

where A is the absorbency of the DNA sample at 260 nm, as determined on an Ultraspec 2000 (Pharmacia-Biotech) spectrophotometer.

2.7. Gel Electrophoresis

2.7.1. Reagents

- 0.7–2.0% (w/v) electrophoresis grade agarose (Sigma
- 1 x TAE (40 mM Tris-Acetate, 2 mM EDTA)
- 10 x loading buffer [20% (v/v) Ficoll 400, 0.1 M Na_2EDTA, 1% (w/v) SDS, 0.25% (w/v) Bromophenol Blue, 0.25% (w/v) Xylene Cyanol]
- 1 µl of 10 mg/ml Ethidium Bromide per 25 ml of agarose gel
- 10 µg of 1 kb DNA ladder solution (10 µl of 1 kb DNA ladder New England Biolabs, 100 µl of 10 x loading buffer, 890 µl of DDW)

2.7.2. Size specific separation of DNA by agarose gel electrophoresis

Electrophoresis grade agarose was electrophoresed in 1 x TAE in a microwave oven until no granules were visible. Agarose gel concentration varied according to DNA fragments to be resolved, with higher concentration of agarose allows for better resolution of smaller DNA fragments. After cooling the agarose to ~50°C, ethidium bromide was added and gently mixed with agarose solution. The gel was poured into a casting tray with appropriately sized combs to yield sufficient wells for samples plus one and capable of accommodating the volume of DNA solution to be loaded. When the gel had solidified it was placed into a horizontal chamber and immersed with 1 x TAE buffer.

A 10× loading buffer was added to samples to a final concentration of 1 x, and the samples were loaded into a well. For sizing DNA 5-10 µl of DNA solution was loaded. To isolate DNA fragments, the entire DNA solution was loaded. The extra well was loaded with 10 µg of 1 kb DNA ladder. Once all the samples and ladder were loaded the electrophoresis was started. Electrophoresis was performed at 100 V in a horizontal chamber. Long wavelength UV light was used to visualize DNA fragments for photography or gel extraction (section 2.9.4).

2.8. Polymerase Chain Reaction (PCR) amplification of DNA.

To amplify specific DNA fragments, to 0.5–1.0 ng of template DNA the following was added:

- 5 µl of 10 x PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]
- 2 µl of 50 mM $MgCl_2$
- 20 pmoles of each primer
- 1 µl of Polymerase enzyme mix [1 part of *Pfu* DNA Polymerase (Stratagene) and 9 parts *Taq* recombinant DNA Polymerase (Gibco)]
- 4 µl 10 mM dNTPs (2.5 mM of each dATP, dCTP, dGTP, dTTP)

The reaction was brought to 49 µl total volume with sterile DDW. 1 µl of Polymerase enzyme mix was added last. The PCR was performed with the following settings:

- DNA denaturation at 94°C for 45 s
- Primer annealing for 30 s at 55°C
- Strand elongation at 72°C for 90 s.

To amplify DNA for the purposes of cloning, the PCR settings were cycled 35 times. A negative control, without template DNA, was always included in every PCR

experiment. Following the amplification reaction the samples were electrophoresed on an agarose gel (section 2.7.2) and the desired DNA product was then isolated and purified with QIAEX II DNA Purification kit (Promega), section 2.9.4.

2.9. Preparation of plasmid DNA for cloning

2.9.1. Restriction endonuclease digestion

All restriction endonucleases were obtained from New England Biolabs and were utilized according to manufacturer's protocol. One to 10 μg of DNA was digested in a total volume of 20 μl containing 1 x reaction buffer and 0.5–1.0 μl of each enzyme, the samples were then incubated at the optimal temperature for the restriction endonuclease(s) (Table 7). When recommended by manufacturer, bovine serum albumin was added to a final concentration of 0.1 μg/μl. After digestion of the sample from 1 to 4 hours the reaction was stopped by adding 2 μl of 10 x loading buffer (section 2.7.1). Vector DNA fragments digested with one restriction endonuclease have their 5' phosphate groups removed by calf-intestinal alkaline phosphatase before the reaction was stopped (Section 2.9.3). The DNA fragments were separated by agarose gel electrophoresis (section 2.7.2) and purified with QIAEX II DNA purification kit (section 2.9.4).

Table 7. Restriction endonucleases utilized in this project.

Restriction endonuclease	Cleavage site	Incubation Buffer	Reaction temperature
BamHI	G/GATCC	BamHI	37°C
BglII	A/GATCT	NEB 3	37°C
EcoRI	G/AATTC	EcoRI	37°C
SacII	CCGC/GG	NEB 4	37°C
SmaI	CCC/GGG	NEB 4	25°C
XhoI	C/TCGAG	NEB 2	37°C

NEB 2: 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT (pH 7.9 at 25°C)
NEB 3: 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT (pH 7.9 at 25°C)
NEB 4: 20 mM Tris-Ac, 10 mM magnesium acetate, 50 mM potassium acetate (pH 7.9 at 25°C)
EcoR1: 100 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 0.025% Triton X-100 (pH 7.9 at 25°C)
BamH1: 10 mM Tris-HCl, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT (pH 7.9 at 25°C)

2.9.2. Blunt ending 5' overhangs following endonuclease digestion.

To the restriction endonuclease digestion reaction from section 2.9.1 the following was added, and the reaction was incubated at 30°C for 15 min:

- 2.5 µl of 10 x Klenow buffer [100 mM Tris-HCl (pH 7.5), 50 mM $MgCl_2$, 75 mM DTT] buffer (New England Biolabs)
- 2 µl of 2.5 mM dNTPs (section 2.8)
- 1 µl of Klenow enzyme (at a concentration of 5 U/µl, New England Biolabs).

Termination of the reaction was achieved by adding 3 µl of 10 x loading buffer (Section 2.7.1).

2.9.3. Phosphatase treatment of DNA fragments

To prevent intermolecular and intramolecular ligation of vector DNA the 5' terminal phosphate groups were removed with calf-intestinal alkaline phosphatase, CIAP (Boehringer-Mannheim). This method was chosen every time the vector DNA was digested with a single restriction endonuclease that yields cohesive ends and for blunt end

reactions. Before incubating the sample at 37°C for 45–60 min, the following reagents were added immediately to the reaction immediately after restriction endonuclease treatment (section 2.9.1):

- 2.4 µl of 10 x dephosphorylation buffer [0.5 M Tris-HCl, 1 mM EDTA, pH 8.5 (20°C)] (Boehringer-Mannheim)
- 1 µl of CIAP (at a concentration of 1 U/µl, Boehringer-Mannheim)

Termination of the dephosphorylation reaction was achieved by incubating the sample for 15 min at 75°C, as CIAP is heat sensitive, or by adding 2.5 µl of 10 x loading buffer (Section 2.7.1).

2.9.4. Purification of DNA from an agarose gel

Purification of DNA fragments was achieved by using QIAEX II DNA Purification kit (Qiagen) and following the manufacturer's recommended protocol. Separation of DNA fragments after endonuclease digestion or PCR was achieved with agarose gel electrophoresis containing ethidium bromide (section 2.7). The desired DNA fragments were visualized under long wavelength UV light and cut out from the agarose gel with a scalpel and placed in a microfuge tube. Volume of solid gel slice was estimated by weight (0.1 g = 0.1 ml).

3 volumes of QX I buffer and 10 µl of QIAEX II resin was added to the gel slice. The sample was then vortexed for 30 s and the gel slice was melted by incubation at 50°C for 10 min. The resin/DNA slurry was then washed one more time with 500 µl of QX I buffer and 2 times with 500 µl of PE buffer. Each time the resin/DNA slurry was re-

suspended by vortexing and precipitated by centrifugation for 30 s at maximum speed, and the supernatant was carefully aspirated and discarded.

After the final wash the resin/DNA was allowed to air dry at room temperature for 10 min. Finally the DNA was eluted from the resin by re-suspension in sterile DDW. The resin was collected by centrifugation and the eluted DNA, contained within the supernatant, was transferred to a labeled sterile microfuge tube. The concentration of the eluted DNA fragments and plasmids was determined by spectrophotometry as per section 2.6, before proceeding to DNA ligation reaction.

2.10. DNA Ligations

For each ligation reaction of DNA fragments with cohesive-ends approximately 100–200 ng of linearized plasmid (as determined in section 2.6) was ligated to an equimolar amount of the desired DNA insert. To this DNA mixture, the following was added:

- 10 x ligation buffer [60 mM Tris-HCl (pH7.5), 60 mM $MgCl_2$, 50 mM NaCl, 1 mg/ml BSA, 70 mM β–mercaptoethanol, 1 mM ATP, 20 mM DTT, 10 mM spermidine]
- 4 Weiss units of T4 DNA ligase (New England Biolabs)
- The reaction was brought to a final volume of 10 or 20 µl with DDW.

The reaction was incubated for 4 hours at room temperature.

For ligation reactions of DNA fragments containing only blunt ends, the following changes were made to the above protocol:

- The DNA insert amount was raised to 3 times the molar amount of the vector
- 40 Weiss Units of T4 DNA Ligase (New England Biolabs)

- The reaction was incubated at 14°C overnight.

2.11. Transformation of chemically competent *E. coli* cells

A 25 µl aliquot of chemically competent *E. coli* cells was thawed on ice. 2 µl of DNA ligation mix was then added from section 2.10; the mixture was gently stirred and incubated for 30 min on ice. The *E. coli* cells were then 'heat shocked' at 42°C for 45 s. After cooling the cells on ice for 5 min, 250 µl of S.O.C. medium was added and the mixture was incubated at 37°C for 1 hour to allow the bacterial cells time to recover. Various aliquots of 50–200 µl of the mixture was spread plated to select for cells that took up the desired plasmid on selective plates with 50 µg/µl of appropriate antibiotic, and incubated at 37°C overnight.

For DH5α *E. coli* cells, the heat shock was performed at 37°C for 60 s, as suggested in manufacturer's protocol. When selecting for cells containing plasmids with the ampicillin resistance, cells were grown on plates containing 100 µg/µl for ampicillin.

2.12. Isolation of nucleic acids from *E. coli* cells

2.12.1. Growth and lysis of *E. coli* cells

Two ml of LB medium containing 50 µg/µl of appropriate antibiotic was inoculated with a single colony containing the desired plasmid and incubated with shaking at 37°C for 16 to 20 hours. Cells from 1.5 ml of this overnight culture were then collected by centrifugation at 13,000 rpm for 2 min in a 5417C microfuge (Eppendorf). The spent medium was removed by aspiration and discarded. A cell pellet was then re-

suspended by vortexing in 200 µl of lysis buffer [25mM Tris-HCl (pH 8.0), 10 mM EDTA, 50 mM Glucose] (Birnboim & Doly, 1979). To this solution 200 µl of sodium hydroxide/SDS solution [1% (w/v) SDS, 5.0 N NaOH] was added and the tubes were gently inverted 3 times. The cell lysate was neutralized with 200 µl of 3M potassium acetate (pH 4.8) and vortexed again. The nucleic acids were extracted from this mixture with phenol:chloroform.

When selecting for cells containing plasmids with the ampicillin resistance gene the medium contained 100 µg/µl of ampicillin.

2.12.2. Phenol:chloroform extraction of nucleic acids

An equal volume of Tris-buffered phenol:chloroform (1:1) was added to the solution from section 2.12.1 and the samples were vortexed for 20 s. Separation of the organic and aqueous phases was achieved by centrifugation at 13,000 x *g* for 5 min. 500 µl of the aqueous phase was then transferred to a new microfuge tube and nucleic acids were precipitated from this solution using isopropyl alcohol.

2.12.3. Isopropanol precipitation

To the aqueous phase an equal volume of 500 µl of isopropyl alcohol was added, and mixed by inversion. The nucleic acids were allowed to precipitate at room temperature for at least 5 min. The DNA was then collected by centrifugation and the remaining supernatant was carefully aspirated and discarded. The DNA pellet was then washed with 70% ethanol and dried at 37°C for 5 min in a SpeedVac SC–110 evaporator

(Savant). Finally the plasmid DNA was re-suspended in 50 µl of sterile DDW with 10 µg/µl RNAse A.

2.13. DNA sequencing

2.13.1. Primers

Table 8: Primers utilized for sequencing or cloning of DNA.

Name of primer	Sequence of primer (5' to 3')
M13 Reverse	GTA AAA CGA CGG CCA G
M13 Forward	CAG GAA ACA GCT ATG AC
TLS start	AGT AGG ATC CCC ATG GCC TCA AAC GAT TAT ACC
TLS stop	GCT GGA GCT CGC GCT AAT TAA TAC GGC CTC TCC C
Ex4	CGG CTA TGG CAG TAG C
Ex5	TAC CTC CAC CTC CAC CTC C
Ex5-AS	CAC TGC TGC TGT TGT ACT GG
Ex6	GGC AAT CAA GAC CAG AGT GG
Ex8	CAG GTA TCG GTG GCG A
Ex9	ATC TGC TTG AAG TAA TCA GCC A
Ex10	ACA AGA AAA CGG GAC AGC C
Ex11	AAG TTT CAT TTG CTA CCC GC
Ex13	AGG TGC CTT ACA CTG GTT GC
Ex14	GGT AAC TAC GGG GAT GAT CG
11D	GGA ATT CTT ACA CCG TCC TC
12H	GGA ATT CAT CAG GAG ACT TGC
32H	GGA ATT CGA GGA ACT GAG G
86A	GGA ATT CAC TTG GAT TCT CG
pMyr-MCS	AGT AGC AAG AGC AAG CCT
pMyr3'	GCG GAT CCT TAC ATG ATG CG
pSOS5'	AAT TAA CCG CGG CGG CCG
pGAD424	TAC CAC TAC AAT GGA TG
pGBT9	TCA TCG GAA GAG AGT AG

2.13.2. Automated DNA sequencing

To 125 ng of plasmid DNA 2.5 pmole primer was added and the reaction volume was brought up to 3 μl with sterile DDW. To each reaction tube 1.67 μl of dilution buffer and 0.33 μl of BigDye reaction mix (Perkin Elmer) was added. The DNA samples were then PCR amplified with the following settings: 96°C for 10 s, 40°C for 5 s, 60°C for 4 min times 25 cycles.

Following PCR amplification, 95% ethanol was added to a final concentration of 60% (v/v), transferred to a fresh tube, and the DNA was allowed to precipitate at room temperature for at least 1 hour. The plasmid DNA was then pelleted in a microcentrifuge at 13,000 x *g* for 15 min, and the supernatant was very carefully aspirated and discarded. The pellet was then washed with 70% ethanol, and dried for 5 min at 37°C in a SpeedVac SC–110 evaporator (Savant). The pellet was then submitted to the Cancer Care Manitoba core sequencing facility where it was loaded within 36 hours onto ABI Prism 310 automated sequencer (Perkin Elmer). While waiting to be sequenced the DNA samples were stored in 4°C in a light deficient environment. The generated output was then analyzed with blast sequence search at the NIH website (http://www.ncbi.nlm.nih.gov/BLAST/).

2.14. Amplification and isolation of DNA library

The *R. norvetigus* pituitary cDNA library was transformed by electroporation (Section 2.19) into high-efficiency electrocompetent DH5α *E. coli* cells (Table 4), spread onto LB+amp plates and incubated for 16 hours at 37°C (Dower et al., 1988). The

overnight colonies were harvested by scrapping the plates and the plasmid DNA was isolated from bacterial cells as described in section 2.12

2.15. Transformation of *Saccharomyces cerevisiae*

Plasmid introduction into *S. cerevisiae* was performed using a modified method of R.D. Gietz (Gietz et al., 1995b). A 10 ml broth of YPAD or appropriate synthetic complete omission medium (SC–) was inoculated with a single yeast colony and incubated overnight at 24.2°C on a shaker cycling at 120 rpm.

The following day, the overnight culture was sub-cultured to a final concentration of 5 x 10^6 cells (or OD_{600} of 0.25) into a fresh 50 ml broth of YPAD (or SC–) medium. The inoculum was then grown for approximately 8 hours (~12 hours for SC– medium) to a final concentration of 2 x 10^7 cells/ml (or OD_{600} of 2.00). *S. cerevisiae* cells were then harvested by centrifugation at 5,000 x *g* for 5 min at room temperature and spent medium was discarded. *S. cerevisiae* cell pellet was then re-suspended and washed twice with first equal volume and later with half volume of sterile DDW and collected as described above. After the two washes the cells were pretreated with 1 ml of 100 mM LiAc, collected by centrifugation at 13,000 rpm for 5 s in a microcentrifuge (Biofuge A). Finally the cell pellet was re-suspended at a concentration of 2.0 x 10^9 cells/ml with 100 mM LiAc.

For each transformation, a 50 µl aliquot of above cell suspension solution (or 1.0 x 10^8 cells) was collected by centrifugation. The *S. cerevisiae* cells were then re-suspended with 360 µl of transformation mix [66% (v/v) PEG $_{(Mr3350)}$, 10% (v/v) 1 M

LiAc, 5% (v/v) DMSO, 5% (v/v), 95% ethanol and 50 μg of denatured salmon sperm DNA]. 15 μg of DNA to be introduced was added and mixed into *S. cerevisiae* cell re-suspended within the transformation solution. Without pre-incubating the cell transformation mixture, the samples were 'heat shocked' at 42°C for 25 min. After heat-treating the cells they were then collected by centrifugation at 13,000 rpm for 5 s using a Biofuge centrifuge. Transformation mix was carefully aspirated and the cell pellet was gently dissolved in 1 ml of sterile nano-pure water. Various aliquots (between 10 μl and 200 μl) of the transformed samples were then spread plated and incubated at 24.2°C for 2–5 days.

The aliquots were incubated on SC plates lacking appropriate nutrients, as dictated by the experiment being performed, to confer a growth advantage to the cells harboring the desired combination of plasmid(s).

2.16. Library screen transformations

Transformation of the library was performed utilizing the protocol described in section 2.15. A 90 times scale-up of the transformation was performed and the entire cell pellet was diluted in DDW.

The cells were then plated on synthetic complete plates with glucose and lacking leucine and uracil (SC+glc–L–U) and were incubated at 24.2°C for approximately 20 hrs. These plates were then replicated onto plates with galactose and also lacking leucine and uracil (SC+gal–L–U) and incubated at 37°C for 4–6 days. Upon growth, recovery of plasmids from cells deemed positive was performed via method listed in section 2.17.

2.17. Plasmid recovery from *S. cerevisiae*

2.17.1. Reagents

- Glass beads 425 to 600 μm acid washed (Sigma G-8772)
- Yeast cracking buffer [2% (v/v) Triton X-100, 1% (w/v) SDS, 100 mM NaCl, 20 mM Tris-HCl (pH 8.0), 10 mM EDTA]
- 3.0 M Sodium Acetate (pH 5.2)
- 100 % Ethanol
- 70 % (v/v) Ethanol
- 10 μg/μl RNAse A

2.17.2. Isolation of plasmid DNA from *S. cerevisiae*

Plasmid recovery from yeast was performed using the method of Hoffman and Winston (Hoffman & Winston, 1987). Yeast colony harboring the desired plasmid was inoculated into 10 ml of SC–L–U and grown for 16–20 hrs. The overnight culture was then harvested by centrifugation and re-suspended in 200 μl of yeast cracking buffer. Cells were then mixed with equal volume of glass beads. This solution was then vigorously mixed with a vortex for 30 s three times with one-minute incubations on ice in-between. The nucleic acids were extracted using phenol:chloroform as previously described in section 2.12.2. The aqueous phase was then transferred to a fresh tube to be ethanol precipitated, described in the following section (2.18). These DNA solutions were then used for transformation of electrocompetent KC8 *E. coli* cells (section 2.19) for both plasmid amplification and isolation of the pYes2 plasmid containing the library cDNA insert.

2.18. Ethanol precipitation

The aqueous phase solutions containing the nucleic acids form section 2.17 were extracted from the aqueous phase by ethanol precipitation. To this nucleic acid solution $1/10^{th}$ volume of 3.0 M NaAc and 2.5 volumes (after salt addition) of ice-cold absolute ethanol was added. The samples were mixed by vortexing and incubated at –80°C for 15 min, or –20°C for a minimum of 1 hour. The precipitate was then collected by centrifugation at 13,000 x *g* for 15 min at 4 degrees Celsius. The pellet was then rinsed with 70% ethanol, and dried for 5 min at 37°C in a SpeedVac SC–110 evaporator (Savant). Finally plasmid DNA was re-suspended in DDW with 10 μg/μl RNAse A. This re-suspended DNA solution was utilized for electroporation of KC8 *E. coli* to allow both amplification and selection of the plasmid containing the library cDNA insert.

2.19. Transformation of electrocompetent *E. coli* and the selection for plasmids containing library cDNA inserts.

2.19.1. Preparation of electrocompetent *E. coli*

Preparation of electrocompetent *E. coli* was performed as described by Dower *et al.* (Dower et al., 1988). *E. coli* cells were prepared by growing a 1 L culture of LB medium inoculated to an OD_{600} of 0.7 with the strain of interest; in this case KC8 *E. coli* (Table 4). The culture was rapidly chilled in an ice-water bath and harvested by centrifugation at 4,000 x *g* for 15 min at 4°C. Cell pellets were washed twice with sterile ice-cold DDW re-suspended in 1 L and the second time in 500 ml. The cells were then

harvested again and re-suspended in 20 ml ice-cold sterile 10% (v/v) glycerol. The cell glycerol suspension solution was allocated into 25 µl aliquots, and rapidly cryo-preserved with liquid nitrogen, then stored at –80°C.

2.19.2. Electroporation

DNA plasmid solutions obtained from section 2.15 were electroporated into *E. coli* cells (Dower et al., 1988). An aliquot of 25 µl electrocompetent KC8 *E. coli* cells was thawed on ice and mixed with 2–10 µl (up to 3 µg) of DNA. The mixture of cells and plasmid DNA was pipetted into an ice-cold electroporation cuvette (Bio-Rad) with a 1 mm gap. Gene Pulser (Bio-Rad) was set at 1.25kV, 25 µF, with a 400 Ω resistor in parallel and used to electroporate the cells with the pulse duration ranging from 6.5 to 9.6 ms. The KC8 *E. coli* cells were immediately transferred to 1 ml of pre-warmed S.O.C. medium and incubated at 37°C with shaking at 200 rpm for 45 min. Two hundred to 500 µl of the samples were spread onto LB+amp (100µg/µl) plates and incubated for 14–18 hours at 37°C.

2.19.3. Identification of KC8 *E. coli* colonies containing library plasmid DNA.

To select for the *E. coli* colonies harboring the plasmid containing a library cDNA insert, the overnight LB+amp plates were replicated onto M9–U+amp and incubated at 37°C for 14–18 hours. The overnight colonies were then cultured; the DNA was recovered and sequenced as outlined in sections 2.12 and 2.13, respectively.

2.20. Preparation of protein extracts from *S. cerevisiae*

2.20.1. Reagents

- Yeast lysis buffer [50mM HEPES, 200 mM NaCl, 10 mM EDTA, 2 mM $NaVO_4$, 10 mM NaF]
- Protease inhibitors [5 μg/μl Aprotinin, 5 μg/μl Leupeptin, 2.5 μg/μl Pepstatin A, 1 mM PMSF]
- Glass beads, acid–washed 426–600 μm (Sigma G-8772)

2.20.2. Isolation of protein extracts from *S. cerevisiae*

A yeast colony harboring the desired plasmid, and hence protein, was inoculated into 10 ml of SC–L and grown for 16–20 hrs. The overnight culture was then harvested by centrifugation and re-suspended in 2 volumes of yeast lysis containing protease inhibitors. Then one volume of glass beads was added. All remaining manipulations were performed either in a cold-room at 4°C or on ice. The samples were then cycled 6 times through vortexing of 30 s with a break of 60 s in-between. The protein lysates were then centrifuged for 5 min and the protein containing supernatant was utilized in the next step. The protein lysates were then separated by polyacrylamide gel electrophoresis and immunobloted (sections 2.23 to 2.25); but first total protein concentration was determined by the Bradford protein assay (section 2.22) for each sample in order to load equal protein concentrations.

2.21. β – galactosidase assay

Secondary screening of TLS' interacting proteins was performed using the β-galactosidase assay, as detailed (Gietz et al., 1995a). Transformants harboring the desired combinations of plasmids, were freshly re-streaked onto appropriate SC omission

plates, and incubated for 2 days at 30° Celsius. Colonies were then transferred to sterile 90 mm filter paper (Whatmann) by carefully lifting the filter disc from the plates. The cells were lysed by submersing the filter in liquid nitrogen for 15 s, and thawed out for another 15 s. The freeze–thaw cycle was repeated three times. This filter was placed on top of a second filter previously submersed in Z-buffer [100 mM Na2HPO4 (ph7.0) 10 mM KCl, 1 mM MgSO4, 38 mM B-mercaptoethanol, 1 mg/ml X–gal] with the cell side facing up. These filters were then incubated at 37°C for 2–8 hours to allow sufficient time for adequate color development on filters.

2.22. Bradford protein assay

Protein concentrations were assayed by a modified protocol first described in Bradford, M.M. (Bradford, 1976). In a 96 well plate the following was added:

- 198 µl of sterile DDW
- 2 µl of cell lysate (from section 2.20)
- 50 µl of Bradford agent (Bio–Rad)

The absorbency was measured in triplicate for each sample at 595 nm using the Ultraspec 2000 (Pharmacia–Biotech) and the protein concentration was extrapolated from a standard BSA curve, with the absorbency of the BSA samples assessed simultaneously.

2.23. SDS–PAGE

2.23.1. Reagents

To make 6% SDS–PAGE:

- 2.0 ml of 30% acrylamide mix [29 % (w/v) acrylamide, 1 % (w/v) N,N'-methylenebisacrylamide]

- 2.5 ml 1.5 M Tris HCl (pH 8.8 at 25°C)
- 0.1 ml 10% Ammonium persulfate
- 0.1 ml 10% SDS
- 8 µl TEMED (N, N, N', N'-Tetramethylethylenediamide, electrophoresis grade)
- Bring to final volume of 10 ml with DDW.

Stacking gel:

- 0.63 ml 1.0 M Tris HCl (pH 6.8 at 25 °C)
- 0.05 ml 10% Ammonium persulfate
- 0.05 ml 10% SDS
- 0.83 ml 30% acrylamide mix
- 0.004 TEMED
- Bring to 5 ml with DDW.

In addition, other reagents necessary for SDS–PAGE:

- 10 x protein loading buffer [50% (v/v) glycerol, 500 mM DTT, 250 mM Tris-HCl (pH6.8), 10% (w/v) SDS, 0.5% (w/v) Bromophenol blue]
- 1 x protein running buffer [2% (w/v) glycine, 0.3% (w/v) Tris base, 0.1% (v/v) SDS]
- 5 µl Protein Ladder (New England Biolabs catalogue # 7708)

2.23.2. Separation of proteins through SDS–PAGE

Sodium dodocyl sulfate electrophoresis (SDS–PAGE) was utilized to separate proteins by molecular weight. Reagents from section 2.23.1 were premixed in 10 ml of DDW without TEMED. After assembling the mini-gel casting tray (Bio–Rad), the tightness of seals was checked with pure water. Once satisfied with the set-up, TEMED was added, mixed, and the polyacrylamide gel was immediately poured into the casting tray to 2/3 full. 95% ethanol was overlayed to smooth out the polyacrylamide gel and let set for 10–20 min. Ethanol was decanted and the stacking gel, previously premixed as described above, was overlayed on the running gel. Appropriately sized comb was inserted taking into consideration a sufficient number of wells for samples and the

volume of protein solution to be loaded. Once the polyacrylamide gel was set, 10–20 min, the comb was removed and the mini-gel apparatus was assembled into a vertical chamber and filled with sufficient 1x protein running buffer to immerse the wells completely.

Ten μg of total protein extract from section 2.20 was loaded per well onto 6% SDS-PAGE. To each sample 10 x protein loading buffer was added to a final concentration of 1 x. Samples were denatured in a boiling water-bath for 5 min, and then cooled to room temperature before loading into wells. The extra well was loaded with 5 μg of protein ladder. Once all the samples and ladder were loaded the electrophoresis can commence. SDS–PAGE reactions were performed in protein running buffer at 100 V for 45–60 min. The time of electrophoresis varied according to the molecular weight of the proteins, with longer electrophoresis reactions required for better resolution of larger proteins. The protein ladder was used to guide the length of electrophoresis as individual protein markers, corresponding to the expected size of sample proteins, become sufficiently separated.

2.24. Western blotting

2.24.1. Reagents:

- Blocking solution [5% (v/v) FBS in PBS, or 3% BSA and 0.2% (v/v) Tween–20 in PBS].
- Nitrocellulose membrane (0.45 μm, Bio–Rad 162-0115)
- PBS [140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, (pH 7.3)]
- Transfer buffer [20% (v/v) methanol, 192 mM glycine, 25 mM Tris base, 4% (w/v) SDS]

- Wash solution [PBS + 0.1% (v/v) Tween–20]
- ECL kit (Amersham, RPN2106)

2.24.2. Protein transfer to nitrocellulose

The transfer of proteins from a SDS–PAGE to a nitrocellulose membrane was performed in a mini–transblot transfer cell (Bio–Rad) as outlined in manufacturers' protocol. The cassette was immersed in a transfer buffer and transferred for 90 min with constant current of 0.30 mA and the voltage not exceeding 180 kV. The cassette was then recovered, components carefully removed, separated, and the protein ladder was marked on the membrane before proceeding with immunodetection.

2.24.3. Antibody mediated protein detection

Following the transfer of proteins to a nitrocellulose, the membrane was placed in a small container and immersed in blocking solution Blocking was performed for 1 hour at room temperature with shaking at 150 rpm, or overnight at 4°C without shaking. The membrane was then washed 3 times for 10 min each with shaking. Then the primary antibody was added and incubated with the membrane for 1 hour on a rocker as above. To remove any unbound 1° antibody the membrane was washed again for 10 min 3 times with shaking. Secondary antibody conjugated to HRP (Table 6) was then appropriately diluted and incubated with the membrane for 1 hour with shaking. Again the unbound secondary antibody was removed by washing as above. The proteins on the blot were then made incandescent via enhanced chemiluminescence.

2.24.4. Enhanced chemiluminescence (ECL)

Equal volumes of reagent A and B of the ECL kit (Amersham) were premixed immediately prior to their addition to the membrane. The membrane was exposed for 90 s with constant shaking at 150 rpm. The blots were then wrapped in cellophane, taped inside the exposure cassette to prevent shifting, and the membranes were exposed for 5 min in absolute darkness with ECL Hyperfilm (Amersham). If required the exposure time was increased to 10, 15, or 20 min. The film was then processed in CP1000 film processor (AGFA), and aligned with the blots inside the exposure cassette. Once there, the molecular protein markers were labeled on the film from the nitro cellulose membrane, thus allowing the results to be analyzed with respect to each other and the standard.

3. Results and Discussion

3.1. Cloning of full-length human TLS cDNA

Our laboratory is interested in the function of *TLS* and how it contributes to cancer. To begin a molecular analysis it was necessary to clone the cDNA for *TLS* as a first step. As our main goal is to determine the role TLS plays in human neoplasms, we rationalized that the human gene was the most relevant homologue to evaluate. In 1996 Aman *et al.* Published the first complete cDNA sequence of the *tls* gene (Aman et al., 1996). The corresponding GenBank Accession, S62140, was chosen as our reference sequence (Crozat et al., 1993b).

TLS cDNA was cloned from an adult *H. sapiens* brain cDNA library (a generous gift from Dr. D. Bosc) (Bosc et al., 1999) by RT-PCR, as detailed in Section 2.8. Briefly, TLS-specific primers "TLS Start" and "TLS Stop" (Table 8) that contain a BamHI and SacI restriction sites, respectively, were used for the cDNA and amplification steps. The resulting PCR product was ligated into the pZero cloning vector (Table 2) and insert containing plasmids were selected and amplified in DH5α *E. coli* cells (Table 4). TLS cDNA from isolated plasmids clones was then sequenced in its entirety in both directions. From seven clones, a single clone containing full-length *TLS* gene (nucleotides 87-1683; amino acids 1-526; Figure 1) was identified. Sequence analysis of the cloned cDNA was determined to be identical to the S62140 *TLS* reference sequence. This clone was denoted

as pZero-TLS^{FL}, and will be referred to as such thereafter. The complete annotated sequence of the human *TLS* cDNA is shown in Figure 3.

3.2. Rationale for the yeast two-hybrid system

Interactions between two proteins form the basis for a variety of biochemical reactions and novel methods to identify such interactions have gathered much interest recently (Uetz & Hughes, 2000). One of the foremost approaches is the yeast two-hybrid screen. The main advantage of the RRS over the original yeast two-hybrid screen described by Fields and Song is that it relocates the protein interaction out of the nucleus and into the cytoplasm (Broder et al., 1998). This, in theory, should allow for a cleaner screening of libraries and bait proteins, especially when the protein of interest is in itself a transcription factor or contains an activation domain, such as TLS (Barak et al., 2001).

3.3. Components for the Ras Recruitment Screen

In human cancer, the two most common *TLS* chromosomal translocation breakpoints occur within intron 5 or 7, corresponding to amino acids 212 and 242 of TLS, respectively (Crozat et al., 1993a; Rabbitts et al., 1993). To better determine role of *TLS* gene and its contribution to carcinogenesis, the amino terminus of *TLS* was cloned into the pSOS vector as bait for interacting proteins.

```
   1 atgctcagtc ctccaggcgt cggtgctcag cggtgttgga acttcgttgc ttgcttgcct
  61 gtgcgcgcgt gcgcggacAT Ggcctcaaac gattataccc aacaagcaac ccaaagctat
 121 ggggcctacc ccacccagcc cgggcagggc tattcccagc agagcagtca gccctacgga
 181 cagcagagtt acagtggtta tagccagtcc acggacactt caggctatgg ccagagcagc
 241 tattcttctt atggccagag ccagaacaca ggctatggaa ctcagtcaac tccccaggga
 301 tatggctcga ctggcggcta tggcagtagc cagagctccc aatcgtctta cgggcagcag
 361 tcctcctacc ctggctatgg ccagcagcca gctcccagca gcacctcggg aagttacggt
 421 agcagttctc agagcagcag ctatgggcag ccccagagtg ggagctacag ccagcagcct
 481 agctatggtg gacagcagca aagctatgga cagcagcaaa gctataatcc ccctcagggc
 541 tatggacagc agaaccagta caacagcagc agtggtggtg gaggtggagg tggaggtgga
 601 ggtaactatg gccaagatca atcctccatg agtagtggtg gtggcagtgg tggcggttat
 661 ggcaatcaag accagagtgg tggaggtggc agcggtggct atggacagca ggaccgtgga
 721 ggccgcggca ggggtggcag tggtggcggc ggcggcggcg gcggtggtgg ttacaaccgc
 781 agcagtggtg gctatgaacc cagaggtcgt ggaggtggcc gtggaggcag aggtggcatg
 841 ggcggaagtg accgtggtgg cttcaataaa tttggtggcc ctcgggacca aggatcacgt
 901 catgactccg aacaggataa ttcagacaac aacaccatct ttgtgcaagg cctgggtgag
 961 aatgttacaa ttgagtctgt ggctgattac ttcaagcaga ttggtattat taagacaaac
1021 aagaaaacgg gacagcccat gattaatttg tacacagaca gggaaactgg caagctgaag
1081 ggagaggcaa cggtctcttt tgatgaccca ccttcagcta aagcagctat tgactggttt
1141 gatggtaaag aattctccgg aaatcctatc aaggtctcat ttgctactcg ccgggcagac
1201 tttaatcggg gtggtggcaa tggtcgtgga ggccgagggc gaggaggacc catgggccgt
1261 ggaggctatg gaggtggtgg cagtggtggt ggtggccgag gaggatttcc cagtggaggt
1321 ggtggcggtg gaggacagca gcgagctggt gactggaagt gtcctaatcc cacctgtgag
1381 aatatgaact tctcttggag gaatgaatgc aaccagtgta aggcccctaa accagatggc
1441 ccaggagggg gaccaggtgg ctctcacatg gggggtaact acggggatga tcgtcgtggt
1501 ggcagaggag gctatgatcg aggcggctac cggggccgcg gcggggaccg tggaggcttc
1561 cgaggggccc ggggtggtgg ggacagaggt ggctttggcc ctggcaagat ggattccagg
1621 ggtgagcaca gacaggatcg cagggagagg ccgtatTAAt tagcctggct ccccaggttc
1681 tggaacagct ttttgtcctg tacccagtgt taccctcgtt attttgtaac cttccaattc
1741 ctgatcaccc aagggttttt tttgtgtcgg actatgtaat tgtaactata cctctggttc
1801 ccattaaaag tgaccatttt agtt
```

Figure 3. The cloned sequence of the human *TLS* cDNA. The 1824 bp fragment containing an ORF encoding the 526 amino acid TLS protein. The start (ATG) and stop (TAA) codons are capitalised and shown in bold. Double triangles indicate exon boundaries. Asterisks indicate introns 5 and 7, the common sites of chromosomal translocations in human neoplasias.

In our screen, TLS cDNA corresponding to amino acids 1–242 of TLS was sub-cloned into the pSOS vector, as described in the following section. The cDNA expression library to be screened was a generous gift from Ami Aronheim (Aronheim et al., 1994), which at the time was a convenient and only available source. This allowed for a prompt way to commence our search for interacting proteins of TLS.

3.3.1. Sub-cloning of amino terminus of *TLS* into the pSOS vector.

To screen for interacting partners of TLS in a two-hybrid system a bait plasmid encoding an in-frame human SOSΔTM–TLS chimera protein was constructed (Figure 4). As not all chimera proteins were expressed by *S. cerevisiae,* two strategies were utilized to sub-clone the amino terminus of the *TLS* gene (R.D. Gietz, personal communication). The expectation was that at least one of the two constructed plasmids would be readily transcribed and translated with stable chimera protein expression upon transformation of the plasmid into *S. cerevisiae*.

The first strategy involved sub-cloning cDNA corresponding to amino acids 1–216 taking advantage of convenient restriction endonuclease cleavage sites. The second strategy involved PCR amplification of cDNA corresponding to the first 242 amino acids of *TLS* gene, which included appropriate restriction endonuclease site in the primers. In both strategies, the bait gene was cloned into an 11.3 kb pSOS vector (Invitrogen) that contains the *SOS*ΔTM upstream of the multiple cloning site. The *SOS*ΔTM cDNA encodes for amino acids 1–1024 of the human Son of Sevenless (SOS) protein, which lacks its native transmembrane domain.

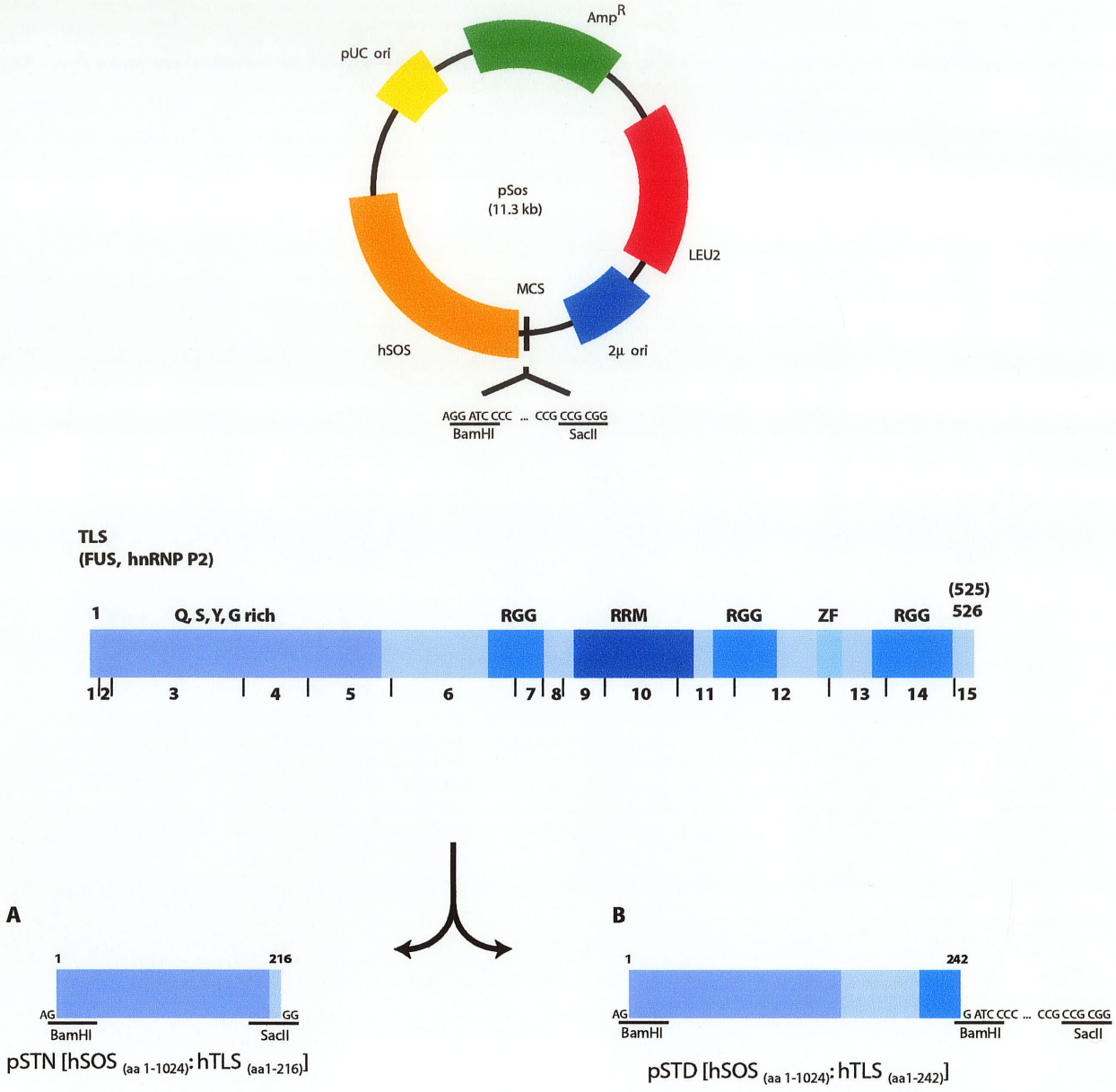


Figure 4. Sub-cloning of *TLS* amino terminus into pSos vector. Sub-cloning of *TLS* amino terminus into pSos vector, corresponding to the *tls* domains of the two common chromosomal fusion protein products. **A.** In the enzymatic digest approach, the vector pSos is first digested with BamHI and SacII restriction endonucleases yielding an 11.3 kb fragment. The $pTLS^{FL}$ (section 3.1.1.) is digested with the same 2 restriction endonucleases as the vector, liberating a 650 nucleotide long insert containing the amino terminus of the *TLS* gene. The two fragments are then isolated and ligated creating a pSTN [SOS (aa 1-1024): TLS (aa1-216)]. **B.** In the PCR strategy, the $pTLS^{FL}$ is used as template with two primers, TLS start and Ex4 (see table 8 for primer details), both containing BamHI restriction sites. Following amplification and endonuclease digestion, the 737 base pair amino terminal fragment of the *tls* gene is isolated and ligated to the pSos vector previously digested with BamHI and dephosphorylated with CIAP. This clone yielded pSTD [SOS (aa 1-1024):TLS (aa1-242)]. The creation of a viable ORF is confirmed in both cases via sequencing with the SOS3′ primer (Table 8).

In the enzymatic digestion strategy, the vector pSOS was first digested with BamHI and SacII restriction endonucleases yielding an 11.3 kb linear fragment. The TLS^{FL} (section 3.1.1.) was digested with the same two restriction endonucleases, yielding a 650 nucleotide insert containing the amino terminus of the *TLS* gene. The two fragments were then isolated and ligated creating the bait expression plasmid, pSTN [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}216)}$]. In the PCR strategy, $pTLS^{FL}$ was used as template for PCR amplification using the primers, "TLS start" and "Ex4" (Table 8). BamHI restriction sites were engineered into each *TLS*-specific primer. Following amplification and endonuclease digestion, the 737 bp *TLS*-amino terminal fragment was isolated and ligated to the pSOS vector previously digested with BamHI and dephosphorylated with CIAP, creating the bait expression plasmid, pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$].

Fusion open reading frames were confirmed in both cases by DNA sequencing with the "SOS3'" primer (Table 8). Both pSTN and pSTD constructs were also assayed for their ability to express a stable SOSΔTM:TLS chimera protein within the cdc25H *S. cerevisiae* strain (Section 3.4).

3.3.2. Amplification of *R. norvetigus* pituitary cDNA library.

A major focus of our laboratory is the elucidation of the role and contribution of the *TLS* gene to cancer, and particularly in human leukemias. Our knockout approach revealed an integral function in the immune system, especially B-lymphocyte development, maturation and activation (Hicks et al., 2000). With this in mind, a cDNA library derived from immature B-cells, at the pro-B stage of development, would have

been the preferred choice. However, the convenience and rapid availability of a *R. Norvetigus* pituitary cDNA library played a major role in choosing the latter. We rationalized that while one may not capture rarer B cell-specific interacting proteins, we would capture more universally important TLS-interacting proteins that may provide insights into the molecular mechanisms of TLS function.

The expression library was generated with the pituitary gland of *R. Norvetigus* and was a generous gift from Ami Aronheim (Aronheim et al., 1994). The cDNA library was cloned uni-directionally into the pYes2 vector utilizing EcoRI and XhoI restriction endonuclease sites. The library has a complexity of 3×10^6 independent clones containing an average insert size of 0.9 kb with an estimated insert frequency of greater than 90% (Aronheim et al., 1994). The pYes2 library was designed to have cDNA inserts cloned downstream of a signal peptide, consisting of a 12 amino acid myristoylation polypeptide sequence (MGSSKSKPKNPSQRR). This post-translational modification of library proteins is an essential part of the Ras Recruitment System strategy as it targets library proteins to the inner leaflet of the cells' plasma membrane (Section 3.4).

For amplification, the library was introduced into DH5α *E. coli* cells through electroporation (Section 2.19), and plated on LB+amp plates for two days at 37°C. The resultant colonies were harvested by scraping, and the plasmid DNA is isolated as described in Section 2.12. This yielded a total of over 500 μg of plasmid DNA, which was later diluted to a final concentration of 0.74 μg/μl. In the screen, a total of 225 μg of library DNA was used to screen for interacting proteins of TLS.

3.4. Verification of protein expression from pSTN and pSTD vectors.

Before proceeding with the Ras Recruitment Screen, pSTN and pSTD vectors were verified for the expression of the in-frame SOSΔTM–TLS chimera protein, and secondly, then to ensure the chimera protein itself does not recruit or interact with the Ras protein or any other member in the MAP kinase signaling cascade.

3.4.1. pSTN or pSTD chimera proteins do not auto-activate Ras.

In the Ras Recruitment System the protein to be investigated (bait) is fused to a human SOS protein, while the cDNA library contains a 5' signal peptide that directs the library protein to the cytoplasmic aspect of the plasma membrane. The RRS relies on the activation of the MAP kinase cascade through the recruitment of the Ras protein to the inner leaflet of plasma membrane (Aronheim et al., 1994). One such protein that is capable to localize Ras to the plasma membranes' cytoplasmic surface is SOS. In RRS, human SOS protein containing the amino acids 1–1024 is lacking its membrane domain (SOSΔTM), hence SOSΔTM can only localize to the plasma membrane is through the interaction between the bait and the library cDNA protein. The screen is performed in *S. cerevisiae* cdc25H, which were deficient for endogenous yeast SOS homologue, cdc25, rendering the MAP kinase pathway defective.

The strategy of the screen presumes activation of the MAP kinase pathway will occur only in cells where the bait and library proteins interact. Should the bait chimera protein interact directly with Ras, this requirement would be by-passed and result in a

significant increase in false positives. Therefore, we wished to determine that the SOSΔTM–bait chimera protein is unable to directly interact with Ras (or another protein part of the MAP kinase cascade) in the absence of a library-expressed chimera protein. Obviously such a protein would be incompatible with the Ras Recruitment System.

Constructs containing SOSΔTM–TLS chimera proteins were tested for auto-activation of the RRS. A chemically competent *S. cerevisiae* cdc25H strain was transformed with pSTN or pSTP plasmids (Section 3.3.1) and grown on SC+glc–L plates for 2 days at 24.2°C. Plates were then replicated onto SC+glc–L and SC+gal–L plates and incubated at 37°C for up to 7 days.

Yeast cells transformed with either of the pSTN or pSTP constructs grew at the permissive temperature of 24.2°C on plates of SC+glc–L. However, neither of cdc25H cells transformed with pSTN or pSTP constructs were able to grow at 37°C on plates of SC+gal–L. Lack of growth on the SC+glc–L plates indicates two important points. First, that the cdc25H cells expressing either of the two plasmids did not develop a second mutation complimentary to the original one, thus reverting the temperature sensitive phenotype of the cdc25H reporter strain. Secondly, our results verify that neither of the SOSΔTM–TLS chimera proteins is able to interact with the Ras protein itself nor other downstream members of the MAP kinase cascade.

While these results were expected, it is also possible that the lack of auto-activation by the pSTN or pSTP constructs may be due to non-expression of the chimera protein itself, as opposed to the lack of interaction between the bait-BD and Ras/MAP kinase pathway proteins. To rule this out, Western blotting was performed to verify that

the chimera SOS-TLS proteins were in fact expressed in the cdc25H reporter strain from the pSTN or pSTP constructs.

3.4.2. Expression of human SOSΔTM–TLS chimera proteins in S. cerevisiae

To evaluate bait-BD protein expression from the pSTN [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}216)}$] and pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$] vectors, competent *S. cerevisiae* cdc25H cells were transformed with the plasmids (Section 2.15; Table 5). For each transformation 6–8 colonies were picked and incubated overnight at 24.2°C in 10 ml of SC–L broth. The cultured cells were collected, and cellular proteins were isolated and quantified as outlined (Section 2.20 and 2.22, respectively).

Detection of chimera protein expression was determined by loading 10 μg of total cell protein extract per sample on an SDS-PAGE gel (Section 2.23) followed by Western blot analysis (Section 2.24). The Western blotting was performed using a monoclonal mouse anti–SOS antibody followed by goat anti-mouse secondary antibody conjugated to horseradish peroxidase (HRP; Table 6) and visualized by enhanced chemiluminescence, as described (Section 2.24.4). A second parallel membrane was Western blotted using a primary anti-TLS polyclonal antibody followed by goat anti-rabbit conjugated HRP as the secondary antibody (Zinszner et al., 1997b). For antibody dilutions and details please refer to Table 6.

Chimera proteins were expressed from both pSTN and pSTD fusion construct (164 or 172 kDa, respectively; Figure 5). These proteins were absent in the mock transformed cdc25H cells (lane W). Protein extracts from murine C57 MEF cells were

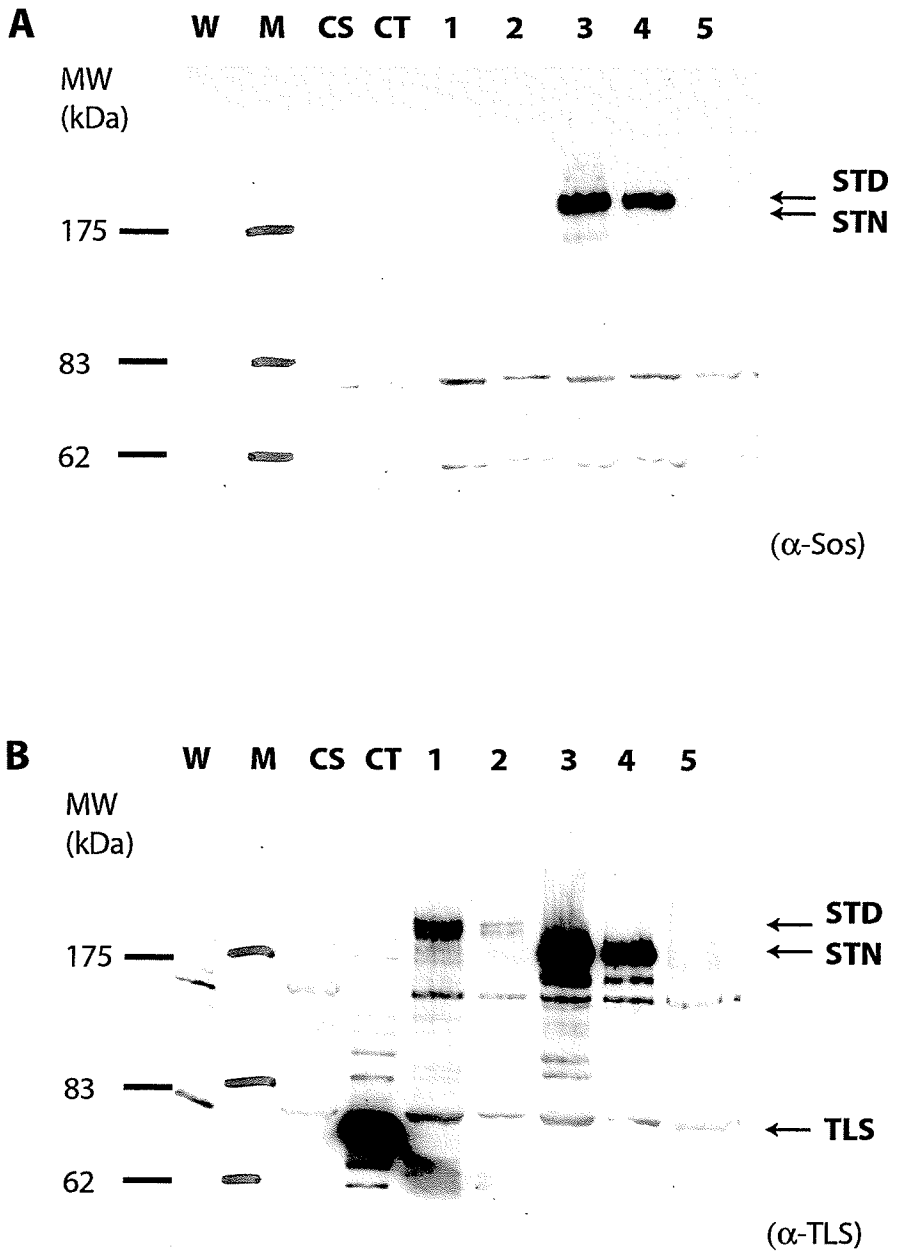


Figure 5. Expression of SOSDTM-TLS chimera protein. A. Western blot with primary antibody of monoclonal anti-SOS at 1;2000 dilution and goat anti-mouse horse radish peroxidase conjugate (HRP) secondary antibody at 1:10,000 diution. **B.** Western blot with polyclonal anti-TLS as primary antibody, and goat anti-rabbit HRP conjugate as secondary antibody; both at 1:10,000 dilution. W, wild-type S. cerevisiae cdc25H; M, molecular marker; CS, SOSD-MafB; CT, wild-type C57 MEF's; 1-5, S. cerevisiae cdc25H transformed with either pSTN [SOS (aa 1-1024):TLS (aa1-216)], lanes 1 and 2, or pSTD [SOS (aa 1-1024):TLS (aa1-242)], lanes 3-5.

Used as a positive control for the Western blot with α–TLS as the primary antibody and shows the expected 78 kDa TLS protein. The positive control for the α–SOS Western blot expressed the 152 kDa human SOSΔTM–MafB chimera protein (Invitrogen), although at a lower level of expression.

The chimera protein from the pSTD construct generated a stronger signal in both anti–TLS and anti–SOS Western blots indicating STD is expressed at higher levels or the chimera protein is more stable. As pSTD also contains the full translocated TLS amino terminus, we chose the pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$] construct to screen the *R. norvetigus* pituitary library as bait in the Ras Recruitment Screen.

3.5. The Ras Recruitment Screen using pSTD [hSOS $_{(aa\ 1\text{-}1024)}$: hTLS $_{(aa1\text{-}242)}$] to screen a pituitary *R. norvetigus* cDNA library.

Having verified that the STD bait protein does not auto-activate the Ras/MAP kinase pathway (section 3.4.1), and confirmed that the SOSΔTM–TLS chimera protein is stably expressed in cdc25H cells (section 3.4.2), we were ready to commence the Ras Recruitment Screen.

It is generally accepted that cotransforming both the bait–BD and library–AD plasmids simultaneously into yeast results in poorer efficiency than performing two sequential transformations (R.D. Gietz, personal communication). Therefore, to increase the transformation efficiency we first established an *S. cerevisiae* cdc25H strain containing the pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$] plasmid that was then used for the cDNA library transformation. 225 μg of library plasmid DNA was used to transform a

cdc25H reporter clone already containing the bait construct pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$]. This large-scale transformation resulted in 7.25 million transformants plated and incubated at 24.2°C on Sc+glc–L–U. After 48 hours the glucose plates were replica-plated onto SC+gal–L–U and incubated at 37°C for up to 7 days.

138 yeast clones capable of growth at 37°C on SC+gal–L–U plates were identified, and thus considered to express putative TLS interacting proteins. Each clone was numbered and sub-cultured into 10 ml of fresh broth. Cell pellets from 1 ml of each culture was re-suspended in 50% glycerol solution and cryopreserved at –80°C. The remaining 9 ml of the SC+gal–L–U broth was used to isolate the library plasmid DNA containing the cDNA for the putative interaction protein (Section 2.17).

It is important to note that this was a large scale and high throughput primary screen. For the 138 positive clones, it is necessary to confirm the results by individually re-introducing the cloned pYes2 plasmid containing the library cDNA insert into *S. cerevisiae* cdc25H cells carrying the pSTD plasmid to reconstruct the yeast two-hybrid interaction (Section 3.6). Library constructs capable of growth at 37°C on SC+gal–L–U plates with the pSTD plasmid during the reconstruction process would then be considered true positives.

3.6. Reconstruction of yeast two-hybrid interaction between TLS and the putative interacting proteins.

For a putative interacting protein originally isolated in a large-scale library screen to be considered a true interacting protein, the interaction between the bait and the library protein must be re-constructed. This is accomplished by first isolating the plasmid

carrying the cDNA insert. Secondly, re-introducing it through a subsequent de novo transformation into the same *S. cerevisiae* cdc25H reporter strain already containing the same plasmid bait used in the large-scale library screen in section 3.5. And thirdly, re-assaying for the presence of growth on nutritionally selective media.

3.6.1. Isolation of pYes2 plasmid containing the library cDNA insert from *S. cerevisiae* putative TLS interacting proteins.

Prior to re-assaying and confirming an interaction between the bait and library proteins, the respective library construct must be isolated from the bait plasmid in the original yeast clone. Since both parent library and the bait plasmids, pYes2 and pSOS, respectively, carry the gene for β-lactamase, the isolation strategy must rely on nutritional selection, and not the more stringent antibiotic resistance approach.

The *E. coli* KC8 strain (Table 4) was chosen because it lacks a functional *pyrF* gene, which is homologous to the *S. cerevisiae URA3* gene. *URA3* is the selectable marker on the pYes2 plasmid and permits growth of *E. coli* cells harboring the pYes2 plasmid on media lacking uracil. Therefore, when *E. coli* KC8 cells were transformed with plasmid DNA containing the bait and library vectors, only *E. coli* KC8 cells transformed with the pYes2 vector were able to grow on M9+amp–U (uracil deficient) minimal media plates.

Plasmid DNA from each of the 138 putative yeast clones was recovered from a 9 ml SC+gal–L–U broth culture, as previously described (Section 2.17). To isolate the library plasmid, extracted plasmid DNA was electroporated into KC8 *E. coli* cells

(Section 2.19), plated on M9+amp–U minimal media plates and incubated at 37°C for up to 2 days. PYes2 plasmids were isolated for 130 of the original 138 putative TLS' interacting protein *S. cerevisiae* transformants. Despite repeated attempts, uracil-independent *E. coli* KC8 clones could not be established for the remaining 8, and were not pursued further.

Occasionally, plasmid DNA isolated from individual yeast clones in a primary screen may contain more than one library-derived plasmid clone, two or more library clones that were originally co-transformed in a single yeast cell (R.D. Gietz, personal communication). To ensure we would be able to identify such instances, plasmid DNA was prepared from each of 4 well-isolated *E. coli* KC8 colonies that were selected on M9+amp–U min media plates derived from each of the original 130 *S. cerevisiae* clones (Section 2.12).

Once isolated, cloned library plasmids were enzymatically digested with BamHI and XhoI to release the respective cDNA inserts. This allows for a rapid screening of cDNA inserts contained within the library when separated by agarose gel electrophoresis (Section 2.7). Plasmid DNA with non-identical digestion patterns for all four clones indicated the presence of multiple library plasmids. In such cases each of the different library constructs would be assayed for the ability to re-construct the bait-library interaction.

After analyzing the restriction digest patterns for KC8-isolated plasmid derived from all of the 130 putative positive yeast two-hybrid clones, 96 clones contained single library plasmids, 21 clones contained two digest patterns, 11 clones contained three

digest patterns and 2 clones contained four digest patterns (Figure 6). In total, 179 isolated pYes2 library plasmids were cloned and prepared for reconstruction of the yeast two-hybrid interaction.

3.6.2. Confirmation of true TLS interacting protein through reconstruction of a TLS: library protein interaction in *S. cerevisiae* cdc25H cells.

Reconstruction of the yeast two-hybrid interaction utilizes the same approach as the primary screen itself, but on an individual library plasmid scale instead of larger scale pooled transformations. First, a *S. cerevisiae* cdc25H reporter strain carrying the bait plasmid pSTD [SOS $_{(aa\ 1\text{-}1024)}$: TLS $_{(aa1\text{-}242)}$] is transformed with each of the 179 putative TLS interacting protein library pYes2 constructs (Section 3.6.1), on an individual basis. Following the transformation reaction (Section 2.15), the cells were plated on Sc+glc–L–U at 24.2°C for two days, to allow sufficient recovery. After 48 hours, the colonies were patched onto fresh Sc+gal–L–U plates at 37°C for up to 7 days, along with positive and negative controls.

Controls for the reconstruction assay include four plasmids, two of each pSOS and pMyr vectors (Stratagene). The positive control is colony growth following co-transformation of pSOS-MafB and pMyr-MafB. The vectors each contain a fused full length MafB cDNA. MafB is a known homo-dimerizing protein and will activate the Ras Recruitment System assay (Hanamura et al., 2001). For negative control, pSOS-LaminC and pMyr-Collagenase expressing vectors were co-transformed. Human Lamin C and

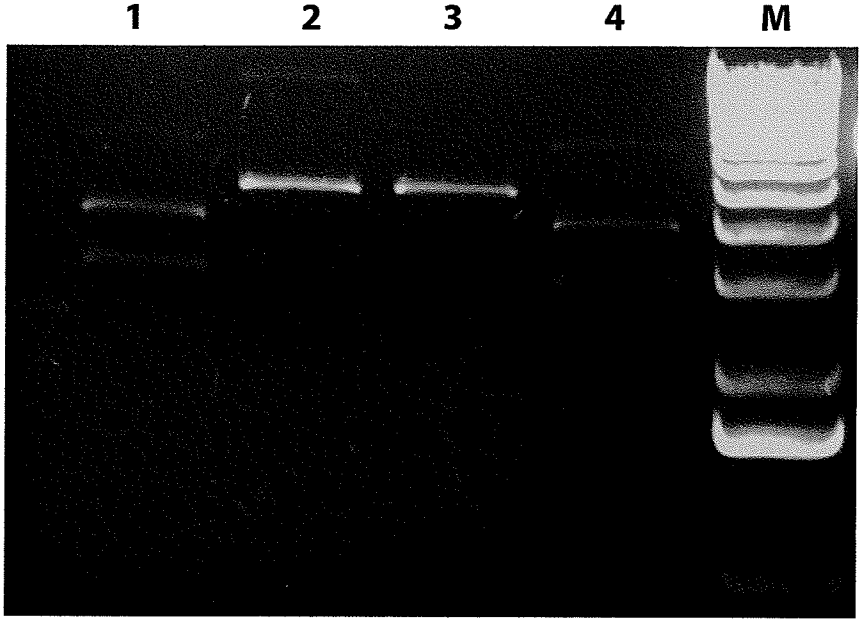


Figure 6. A putative yeast two-hybrid clone harbors multiple cDNA plamsid clones. Plasmid DNA from a single putative-positive *S. cerevisiae* clone was isolated by plasmid shuttling into *E. coli* KC8. Following transformation of the *E. coli* KC8, well isolated colonies were selected on M9+amp–U minimal media plates. Plasmid preparations from each of the bacteria clones show a double restriction endunuclease digestion pattern indicating the original yeast clone harbored two cDNA expression plasmids. 1-4, lanes from 4 separate *E. coli* KC8 colonies plasmid digestions; M, DNA ladder.

Collagenase have previously been shown not to interact, and thus should not result in colony growth. Control transformations were performed at the same time and under the same experimental conditions as the test transformations.

For a putative positive interaction to be counted as a true yeast two-hybrid interaction, a number of reconstitution criteria must be met. First, the interaction between the two proteins must allow for growth on Sc+gal–L–U plates at 37°C. Second, this interaction must occur in the similar time frame as the controls – colonies appearing on test plates around the time as the positive control, but not later than the negative control. The later is important as a negative control can often revert to a wild-type phenotype by growing at the non-permissive temperature of 37°C in the presence of galactose after about 7 days. This is often due to a complimentary mutation for the cdc25H phenotype, thus effectively removing the temperature based selection process. Third, the interaction must only occur on plates with galactose as the carbon source. The ability to grow on glucose plates indicates loss of the temperature selective phenotype of the reporter strain. And lastly, transforming pYes2 plasmid containing the library cDNA insert into cdc25H strain by itself should not result in growth on Sc+gal–U plates at 37°C, as this would indicate a false positive. For example, if a putative TLS-interacting protein is able to confer nutritional and temperature selection to the reporter strain without the presence of the SOSΔTM–TLS chimera protein, it obviously does not require the interaction between it and TLS. Expected false positive interactions would involve proteins capable of interacting with Ras, or any other member of the *S. cerevisiae* MAP kinase pathway. For

example, GTP exchange proteins other than SOS would be capable of such action and a small number of such proteins were expected.

As expected, a majority of putative interactions were ruled out because they failed to reconstitute the yeast two-hybrid interaction when assayed for colony growth. However, despite an entire plethora of modifications to the process of culturing the cdc25H reporter strain and transformation reaction, the nutritional selection of the Ras Recruitment System was never successfully achieved. Specifically, the control for no growth on glucose-based medium failed to provide convincing or reproducible results. Discussion with other laboratories utilizing the Ras Recruitment System made it apparent that difficulty with the glucose selection during reconstitution was not uncommon, and moreover, the inability to ascertain reproducible results from this control in many cases did not preclude true two-hybrid interactions (as identified by the other controls). We reconsidered strategic options available to us and rationalized that protein-protein interactions could be assessed directly if the number of candidate TLS-interacting proteins was smaller. To help in making better-informed decisions as to which candidates to pursue, we reasoned there would be informative value in sequencing the putative clone cDNAs. A strategy to combine the identity of the putative interacting proteins with the two-hybrid reconstruction results was undertaken.

3.7. Identification of pYes2 cDNA inserts for 179 putative library clones through DNA sequencing.

The prey vector contains *R. norvetigus* pituitary cDNA library in a pYes2 vector (Figure 2). Direct sequencing the putative positive library cDNAs was performed using the pMyr primer (Table 8). The pMyr primer is located 45 base pairs upstream of the cDNA cloning site. Subsequent analysis of cDNA sequences would identify the putative interacting protein and allow quick determination of whether a viable open reading frame with the upstream myristoylation sequence exists. We expected that a majority of sequences identified would be previously known genes or expressed sequence tags and a number of these would contain out-of-frame sequences. Another advantage of this approach is that we would be able to quickly identify cDNA clones that were represented multiple times. cDNAs with multiple hits may indicate independent validation of the two-hybrid interaction, and would certainly reduce the number of interactions that needed to be reconstituted.

To manage the data generated by this sequencing project and to streamline integrative sequence analysis, Dr. Songyan Liu in our lab developed the Yeast Two-Hybrid TLS-Interacting Protein website, Y2HTIP (http://140.193.242.7/y2htip/index.html). For the sake of convenience, raw and annotated sequence data for all 179 putative cDNA clones can be accessed online through the Y2HTIP homepage (Figure 7). Curated cDNA insert sequence and gene identity were provided in Appendix II.

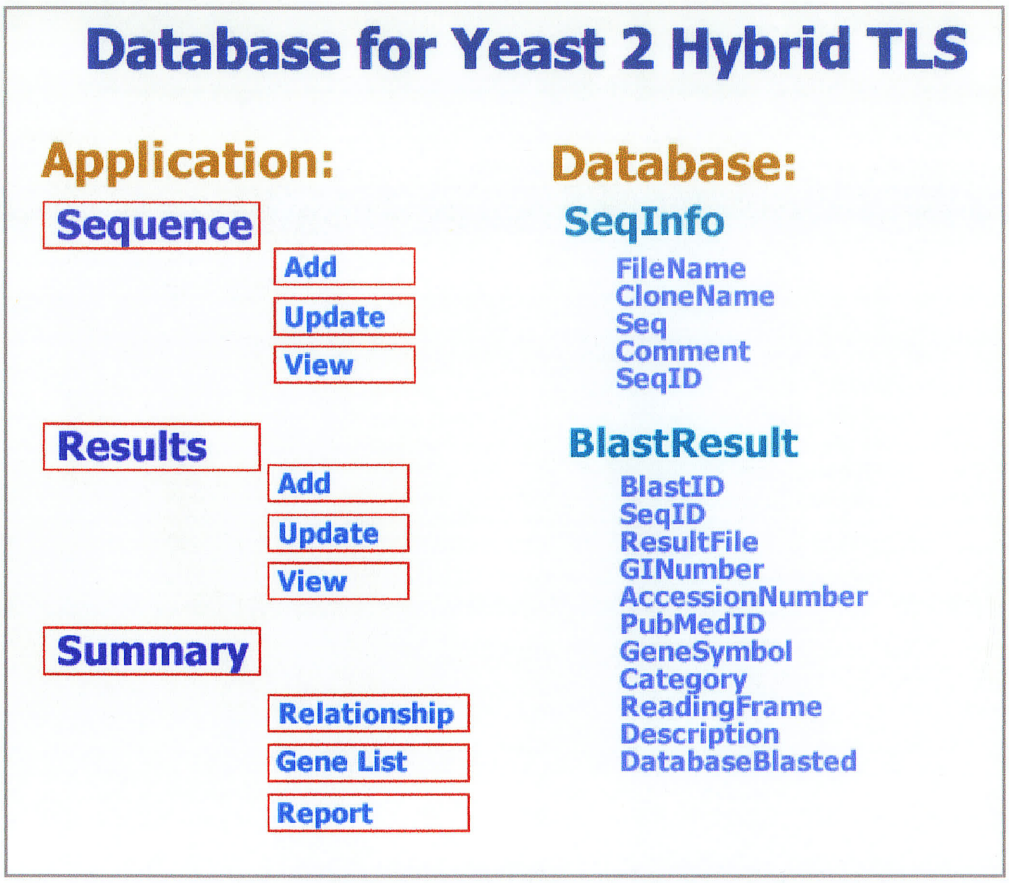


Figure 7. The Yeast Two-Hybrid TLS Interacting Protein website. Screenshot of the Y2HTIP homepage is show and can be accessed online using the hyperlink: http://140.193.242.7/y2htip/index.html.

Sequence analysis of the 179 cDNA clone inserts revealed that 125 sequences were redundant and repetitive within the dataset. The sequences of the unique 44 cDNA inserts were then analyzed for their ability to maintain on open reading frame. 15 clones were identified to be in-frame with the upstream myristoylation signal in the pYes2 vector, and are summarized in Table 9. Only those cDNA inserts containing in-frame open reading frames were pursued further.

Table 9: Sequence analysis identifies 15 putative interacting TLS proteins.

Gene Symbol	Gene Name	Accession Number	GI Number	Description
LOC291677	DNA J	XM_214587.2	34878666	Similar to DNAJ protein homolog 1
J207		AF414190.1	15778613	seven-span transmembrane protein-like protein
LOC303763		XM_226525.2	34851812	similar to RIKEN cDNA 4632415K11 gene
MIF	MIF	S73424.1	663139	macrophage migration inhibitory factor
LOC295064	My004	XM_215578.2	34856969	similar to HSPC042 protein
KIAA1826		AK032884.1	26083084	EST
p60		XM_214816.1	34854549	Glioma tumor suppressor candidate region gene 2
LOC360754	Mzf 13	XM_341026.1	34870077	similar to zinc finger protein ZFEND
KIAA1536		NM_139190.1	21070933	EST
LOC296178		XM_215825.2	34858767	similar to MCM2/3/5 family member
LOC299821	Man1	XM_216900.2	34865681	similar to integral inner nuclear membrane protein
Hspca		NM_175761.2	28916694	*Rattus norvegicus* heat shock protein 1, alpha (Hspca)
LOC312398	SMARCAD 1	XM_231860.2	34855955	similar to etl1 (M. musculus) and SMARCAD1 (H. sapiens)
LOC362742	pinin	XM_343067.1	34865261	similar to pinin, desmosome associated protein
LOC362579	Yb-1	XM_342898.1	34870881	YB-1, nuclease sensitive element binding protein 1

As we had anticipated, sequence analysis of the 179 putative cDNAs allowed us to reduce the number of cDNAs to 15 that would be required to reconstitute the yeast two-hybrid interaction. Since the number of remaining clones was so significantly diminished through this process, an option previously not considered is now available and eventually pursued. This option was to switch from the Ras Recruitment System to the classic yeast two-hybrid screen as described by Fields and Song. We rationalized that the time required to subclone 15 cDNA inserts and the bait DNA would be well balanced by the tremendous advantage of reconstituting in a simpler yeast two-hybrid interaction, especially on previously tested in our hands and with a greater range of experience from which to draw should troubleshooting be required.

3.8. Sub-cloning TLS and 15 library clones into reverse yeast two-hybrid system.

To address our original concerns regarding the potential for the amino-terminus of TLS to function as a transcriptional activator (Vidal & Legrain, 1999), we undertook one small modification of exchanging the bait and prey vectors, that is, the bait was in-frame with the Gal4 activation domain and the prey with the Gal4 binding domain.

3.8.1. Sub-cloning of the human TLS amino terminus gene, corresponding to amino acids 1-242, into pGAD424 vector.

To reconstruct the interactions between the *TLS* gene and the putative interacting proteins, cDNA for the TLS amino terminus was sub-cloned into the pGAD424 vector (Table 2 and Figure 2). The pSTD construct (section 3.2) was digested with BamHI

(section 2.7.1) to liberate the amino terminal fragment of cDNA encoding the first 242 amino acids of human *TLS* gene. The vector, pGAD424, was digested with BamHI and BglII endonucleases liberating a 6.6 kb fragment. The two DNA fragments were then ligated to produce the pGAD424-TNT $_{(aa\ 1-242)}$ construct (Figure 8). A viable open reading frame was confirmed by DNA sequencing using the AD primer (Table 8).

3.8.2. Sub-cloning of putative TLS interacting proteins into pGBT9 vector from the pYes2 vector.

Two separate strategies were developed for the sub-cloning process based on ease of cloning and convenience. The first strategy was based on sub-cloning using existing restriction endonuclease sites. cDNA inserts were digested with EcoR1 and Xho1 restriction endonucleases (Section 2.9.1) and the 3' overhangs were filled in using the Klenow fragment of DNA Polymerase I to create blunt-ends (Section 2.9.2). The pGBT9 vector was digested with the Sma1 restriction endonuclease and dephosphorylated using CIAP (Section 2.9.2). The two DNA fragments were then blunt-end ligated (Section 2.10).

A second strategy involved designing sequence specific primers and amplifying the desired library cDNA fragment using a polymerase chain reaction. This strategy was only used for five clones. The obtained PCR fragments were then digested with EcoRI and BamHI restriction endonucleases and purified. The pGBT9 vector was digested with the two corresponding restriction endonucleases to yield a 4.5 kb fragment. The two DNA fragments were ligated (Figure 9). In all instances the viability of open reading frame was confirmed by DNA sequencing using the T9 primer (Table 8).

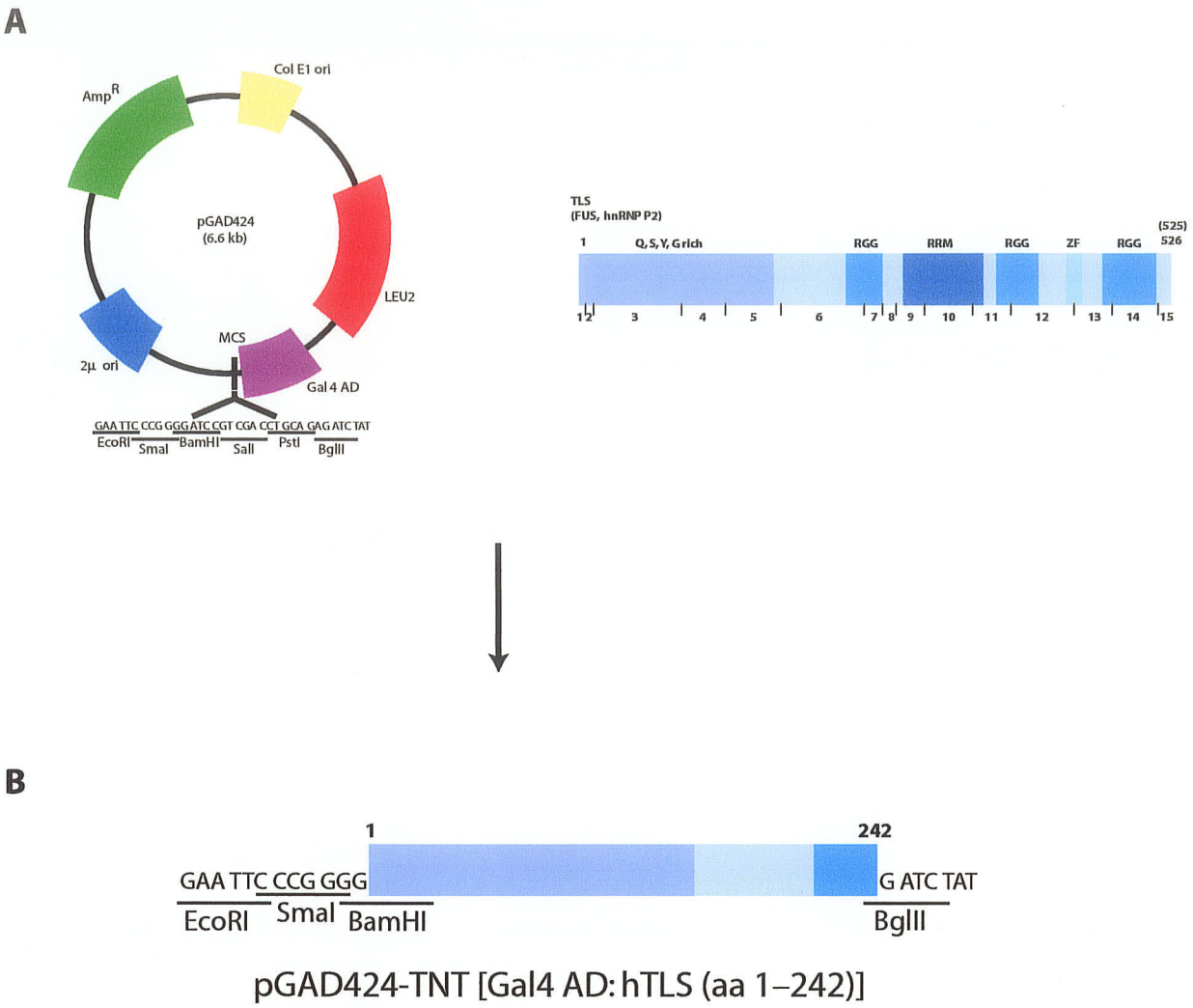


Figure 8. Sub-cloning of the amino terminal *TLS* domain into the pGAD424 vector.
A. Map of the vector and TLS protein. **B.** Multiple cloning site of pGAD424-TNT construct containing pGAD424 vector with *H. sapiens TLS* nucleotides 78- 824 (amino acids 1- 242). The pSTD construct, from section 3.2, is digested with BamHI enzyme (section 2.7.1) to isolate the amino terminal fragment of DNA encoding the first 242 amino acids of human *TLS* gene, of 727 base pairs. The vector, pGAD424, is also digested with the BamHI and BglII endonucleases yielding a 6.6 kilobase fragment. The two DNA fragments are then ligated to give pGAD424-TNT [Gal4 AD: TLS (aa 1–242)] construct. Continuation of a viable ORF of the pGAD424-TNT construct is later confirmed through sequencing using the AD primer (see table 8).

3.9. Determination of true TLS interacting proteins through reconstruction by the classic yeast two-hybrid method.

The yeast two-hybrid system relies on the reconstitution of the DNA binding domain and a transcriptional activation domain of the Gal4 transcription factor. The first is fused to the 15 putative positive cDNA inserts from the primary screen, while the latter is fused to amino acids 1-242 of TLS. If an interaction takes place between the library and the TLS proteins, the two domains of Gal4 were approximated, and functional transcription factor, *GAL4*, is reconstituted.

The *S. cerevisiae* KGY37 reporter strain was chosen as it contains a genomic alteration that places Gal4 specific upstream activation sequences (UAS) within the promoter of *HIS3* gene. It also has a stable insertion of the *E. coli lacZ* gene, which is also under the same *GAL4* UAS control. Reconstituted Gal4 expression in KGY37 cells allow nutritional selection for ability of transformants to grow on medium lacking histidine, or a color-based assay screening for β-galactosidase to identify cells that were able to convert the colorless X-gal substrate its blue by-product.

Controls for this system were a generous gift from Dr. R.D. Gietz. For positive control, pGAD424 expresses a full-length human insulin receptor gene and pGBT9 expressing a full-length Grb14 gene. In the assay, interaction of insulin receptor and Grb14 proteins will activate the Gal4 reporter system. The negative control consisted of the same insulin receptor construct and a pGBT9 vector in which the Grb14 gene carries a small deletion mutation that prevents the protein interaction between the insulin receptor and Grb14 from occurring (Hemming et al., 2001).

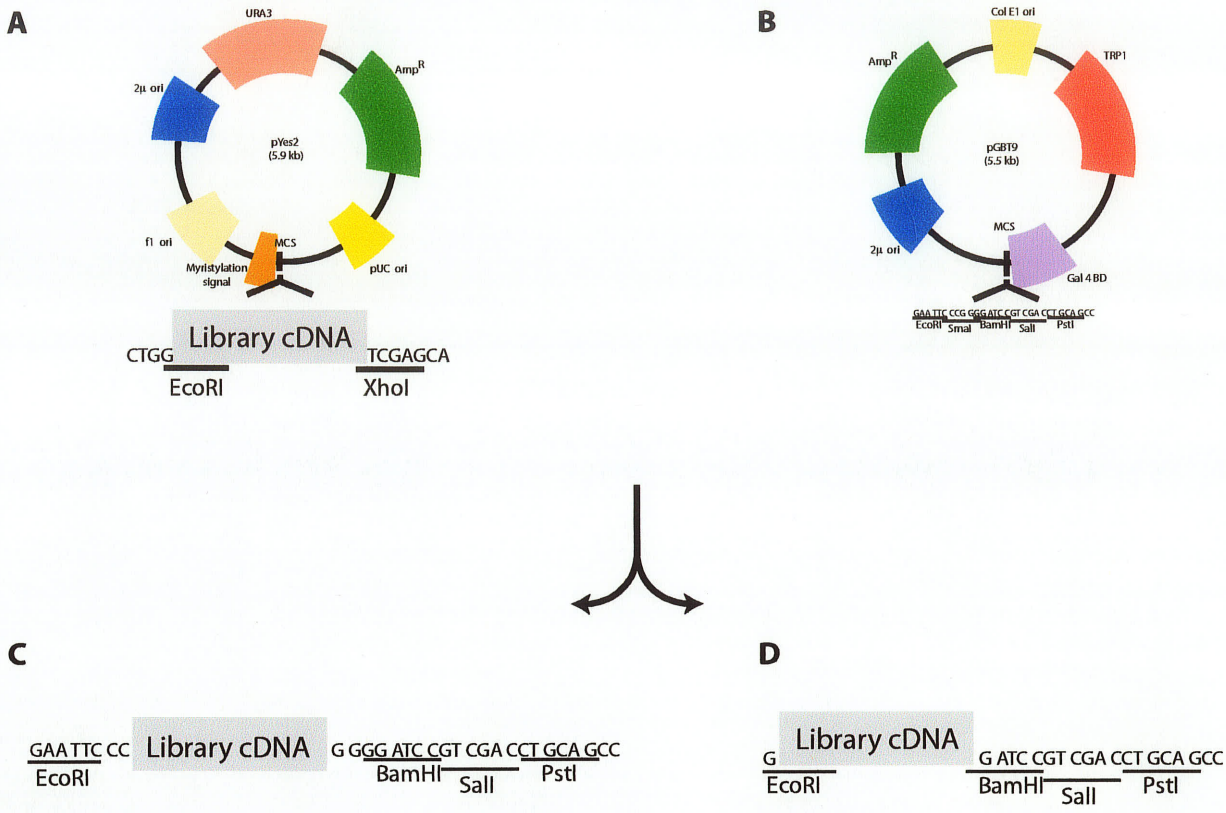


Figure 9. Strategies for sub-cloning library cDNA from the pYes2 to the pGBT9 vector. **A.** Map of library cDNA within the pYes2 vector prior to sub-cloning into the pBT9 vector. **B.** Map of pGBT9 vector. **C.** The first strategy is based on sub-cloning by digestion with restriction endonucleases. The library cDNA, contained within the pYes2 vector, is digested with EcoR1 and Xho1 restriction endonucleases (section 2.9.1) and the 3' overhangs are filled in via incubation with the Klenow fragment of Eco Polymerase I (section 2.9.2) to create blunt-ends. Meanwhile, the pGBT9 vector is digested with the Sma1 enzyme, and dephosphorylated using the CIAP enzyme (section 2.9.3). The two DNA fragments are then ligated via their blunt-ends (section 2.10). **D.** The second strategy involved designing sequence specific primers and amplifying the desired library cDNA fragment by using a polymerase chain reaction (see table 8 for primer details). The obtained PCR fragments are then digested with BamHI and EcoRI restriction endonucleases. The pGBT9 vector is also digested with the two corresponding enzymes to yield a plasmid fragment of 4.5 kilobases. The two DNA fragments are ligated. In all instances the viability of the new ORF within the pGBT9 constructs is later confirmed with the T9 primer (see table 8).

3.9.1. Nutritional selection on Triple knockout + 3-AT plates

After verifying that the bait construct, pGAD424–TNT $_{(aa\ 1\text{-}242)}$, was unable to activate transcription from the *his3* gene promoter by itself when transformed alone into *S. cerevisiae* KGY37 cells, the reconstitution assays were performed. Each of the putative 15 library cDNA clones was co-transformed into the *S. cerevisiae* KGY37 reporter strain with the pGAD424–TNT $_{(aa\ 1\text{-}242)}$. The transformants were then plated onto SC–W–L, and SC–W–L–H+3–AT plates and incubated at 30°C for 48–72 hours. The SC–W–L–H+3–AT plates were assayed for nutritional selection, or the ability of the library protein to interact with TLS $_{(aa\ 1\text{-}242)}$ when co-expressed, thus allowing for growth on media lacking histidine. The positive and negative controls were co-transformed under the same conditions, at the same time.

None of the 15 putative TLS interacting proteins tested were capable of growth on Sc–W–L–H+3–AT media when co-transformed with the bait construct, pGAD424–TNT $_{(aa\ 1\text{-}242)}$, into the *S. cerevisiae* KGY37 reporter strain (Figure 9).

3.9.2. Interaction confirmation through the LacZ assay

The β-galactosidase assay was performed on 80 mm 3M Whatmann filters colony lifts from Sc–W– L plates following incubation at 30°C for 48–72 hours. After lysing the *S. cerevisiae* cells with liquid nitrogen the filter was placed on a second filter previously soaked with Z-Buffer (Section 2.19). The two filters were then incubated at 37°C for 2–8 hours. The colonies capable of turning blue after 2 hours were considered strong

positives, as that was the necessary time for the positive control to do so. Also, the bait construct, pGAD424–TNT $_{(aa\ 1\text{-}242)}$, was unable to activate transcription from the *lacZ* gene promoter by itself when *S. cerevisiae* KGY37 cells transformed with that plasmid by itself and subjected to the color-based assay. None of the 15 putative TLS interacting proteins tested were positive for the *lacZ* assay at 37°C after 2–8 hours when co-transformed with the bait construct pGAD424–TNT $_{(aa\ 1\text{-}242)}$, into the KGY37 reporter strain (Figure 10).

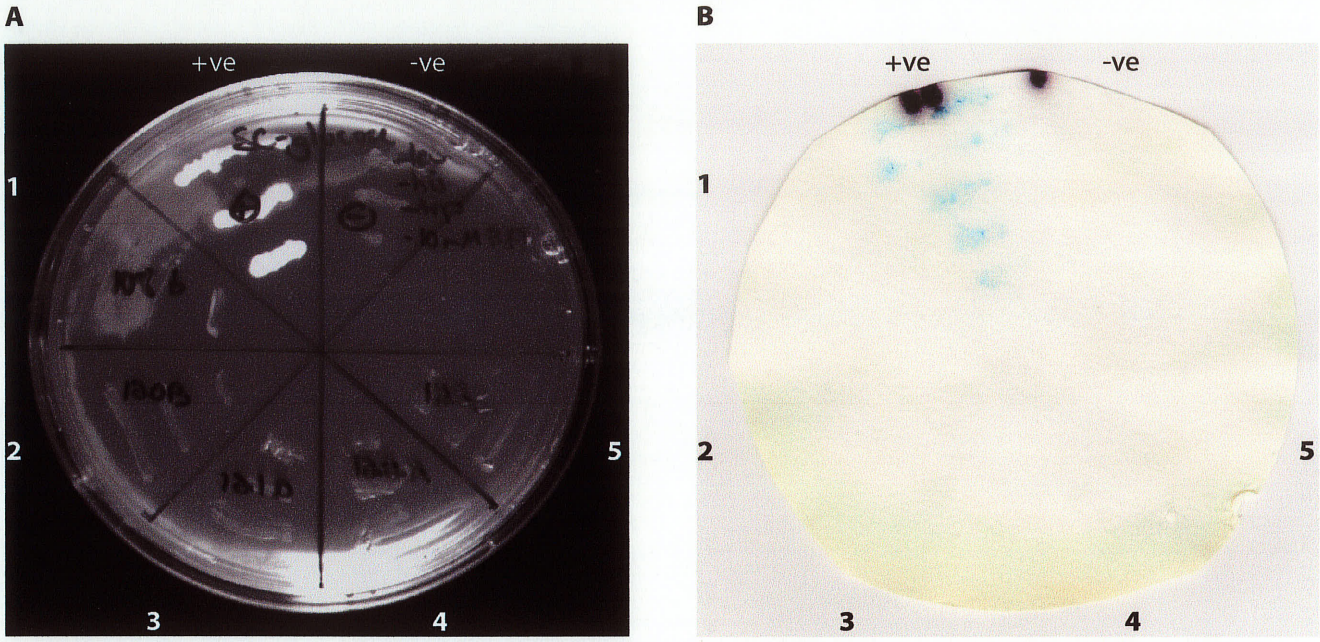


Figure 10. Nutritional selection and colorimetric assays to confirm true TLS interacting proteins. A. Nutritional selction through selctive media plate assay. Each of the library's 15 cDNA clones together with the pGAD424–TNT (aa 1-242) are co-transformed into the *S. cerevisiae* KGY37 reporter strain. The cells are then plated in equal aliquots onto each SC–W–L, and SC–W–L–H+3–AT plates at 30C for 48–72 hours. The SC–W–L–H+3–AT plates are scored for the growth on media lacking histidine; thus indicating an interaction of the library protein and TLS (aa 1-242). The positive and negative controls are co-transformed simultaneously to maintain a point of reference. **B.** Colorimetric assay. The β-galactosidase assay is performed on 80 mm 3M Whatmann filters colony lifts from Sc–W– L plates following incubation at 30∞C for 48–72 hours from part A above. After lysing the *S. cerevisiae* cells with liquid nitrogen the filter is placed upon a second previously soaked with Z-Buffer (see section 2.19) filter. The two filters are then incubated at 37 degrees Celsius for 2–8 hours. The colonies capable of turning blue after 2 hours are considered strong positives, +ve, positive control; -ve, negative control; 1-5; library cDNA clones within pGBT9 vector.

4. Summary and Conclusions

The main focus of our laboratory is the identification of the wild-type function of TLS. A more complete understanding of the role of TLS in normal cellular processes is expected to enhance our understanding of TLS contribution to tumor progression in human cancer.

TLS knockout studies performed in our lab have shown that TLS is essential in the maintenance of genomic stability and in the maturation and activation of B cells in vivo (Hicks et al., 2000). The goal of this thesis project is to identify TLS-interacting proteins with the expectation that this approach will lead to identifying the molecular mechanisms that underlie the functional defects we observed in the *TLS* knockout mice. A powerful approach to identify interacting proteins is through the use of the yeast two-hybrid system. This strategy has already been successful in identifying protein partners for TLS. However, in those experiments the carboxy-terminus of TLS was used, the part of the gene which is lost in oncogenic TLS translocations (Yang et al., 1998). We reasoned that identification of proteins that interact with the TLS amino-terminus, which is present in TLS oncogenic fusion proteins, would be more informative with regard to the molecular mechanisms by which TLS contributes to the initiation and maintenance of several human cancers.

The N-terminus of TLS is a known to have transcriptional activation potential, and as such, would be poorly suited for the yeast two-hybrid screen as described by Fields and Song. The "classic" yeast two-hybrid screen relies on an interaction between

bait and prey inside the nucleus to reconstitute the Gal4 transcription factor, which may easily be short-circuited if the bait protein (TLS) also contributes a functional transcriptional activation domain.

To eliminate potential trouble with a high number of false positives when using the classic two-hybrid system, a modified cytoplasmic two-hybrid system was chosen (Aronheim et al., 1994). The Ras Recruitment System functions like any other variant of two-hybrid screen, and is also made up of two components, the bait and the library. The library is localized to the plasma membrane and the bait is fused to a Ras-GDP exchanger. Upon interaction between the bait and a library, the MAP kinase pathway is activated, mimicking upstream activation of the *Ras* gene and rendering that cell with phenotypic advantages that may be selected for.

Using the amino-terminus of TLS (aa 1-242) as bait, we screened a pituitary cDNA expression library for TLS-interacting proteins. The initial large-scale screening identified 179 putative positive clones. These are deemed putative as the interaction between the cDNA expressed protein and TLS must be repeated in order to rule out potential false positives. During the process of confirmation through reconstruction the putative positives are assayed under two selective criteria to increase the stringency, and thus confidence that the interaction is a true one.

Unfortunately, the loss of the nutritional selection within the screen itself during reconstruction made it difficult to confirm the putative positives as true interacting proteins of TLS in Ras Recruitment System. Despite numerous attempts and modifications to retain stringency and the selective phenotype of the cdc25H *S.*

cerevisiae strain, it was not feasible to maintain selective pressures necessary to recover the RRS and obtain data that would allow for relevant and reliable interpretation of the results for the putative TLS interacting proteins.

A sequence-based approach was then undertaken to further analyze the 179 putative positives. Through DNA sequencing of the putative cDNAs was is possible to quickly ascertain the identity of the gene or EST be a simple nucleotide Blast search against the GenBank DNA databases. By discovering the identity of all 179 positive cDNAs it was possible to establish, and thus eliminate, a significant number of putative positives as they are identified in multiple instances. This process reduced the actual number of unique putative positive cDNAs to 44 sequences.

Since the cDNA sequence encoding the prey protein product was cloned downstream of a signal peptide, it is possible that a number of the 44 unique putative positives may not maintain an open reading frame and would therefore be false-positives. This offered another opportunity to reduce the number of putative positives that need to be reconstructed. After such analysis, the number of putative positives with viable open reading frames decreased to only 15 cDNA.

Given our previous difficulty in reconstructing the yeast two-hybrid interactions using the Ras Recruitment System, we chose to undertake another approach to reconstruct the interaction between TLS and the 15 unique and in-frame putative positives. After sub-cloning the amino terminal fragment of the *TLS* gene and the 15 putative positives from the RRS screen into the necessary vectors for a reverse yeast two-hybrid system, they were all tested simultaneously in two assays. The first was a

nutritional selection assay in which yeast cells harboring the bait and prey protein products are required to grow in the absence of the amino acid histidine. The second assay is a colorimetric one, testing for the expression of the LacZ gene. Unfortunately, neither of these assays was able to establish a true two-hybrid interaction with the amino terminal TLS protein domain.

Should the interactions have been reconstituted, the next step would be to validate the protein-protein interactions of full-length wild-type TLS and candidate gene proteins. Rather than repeating or trouble-shooting the classic yeast two-hybrid screen, we considered simply moving to the validation step for candidate genes of interest. From the 15 putative interacting proteins a number of them looked particularly interesting with regard to our labs interest in the DNA repair and B cell functions of TLS. Surprisingly, several predicted genes fit these criteria. DNA J, My004 and Hspca are heat shock proteins that are likely related to TLS' function in stress response. *MAN1* and *MIF* are two genes with implications in immune response, the first in repressing signals following cytokine activation (Lin et al., 2005; Pan et al., 2005; Wu et al., 2002), while transgenic mice of the latter demonstrate decreased response when stimulated by lipopolysaccharide challenge (Ohkawara et al., 2005; Pan et al., 2004).

Finally, two other genes are known to DNA damage induced and involved in maintaining genomic stability. SMARCAD1 is a member of DEAD/H box-containing family of proteins that is essential to genome replication, repair and expression, and is known to interact with ATM (Adra et al., 2000). Our own lab has demonstrated that TLS is phosphorylated by ATM *in vitro* (Hicks and Miller, unpublished results), and this

suggests regulation of TLS by ATM may be part of the cells major response to DNA damage. The SMARCAD1 interaction, if true, may reveal important clues as to how TLS functions in the DNA damage response at a molecular level. Validation of the TLS-SMARCAD1 interaction is currently underway by others in the lab, particularly as SMARCAD1 is a SWI/SNF related matrix associated actin dependent regulator of chromatin. Together with the Davie lab, we are quite interested in how TLS may function to link chromatin structure and the regulation of gene expression. We also know from colleagues that a functional interaction between a closely related DEAD-box protein and the closely related TLS proto-oncogene EWS appears to be important in Ewing sarcoma. Taken together, it is likely that discovery of the TLS-SMARCAD1 interaction will lead to important functional discoveries for understanding the molecular mechanisms by which TLS participates in maintaining genomic stability.

A second DNA damage responsive gene that is a putative TLS-interaction partner is the Yb-1 transcription factor (Kajino et al., 2001; Shibahara et al., 2004; Uramoto et al., 2002). Numerous recent studies provide a strong link between YB-1 misregulation and cancer (Bader & Vogt, 2004; Bergmann et al., 2005; Gessner et al., 2004). Interaction between TLS and Yb-1 has been previously identified in a two-hybrid screen, but with the carboxy-terminal domain of TLS used in this screen (Yang et al., 1998). In this study, our data support an interaction with the amino-terminal domain, suggesting that both TLS domains may be involved in YB-1 interaction, and moreover, that the conserved hinge region between the two domains might provide an important mechanism for the regulation of this interaction. While interaction with YB-1, a known TLS-

interacting protein, provides internal validation that our yeast two-hybrid screen is successfully identifying TLS-interacting proteins, the fact that both TLS amino and carboxy domains may be required for some protein-protein interactions may explain why other known transcription factors like NF-κB and PU.1 were not identified within our screen. None-the-less, since YB-1 was identified in our screen and is a known DNA damage responsive gene, our lab has gone on to demonstrate that TLS and YB-1 proteins do interact, as determined by co-immunoprecipitation studies using wild-type full length cDNA expression vectors (Bosc and Hicks, unpublished results) and that this interaction modulates YB-1 directed gene expression (Law and Hicks, unpublished results). TLS modulation of transcription-factor-dependent gene expression is currently a major funded research program in our lab.

5. References:

Adra, C.N., Donato, J.L., Badovinac, R., Syed, F., Kheraj, R., Cai, H., Moran, C., Kolker, M.T., Turner, H., Weremowicz, S., Shirakawa, T., Morton, C.C., Schnipper, L.E. & Drews, R. (2000). *Genomics*, **69,** 162-73.

Aman, P., Panagopoulos, I., Lassen, C., Fioretos, T., Mencinger, M., Toresson, H., Hoglund, M., Forster, A., Rabbitts, T.H., Ron, D., Mandahl, N. & Mitelman, F. (1996). *Genomics*, **37,** 1-8.

Aronheim, A. (2001). *Methods Enzymol*, **332,** 260-70.

Aronheim, A., Engelberg, D., Li, N., al-Alawi, N., Schlessinger, J. & Karin, M. (1994). *Cell*, **78,** 949-61.

Aronheim, A. & Karin, M. (2000). *Methods Enzymol*, **328,** 47-59.

Bader, A.G. & Vogt, P.K. (2004). *Oncogene*, **23,** 3145-50.

Baechtold, H., Kuroda, M., Sok, J., Ron, D., Lopez, B.S. & Akhmedov, A.T. (1999). *J Biol Chem*, **274,** 34337-42.

Bailly, R.A., Bosselut, R., Zucman, J., Cormier, F., Delattre, O., Roussel, M., Thomas, G. & Ghysdael, J. (1994). *Mol Cell Biol*, **14,** 3230-41.

Barak, O., Aronheim, A. & Shaul, Y. (2001). *Virology*, **283,** 110-20.

Baskaran, R., Wood, L.D., Whitaker, L.L., Canman, C.E., Morgan, S.E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M.B. & Wang, J.Y. (1997). *Nature*, **387,** 516-9.

Bergmann, S., Royer-Pokora, B., Fietze, E., Jurchott, K., Hildebrandt, B., Trost, D., Leenders, F., Claude, J.C., Theuring, F., Bargou, R., Dietel, M. & Royer, H.D. (2005). *Cancer Res*, **65,** 4078-87.

Bertolotti, A., Lutz, Y., Heard, D.J., Chambon, P. & Tora, L. (1996). *Embo J*, **15,** 5022-31.

Bertrand, P., Akhmedov, A.T., Delacote, F., Durrbach, A. & Lopez, B.S. (1999). *Oncogene*, **18,** 4515-21.

Birnboim, H.C. & Doly, J. (1979). *Nucleic Acids Res*, **7,** 1513-23.

Bishop, J.M. (1985). *Cell*, **42,** 23-38.

Bosc, D.G., Luscher, B. & Litchfield, D.W. (1999). *Mol Cell Biochem*, **191,** 213-22.

Bradford, M.M. (1976). *Anal Biochem*, **72,** 248-54.

Broder, Y.C., Katz, S. & Aronheim, A. (1998). *Curr Biol*, **8,** 1121-4.

Brown, A.D., Lopez-Terrada, D., Denny, C. & Lee, K.A. (1995). *Oncogene*, **10,** 1749-56.

Cahill, D.P., Kinzler, K.W., Vogelstein, B. & Lengauer, C. (1999). *Trends Cell Biol*, **9,** M57-60.

Calvio, C., Neubauer, G., Mann, M. & Lamond, A.I. (1995). *Rna*, **1,** 724-33.

Cleary, M.L. (1991). *Cell*, **66,** 619-22.

Cooper, G.M., Okenquist, S. & Silverman, L. (1980). *Nature*, **284,** 418-21.

Courey, A.J. & Tjian, R. (1988). *Cell*, **55,** 887-98.

Crozat, A., Aman, P., Mandahl, N. & Ron, D. (1993a). *Nature*, **363,** 640-4.

Crozat, A., Aman, P., Mandahl, N. & Ron, D. (1993b).

Delattre, O., Zucman, J., Plougastel, B., Desmaze, C., Melot, T., Peter, M., Kovar, H., Joubert, I., de Jong, P., Rouleau, G. & et al. (1992). *Nature*, **359,** 162-5.

Dower, W.J., Miller, J.F. & Ragsdale, C.W. (1988). *Nucleic Acids Res*, **16,** 6127-45.

Ewing, J. (1972). *CA Cancer J Clin*, **22,** 95-8.

Fearon, E.R. & Vogelstein, B. (1990). *Cell*, **61,** 759-67.

Fields, S. & Song, O. (1989). *Nature*, **340,** 245-6.

Gessner, C., Woischwill, C., Schumacher, A., Liebers, U., Kuhn, H., Stiehl, P., Jurchott, K., Royer, H.D., Witt, C. & Wolff, G. (2004). *Eur Respir J*, **23,** 14-9.

Gietz, R.D., Graham, K.C. & Litchfield, D.W. (1995a). *J Biol Chem*, **270,** 13017-21.

Gietz, R.D., Schiestl, R.H., Willems, A.R. & Woods, R.A. (1995b). *Yeast*, **11,** 355-60.

Gietz, R.D., Triggs-Raine, B., Robbins, A., Graham, K.C. & Woods, R.A. (1997). *Mol Cell Biochem*, **172,** 67-79.

Gill, S., McManus, A.P., Crew, A.J., Benjamin, H., Sheer, D., Gusterson, B.A., Pinkerton, C.R., Patel, K., Cooper, C.S. & Shipley, J.M. (1995). *Genes Chromosomes Cancer*, **12,** 307-10.

Hackl, W. & Luhrmann, R. (1996). *J Mol Biol*, **264,** 843-51.

Hahn, W.C. & Weinberg, R.A. (2002). *N Engl J Med*, **347,** 1593-603.

Hanahan, D. & Weinberg, R.A. (2000). *Cell*, **100,** 57-70.

Hanamura, I., Iida, S., Akano, Y., Hayami, Y., Kato, M., Miura, K., Harada, S., Banno, S., Wakita, A., Kiyoi, H., Naoe, T., Shimizu, S., Sonta, S.I., Nitta, M., Taniwaki, M. & Ueda, R. (2001). *Jpn J Cancer Res*, **92,** 638-44.

Hardin, J.D., Boast, S., Schwartzberg, P.L., Lee, G., Alt, F.W., Stall, A.M. & Goff, S.P. (1995). *Cell Immunol*, **165,** 44-54.

Hemming, R., Agatep, R., Badiani, K., Wyant, K., Arthur, G., Gietz, R.D. & Triggs-Raine, B. (2001). *Biochem Cell Biol*, **79,** 21-32.

Hicks, G.G., Singh, N., Nashabi, A., Mai, S., Bozek, G., Klewes, L., Arapovic, D., White, E.K., Koury, M.J., Oltz, E.M., Van Kaer, L. & Ruley, H.E. (2000). *Nat Genet*, **24,** 175-9.

Hoffman, C.S. & Winston, F. (1987). *Gene*, **57,** 267-72.

Ichikawa, H., Shimizu, K., Hayashi, Y. & Ohki, M. (1994). *Cancer Res*, **54,** 2865-8.

Ichikawa, H., Shimizu, K., Katsu, R. & Ohki, M. (1999). *Mol Cell Biol*, **19,** 7639-50.

Immanuel, D., Zinszner, H. & Ron, D. (1995). *Mol Cell Biol*, **15,** 4562-71.

Jeon, I.S., Davis, J.N., Braun, B.S., Sublett, J.E., Roussel, M.F., Denny, C.T. & Shapiro, D.N. (1995). *Oncogene*, **10,** 1229-34.

Kajino, K., Yamamoto, T., Hayashi, J., Umeda, T., Takahara, T. & Hino, O. (2001). *Intervirology*, **44,** 311-6.

Kuroda, M., Sok, J., Webb, L., Baechtold, H., Urano, F., Yin, Y., Chung, P., de Rooij, D.G., Akhmedov, A., Ashley, T. & Ron, D. (2000). *Embo J*, **19,** 453-62.

Ladanyi, M. & Gerald, W. (1994). *Cancer Res*, **54,** 2837-40.

Lessnick, S.L., Braun, B.S., Denny, C.T. & May, W.A. (1995). *Oncogene*, **10,** 423-31.

Lin, F., Morrison, J.M., Wu, W. & Worman, H.J. (2005). *Hum Mol Genet*, **14,** 437-45.

Liotta, L.A. & Stetler-Stevenson, W.G. (1991). *Cancer Res*, **51,** 5054s-5059s.

Look, A.T. (1997). *Science*, **278,** 1059-64.

Mastrangelo, T., Modena, P., Tornielli, S., Bullrich, F., Testi, M.A., Mezzelani, A., Radice, P., Azzarelli, A., Pilotti, S., Croce, C.M., Pierotti, M.A. & Sozzi, G. (2000). *Oncogene*, **19,** 3799-804.

May, W.A., Lessnick, S.L., Braun, B.S., Klemsz, M., Lewis, B.C., Lunsford, L.B., Hromas, R. & Denny, C.T. (1993). *Mol Cell Biol*, **13,** 7393-8.

Meissner, M., Lopato, S., Gotzmann, J., Sauermann, G. & Barta, A. (2003). *Exp Cell Res*, **283,** 184-95.

Morohoshi, F., Arai, K., Takahashi, E.I., Tanigami, A. & Ohki, M. (1996). *Genomics*, **38,** 51-7.

Ohkawara, T., Miyashita, K., Nishihira, J., Mitsuyama, K., Takeda, H., Kato, M., Kondo, N., Yamasaki, Y., Sata, M., Yoshiki, T., Sugiyama, T. & Asaka, M. (2005). *Clin Exp Immunol*, **140,** 241-8.

Pan, D., Estevez-Salmeron, L.D., Stroschein, S.L., Zhu, X., He, J., Zhou, S. & Luo, K. (2005). *J Biol Chem*, **280,** 15992-6001.

Pan, J.H., Sukhova, G.K., Yang, J.T., Wang, B., Xie, T., Fu, H., Zhang, Y., Satoskar, A.R., David, J.R., Metz, C.N., Bucala, R., Fang, K., Simon, D.I., Chapman, H.A., Libby, P. & Shi, G.P. (2004). *Circulation*, **109,** 3149-53.

Panagopoulos, I., Aman, P., Fioretos, T., Hoglund, M., Johansson, B., Mandahl, N., Heim, S., Behrendtz, M. & Mitelman, F. (1994). *Genes Chromosomes Cancer*, **11,** 256-62.

Panagopoulos, I., Hoglund, M., Mertens, F., Mandahl, N., Mitelman, F. & Aman, P. (1996). *Oncogene*, **12,** 489-94.

Pereira, D.S., Dorrell, C., Ito, C.Y., Gan, O.I., Murdoch, B., Rao, V.N., Zou, J.P., Reddy, E.S. & Dick, J.E. (1998). *Proc Natl Acad Sci U S A*, **95,** 8239-44.

Perez-Losada, J., Sanchez-Martin, M., Rodriguez-Garcia, M.A., Perez-Mancera, P.A., Pintado, B., Flores, T., Battaner, E. & Sanchez-Garcia, I. (2000). *Oncogene*, **19,** 6015-22.

Perez-Mancera, P.A., Perez-Losada, J., Sanchez-Martin, M., Rodriguez-Garcia, M.A., Flores, T., Battaner, E., Gutierrez-Adan, A., Pintado, B. & Sanchez-Garcia, I. (2002). *Oncogene*, **21,** 1679-84.

Peter, M., Couturier, J., Pacquement, H., Michon, J., Thomas, G., Magdelenat, H. & Delattre, O. (1997). *Oncogene*, **14,** 1159-64.

Petermann, R., Mossier, B.M., Aryee, D.N., Khazak, V., Golemis, E.A. & Kovar, H. (1998). *Oncogene*, **17,** 603-10.

Powers, C.A., Mathur, M., Raaka, B.M., Ron, D. & Samuels, H.H. (1998). *Mol Endocrinol*, **12,** 4-18.

Prasad, D.D., Ouchida, M., Lee, L., Rao, V.N. & Reddy, E.S. (1994). *Oncogene*, **9,** 3717-29.

Rabbitts, T.H. (1991). *Cell*, **67,** 641-4.

Rabbitts, T.H. (1994). *Nature*, **372,** 143-9.

Rabbitts, T.H. (1999). *J Pathol*, **187,** 39-42.

Rabbitts, T.H., Forster, A., Larson, R. & Nathan, P. (1993). *Nat Genet*, **4,** 175-80.

Shafman, T., Khanna, K.K., Kedar, P., Spring, K., Kozlov, S., Yen, T., Hobson, K., Gatei, M., Zhang, N., Watters, D., Egerton, M., Shiloh, Y., Kharbanda, S., Kufe, D. & Lavin, M.F. (1997). *Nature*, **387,** 520-3.

Shibahara, K., Uchiumi, T., Fukuda, T., Kura, S., Tominaga, Y., Maehara, Y., Kohno, K., Nakabeppu, Y., Tsuzuki, T. & Kuwano, M. (2004). *Cancer Sci*, **95,** 348-53.

Sjogren, H., Meis-Kindblom, J., Kindblom, L.G., Aman, P. & Stenman, G. (1999). *Cancer Res*, **59,** 5064-7.

Sorensen, P.H., Lessnick, S.L., Lopez-Terrada, D., Liu, X.F., Triche, T.J. & Denny, C.T. (1994). *Nat Genet*, **6,** 146-51.

Storlazzi, C.T., Mertens, F., Nascimento, A., Isaksson, M., Wejde, J., Brosjo, O., Mandahl, N. & Panagopoulos, I. (2003). *Hum Mol Genet*, **12,** 2349-58.

Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S. & Rothberg, J.M. (2000). *Nature*, **403,** 623-7.

Uetz, P. & Hughes, R.E. (2000). *Curr Opin Microbiol*, **3,** 303-8.

Uramoto, H., Izumi, H., Ise, T., Tada, M., Uchiumi, T., Kuwano, M., Yasumoto, K.,

Funa, K. & Kohno, K. (2002). *J Biol Chem*, **277,** 31694-702.

Urano, F., Umezawa, A., Hong, W., Kikuchi, H. & Hata, J. (1996). *Biochem Biophys Res Commun*, **219,** 608-12.

Vidal, M. & Legrain, P. (1999). *Nucleic Acids Res*, **27,** 919-29.

Vogelstein, B. & Kinzler, K.W. (1993). *Trends Genet*, **9,** 138-41.

Waters, B.L., Panagopoulos, I. & Allen, E.F. (2000). *Cancer Genet Cytogenet*, **121,** 109-16.

Weinberg, R.A. (1988). *Cancer*, **61,** 1963-8.

Weinberg, R.A. (1989). *Cancer Res*, **49,** 3713-21.

Weinberg, R.A. (1996). *Sci Am*, **275,** 62-70.

Wu, W., Lin, F. & Worman, H.J. (2002). *J Cell Sci*, **115,** 1361-71.

Yamaguchi, S., Yamazaki, Y., Ishikawa, Y., Kawaguchi, N., Mukai, H. & Nakamura, T. (2005). *Genes Chromosomes Cancer*, **43,** 217-22.

Yang, L., Embree, L.J. & Hickstein, D.D. (2000). *Mol Cell Biol*, **20,** 3345-54.

Yang, L., Embree, L.J., Tsai, S. & Hickstein, D.D. (1998). *J Biol Chem*, **273,** 27761-4.

Young, K.H. (1998). *Biol Reprod*, **58,** 302-11.

Zinszner, H., Albalat, R. & Ron, D. (1994). *Genes Dev*, **8,** 2513-26.

Zinszner, H., Immanuel, D., Yin, Y., Liang, F.X. & Ron, D. (1997a). *Oncogene*, **14,** 451-61.

Zinszner, H., Sok, J., Immanuel, D., Yin, Y. & Ron, D. (1997b). *J Cell Sci*, **110 (Pt 15),** 1741-50.

Zucman, J., Delattre, O., Desmaze, C., Epstein, A.L., Stenman, G., Speleman, F., Fletchers, C.D., Aurias, A. & Thomas, G. (1993). *Nat Genet*, **4,** 341-5.

6. Appendix I

When preparing media, all components are mixed in double distilled water to a final volume of 600 ml. If liquid media is desired, Difco Bacto-agar was omitted. All media were sterilized through autoclaving for 20 min at 121°C and 20 pounds per square inch.

6.1. Bacterial Media

Luria Bertani (LB)

Reagent	Mass (g)/ 600 ml	Final concentration g/L
Difco Bacto-yeast extract	3	5
Difco Bacto-tryptone	6	10
NaCl	6	10
Difco Bacto-agar	10	16.67

Antibiotics were added after the autoclaving process at a concentration of 50 μg/ml, except for ampicillin, which is added to a concentration of 100 μg/ml.

6.1.1. S.O.C.

Reagent	Mass (g)/ 600ml	Final concentration g/L
Difco Bacto-yeast extract	12	20
Difco Bacto-tryptone	3.0	5.0
NaCl	0.36	0.6
KCl	0.108	0.18
Dextrose	1.2	2.0

6.1.2. M9 Minimal medium

Reagent	Mass (g)/ 600 ml	Final concentration
Na_2HPO_4	3.6	6.0
KH_2PO_4	1.8	3.0
NaCl	0.3	0.5
NH_4Cl	0.6	1.0

$MgSO_4$	0.6	1.0
$CaCl_2$-$2H_2O$	3.3 mg	5.5 mg/L
$FeCl_3$	0.32 mg	530 mg/L
Dextrose	1.2	2.0
Thiamine	1.2 mg	2.0 mg/L
Supplement*	0.46	0.77
Difco Bacto agar	10	16.67

* Supplement is a complete supplement mixture without Uracil, CSM-Ura (BIO 101).

6.2. Yeast Media

6.2.1. Yeast, Peptone, Adenine, Dextrose (YPAD)

Reagent	Mass (g)/ 600 ml	Final Concentration g/L
Difco Bacto-yeast extract	6	10
Difco Bacto-tryptone	12	20
Dextrose	12	20
Adenine Hemisulphate	60 mg	100 mg/L
Difco Bacto-Agar	10	16.67

6.2.2. Synthetic Complete omission media (SC–)

Reagent	Mass (g)/ 600 ml	Final concentration g/L
Difco yeast nitrogen base*	1.0	1.67
$(NH_4)_2SO_3$	3.0	5.0
Dextrose#	12	20
Dicfo Bacto agar	10	16.67

* Yeast nitrogen base is without amino acids or ammonium sulfate.

If desired, dextrose is substituted with galactose at the same concentration to create Sc+gal media, instead of Sc+glc.

After the above contents were dissolved in distilled water to a final volume of 600 ml, the solution was then titrated to a final pH of 5.6 and the media was sterilized. Following autoclaving, the appropriate amino acid drop out mix (CSM) minus desired amino acids was added according to manufacturer's recommendations (BIO 101).

7 Appendix II: Sequence and analysis of putative interacting proteins of TLS.

FileName: 001E.Seq
CloneName: 001
Seq: GGCACGAGCT ACAACCAACC AACATAACTA ACCCCCCCCC CCCCCATAAA CTAAAACATT
TAACTCAAAA AGTATTGGAG AAAGAAATTT ACTTACCAGG AGCTNTTTTT TTAAAGTACC
GCAAGGGAAT GGTGAAAGAC TAATTTAAAG TAAAAATAAG CAAAGATTAA ACCTTGTACC
TTTTGCATAA TGAATTAACT AGAAAATCCT TAACAAAAAG AATTTAACTA AGAACCCCGA
AACCAAACGA GCTACCTAAA AACAATTTCA TGAATCAACC CGTCTATGTA GCAAAATAGT
GGGAAGATTT TTAGGTAGAG GTGAAAAGCC TATNGAGCTT GGTG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 001N.Seq
CloneName: 001
Seq: GGCACGAGCT CGTGCCGCGC GAGTCAGGGG CTCGTCCGAA AGCCGCCGTG GCGCAATGAA
GGTGAAGGGC CCCGTTCCCG GGGGCCCCGA GGTGGGATCC CNNTTTTTTT NCAGTCCGCT
GAGGGCGCAC CACCGGCCCG TCTCGCCCGC CGCGCCGGGG AGGTGGAGNA CGAGCGTACG
CTTTTTGACC CGAAAGATGG TGAACTATGC CTGGTCANGT CNAANTCAGA NGAAAACTNT
AccessionNum: V01270.1
GeneSymbol: 18S rRNA
Description: 18S ribosomal RNAs

FileName: 002A.Seq
CloneName: 002
Seq: GGCACGAGGG TGACTCTAGA TAACCTCGGG CCGATCGCAC GCCCTCCGTG GCGGCGACGA
CCCATTCGAA CGTCTGCCCT ATCAACTTTC GATGNTTTTT NGCCGTGCCT ACCATGGTGA
CCACGGGTGA CGGGGAATCA GGGTTCGATT CCGGAGAGGG AGCCTGAGAA ACGGCTACCA
CATCCAAGGA AGGCAAGCAG GCTTACCTCG AGCATGCATC TAGAGGGCCG CATCATGTAA
TTAAGTTATG TCACGCTTAC ATTCACGCCC TNCCNCCACA TNCGNTCTAA CCGAAAAGGA
AGGAGTTAGA CAACCTGAAG TCTAGGTCCC TATTTATTNN TTTATAGTAT GNTAGTATTA
AccessionNum: M11188.1
GeneSymbol: 18S rRNA
Description: 18S rRNA gene

FileName: 003E.Seq
CloneName: 003
Seq: GGCACGAGGC AAGATGGGTC ACCAGCAGCT CTACTGGAGT CACCCGCGGA AGTTCGGCCA
GGGTTCTCGC TCTTGCCGCG TCTGCTCTAA CCGCCCGGTC TNTTTTTTAA ATACGGGCTG
AACATGTGCC GACAGTGCTT CCGTCAGTAC GCGAAGGACA TAGGCTTCAT TAAGTTGGAC
TAAGCGACCT GAATGGATGA TTCNACTGTC TACNNACTGA NNCNNNCTGT NTACNNTTGN
GNTACNANTN CTGCTANTNT TTGT
AccessionNum: NM_012876.1
GeneSymbol: Rps29
Description: Ribosomal protein S29

FileName: 003F.Seq
CloneName: 003
Seq: GGCACGAGGC AAGATGGGTC ACCAGCAGCT CTACTGGAGT CACCCGCGGA AGTTCGGNCA
GGGTTCTCGC TCTTGCCGCG TCTGCTCTAA CCGCCACGGT CTGNTTTTTA AATACGGGCT
GAACATGTGC CGACAGTGCT TCCGTCAGTA CNCGAAGGAC ATAGGCTTCA TTAAGTTGGA
CTATNCGANN TGAATGGATC ATTNNNANTG TGTANNNTNN GANTCNG
AccessionNum: NM_012876.1
GeneSymbol: Rps29
Description: Ribosomal protein S29

FileName: 003I.Seq
CloneName: 003
Seq: GGCACGAGCG GGGACTCGCA TTCCCCGGTC CCCCCTCCGC CCCACGCGGC TGGGCCATGG
ACGCCAGATG GTGGGCAGTA GTGGTACTCG CCACGCTCCC TTCCTTGNGA GCAGGTGGAN
AGTCACCCGA AGCCCCTCCG CAGTCCTGGA CACAGCTGTG GCTCTTCCGC TTTTTGTTGA
ATGTAGCTGG CTATGCCAGC TTTATGGTAC CTGGCTACCT NCTGGTGCAG TACTTANGAC
GGAAGAACTA CCTGNAGACA NGNANGGGNN TNTGTTNCNN NTGNN
GiNum: 15778613
AccessionNum: AF414190.1
GeneSymbol: J207
Description: seven-span transmembrane protein-like protein

FileName: 003N.Seq
CloneName: 003
Seq: GGCACGAGTG ACTCTTTTCA ACTAACCACA AAGATATCGG AACCCTCTAC CTATTATTTG
GAGCCTGAGC AGGAATAGTA GGGACAGCTT TAAGTATTNT TTTTTTNAGC TGAACTAGGA
CAGCCAGGCG CACTCCTAGG AGATGACCAA ATCTATAATG TCATCGTNAC AGCCCATGCA
TTNGTAATAA TNTTCTTTAT AGTAATACCT ATAATAATTG GAGGCTTCGG GAACTGACTT
GACCNCTAAT AATTGGAGCC CNTGATATAN CATTCCCACN AATNNANAAC ATAAGCTTTT
AGANTGCTTG CTNAATNATT NAA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 004D.Seq
CloneName: 004
Seq: GGCACGAGTA AACCCAAGCC CATGACCACT AACAGGAGCC CTATCAGCTC TTCTACTCAC
ATCCGGCTTA GTAATATGAT TCCATTACAA CTCCNTTTTT NTCCTATCAT TAGGCCTNCT
GACAAACATC CTAACTATAT ATCAATGATG ACGAGATATC ATCCGTGAAG GAACATACCA
AGGCCACCAC ACCCCTATTG TACAAAAAGG CCTCCGATAC GGAATAATCC TGTTTATTGT
CTCCGAAGTA TTCTTCTTTG CCGGATTTTT CTGAGCATTT TATCATTCCA GCCTAGTTCC
TACCCACGAC CTAGGCGGTT GCTGACCCCC AACAGGAATN CCCCTTTAAA TCCCCTAGAA
GTACCCCTTC TAAATACATC AAGTCCTCTT AGCATCAGGA GT
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 005B.Seq
CloneName: 005
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCANTTTTT TTGTCCTCCT GGTTCTTANA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGTG GGATGAATCT GCCAAGCACA ATTGTTCTCT GAGTGGAACC
AAAGAAGTGA GGAGCTAGGC CGCCTGGCCT GGCACCTAGG AACACAGGAG CAGGTGGGTA
TAGAAGTCAT CGGCTGNAAC TGCAGGCCCT GGGCATGGAC
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 006F.Seq
CloneName: 006
Seq: GGCACGAGGT CTGATATAGT ATTTTTAACA ATGTGCTCAA GAAACAGTAA GCAGGTATTC
TCCTTTGTGA TTTTGTCGTA GAACTTTATG TAGCAGTTAT TTGGGNAAGG GGTGTGTCTG
CATCTAAGAA ATGTTTTTAA GAAAGGAGAA AAAAGAACTA GAATTTGGAT GTTTGACCAA
AGAACATATT GGAGTAGTAT ATAAATGGTT TNCAGGAAAT GTACCANATG ATTANAATGG
NTATACNTGT TGNNANTGNN TTGNANNATG ATNG

FileName: 006I.Seq
CloneName: 006
Seq: GGCACGAGTA AAAAGTTAAG CTTTGCTTAC CTTCAGGCGT GTGTGTCAGC CTGTGTGCTA
ATATTGCCAG TTTTGTTGTT TCCATCCATC CCTGCACTTT GGNTTTTTCT TGTTCAGTTT
NTTAGGAATT GCAGCAGACT CATTGGGCTA CATTTANTAC ANGAACCACA TATTGATGTT
AAAGGACACA GTNTANTGAT GCCNNATNCT TGANT
GiNum: 8394164
AccessionNum: NM_009035.1
GeneSymbol: Rbpsuh
Description: recombining binding protein suppressor

FileName: 006J.Seq
CloneName: 006
Seq: TTAAGGNTTN CTTANAGACC NTTCTANTTC TTGGGGAGGA CNNAAGAGCA AGCCTAAGGA
CCCCAGCCAG CGNCGGCCCG GGAGATCCNC TAGTAACGGC CGCCAGTGTG CTCGGAATNC
GGCACGAGAC ATGGAGANGG CNCCAAGGCG CCGNGCAANG NAGGCNATNN NGAACCAGNA
ACANAGAAAG GNAANCANAN AAGNCNAGGN GAGNANGACA NAANNNNCNC AANTTTTTTT
TTTTTTAANC NNNAGNGGGG GGGGGGGNCC CCAANNNNCA ANNAANNGNG AANGANGNGN
ANAANAAAAC NNAGACNCGA ANGNGGGGGG NGGNGGCNCC NCNNNNANAN TTACAANGNN
ANGANAAAGA AANAAANNAN GAAAAGNNNN AANNNGGGCN AGGACACANA CNNNNANNNA
NAGNNNNAGG NANNAAGCGG GGNAANGANN ANNGNNAAAC ANGANNAGCC AAGANA
AccessionNum: X84074.1
GeneSymbol: src

FileName: 006K.Seq
CloneName: 006
Seq: GGCACGAGCT GCACTGTCGG GTGGTTGGAC TCTTTCCTCC CGGTCCTGCG GNCCTCTTTC
TCCGGCGCGC CGCCCNTCGG NTGNTCATAN TTTTGTTTTT TTTTTTTTAA NGNTANTTTG
GGGGCCNNTN AANANNNNAA AGCCAGTTNG NNAAAAATNA ANGNTTNNGG GGGNGGGNNN
NCCCCCNTTT TTNAANANCA NANNATNNNN TNNNNNNATA NANANNNNAN NGGGNANCNT
AANGANAGAN TANCNATGNA NNANNANNAN ANGAAAATGN NCNCCCCNNA TGNNAGGANA
NCNNANGANT NTCNATNAGA TNNNAATGAN NANNNNTNAA GTTCCTCNTT CCNNNACNAC
AAN
GiNum: 42476131
AccessionNum: NM_030835
GeneSymbol: RAMP4
Description: ribosome associated membrane protein 4

FileName: 006M.Seq
CloneName: 006
Seq: GGCACGAGAA AAGACACACA TAACCTTTAT GTTTACAGTA AACCTCGGAC AGCACAGGAG
CTGGGCAGAT TCTACCCGTA TGGCCTTTCC TATCGATTAT TTTTTGTTAT CACCTACTAT
TGACTATGTT CTTCTAGGGT GCTCTCAACT CCCACTGACC AGCCCTCAGG ACCATGTCTC
ATGACTCACC TACCCCATGA TGGCTTCTCT TCCTCCTTGC ACCTTCTTCC TGTGGNTNTC
ATCAGATCCG CNGNCTGGGG AAAACCTAAA NCCCANCTNT GGATNTTNAG NTCAGCTCTT
AACTTGTNAN NGGGCTTTGA TANTTGGCCT TGTAGNNANN GAA
AccessionNum: AC079445.28
Description: BAC clone

FileName: 006N.Seq
CloneName: 006
Seq: GGCACGAGTG AATGGGGTAC ATTCTCCTTG AAAACTGCTC AGATTTTACA TCTCGTTTGG
GTTTAGTTTA CATAAGTATG TGGGTGTCTT GTGGGGGAAN NTTTTTTGCT ATTGTCTTTG
NTAATTTATT GGGACTCTGT GTAAGGCCAT ATTTTANTGG CTGTGTGANA CCCCCTGTGT
TNTTTGNCNN NTTGCNNANN ANGGGTGNGG T
GiNum: 26353093
AccessionNum: AK088154.1
Description: sortilin-related receptor

FileName: 007B.Seq
CloneName: 007
Seq: GGGNNNNNNN GNNTNNNNTT TGGGTTTACT GGGGCANATC NNTTTTGGGG AGTAGCAAGN
GCAAGCCTAA GGACCCCAGC CAGNGCCGGC CCGGGTAGTA CCACTAGTAA CGGCCGCCAG
TGTGCTGGGA CCCCGCTGGA GGTGGAGACC CAATTCTCTA TCAACACCTA TTCTGATTCT
TCGGCCACCC AGAAGTGTAC ATCTTAATTC TTCCAGGGTT TGGAATTATT TTACATGTAG
TTACCTATTA CTCTGGAAAA AAAGAACCCT TCGGATATAT AGGTATGGTA TGAGCCATAA
TATCTATTGG CTTCCTAGGG TTTATTGTAT GAGCACATCA CATATTCACA GTAGGCCTAG
ATGTAGACAC CCGAGCCTAC TTTACATCTG CCACTATAAT TATCGCAATT CCTACAGGCG
TAAAAGTATT CAGCTGACTC GCTACACTAC ATGGAGGGAA TATCAAATGA TCCCCCGCCT
ATTATGAGCC TTAGGGTTAA TCTTCTTATT CACAGTAGGG GGCCTAACAG GGATCG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 008C.Seq
CloneName: 008
Seq: GGCACGAGGG AAGGAGCTCG GTCCCTGTAG GCCCAGCTGC TCTCAGAGAT GAAGTTTCAA
GACGGGCAGA AGTTTCTGGG CCCTCAGTGC CTGGACNTNT GTGCGACCAA GCCATGATCG
AGGAGTGGGT GTTCCATGTC CCTCTCGTGG GCGTGTTTCT GAGCGTGGTC ATCCACAGGG
GCCTGTGCCT CCTGGCCTCA TCCTTTGACC TCTCCACGCT GGTCCCCGAG TGCCACGTGG
ACGGAGGGAG GCCGTCTGAG AGCATCCTGG ATGTGCTGTC CGTCATCTAC CTCAGTCCCA
CCTGGCAGTG GAACACCGGC AGTGCTGGCG CCTGCTGTTC TCCACGCAGC TCACGGACAG
AGCTTCTNCC AGCTCTGCAG CCTATNCGAG CCAGGGGC
GiNum: 34851812
AccessionNum: XM_226525.2
GeneSymbol: LOC307901
Description: similar to 4632415K11 gene [Mus musculus]

FileName: 009B.Seq
CloneName: 009
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG NGCATTTTTT TTGTCCTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGTG GGATGAATCT GCCAAGCACA ATTGTTCTCT GAGTGGAACC
AAAGAAGTGA GGAGCTAGGC CGCCTGCCTG GCACCTANGA ACACAGGAGC AGGTGGGTAT
AccessionNum: XM_217467.1
GeneSymbol: Itm2cDescription: Integral membrane protein 2C

FileName: 010B.Seq
CloneName: 010
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TNTTGCACCG ANNTTTTTTT TTGTCCTNAT GGTTNTTNTT
ANNTAANTGT GGACTANTGA TGNGGAANAT GNNTCCTGCG TTNGACACTG AGAGCACANT
TATAATNTNN CTAANNTAAG TTNTTNANAN AANTNNTGAC AATGNCTNAA TNATTNANTN
GNGANATTGA ANNTGGNANT AAAGNATGAA ANNNNAATNT NNAAAANNTA TNNGAATTAA
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C

FileName: 011D.Seq
CloneName: 011
Seq: GGCACGAGGG TCACACCGCG CTTTACACCG TCCTCCGGCC GTCGCTCGCA GTCTCTCCGC
CACCATGCCT ATGTTCATCG TGAACACCAA TGTTCCCCGC GCNTTTTTGC CAGAGGGNTT
TNTCTCCGAG CTCACCCAGC AGCTGGCGCA GGCCACCGGC AAGCCGGCAC AGTACATCGC
AGTGCACGTG GTCCCGGACC AGCTCATGAC TTTTAGCGGC ACGAGCGACC CCTGCGCCCT
CTGCAGCCTG CACAGCATCG GCAAGATCGG TGGCGCCCAG AACCGCAACT ACAGCAAGCT
GCTGTGCGGN CTGCTGTCCG ATCGNCTGAC ATNANNCCGG ACCGGGTNTA ATNAACTATT
GiNum: 663139
AccessionNum: S73424.1
GeneSymbol: MIF
Description: macrophage migration inhibitory factor

FileName: 011M.Seq
CloneName: 011
Seq: GGCACGAGCG AGAACGGGTG AAAGTGGGTG CAAGTTTGGC TGATGTTGAT CCGATGAACA
TTGATAAATC AGTGCGGTTT GATANNNTAN GTGGTTTNNT TTTTTTTTTT NTGGTTTNNT
NGGNGNTNCT NNNNTTTTNT NNTTNNNTTN NANNNNNNTT TTTTNNGGGG GGGGTNTTT
AccessionNum: XM_233953.2
GeneSymbol: LOC313940
Description: similar to hypothetical protein KIAA1240

FileName: 012H.Seq
CloneName: 012
Seq: GGCACGAGGA AGTCAGGAGA CTTGCTAAGA TGCTGCTGGA TTTGTCCGAG GAGCACAAGG
AGCACCTGGC CTTCCTGCCG CAAGTGGACA GTGCGGTGGT CNTTTTTTTG GGAGGATTNT
CGNGNAGTTN T
GiNum: 34856969
AccessionNum: XM_215578.2
GeneSymbol: LOC295064
Description: similar to HSPC042

FileName: 012I.Seq
CloneName: 012
Seq: GGCACGAGGT TGACTCTTTT CAACTAACCA CAAAGATATC GGAACCCTCT ACCTATTATT
TGGAGCCTGA GCAGGAATAG TAGGGACAGC TTTAAGTATT CTAATTCGAG CTGAACTAGG
ACAGCCAGGC GCACTCCTAG GAGATGACCA AATCTATAAT GTCATCGTCA CAGCCCATGC
ATTCGTAATA ATTTTCTTTA TAGTAATACC TATAATAATT GGAGGCTTCG GGAACTGACT
TGTACCACTA ATAATTGGAG CCCCTGATAT AGCATTCCCA CGAATAAATA ACATAAGCTT
TTGACTGCTT CCTCCATCAT TTCTACTCCT TTTAGCATCC TCATAGTAGA AGCTGGACTG
GACAGGATGA ACAGATNCCC CCTTANCCGG AT
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 013D.Seq
CloneName: 013
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG NGCACTCNNT TTTTTTTTTT GGTTCTTAGN
NNTAAGTGTG GACTAANGAT GGGGAAGTGT GTTCTGTGTT AGANCCATCA NAGNACAATA
TNNTNTGNNT AATNTGGGNT ATT
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C

FileName: 013F.Seq
CloneName: 013
Seq: GGCACGAGAT CTTGAAGAAG TTACTAGGCA TGGTTGTTAC ATATAGTAAA ACTGAGAGTA
ATTCATCTGT GAATCTGCAT ATTAATTACC TGCTGATTGA CTTNTTTTTT ANATGTGATT
TTATACATGG ACATTTTTGA ATATGCTNTT AATTTANANA CTGATAAANA TNNANNTANA
TNATTNTGGN TGATTNT
AccessionNum: NM_019491.4
GeneSymbol: v-ral
Description: v-ral simian leukemia viral oncogene homolog A

FileName: 013L.Seq
CloneName: 013
Seq: GGCACGAGGT TCTAGAGACA GCCGCATCTT CTTGTGCAGT GCCAGCCTCG TCTCATAGAC
AAGATGGTGA AGGTCGGTGT GAACGGATTT GGCCGTATCG GACGCCTGGT TACCAGGGCT
GCCTTCTCTT GTGACAAAGT GGACATTGTT GCCATCAACG ACCCCTTCAT TGACCTCAAC
TACATGGTCT ACATGTTCCA GTATGACTCT ACCCACGGCA AGTTCAACGG CACAGTCAAG
GCTGAGAATG GGAAGCTGGT CATCAACGGG AAACCCATCA CCATCTTCCA GGAGCGAGAT
CCCGCTAACA TCAAATGGGG TGATGCTGGT GCTGAGTATG TCGTGGAGTC TACTGGCGTC
TTACCACCTG GANAAGGCTG GGGCT
AccessionNum: XM_216453.1
GeneSymbol: gapdh
Description: glyceraldehyde-3-phosphate dehydrogenase

FileName: 014A.Seq
CloneName: 014
Seq: GGCACGAGCT CTTTTCAACT AACCACAAAG ATATCGGAAC CCTCTACCTA TTATTTGGAG
CCTGAGCAGG AATAGTAGGG ACAGCTTTAA GTATTNTNAT TCGAGCTGAA CTAGGACAGC
CAGGCGCACT CCTAGGAGAT GACCAAATCT ATAATGTCAT CGTCACAGCC CATGCATTCG
TAATAATTTT CTTTATAGTA ATACCTATAA TAATTGGAGG CTTCGGGAAC TGACTTGTAC
CACTAATAAT TGGAGCCCCT GATATAGCAT TCCCACGAAT AAATAACATA AGCTTTTGAC
TGCTTCCTCC ATCATTTCTA CTCCTTTTAG CATCCTCCAT AGTAGAAGCT GGAGCTGGAA
CAGGATGAAC AGTATACCCC CCCTTAGCCG GAAACCTAGC C
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 015.Seq
CloneName: 015
Seq: GGCACGAGCA AAATGTAAAC TTAAAATATA ACCAAAAGAG GGACAGCTCT TTAGGAAAAG
GAAAAAACCT TAAATAGTGA ATAAACAACT ACAACCNNTT TTCCATTGTA GGCTTAAAAG
CAGCCATCAA TAAAGAAAGC GTTCAAGCTC AACATACATA CTTACACACA CTAATTCCAC
AAACCTCAAT AAATTCCTAT ATTACAAATT GGGCTAATCT ATAGACCCAT AGATGAAATA
CTGTTAATAT GAGTAACAAG AACCAATTCT CCTAGCACAA GTGTATGACA ACCCGGATAA
CCATTGTCAA TTATCGAATC ATAGGTACTA ACCCAACAAT AAAATTACCT ATCCCTAACT
CGTTAGCCCA TACCTCGAGC ATGCTCTAGA GGGCCGATCA T
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 016B.Seq
CloneName: 016
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG NGCNTTTTTT TTGTCCTCCT GGTTCTTANA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTNAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGNTG GGATGAATNT GTCAAGCACA ATTGTTNTNT GAGTGGAACC
AANNAAGTGA GGANCTANGN CGNNNTGNCT GNACNTANGA ACACANGANC
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 017A.Seq
CloneName: 017
Seq: GGCACGAGGA AGGGCTTTAG TCGCCAGTCT TCACTCCTGT TCAAGATCCT TTCCAGTGTT
CGGAATCACC AGATCAATTC AGATTTGGCG CAGCTACTGC TCNNNCTAGA CTATAACAAA
TATTATACCC AGGCTGGTGG GACTCTGGGC AGTTTTGGGA TGTGAAAACT CAGCTTCATA
AAATGAAGCA GCAGTCAGCC CTACCACGTT CTGATGTCTG TAACAAACCT CTCTAGTCAT
TCATCAAATG CCTGCAGACT GCTGAAAGGG TCTTCTAGAA GAAGTCCAGA GGTATAAATC
CTTCCTGCCA TCCCTGTGGG AAACTGGGGT CTGAGATCCC TCTGCTG
GiNum: 26340967
AccessionNum: AK050250.1
GeneSymbol: 76P
Description: gamma tubilin ring complex

FileName: 018A.Seq
CloneName: 018
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCANTTTTT NTGTCCTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGTG GGATGAATCT GCCAAGCACA ATTGTTCTCT GAGTGGAACC
AAAGAAGTGA GGAGCTAGGC CGCCTGCCTG CACCTAGGAA CACAGGAGCA GGTGGGTATA
GAAGTCATCG GNTGTGAACT GCANGCCCTG GGCAGT
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 019C.Seq
CloneName: 019
Seq: GGCACGAGCG GCACGAGCGA TCACTGAGTG GCGGCGGCTG CTGATTGTGT TCTAAGGGTC
GGAGTGGGGT CNAACGCTTN NTCNATNTNT NTNTNTTTTT TTTTTTNNNC CNTTTCNNTT
NNTGNTNTNN ANANTNNTTN ATTTTNTNTN ATTTCNT
AccessionNum: X15744.1
GeneSymbol: RAB1
Description: ras-related GTP-binding protein

FileName: 020D.Seq
CloneName: 020
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCATTTTTT NTGTCCTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGNTG GGATGAATCT GNCAAGCACA ATTGTTNTNT GAGTGGAACC
AAAGAAGTGA GGAGCTAGGN CGCCTGNCTG GNACCTANGA ACACANGAAN ANGTGGNTAT
ANAANTATNN NTGTANCTGA NGNCNTGNNN ANNGAA
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 021F.Seq
CloneName: 021
Seq: GGCACGAGGC GGTCAGCATT GCATNTCCAC CTGGCTCTTT CTGTTTGGCG CTGTGCGGGG
CAGGCTGTAT GGCCGCAGGT CCCAGCTACA GCCTGAGAGG GATTTTNNGG GTGAGCAACG
GGAATAGTTC CCTGGAGCTG CCGGCTACTG TCCCTGGCTA TGTGCACAGC GCCCTGCAGC
AGCACGGGCT GATCCAGGAT CCTTACTACA GATTTAATGA CCTGAACTAC AGATGGATTT
CCTTAGATAA CTGGACCTAC AGCACAGAAA TTTAAAATCC CCTTTAATCG CAGTGAATGG
CAAAAAGGTA AAGTTGATTT TGACGNA
AccessionNum: BC031409.1
GeneSymbol: Manba
Description: mannosidase, beta A

FileName: 021G.Seq
CloneName: 021
Seq: GGCACGAGGC CGGTAGAAGC AAGATGACGA AAGGAACGTC ATCCTTTGGA AAGCGTCGCA
ATAAGACGCA CACGCTGTGC CGCCGCTGTG GNTCCANTTT TTTTTNGNTT NANNAAGNNN
GGGATGNGNG CAAATGTGGN GTATCGNTGT NANTNTNCGA TAAANTTTNC CGNAGGANNN
CCCCTGTTTT TTAANAGAAA NANNTANANG TANAATAGNA ANANGANCNT NANTAACTAA
AATNANNANN ANNATNNTNN NNNNAANNGG AATTTCNNNG TTGCTGCNTC GCNTATNNNG
GiNum: 1839336
AccessionNum: S79981.1
GeneSymbol: Rpl37
Description: ribosomal protein L37

FileName: 022D.Seq
CloneName: 022
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTNNTNATN TTCTNGCANA NTNNACTTTT TTTTTTTTTC TGGTTNTNNG
NGNCGAGNGA GNACNANGGN ATGGGGAAGT GNNTNCTGGG CNANANNNAN GGNGACCCCC
CCNTNTNANG NGNGNANAAG CAAGGNTANG CCTGNGGAAT ANGGGGGGGG NNAAGNNNNA
GNNNNACAGA ACGGTANNNN NGNNGTGGGA NTCTGNANAT ANNGGGNAGA NACNNNAANN
ANNNANNTAA AAGNNANAAN AAAANGGNNN AAANGNANCC NACCNNCGAG
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 022H.Seq
CloneName: 022
Seq: GGCACGAGGN NCNGNAAAGA CCATATTACA NATAACGAAG CTTTGTATGA TTTAAATCCA
TTAATAATTT TGNAANATAG GACTATANTA TGTATGTTTT NTNTATTTTT TTTTTTTANT
ATTTNTTTNN ANCNTNTTAT NTNTTTNNTN TTNNNNNNAT TTTNNNTTTN TTNNNNNTTN
NTTTTTNNTN TNTNTTTTTN TANNTTTTTT TTTNNNTTNN TNTANTNTTT TTTTTNTTTT
TNTNTTTTNT NNATTTTNNT TTTTTNNTNN TTTNTTNTTN TTTTTTNNTT TTNTTTTTTN
TNNTTAATTN NNTNNTATNN NNTNTTATTN TNTNTTTTNN NTTTNTTNNT TNTTTNTNNT
TNTNTNTTNN TTTGTTNTNN TNTNTTTTNT NAT
AccessionNum: AL929322.6

FileName: 022L.Seq
CloneName: 022
Seq: GGCACGAGGC GCGGTGAAGC CAGATTAGGA TCAGCGAGCA CTTGAGGACT TAGGGCCACA
AAAAAACCGC ACAAGATCGA CAGACTATTT CTGGAGAGCT GCAGAACGGG CACGCTGGGG
TCGCTGGTGC TGGCCATGGT GATGGAGGTG GGCATCCTGG ACGCCGGGGG GCTGCGCGCG
CTGCTGCGAG AGCGCGCCGC TCAGTGCCTG CTTCTGGATT GTCGCTCCTT CTTCGCCTTC
AACGCCGGCC ACATCGTGGG CTCAGTGAAC GTGCGCTTCA GCACCATCGT GCGGCGCCGN
GCCAAGGGCG CCATGGGCCT GGAGCATATC GTGCCGAACA CCGAACTGCG CGGNCGCTGT
GGCCGGACCT ATANGCCTAN TGNTGT
GiNum: 14164984
AccessionNum: AF357203.1
GeneSymbol: Mkp1
Description: MAP kinase phosphatase-1

FileName: 023B.Seq
CloneName: 023
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TATTGCACCG CGCATTNCCT TTTTTTTTCT GGTTCTTANA
NCTAANTGTG GACTAANGAT GGGGAAGTGT GTCCTGGGTT AGACCNATNA GAGCACAAGA
TANGGTGGNT AACCTGNGTT NTTCCCAGGA CTNNTGTNAG TGNCTTNANN TNNNCANTAN
NATNCTGTAA TTNNNNNNTN ANATGNGTAT GANTANNNAT TGTTTNTNAA TNNAANNNNA
NANNTTTAAN NNATTTNNTN TTNTTNTNTN TNTNGANATT TNANCNTTAT TTCNNNNTTT
TTTNTTTTTN TNNTANTATA
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C

FileName: 023D.Seq
CloneName: 023
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG NGCATTTNCT NTTTTTTTTT GGTTCTTATT
TNTAACTGTG GACTAANGAT GGGGAAGTGT GTNCTGNTTT ATANNNATNA NANNACANTA
TNTTGTGNNT NANNTGNNTT ATT
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 024B.Seq
CloneName: 024
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCACTCCCT CTGTNCTCCT GGTTCTTANT
ACCTAAGTGT GGACTAAGGA TGGGGAAGTG TGTCCTGGGT TAGACCCATC AGAGCACAAG
ATAAGGTGGC TAACCTGGGT TCTTCCCAGG ACTCCTGTCA GTGCCTTCAG CCCACCAGTA
GGAGCCTGGA GTNGGCGGGT GGGATGAATC TGCCAAGCAC AATTGATCTC TGAGTGGAAC
CNANGAAGTG AGGAGCTAGG CCGCCCTGGC CTGANACCTA AGAACNANGA ACAN
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 025B.Seq
CloneName: 025
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCACTCCCT CTNTNNTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTNAGC CCACCAGTAN
GAGCCTGGAG NTGGCGGGTG GGATGAATCT GCCAAGCACN ATTGTTCTCT GAGTGGAACC
NAAGAAGTNA GNGAGCTANG TCGNCCTGGG NCTGGCACCT ANNAACACAN GAN
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 026M.Seq
CloneName: 026
Seq: GGCACGAGGG TTGCTGAGGN CTNNCCNTCG NCGGNNGTNN NATNTTTTTN TTANTNNTTT
TTNTNTTNNN TNNTTTNTTN NTNNNTTTTT TTTTTTTTNN TTTTTTTTTT TTTTTNTNTG
GNTNNNNGGG CCCNTTTNTN TAANANNNNN ATTTTTNTNT TANTNTNNNT NNTNNTTNGG
GGGGGTANGT NTTTTTTTTT TTGNTTATTT NTTTTTNNNN TNNNNNTTTN GTTTGTNNTN
TNTNTNTNGT ATNTNNNNGN GTNTTTTGTN TNNTACNNGN TTNNTANTTT TTTNTTNNTN
NNNNTNNTNA TTTTNTTNNT TNNNTNTNTT NNTNGTNNAN NNTGNTTNTN NATATTNTNT
NTTATNTGTT NNNTNTTTTT AT

FileName: 027A.Seq
CloneName: 027
Seq: GGCACGAGGA CGGAGGTGCC CTGGGGTCGC AGGTATCAAG CTGGCCTCCA GAATTATATG
ATTGGAAACC TCAGTGATTT GAACTAAGAG ACAAAAAGAG ATTNTNCTTG CGTTTTGTTT
TGTAGCACGT TGTTCACATT ATTTGCGCAG CTCTTTTTGC CATTAAAACC TGCACTGAAT
TTTTTAAAAT GAAGCAATTG AAAAGGAAAA GGAAAAGCAA TTTTAGTGTT CAGGAGACTC
AGACCCTTTT GAAAGAAATT ACCAAGAGGA AGGAAGTCAT TTTTTCTAAG CAGCTCATCA
CAACAATANA CGTAATGAAG CNGAATGGCN CTGNGAGGAG ATCGCACAGT GTGTGAATGN
GiNum: 26083084
AccessionNum: AK032884.1
Description: similar to FLJ14884

FileName: 028C.Seq
CloneName: 028
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCACTCNNT TTGTCCTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGTG GGATGAATCT GCCAAGCACA ATTGTTCTCT GATTGGAANC
AANNANGTGA NGAGCTAGGC CNNACTGTCT GACACCTANT AACANCNGAA CANGTTGNTN
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C

FileName: 029B.Seq
CloneName: 029
Seq: GGCACGAGGN AAGAGTTCAA CATTGAGAAA GGTCGTCTTG TGCAAACCCA GAGATTGAAG
ATTATAGGAA TACTATGAGA AGAAGGAAAA GCAGATCGAG NTTTTTTTTT AAAATTCAAA
TGNGGCAACT TGATGAATCA AGCAAGACTC ANAGNCCTCA GAGCAAGAGA TGACCNCATC
ACTGATCTGC TTGAATGAGG CCAACAGAGA CTCGCNAAGG TGGNAAAAGA TNCGANACAN
GGCCTCAAGC ATGNANTNTN GAAGGGCCNC ATNANGTNAG GANNTNATCN GNNGCNTTAN
GiNum: 37589623
AccessionNum: XM_216251.1
GeneSymbol: LOC297566
Description: ATPase, H+ transporting

FileName: 029L.Seq
CloneName: 029
Seq: GGCACGAGTG AGAATGCCGC CCTGGAGAAA GTTGTTCAGA AATCCACCCA GAGGCCCTCT
CACTCCTGAG TCTAGACGCC TAAAGACAGT AAGGAAGAAC TTTTTTNGCA ACAAGCCCGC
CCGTGTGACG ACATCACCAG CCATACGCTT TGTTTGAATA TGGTTTTTAA ATGACCCAGC
TCTTTTTTCT TTTCAAGCAT TTGAAAGATG TTTGTCAAGC CACTTCACAG NANGCTATTG
TTTGTCCNCA AATNCCAGNG TNCCCTTTAA ACTANGTTAG GATACANTTT ANATTNTCAT
GiNum: 6978720
AccessionNum: NM_012939.1
GeneSymbol: Ctsh
Description: Cathepsin H

FileName: 030A.Seq
CloneName: 030
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCACTCCCT TTTTTNTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGNG GGATGAATCT GCCAAGCACA NTTGTTCTCT GAGTGGAACC
AAAGAACTGA GGAGCTAGGN CGTCCTGGNC TTGGCACCTA GGAACACAGG ANCAGGT
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 031A.Seq
CloneName: 031
Seq: GGCACNAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTAGAAA GACTTTTATT TTCTTGCACC GCGCACTCCC TCTGTCCTCC TGGTTCTTAN
CACCTNNTTT TTTCCCNNNG CATGGGGAAG TGTGTCCTGG GTTAGACCCA TCAGAGCACA
AGATAAGGTG GCTAACCTGG GNNCNTCCCA GGACTCCTGT CAGTGCCTTC AGCCCACCAG
TAGGAGCCTG GAGTTGGCGG GNGGGATGAA ACTGCCAAGC ACAANNGGNN TCTGAGNGGA
ACCAAAAGAA GNGAAGAACT ANGCCCNCCT GGNCCNGGCA CCTAAGAACC CAGGAACANG
GGGGNAAAAN AANNNAANNG ACNGNAACCN NNANNCCCNG GGGCCANNGA NCGGANNCCA
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 032H.Seq
CloneName: 032
Seq: GGCACGAGCG GTGATAAGCG CGGCTCGAAA AGCCAGGCGG ACTCTAACTT TCTGGGGCTG
CGGCCCACCT CGGNAGGATC CNGNNCGTGA CGNNNNNTTT TTTTTTTNNN GAGNANNTTG
NGGGGNGNNT NTNANNAAAA NCANNCTNGN AGAGAAANTN NTNNTNTNCA GGGNAGNGAG
AGCCNCCNNT TTTTTTTAGA NGNNNACNTN ANGAGCNANN NACNNNANNN TNCNNGANNN
ACGNCNNTAN AANTNGANCN ATGNNNNANG ANAGACANAG NTAGAGANTA GTGAANNNNN
GAANNNNTNA TTNNNATTNG NNNATNAAAN TNANGNAAGN NAAANNNNGN GTGNTGNAAA
AAANGNNAGN ANNNGGNNNA TCANNAA
GiNum: 34854549
AccessionNum: XM_214816.1
GeneSymbol: p60
Description: Glioma tumor suppressor candidate region gene 2 protein

---

FileName: 032N.Seq
CloneName: 032
Seq: GGCACGAGTC CGGTTCCGAG GTCTTTCTGT GAGGGTGTTT NNGNCTNNNA ANAACNNANA
CANNNTTNTC ANGANTACAG TANNATCNNC TNTTNNTTAT TTTTTTTTTT TTTNGNNAGN
NNNNNTGGGN NCCCNAANAN NTATATANCT GANNNNNNNA ANANTTNNNN NANNNTTNGT
GGGGGNGANG GNCCCCNTNT TTTGNNACNA GANANAGNNG TANNGCGNGA NNTNNNANGN
ANAAAGNNGG NANNNANNNA NNANCTATTA NNNGNACGAA AGCTNTNNAN NNANANTNNA
GiNum: 37805415
AccessionNum: BC060312.1
GeneSymbol: Polyubiquitin
Description: Rattus norvegicus polyubiquitin, mRNA

FileName: 033A.Seq
CloneName: 033
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTTTGAAAG ACTTTTATTN TCTTCGGACC GNANNAGTCC CTTTTTTTTN CNGGANCTTA
GTACCNAAGT GNGGACTATA GGATNGGGAA GNGAGTNNCT GAGNTNCANN NNTCAATATN
NCAANATNAA GNGTNCTAAC TANGANANTN TTTNTAAGAC TNNNTNTNAA ANNANTACAG
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 034A.Seq
CloneName: 034
Seq: CTCNTTACCC CCGCGNCGNN CGCNACNGCC TGGGCATTCC CTAGTAACGG CCGCCAGTGT
GCTGGANTTC GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA
CATATGGGGA AGTCTGAAAG ACTTTTATTT TCTTGCACCG CGCACTCCCT CTGTCCTCCT
GGTTCTTANA CCTTTTTTTT TCCCCCNGCA TGGGGAATTN TGCCCTGNNT TANACCCACA
NACACAANAN CCGTNTCTAA C
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 035C.Seq
CloneName: 035
Seq: GGCACGAGGC CGATCTCGCG CACGCTACTA GCTGCTGCTC GCCCGTCGTC CCCCATCGTG
CACTAAGCGG TCCCAAAAGA TTCAAAGTCC AAGATGGCAN TTTTTTNGGG ACCAGCTGAT
TGNGAATCTT CTTAAGGAAG AACAGGTCCC CCAGAACAAG ATTACAGTTG TTGGGGNCGG
TGCTGTTGGC ATGGCTTGTG CCATCAGTAT CTTAATGAAG GACTTGGCTG ATGAGCTTGN
CCTTGCTGAT GTCATAGNAC GANAAGCTAA AGGGAGAAAA TGATGGATCN TTNAGCCATG
GNAANCCNTT TNCCTAAGAC ACCNAANTGG TATCCAAGNA AANAA
GiNum: 8393705
AccessionNum: NM_017025.1
GeneSymbol: Ldha
Description: lactate dehydrogenase A

FileName: 035I.Seq
CloneName: 035
Seq: GGCACGAGTA CAATCATCTC CTCAATAGCC ACACTATTTA TTTTATTTCA ATTAAAAATT
TCTTCCCAAA CCTTTCCTGC ACCTCCCTCA CCCAAAACTA TTTTTNCAGA AAAAACGANT
AACCCTTGAG AATCAAAATG AACGAAAATC TATTTGCCTC TTTCATTACC CGCACAATAA
TAGGTCTACC AATTGTTGTA ACCATTATTA TGTTCCCATC AATTCTATTC CCATCATCAA
AACGCCTAAT NAGNAACCGG ACTNCACTCA TTNNCAACAC NTGACTATCA AAACNTATTC
ATNAACCAAC TNATTGNTAA TTTCANNNCC CAAAANGACC AAA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 036C.Seq
CloneName: 036
Seq: GGCACAAGCG GCACGAGGGG GTTTGGCATG AGGTTTNNNA AATGGCCCCC ACATATGGGG
GAAGTCTGAA AGAATTTTAT TTNTTTGNAC CGNGCACTCC TTTTGTCCTC CTGGTTCTTA
GACCTTAAAG TGTGGGACTA AGGATGGGGG AAANTGTGGT CCTGGGTTNA ACCCCATTCA
AGAACCCAAG GATNAAGGTG GGCTTAACCT TGGGGTTTNT TTCCAGGAAC TTCTTGTNAA
TGGCCTTTNA NCCCCAACCA NTTNGGAAAC CNTGNAATTT GGCCNGNTTN GGNAATANAA
ATTTTGCNAN GNCCNAAATT TNGTTCTTTT AANTGGAAAC CCCATAANTT TNGGGAANTT
NGNCNNNCTT TANTTNNGGN CNNTTNNGAA CNCNGNNNNC NTGGGGTTTT AAAAATNTAT
NNTTTTAANN TTNNNTNNNT TTNNNTTNNC ANTNNNTCAT NNTTNTTTTT TACA
AccessionNum: XM_217467.1
GeneSymbol: itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 037A.Seq
CloneName: 037
Seq: GGCACGAGCG GCACGAGGGG GTTTGGCATG AGGTTCACAA ATGGCCCCCA CATATGGGGA
AGTCTGAAAG ACTTTTATTT TCTTGCACCG CNNNTTTTNT CTGTCCTCCT GGTTCTTAGA
CCTAAGTGTG GACTAAGGAT GGGGAAGTGT GTCCTGGGTT AGACCCATCA GAGCACAAGA
TAAGGTGGCT AACCTGGGTT CTTCCCAGGA CTCCTGTCAG TGCCTTCAGC CCACCAGTAG
GAGCCTGGAG TTGGCGGGTG GGATGAATCT GCCAAGCACA ATTGTTCTCT GAGTGGAACC
AAAGAANTGA GGAGCTAGGC CTNCTGNCTG CACCTANGAC ACANGNNCAG GTGGGTATTG
AANTTATNTN NTTGNAA
AccessionNum: XM_217467.1
GeneSymbol: Itm2c
Description: Integral membrane protein 2C (Transmembrane protein BRI3)

FileName: 038C.Seq
CloneName: 038
Seq: GGCACGTGAC CACACCCTAA TAATTGTATT CCTCATCAGC TCCCTAGTAC TTTATATTAT
TTCACTAATA CTAACAACAA AACTAACACA CACAAGCACN TTTTTTNCCC AAGAAGTAGA
AACAATTTGA ACAATCCTCC CAGCTGTCAT TCTTATCCTA ATTGCCCTTC CCTCCCTACG
AATTCTATAC ATAATAGACG AGANTAATAA CCCAGTTCTA ACAGTAAAAA CTATAGGGAN
ACCAATGATN CTGGAACCTA TGAAATATAC TGGCTATNGA AGAACCTATG CTTTTNNACT
TCTANANAAN NCNAAGCGTT TAGCCATGNC TTNTNAAAGG GGCNGNTAN
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 038L.Seq
CloneName: 038
Seq: GGCACGAGAG AAATCCAGAA CAAAGAGCAC ACCGCTCCTG CTTCTTCAGA AGGCCTTGCT
GAGCCCGTTG TTAACAGGAG GGCTGAGGGC AGTGAGCNTT TTTTGCAGAA CTCAANGAGT
ACCCAGAGGC TCCTGTTCAA AGGAAACAGA NGAAGATAAG ACCANCTCCT GAGNNGGAGA
CTTCCTTCAC TGAAAAGGCA TCCAGTNCGT CTTTACTACG GAATGAAAAA NGGCATTGGA
NGGTGGAGCC TNCTGANGAA NANGNTATTN NNANNGNTCG ANNGANGTNA ANGANNNCTN
GTNNCTTGTN NNNNNCNTAT GNNANNAAGN NNAGAAATNA A
GiNum: 8393705
AccessionNum: XM_237179.2
GeneSymbol: LOC316412
Description: hypothetical protein FLJ33282

FileName: 039B.Seq
CloneName: 039
Seq: GGCACGAGTA ACCCCCCCCC CCCATAAACT AAAACATTTA ACTCAAAAAG TATTGGAGAA
AGAAATTTAC TTACCAGGAG CTATAGAGAA AGTACCGCAA GGGAATGGTG AAAGACTAAT
TTAAAGTAAA AATAAGCAAA GATTAAACCT TGTACCTTTT GCATAATGAA TTAACTAGAA
AATCCTTAAC AAAAAGAATT TAAGCTAAGA ACCCCGAAAC CAAACGAGCT ACCTAAAAAC
AATTTCATGA ATCAACCCGT CTATGTAGCA AAAATAGTGG GAAGATTTTT AGGTNAGGTG
AAAAGCCTAT CNAGCTTGGA GATANCCTGG TTTGCCCCAN TNAANAATTT CATTNANNCT
TTANNNTTTN CNTTNNTAAN NNTATTAAN
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 040H.Seq
CloneName: 040
Seq: GGCACGAGCC AACATAACTA ACCCCCCCCC CCATAAACTA AAACATTTAA CTCAAAAAGT
ATTAGGAGAA AGAAATTTAC TTACCAGGAG CTATAGAGAA NTTTTTTNNA AGGGAATGGT
GAAAGACTAA TTTAAAGTAA AAATAAGCAA AGATTAAACC TTGTACCTTT TGCATANTGA
ATTANCTAGA AAAATCCTTA ACAAAAAGAA TTTAAGCTAA GAACCCCGAA ACCAAACGAA
GCTACCTAAA AAACANTATC TGAAATAACC CCGGTCTATG GNNNAAAAAT AGTTGGGAAA
GATTTNTTAC NGNAANANGG TCNAAAAGCN TNNTNAAANN TAGGNNNNAA GC
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 040J.Seq
CloneName: 040
Seq: GGCACGAGAT TTATTACAAT CATCTCCTCA ATAGCCACAC TATTTATTTT ATTTCAATTA
AAAATTTCTT CCCAAACCTT TCCTGCACCT CCCTCACCCN TTTTTNTAGC TACAGAAAAA
ACGAATAACC CTTGAGTAAT CAAAATGAAC GAAAATCTAT TTGCCTCTTT NATTACCCCC
ACAATAATAG GTCTACCAAT TGTTGTAACC ATTATTATGT TCCCATCAAT TNTATTCCCA
TCATCAAAAC GCCTAATGAG CAAACCCGAC TACACTNAAT TTTAACCACT TGACTAATNA
AANCTTATCA TAAAACCAAA TATGNNAANN NAAGACACCN CGA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 041H.Seq
CloneName: 041
Seq: GGCACGAGCT CGTGCCGCTT TTCAACTAAC CACAAAGATA TCGGAACCCT CTACCTATTA
TTTGGAGCCT GAGCAGGAAT AGTAGGGACA GCTTTAAGTA TTCTAATTCG AGCTNTTCTA
GGACAGCCAG GCGCACTCCT AGGAGATGAC CAAATCTATA ATGTCATCGT CACAGCCCAT
GCATTCGTAA TAATTTTCTT TATAGTAATA CCTATAATAA TTGGAGGCTT CGGGAACTGA
CTTGTACCAC TAATAATTGG AGCCCCTGAT ATAGCATTCC CACGAATAAA TAACATAAGC
TTTTGACTGC TTCTCCATCA TTTCTACTCC TTTTAGCATC CTCCATAGTA GAG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 041I.Seq
CloneName: 041
Seq: GGCACGAGCC TGTAATCACT ATATCTAGCT CCAACTTACT CCTAATATGA GTAGGATTGG
AAATAAGCCT TTTAGCTATC ATCCCACTTC TAGCCAACAA AAAAAGCCCA CGATNNTTTG
AAGCAGCAAC AAAATATTTT CTAACCCAAG CTACAGCCTC AATAATTATC CTACTAGTCA
TCATCCTCAA CTACAAACAA TCAGGAATTG AACCCTCCAA CAACAAACCA ATAACATACT
ACTCAACATA ATACTCATTT CACTAGCCAT AAAACTTGGA CTAGCCCCAT TCCACTACTG
ACTACCCGAA GTCACCCAAG GAATTCCCCT ACACATTGGA TTAATCTTAC TAACATGACA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 042A.Seq
CloneName: 042
Seq: GGCACGAGCA TTCCCCTACA TGGGGGCATT GAGCCTTGGC AGGCAGGATG AAGGAATTCT
CCCACTGATG CCCAACAAGG CCATCCTCTG CTACATATGC AGCTGGAGCC AGGGGTCTGT
CCATGTGTAG TCTTTGGAAT CTAACTTCTT GAGTTCTTTG TATATTTTGG ATATAAGGCC
TCTATCAGAT GTAGGATTAG TAAAGATCTT TTTCCAATCT GTTGGTTGCC ATTTTGTCCT
ATTGATAGTG TCCTTTTCCT TACAGAAACT TTGCAGTTTT ATGAGGTTCC ACTTTGGTGA
TTCTTAATNT TAGAGCATAA AGCCAGTGGT GTCTGTTCAG GAAATTTTCC CTTGNGCCAT
GTGTTTGAGG CTTTCCCCAN T
AccessionNum: AC145398.2
Description: chromosome 1q35-q36 DNA

FileName: 043F.Seq
CloneName: 043
Seq: GGCACGAGCG GCACGAGTTT ATGAAACACT GCCAGTGGCC ATCAATGGGA ATGGGCCCAC
CAAAATTCTC TTGAATTACT TTGGAAACTA TGTTCCAAAT TCATGGACAC AGGATTTGGC
TGTGCTCTTT GTGACTTTGA CACAATTGAC CTGTCTACAG TAGATAAAAT GGTGGTGTCC
TCTGGAAGGA GAATCAATTC CTTCTGTTTG GATTTTCCTT TCTAATATAA ACTTTCTGGT
TGAAGGAGAA AATGTTCATG TTGAGGGTTN TATNNATTAT T
GiNum: 31343608
AccessionNum: NM_175869.2
GeneSymbol: Plod2
Description: procollagen lysine, 2-oxoglutarate 5-dioxygenase 2

FileName: 043H.Seq
CloneName: 043
Seq: GGCACGAGAA CTGCTNANGT CCTGTGGACA GATCACTGAG TGGCCGATGG NTGCAGACTC
TCANACTCCC TGGCTCCTGA CCTTCAGCCT GCTCTGCCTG NTGTGGCCTA AAGNNNNTGN
TGCTTTNCCT GCCATGCCCT TGTCCAGTCT GNTTGCCAAT GCTGNGCTCC NAGCCCAGCA
CCTGCACCAG TTGGNTGNTG ACACCTACAA ANAGTTCGAG CGTGCCTACA TTCCCTAAGG
GACAGTGCTA TTCCATTCAA AATGCCCAGG CTGCGTTCTG CTTCTCAGAN ACCATTCNAG
TCCAACCGGC AAGGANGAGG CCCAGCANAG AACTGACATG GAATTGTTCN NTNNTCGT
GiNum: 1432170
AccessionNum: U62779.1
GeneSymbol: presomatotropin
Description: presomatotropin

FileName: 043L.Seq
CloneName: 043
Seq: GGCACGAGGC GGAGCGAGTC GACTTTGGAC TACTCTCTTA GCGTGGCGTC GCGCGAGGCT
GCGGCGGAGC CTCTGTCAGG AAGGCGGGAG CTTGACAGCT TCAGTNTNGA NTTTTTTTTN
GTGACTGTNA CCCGGATCAT GAGCGACAGC GGNNATCATA ACTACGGNNA GNNGGGGGNG
GTG
GiNum: 16923965
AccessionNum: NM_057119.1
GeneSymbol: Sfrs10
Description: splicing factor, arginine/serine-rich 10

FileName: 043M.Seq
CloneName: 043
Seq: GGCACGAGCG GCACGAGTTT ATGAAACACT GCCAGTGGCC ATCAATGGGA ATGGGCCCAC
CAAAATTCTC TTGAATTACT TTGGAAACTA TGTTCCAAAT TCATGGACAC AGGATTTTNG
CTGTGCTCTT TGTGACTTTG ACACAATTGA CCTGTCTACA GTAGATAAAA TGGTGGTGTC
CTCTGGAAGG AGAATCAATT CCTTCTGTTT GGATTTTCCT TTCTAATATA AACTTTCTGG
TTGAAGGAGA AAATGTTCAT GTTGAGGGTT TNATTNATTN AT
GiNum: 31343608
AccessionNum: NM_175869.2
GeneSymbol: Plod2
Description: procollagen lysine, 2-oxoglutarate 5-dioxygenase 2

FileName: 044C.Seq
CloneName: 044
Seq: GGCACGAGCA TTATTCGAGA CAGCCTCCTT CCTGTCACCC TGCAGTGTAA CCTCACTTCC
AGCTCTCACA CCCTTATGTA CAGCTACTGG ACAAAGAATG GGGTAGAACT CANTNTTTNC
CGTAAGAATG CCAGCAACAT GGAATACAGG ATCAATAAGC CAAGAGCTGA GGATTCAGGC
GAATACCACT GTGTATATCA TTTTGTCAGC GCTCCTAAAG CAAATGCCAC CATTGAAGTG
AAAGCTGCTC CTGACATCAC TGGCCATAAA CGCAGTGAAA ACAAAAATGA AGGGCAGGAT
GCTATGATGA TTGCAAGTCT GTTGGCTACC CCCACCCGGA ATGGATGTGG CGCAAGAAG
GiNum: 9507072
AccessionNum: NM_019380.1
GeneSymbol: Sdfr1
Description: stromal cell derived factor receptor 1

FileName: 044I.Seq
CloneName: 044
Seq: GGGNNGNNCA CNANATACGC NTCTAGACCN TGGTGGAGGA GCCACAAGCA AGCCTAAGGA
CCCCAGCCAG CGCCGGNCCG GGAGNANCCA TAAACCGGCT NTNAGAAAAA ACNACAAAAN
ANNNGNNAAN CNAACGANNA ACNAANGCAG NCNNANNAAN ACAGAAANCC CCCANCCCGG
NANNANNNAN NNAAANACAN AACACNANNN ANCAAAAATC NNCNANNCNC NCCCNANNNA
NAGAAATGCN CGACANNANN NNNACNNNNN AGTTTTTTTT TTNANGGGAG GGGGGGGNGG
AccessionNum: X84074.1
GeneSymbol: v-src
Description: Rous sarcoma virus v-src gene

FileName: 044J.Seq
CloneName: 044
Seq: GGCACGAGAG TTACTGAAGA GAGACCAGTG AACAATGTCA GCCCCAAACA TTTGCCTGCA
AAGCTGGCTA AGGTTTNTCA AGGAANNTAG GCTTTGACTA TTCATGTTTA ATTTTTTTTT
GAATGGGNGG GTANNATNNN CNTTTTTTTA TTATNTNAAA AAAANCCNTT NATGGTGGGG
GGGGGGGCGT NANTAAATTT TTTNNNNATT GTAATNAATN AANTCNAANN TNTNTTTTTA
GATANATTNN NCNTNNTNNA TNNNTTATAA NTNNNTACNT TNANANNTNN NNGNNGNCTN
GiNum: 26095084
AccessionNum: AK052241.1
GeneSymbol: irs1
Description: insulin receptor substrate 1

FileName: 044L.Seq
CloneName: 044
Seq: GGCACGAGGG AGGATGGGCC GCTGCTAGGC TCGCAGTGGG AAGNTNANNN AACNNAAANN
AANAGNCAAA AANANNNNNN GANGNANTNA CTAANANCAC NACCGNNAAA NTNTNNNTTT
TTTTTTTANG GGGGNNGNGG NNAAANCNCC CCACANCNAT AANCTNNNTA AAAAAANCNC
NCNACNGNGG NGGGGGGGGG TATANNNACC AATTTTTTTT TNANCCGANN TANNAANGNN
ACNCNTNNNA NNTTTNANCC TTTCNCTNTN ANNANACTCG ACNANATGGA NNANCATGAC
NNGNNNANNN NAGTNTNNNA NAGAGANNTA ATNNGANANN NGNNNGNACN AAANCAAANN
NANNTNNTAA ACAAANNANN AANNNNANAN NNACNANTNA

FileName: 045B.Seq
CloneName: 045
Seq: GGCACGAGCA ATGCCATTTC AATGCACTAC CAAGTGATAC CTGCAGTTCC TAGCTCTTCT
TAGGAAACAA CATTTGTGGC CTTCTCTTAA ATATTTTGCG NTTTTCCTTG CCAGTGTTCG
AGCTTTNCGA TAATCACAGC TTCTGCTCTG TAAGTTACAG ACTATNACAN ATGTGTACCT
AAGTNNNAGG NTATNTCTTG NNTNTAANGA CTAANGAACT GCCAGCTTNC TTGNCNNANA
NGNTAGGTAG NTGANCNTNA TNANTNNGAC NNCTCNTGGT GNAGACCNTN ANACATATNT
GTNAANNNCA ANCNGCTATC TNNNTCNNCA NAGTNATNGN ANANTTATNC AGNAACTNT
GiNum: 19424337
AccessionNum: NM_133618.1
GeneSymbol: Hadhb
Description: hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase

---

FileName: 046A.Seq
CloneName: 046
Seq: GGCACGAGCG GCACGAGCTG CATTTCAGAT ATTTACATTA ATAGTAGCAA AATTATAAAG
TAACTAATTT CCTGGACATG CAGCTGATAC ATATTAGAGG AGGAACTATG TTNTNTTTCG
TTGATCTCAA CCTTTCCTAA TGCTGTGATG TCAACCTTGA GTACAATTCT TCAGGTTTGG
TGACCACCCA ACCATAACAG TATTTTGTTG TCAATATTGT CATTGAACTT GTCACTGGGA
GATTAGTAGA AATCAAAACA NGCATGAAGA TTNTTTTTTT TTTGCCCANA AAGGNTNTTA
ATTTGATTGG GTTNTCANAA GGAAGNTNTT TTGANGATTT ATATNNNANN TGANTGGAAA
AG

FileName: 047A.Seq
CloneName: 047
Seq: CTNNTTTAAA AGGNNCAACN TACTGCCCGG GCCACCATAT AACGGCCGCC ATGTGCTGGA
TTCGGCACGA GGNNGATTCT GGAACAAGGG GATTGGCTGA TTGGAGGAGA TCTTCAAGTC
CTGGACCGGN TTTACTGGAA CGATGGCCTT GACAGTACCG TCTTACCCCT GCGGACTCAA
GCANATTTTN AAGGATATGC TGATGCTGTC TTTGCGTTTC AGTTGCGCAA CCCAGTGCAC
AATGGACACG CTCTGCTAAT GCAGGACACC CACAGCAGCT TCTAGAGAGG GGCTACCGGC
GTNCAGTCCT TCTTCTTCAT TCNNTTGGNG GCTNGACCAA
GiNum: 34860565
AccessionNum: XM_215701.2
GeneSymbol: LOC295443
Description: *R. norvegicus* similar to ATP sulfurylase/kinase

FileName: 048E.Seq
CloneName: 048
Seq: TGGGGGGGTG TACAAAAGAN NGCATCTAGA CNTTGGNGGA GGNGNACAAG CAAGCCTAAG
GACCCCAGCC AGCGCCGGTT NCGGGAGGAN CCATTACCNA CTTGNCGNCA GNGAAGCTAG
GAANNNNGNA CAGAGCATAC NGNNANANAN TAANANGANT AGTACAGNCN NAANAANNNA
AANCTAGGAN CANNNGCNNN NGNNCAANAG CAGNNCNAGG NACGCNNTGN CGANNGACNN
NAAAAGACNA CNTTTANAAN TTTTTTTTTG AGGGGNGGGG GGGGAGAACA NCACCCCNNG
NNACAAGAAA AAGANGANAG GAAAGAAANC CAAGAAAANN GNGGGGGGGG GGGNAAANCN
CACNNNANTT ANTCNAGNAA ANANNNACNN GNAANACAAC AACNAGGNNA ACAACGANNC
NAAAGGAGCN NNGAANNGGN NAAAAANCGA NGCNNAGGAA AGACCCNGNT ANANGANACA
CANGNCANAA NNTNNANNAN
AccessionNum: AF102577.1
Description: Cloning vector pMyr

FileName: 048J.Seq
CloneName: 048
Seq: GGCACGAGGG CTTGAATTAG TTTTATTTTT CTTAACTTTA AATATATATA TGGAAATATA
TATTAAAATG TTCTCTAAGT ATTTTCTGCT TCTTGCAGGT CTCTTTTTAC TAGNTNTTGG
CCACTCTTCC CACCTCATCC CTCTGAAAAC AAACATGTGT TGTCCTTTCT ACCACCCNGA
GCCCGACGAT TAACTTGACT TAGTTCACAG CGAGGATCTC CTGCAGCCCT GCAGAGCCAG
TGTGCAGACG GGATTTGGCC TTGAGGGTCC TGATGGCTTT CTGGTCTTTA ACTGNATGNC
CTGTCTCACA TTCGGNCCCA ACCTNAGGAC TCCTNTGCTT TGANTNCATG GNCTANACAG
AccessionNum: XM_126665.4
Description: hypothetical protein 1110059P07

FileName: 048K.Seq
CloneName: 048
Seq: AAANGGNTTA CAGAANGGAA NAAACNCTCA GAAGCTTNNT GGGGGGGGNA AACAAGGNNN
GAGACNANAG GANNANNAGC GNNNAAGANN ATTTTTGGGG GGGGGGGGGG TTTTAAANCN
TNTTGTGANN NACAAAANAC GANACGNGAN NAAANGACAA NAGGAGGAGN NANNCNNCNN
TTNANGAANN NAAANGCGNN GAAGCACTGN GNACGGNANN ANCANCGCGG NNCAGAAANA
ANANNNGAAN NGGACAAAGA NNNNTAANNA CANNNNAANT GGNNNCAGGC NNNCNNAAGC
NNNGACNGNN ANNNNNAGNG ANGAANTATT NNAGNNNNAN TTTTTTTTTN NGGANNGGGA
TNTTTTTGCG AANNNANNNC CCCNNGGCAN AGAAAACNAG GNANNANAAN NAANAAGNNN
CNNAGNGCGA AAGNAGGGGG GGGGGGGGGN GANNGNCGCN CNACATNTNT NAGNNANNNG
AGGNNCGANN NCAACAGNNG AGANGAANAC AAACGCANAA AGNCAGCTNC CGNNNGAGNA

FileName: 049J.Seq
CloneName: 049
Seq: GGCACGAGCC TTGTTGCCCA CTCCAGGCAC AGAACTTTCC CAGACCTGTG GAGATTGGCA
TTGCAGCTGA ACTTGTGCCT GGACTGCAAA GTCACAGAGA AAGGGGTTCT CTTTTTTTTT
TGGGGNNNTG GGNAANACTC NNTGNGTNGN NNTTAAAAAA AAANTGCNNT NTTTGGNGGG
GGGGGGNNGN NTNAAAAATT TTTACGNNGN TNTNTTCATT NNTCNGTTNN NNTTANTNTT
NNTNCNTTNN NNNCTNATTT NNTTTNNTTN TTTANTATCT NTTTNAANTN NNTGNTTNNT
GiNum: 34862238
AccessionNum: XM_347080.1
GeneSymbol: LOC362822
Description: hypothetical protein XP_347080

FileName: 051F.Seq
CloneName: 051
Seq: GGCACGAGAG TAGATCTCTC CAAAAACCAG ATTCGGAGTA TACCTGACAC AGTCGGGGAG
CTGCAGGCCA TCGAACTCAA TCTCAACCAG AACCAGATAT CTCAGATCTC CGTGNTNATA
TCCTGCTGTC CTCGCCTCAA AGTCCTCCGG CTGGAAGAGA ACTGTCTTGA GCTCAGCATG
CTTCCACAGA GCATCCTCAG TGATTCCCAA ATCTGCCTGC TTGCCGTGGA AGGCAATCTC
TTTGAAATAA AGAAACTTCG AGAACTAGAG GGTTATGATA AGTACATGGA AAGGTTCACA
GCCCCAAGAA GAAATTTGCA TGATGTTCTC CAGGACAATG GGACACTGAC TTGGAACTCT
TTTGG
GiNum: 34856730
AccessionNum: XM_230492
GeneSymbol: LOC311346
Description: RIKEN cDNA 2810002D13

FileName: 051K.Seq
CloneName: 051
Seq: GGCACGAGCC TTTACATTAT CTCTACTAGG TACTTTTATA TTTCGCTACC ACANTAATAT
CTACTNCTNC TNTGCCTAGN AAGGAATAAT ACTATNACTA TTNGTNNTAN TTNNTTTTTT
TTTTANGGNG NGTNGNAACT CCATCCTCNN CATAANCATC CCAANAACCA TTNTAANTTT
NGGGGGGGGC TGNNGANCCC TNNTTTTTTT TNNANCNCNN TTTNAAANNC ACAANNNTAA
NAANNCTTTT NNGCNAAANN NTACATCNNN NNTNNNCTAA CAAANAAACA ANNCNNNATN
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 051N.Seq
CloneName: 051
Seq: GGCACGAGGC ACACCCTNAC CATNACCTCC AAGGGCAAGG ATAATAAACC AAGTTATATC
CACTACCAGC CTGCCCAGGA CGGCAGCAGC CCCACTCCTG GAGATGCTCA TTNTTTTTGC
CGGCCAACTC CGTCACCAAG GTCTCCATCC AGTTTGAACG AGCCCTGCTC AAGTGGACAG
AATACACGCC ACACCCCACC TCGAGCATGC ATCTAGAGGG CCGCATCATG TAATTAGTTA
TGTCACGCTT ACATTCACGC CCTNCCCNCA CATCCGCTCT AACCGAAAAG GANGGAGTTA
GACAACCTGA AGTCTAGGTC CCTATTTATT NNTTTATAGT TATGTTAGTA TTAANAACGT
AccessionNum: AF102577.1
Description: Cloning vector pMyr

FileName: 052I.Seq
CloneName: 052
Seq: GGCACGAGCG CGGCTCNAGT ACACTNAANG ACGCTTTACN CAAANCANNN CCATGTANAN
ANACTNANAA ANGNNGNNAC GNNTNNNNNC NGNNNTANCA TNCNTNNNNA NNNAANGTNT
TTTNTTTTTT TTTTTTTANN ANANATTNGN NGNNAGNNNN NCNCNTANAA CAACAANTNN
CTTTCNANAG ANNCTNCACN TTNTANGGNA GTGTGNGNAN ANNCNANCTA ANTTTNTGNA
GANGNNACNG CNCTNGTNAC NATNANNTNN GNATNCTAAG ATNNNTNTNN CTCANATTNA
NATTGGNCNA NTACNNNAAN TAGTNNGNAC NCNCNGNNNN GNNNNNANNA TNAAANACAA
CAGNANNGNA CGTNANCNAC GCCGNANATC TNTGAANNCN NNACTACGNG ATTNNCNCCG
TNCNNNNNNT TNCNTNNCNA NANACNAGCT ANCNCTAGTN TTACNNNCTA CNNNNANCNA
ANTNTTNNNN NAATNNTCAN TCTGTCATTN NNAANNCANA NNTAATGTGC NCACNTAACT
GNAACNAC

FileName: 053B.Seq
CloneName: 053
Seq: GGCACGAGGG AAACCCTTGG CTTCTCTCGG TCTCGTGCTG TGCGGGAAGG ACCGTGTGTG
CACACGCGGG TCTCCTCGTT CTGGTGTCCT GCTGTTCCCC CCCTCTGGGT GTTTTTNGGC
TTNTCTCTGT CCGTACTCTG ATGTTGGGTA GACTCGCCGG CTTTCCAGGA TGCAGGTTGT
GGAACTTGTG CGCTAAAAAC TACAGGCGAG AGGAAGCTGT GATGTANAAG TCNTAAGTAC
TGCACTACCG GAAGCCGAAG CCAAAACCNC NATNACCAAA ACCGTGNNCA GTATACCNNC
TTCAAAGTNG GTTNCTNACA TTTTCTTCAA NATGAANNGT NACNGGNTAG TTCNTNGANN
ACCTTTACGN AANANNTTGG TNCCTNNACT TACNTTACTA CACNANTATT NCNCNTTNAG
NGNTNNCNAN NACNNTCNAA NTTAAAGGNA NNANTT
GiNum: 34868020
AccessionNum: XM_221564.2
GeneSymbol: LOC304012
Description: Rattus norvegicus similar to hypoth. protein D16Ertd454e

---

FileName: 053G.Seq
CloneName: 053
Seq: GGCACGAGGC CTTATAATTA ATTGGAGGTA AGATTACACA TGCAAACATC CATAAACCGG
TGTAAAATCC CTTAAACATT TGNACTAAAA CTTAAGGAGA GGGCATNTNG CNNTTTTTAT
AGNTCANGNA CNCCTTGNCT ANTCCACACT CTCNACNAGG ACTCATNCAG TCGATNGTAN
ATTAANNNNN TGAACCATAN TNTGNCTAAN NCTATACCTN TCAGGGGTGC NTNNCTTNCT
NNNACAANNC CNANNTGNNG AACNNNCTNN NCTTNCNNNT AAGTTANNNC NCTNANNNCN
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 053J.Seq
CloneName: 053
Seq: TAANGNNNCC ACNGCNANTT NTGGGGGAGC NNAAAGAGCA AGCCTAAGGA CCCCAGCCAG
NTGNGGGCCC GGGCAGANCC ACTANNAACA GGCCGCCAGN GTGNTCGGAA TTACGGCNCG
AGGTCNGACN CNNNTTANCT ACACCACACA NGATACNGNG AAACCNNCNA CCNANACAAT
CAGGNAGCCN GAGCANGAAC AGCGANGGAC ANGNNTNANT TTTTTTTTNA GCNNNGAGNN
GGGNCAGNCC CNCANCGNGA NGNAANNNNC AGNNACNACC NNCCNACANN NGGGGGGGNG
CNNCACCNCA NAGCACAATC NCNCNGANCC CACACGANNG AANCCCANCC ANANCAGNCG
AccessionNum: AF102577.1
Description: Cloning vector pMyrCAM

FileName: 054A.Seq
CloneName: 054
Seq: GGCACGAGAA TTAATTGGAG GTAAGATTAC ACATGCAAAC ATCCATAAAC CGGTGTAAAA
TCCCTTAAAC ATTTGCCTAA AACTTAAGGA GAGGNTTTNA AGCACATAAT ATAGCTCAAG
ACGCCTTGCC TAGCCACACC CCCACGGGAC TCAGCAGTGA TAAATATTAA GCAATGAACG
AAAGTTTGAC TAAGCTATAC CTCTCAGGGT TGGTAAATTT CGTGCCAGCC ACCGCGGTCA
TACGATTAAC CCAAACTAAT TATTTTCGGC GTAAAACGTG CCAACTATAA ATCTCATAAT
AGAATTAAAA ATCCACTTAT ATGTGAAAAT TCATTGGTNG GGACCTAAGC CCATTACCGA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 055B.Seq
CloneName: 055
Seq: GGCACGAGAC TCTTTTCAAC TAACCACAAA GATATCGGAA CCCTCTACCT ATTATTTGGA
GCCTGAGCAG GAATAGTAGG GACAGCTTTA AGTATTCTAA TTCGAGCTNN TTTNNGACAG
CCAGNCGCAC TCCTAGGAGA TGACCAAATC TATNATGTCA TCGTCACAGC CCATGCATTC
GTAATAATTT TCTTTATAGT AATACCTATA ATAATTGGAG GCTTCGGGAA CTGACTTGTA
CNACTAATAA TTGGAGCCCC TGATATAGCA TTCCCACGAA TAAATAACAT AAGCTTTTGA
CTGCTTCTNC ATCATTTCTA CTCCTTTTAG CATNCTCCAT ANTANAAAGT GGAAGCTGGA
ACAGGATNAA CAGTTACCCC CCCTTACCTG NAAA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 055E.Seq
CloneName: 055
Seq: TAGGAATCNA CTTCTANTNN TGGGGAGTNN CAAGAGCAAG CCTAAGGACC CCAGCCAGNG
CCGGCCCGGG AGATCCACTA GTAACGGCCG CCAGTGTGCT GGAANTCGGC ACGAGCGGAA
CTTAAGCGCA AATTTAGAGC AGATGAAGCA AGANAGGATC ANCAGTATAT NANGGTGNAT
TANTNANATG TTGNNNNNTN TTTTTTTTTT TTTTTTNNNN NAGGTNGNTG GGGGGANACC
CTGNNNANNT NANACNATTN NTTNTNANAG TNNCNATNNT TGGGGGGGGG GGGGGGNGNC
CTNCNTTTTN TNAANNCAGG NNCNNANNAA NNATNTACNA CTTTTNNTTT ANTAAACGNN
GGNNANTTGA ATANTNAAAN ATTGANTTAN ANNNTNANTN ATTGAAANNG TNNNNTGNTT
AccessionNum: NM_008774.2
GeneSymbol: Pabpc1
Description: poly A binding protein, cytoplasmic 1

FileName: 056A.Seq
CloneName: 056
Seq: GGCACGAGCA TAACTAACCC CCCCCCCCNC ATAAACTAAA ACATTTAANT CAAAAAGTAT
TGGAGTAAAG AAATTTACTT ACCAGGAGCT ATNAGNAGGA ANGTANTNGN ATGNNTTTTG
GTAGATAAGN ANTNNTTTAT AANCCCAANN TAANGCAACA GTATTANNAN CTTGTACCCT
TNNTGCATAG NNGAGATNAN CTATGAAAAT NCTTAANACN ACGANATNTT CANCTNNCNA
ACNACGAANA ACAAACGAAG GTNNCTANNA NANANNTNCA TGAANGANCC CNATNNATGT
AGCNNNAAAN ANNCGGGNNG ANNNTTANGT GNNNGNGNAA ANGNCCTATC ANNNTTCGNN
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 056D.Seq
CloneName: 056
Seq: GGCACGAGCT CCGGATCTCT CTCTTCTTCA GGCTCTCGCG TGCCGGACCA GGGATCCTGC
AAGCAAGGAA GCAGCCCCGG GGTGACACCC AGCACGTACG TNTGTGNTTT NNNAAAGCCA
AGNGGGGGAC GCTTCGCGGA GGAGTNGNGG NAGGGTNCAG NTNCCTGTGG NNGNANCGAN
CCCCGTTNCT GNNCNNANCA CNGNNGAGGG ACANTTGAAA AATNNAGACC CTAATACCAC
NCATCTGANT GNATATATAC ACGCNTNACN ANGCANCCTN CTGTAACGNN AGGAGANAGA
TCCGAATNAC TAGTNACGCT GCGNACNANN NNCNAGNNGA TANNTGCNAG NAG
AccessionNum: AF110132.1
GeneSymbol: GAD67
Description: glutamic acid decarboxylase

FileName: 057J.Seq
CloneName: 057
Seq: GGCACGACAC GTTTCGTGCG TGCTCATTCT GCCAAGATGC CTGAGGAAAC CCAGACCCAA
GACCAACCAA TGGAGGAAGA GGAGGTCGAN TTTTTTTTTT TTNAGGCAGA NNNNGGGNCA
GNTCCATGTC CTTGATCATC AACANTTTCT ACTCGAANGG GGGAGATCTT TCTGANNTAG
CTCATTTANC ACTNTTANGA CNCTCTGGAT AAGATNAGAT ACGANNGCTG NCNAANCNTA
GNAAANTGNA NTTNGANAAT NACTGNANAT NANAAATNTT NCNTNCNGNA ATNNNNNAAC
TTTCTTATNG GTGTNANANN GANAATTNAA GAAANNAGAA ATATNNGTNA
AccessionNum: NM_175761.2
GeneSymbol: Hspca
Description: heat shock protein 1 alpha

FileName: 058B.Seq
CloneName: 058
Seq: GGCACGAGGC ACGCCGGTAG GATCTGCCTC TAGGAATGCT GGGATCATAC ATGTTCACTG
CAATGCCCAT TTCCCATGGG AGTTTTGGCA TTTTTTACAT TTTACCTTTC CTTTGTATAC
ATCTAAGGCT GGCCTCAGAC GCAAGACGTT CTTCCACCCT ATACACCCTT TTAATCTCAC
TGTGTGTGGG AGGGGGGTCG TTTGCACACG ACGCACGGTG GATGTCTGGT GTGCTGTTGG
CTGNAGCCCC TGTGGCTTAT ACAANTGNGA NCGTATGGAG GTACGAANGG TTCNNANAAC
ANNNACCCTG CTGNTGGCTA CGGTNCNNGC NAANNCTCNG TNATNCNCTN NTA
AccessionNum: NM_031594.1
GeneSymbol: P2rx4
Description: purinergic receptor P2X, ligand-gated ion channel, 4

FileName: 060C.Seq
CloneName: 060
Seq: GGCACGAGGT AAACCCAAGC CCATGACCAC TAACAGGAGC CCTATCAGCT CTTCTACTCA
CATCCGGCTT AGTAATATGA TTCCATTACA ACTTTTTTTT TCTCCTATCA TTAGGCCTCN
TGGACAAACA TCCTAACTAT ATATCAATGA TGACGAGATA TCATCCCGTG AAGGAACATA
CCAGGCCACC ACCCCCTANT TGNACAAAAA ANGNCTACGA NTCGGGAATA ATTNCNNGTT
ANTTGGCTTC NNAAGGTATT ANNTCNTTTG GNGGGGATAT TTNNTNAANC ANNTTNAANN
ATTCNAACCT ANNTTCCTAA CCCANGGAAC TAAGNCGGNT AGNTTAACCC CCNAACAAGA
AATTNCNCCN TTTAAANGCA TNAAAAAG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 069D.Seq
CloneName: 069
Seq: GGCACGAGAA TTGGAGCCCC TGATATAGCA TTCCCACGAA TAAATAACAT AAGCTTTTGA
CTGCTTCCTC CATCATTTCT ACTCCTTTTA GCATCCTCCA TAGTAGAAGC TGGAGCTGGA
ACAGGATNAA CAGTATACCC CCCCTTAGCC GGAAACCTAG CCCATGCTGG AGCATCCGTA
GATTAACTAT TTTTTCCCTC CACTAGCCGG GGTGGCTTCT ATCTTAGGGA GCTATCAACT
TTATCNCCCC TATTNNTAAT ATTAAACCCC CTGGTANNAC CCCAANTANA ACNCNNGTNN
TTGGANGAAC CCGACNTAAT ANCNGCCGNC NAANACTNNN NNAANTGCNG NNTTNCNNNN
GGNNNACTAN ACNCCTANNN ACCGAANNNN AANCNNNNNN NTTNNACCCC
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 070C.Seq
CloneName: 070
Seq: GGCACGAGAA ACGATTTAAT TTTCGTAAGT AAGCATGGTA ATTGGAAAAG CACTTTCCTC
AGAGGTGGAA GGCAAGGGTA CGTGATGCTC CAGAATCTGG AGCCAAAGCA GAGCAATTAC
CAGTTCTAGG CTGGCCTAGG GTTCGAGACG ACGTTCCCAA AATCCAAAGC AGAAAGAAAA
GTAGCGAATA CATTTCTGGT CTTTCTTAGC CTCATTTCAT CTTCTGCCTC ACGTAGCCCA
GGCTGACCTT GAACTTCCAG TGTAGCTAAG GATGACCTTG AACTTCCGAT CCCCCAGACC
CCACCTCCCA ATTCTAGAAT TACAAATGNT CTGCGCCACA CCTGGNTTGN TNGGNTNGNN
NGGCTGG
AccessionNum: AC132325.3
Description: Similar to Mus musculus BAC clone from chromosome 12

FileName: 071D.Seq
CloneName: 071
Seq: GGCACGAGAA ACGATTTAAT TTTCGTAAGT AAGCATGGTA ATTGAAAAGC ACTTTCCTCA
GAGGTGGAAG GCAAGGGTAC GTGATGCTCC AGAATCTGGA GCCAAAGCAG AGCAATTACC
AGTTCTAGGC TGGCCTAGGG TTCGAGACGA CGTTCCCAAA ATCCAAAGCA GAAAGAAAAG
TAGCGAATAC ATTTCTGTCT TTCTTAGCCT CATTTCATCT TCTGCCTCAC GTAGCCCAGG
CTGACCTTGA ACTTCCAGTG TAGCTAAGGA TGACCTTGAA CTTCCGATCC CCCAGACCCC
ACCTCCCAAT TCTAGAATTA CAAATGTTCT GCGCCACACC TGGTTTGTTT GGTTTGTTTG
GCTGGNTTTA ATTGNTGTNT CATTTAATNN AATTNTC
AccessionNum: AC132325.3
Description: similar to Mus Muluscus BAC clone

FileName: 072A.Seq
CloneName: 072
Seq: GGCACGAGGT AAACCCAAGC CCATGACCAC TAACAGGAGC CCTATCAGCT CTTCTACTCA
CATCCGGCTT AGTAATATGA TTCCATTACA ACTCCACAAT TCTCCTATCA TTAGGCCTCC
TGACAAACAT CCTAACTATA TATCAATGAT GACGAGATAT CATCCGTGAA GGAACATACC
AAGGCCACCA CACCCCTATT GTACAAAAAG GCCTCCGATA CGGAATAATC CTGTTTATTG
TCTCCGAAGT ATTCTTCTTT GCCGGATTTT TCTGAGCATT TTATCATTCC AGCCTAGTTC
CTACCCACGA CCTAGGCGGT TGCTGACCCC CAACAGGAAT TACCCCTTTA AATCCCCTAG
AAGTACCCCT TCTAAATACA TCAGNCCTCT C
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 073A.Seq
CloneName: 073
Seq: GGCACGAGCT TCCAGTAACT CGCCAAAATG ACGAACACAA AAGGAAAGAG GAGGGGTACT
CGGTATATGT TCTCTAGACC TTTTAGGAAA CATGGNAGTC GTTCCTTTGG CCACATACAT
GCGAATCTAC AAGAAGGGTG ATATTGTAGA CATCAAGGGA ATGGGCACTG TTCAAAAAGG
AATGCCCCAT AAGTGTTACC ATGGCAAAAC CGCTCGAGCA TGCATCTAGA GGGCCGCATC
ATGTAATTAG TTATGTCACG CTTACATTCA CGCCCTCCCC CCACATCCGC TCTAACCGAA
AAGGAAGGAG TTAGACAACC TGAAGTCTAG GTCCCTATTT ATTNTTTTAT AGTTATGTTA
GTATTAAGAA CGTTATTTAT ATTTCAAATT TTTCC
AccessionNum: XM_213156.1
GeneSymbol: LOC301582
Description: 60S ribosomal protein L21

FileName: 074A.Seq
CloneName: 074
Seq: GGCACGAGGA CATACCGTCG TAGGCTTTCC TACAATACAG CCTCTAACAA AACTAGGCTG
TCTCGAACCC CTGGCAACAG GATCGTTTAC CTTTACACCA AGAAGGTCGG GAAAGCACCT
AAATCCGCAT GTGGCTGTGT GCCCAGGCAG ACTGCGAGGG GTCCGTGCTG TGAGACCCAA
AGTCCTCATG AGATTGTCCA AGACAAAGAA GCACGTCAGC AGGGCCTATG GTGGCTCCAT
GTGCGCCAAG TGTGTCCGGG ACAGGATCAA GCGGGCTTTC CTTATCGAGG AGCAGAAAAT
CGTTGTGAAA GTGTTGAAGG CACAAGCACA GAGTCAGAAA GCAAAATAAA TGGGCAACCT
CGAGCATGCA TCTAGAGGGC CGCATCATGT AATTAG
AccessionNum: XM_225596.1
GeneSymbol: LOC307135
Description: ribosomal protein L34; 60S ribosomal protein L34

FileName: 075A.Seq
CloneName: 075
Seq: GGCACGAGCC ACATGATTTA TTACAATCAT CTCCTCAATA GCCACACTAT TTATTTTATT
TCAATTAAAA ATTTCTTCCC AAACCTTTCC TGCACCTCCC TCACCCAAAA CTATAGCTAC
AGAAAAAACG AATAACCCTT GAGAATCAAA ATGAACGAAA ATCTATTTGC CTCTTTCATT
ACCCCCACAA TAATAGGTCT ACCAATTGTT GTAACCATTA TTATGTTCCC ATCAATTCTA
TTCCCATCAT CAAAACGCCT AATCAGCAAC CGACTACACT CATTTCAACA CTGACTAATC
AAACTTATCA TCAAACAAAT AATGTTAATC CACACACCAA AAGGACGAAC CTGAGCCCTA
ATAATTGTAT CCCTAATTAT ATTTATTGGG CTCAACCC
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 076C.Seq
CloneName: 076
Seq: GGCACGAGCG GCACGAGGCT GCTGGGAGGC GGAAGCAGCC GCAGGTATGG CGGCTGCCAT
GCCGCTGGGT TTATCGTTGC TGTTGCTGGT GCTAGTGGGG CAGGGCTGCT GTGGCCGCGT
GNNTTTTTTN CGCGACAGCC TGCGATAGGA ACTCGTTATC ACTCCGNTGC CTTCCGGCNA
CNTGGNCNNN ACATTCCACC CCCNNANCNT TNGGAATTCN ATNTT
GiNum: 34860688
AccessionNum: XM_215919.2
GeneSymbol: LOC296360
Description: NEURONAL DEVELOPMENT-ASSOCIATED PROTEIN

FileName: 077D.Seq
CloneName: 077
Seq: GGCACGAGAT TAATTGGAGG TAAGATTACA CATGCAAACA TCCATAAACC GGTGTAAAAT
CCCTTAAACA TTTGCCTAAA ACTTAAGGAG AGGGCATCAA GCTCTTAATA TAGCTCAAGT
ACGCCTTGCC TAGCCACACC CCCACGGGAC TCAGCAGTGA TAAATATTAA GCAATGAACG
AAAGTTTGAC TAAAGCTATA CCTCTCAGGG TTGGTAAATT TCGTGCCAGC CACCGCGGTC
ATACGATTAA CCCAAACTAA TTATTTTCGG CGTAAAACGT GCCAACTATA AATCTCATAA
TAGAATTAAA ATCCAACTTA TATGTGAAAA TTCATTGTTA GGACCTAA
AccessionNum: J01438.1
GeneSymbol: 12S rRNA
Description: 12S ribosomal RNA gene

FileName: 078D.Seq
CloneName: 078
Seq: GGCACGAGGG ACAAGCTTCA TTTATACCAT CTCTGGAGAT GTGTATATTG GGCCTGATGA
TGCTCAACAA AAGATAGAAC CGCATCGCAC AGCAGTGCTT GGAGAAGGTG ACACTGTCCA
GTTGGAAAAT AAGGATCCCA AAAGAAGTCA TTTTGTCTTA ATTGCCGGGG AGCCATTAAG
AGAACCAGTT GTCCAACATG GTCCGTTTGT GATGAATACC AATGAGGAGA TTTCTGAGGC
CATTCTTGAT TTCAGGAATG CAAAAAATGG TTTTGAGGGG GCCAAAACCT GNAAGTCANA
GATTGGAAAC CAATGATGGG CTGAAATGCT GNCATTNGAT GANCTGACCA
AccessionNum: AK009757.1
GeneSymbol: pir
Description: pirin

FileName: 079A.Seq
CloneName: 079
Seq: TTTNGNGGCA CANNGNNNTT TTTGGGGGGG CACAGNGTNN AAGNNTANGG NAAAACAGGN
CTTTNTGGGG GGNCGGGAAN ACCCNNGNGC GGNGAGAGCG CGNANANANG GANAANNGNA
NGANNNNCGN NGTGANCCAN NNANGCNGCC GNNNGGACGN NGGGNNGNAG GCGGANANAA
GGNANNNNCA NNNANANCNC ACNNNNGNAN NGNCNNCNGN ANNAGCNAGA ACGNCNCGGC
GANANAGNNA GNNANTAGAG CGNCNATTTT TTNNNAGAGN GCCGCGNGNN NTAGNGGACN
ACNCCCCTNN ANANCGGNGA GNANNCGANN NGNNGGAAAA NAGGAACGNA GGAGGNANGN
NNANCGANGN GCGNCAACNC NANNNGNCNA CNATCNGGNN GACNNNCNNN NCACCACGGG
GAGGAGNGCN NTNNGGNGNG NAAGNCNTNN GNCGNNNGGN AAGNCNANTC AGCNCCGNNA
CTNNNNGGNG CAGGACCNNG NCNANGNGGC ACACAAGCAA NNNAGCACGN NNGNACNCNN
NCNCNGCGCN NNCNNCCNCN AANCGANGGG NNAGTGGCNC AANCCCANGN NCGANTNNNC
GANANAGTGN CGACNNGNNC NGACACGNCG ANNCNCNGCA NGGNGNNCAT

FileName: 080A.Seq
CloneName: 080
Seq: GGCACGAGGT AAACCCAAGC CCATGACCAC TAACAGGAGC CCTATCAGCT CTTCTACTCA
CATCCGGCTT AGTAATATGA TTCCATTACA ACTCCACAAT TCTCCTATCA TTAGGCCTCC
TGACAAACAT CCTAACTATA TATCAATGAT GACGAGATAT CATCCGTGAA GGAACATACC
AAGGCCACCA CACCCCTATT GTACAAAAAG GCCTCCGATA CGGAATAATC CTGTTTATTG
TCTCCGAAGT ATTCTTCTTT GCCGGATTTT TCTGAGCATT TTATCATTCC AGCCTAGTTC
CTACCCACGA CCTAGGCGGT TGCTGACCCC CCAACTCGAG CATGCATCTA GAGGGCCGCA
TCATGTAATT AGNTATGTCA CGCTTACATT C
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 081A.Seq
CloneName: 081
Seq: GGCACGAGTA TTTACTTTAC TATCCTCATA GGGCCTGTAA TCACTATATC TAGCTCCAAC
TTACTCCTAA TATGAGTAGG ANTGGAAATA AGNTTTTTAG CTATCATCNN ACTTCTAGCC
AACAAAAAAA GCCCACGATC AACTGAAGCA GCAACAAAAT ATTNTCTAAC CCAAGCTACA
GCCTCAATAA TNATCCTACT AGTCATCATC CTCAACTACA AAACAATCCA GGAATATGAA
CCCTCCANCA ACAANCCAAT AACNTACTAC TCAACATAAN ACTCANTTCA CTAGCCTNAA
CACTTGGACN AGCCCCAATC CACTACTGAT ACCNGGAAGA NCNAGCAAGG AATAC
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 081B.Seq
CloneName: 081
Seq: GGNACGAGTA TTTACTNTAC TATCCTCCAT AGGGCCTGTA ATCACTATAT CTAGCTCCAA
CTTACTACTA ATATGAGTAG GATNGGAAAT ANGNTTTTTT AGNNATCATC CCACTNGNAG
CCANCAAANN AAGCCCACGA TCAACTGNNG CAGCANCCTN NNATAGCCNA ACCCAAGCTA
CNGCCNGAAN NNANTATGNT ACNANGACAT CANNCCTCNA ANTACNAACA NTCANGAANN
NGANGCCCTN NNACAAGNAN AACANAANCA TTACTACCTC AACATANTCA CTCATNACCA
GTANNCAANA GAACNNGGGG CTNGCNCCAA NTNGGAANAN NGANTANCNG AANACACCCC
NAGGGNNNAN CCGCANN
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 081C.Seq
CloneName: 081
Seq: GGCACGAGTA TTTACTTTAC TATCCTCATA GGGCCTGTAA TCACTATATC TAGCTCCAAC
TTACTCCTAA TATGAGTAGG ATTGGAAATA AGCCTTTTAG CTATCATCCC ACTTCTAGCC
AACAAAAAAA GCCCACGATC AACTGAAGCA GCAACAAAAT ATTTTCTAAC CCAAGCTACA
GCCTCAATAA TTATCCTACT AGTCATCATC CTCAACTACA AACAATCAGG AATATGAACC
CTCCAACAAC AAACCAATAA CATACTACTC AACATAATAC TCATTTCACT AGCCATAAAA
CTTGGACTAG CCCCATTCCA CTACTGACTA CCCGAAGTCA CCCAAGGAAT TCCC
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 082B.Seq
CloneName: 082
Seq: GGCACGAGCT GTTACAAAAT ATAGTTTATG TAGCTTTGTA ACATTCCTCA GTGCCTGTCC
ATACCCGGGA AGTATAACGA GTGCACTTAG GGCCAGATGC ACTNTAAACA CTGCAGGGTT
AAATACAGCA GGAGTCTTTA GGAAAGTCAT TTGAATTGGA GTTTTAGGTT TTAGAATAGA
GCTGACAAAA ATCATATAAA TATATTTTTT GTAATATGAG CCAGAATTCT TCTTTTGACA
ATTTAAGGTT TTTTCCATAG AGCTTATTTA TACCAACTTT TTTTCCTTTT AAACGTGTCA
GCACTGTANT GTAATAGCTC TACAAAACCT TCTTAGTGCG ATTATATTGA GA
AccessionNum: AC130218.4
Description: Mus musculus BAC clone

FileName: 083A.Seq
CloneName: 083
Seq: TANNATANNC TCGCTTTTGC CTTGGANATC TCTAGTAGCG NCCGCCNGTG TGCTGGANTT
CGGCACGAGA GATGATAGGG ATTTGTAAAA TGGAAGAAAA TAATCATGTC TTTTTAAACA
GTTGTATCAT CCTATAATTA ACTATTGATT GATTATTGGA TCTCTTAATG AGAATGATTA
TTTCTTTTTT TTNCCTGNNN NNTNTTTTTT TCACNNCNCN CTNCNNTTNT CCTT
AccessionNum: AC121932.2
Description: Mus musculus BAC clone

FileName: 084A.Seq
CloneName: 084
Seq: CATGCATCTA GAGGGCCGCA TCATGTAATT AGTTATGTCA CGCTTACATT CACGCCCTCC
CCCCACATCC GCTCTAACCG AAAAGGNAAG GAGTTAGACA ACCTGAAGTC TAGGTCCCTA
TTTATTTTTT TATAGTTATG TTAGTATTAA GAACGTTATT TATATTTCAA ATTTTTCTTT
TTTTTCTGTA CAGACGCGTG TACGCATGTA ACATTATACT GAAAACCTTG CTTGAGAAGG
TTTTGGGACG CTCGAAGGCT TTAATTTGCA AGCTGCGGCC CTGCATTAAT GAATCGGCCA
ACGCGCGGNG AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTNCTCGN TCACTGACTC
NNTGNNCTCG G
AccessionNum: AF102577
Description: pMyrCAM Cloning vector

FileName: 085B.Seq
CloneName: 085
Seq: GGCACNCAGG NNNNTNNGNN CANCATACTN TANNANNATA NACCTANANA ANNANCCCTN
NANTNCNNNA ACCNNTNGCN NCCCCCCANC ATNTNANNTN AANNNACNNN TNGNCCCAAA
CNCAAACACN ANANANNTCN CNCNACNNNC NCANNNNTTT TTTTTTTCC NCCCCCCNCN
CNNNNTTTTT TTGNNCGNAN CTACACAANA ANNNACNANC TTCNTNAACC CAANCNCCCA
NNNTTTCNCA NNCCAANANA ACTCCGCACN ACNCTTTNTT TNCCNNCCAA NANGACNACC
CCNCCAAAGC CANCGCNANC NCANGACCTN ATCANACGCN CCANACTCNT ACNCCTCATN
CNACNCNAAC GNACANCANN NANACNNCAC CNCAGNAGNN NCNNNNTNTN NACNCCAANN
ACNCNCTCNN NNCACNNCCN GCACCCACCA CNCCGTANCC CCAACCNCAC ANGNANNNCN
NACCNCAGNC CCNCNNACGC NCCAATCNNC ACNNAAACCA NACGNNTCAC ANCNNNAAAN
CNNAANCACN NCACANTNCT ACCNTCNNNN CNATCNACAC NACACCCACN NNTACNNCCG

FileName: 086A.Seq
CloneName: 086
Seq: GGCACGAGCA GACTTGGATT CTCGAGATCT GGATCCCACG TCTTCAAGTT TCGACCTGGA
TCCTGATGTG ATTGGCCCGG TGCCACTAGT TCTCGACCCA NNNTTATGAC ACCCTCAGCC
CTGCTGCTCC ACATGTGGAT TCCCTTCCCT CTAGCCTCAC TGCCACCCCC GAAATCTTGG
CCACCAGCTC AGCGGTGGTG CTTCCTGCCC CTGCCAGTNC CCTCGNCCCT TCTCCTGTCC
TGATTGCNGG CGAGCCTTTC GCCGAGCTCT GGGCTGAGCC AGCACCTACN CACGCACANC
GGAGAGAAGC CTTACCGCTG ACCTGACTGT GGCAAGGCAN TTCANNCA
GiNum: 34870077
AccessionNum: XM_341026.1
GeneSymbol: LOC360754
Description: similar to zinc finger protein ZFEND

FileName: 087C.Seq
CloneName: 087
Seq: TTGNTCNCTG GCNCCNTCNC GTTCGGCCCG GGCGGTCCAT AGTAACGGCC GCCAGTGTGC
TNGGNATTCG GCACGAGCGG CACGAGCGGG ACGCCGGGCT CGGGGGCGGG GGCGGGGGGC
GCGGGCACNG GA

FileName: 088A.Seq
CloneName: 088
Seq: GGCACGAGCT GAAGGCAAGN TGGGTCACCA GCAGCTCTAC TGGAGTCACC CGCGGTAAGT
TCGGCCAGGG TTCTCGCTCT TGCCGCGTCT GCTCTAACCG CCACGGTCTG ATCCGTAAAT
ACGGGCTGAA CATGTGCCGA CAGTGCTTCC GTCAGTACGC GAAGGACATA GGCTTCATTA
AGTTGGACTA AGCGACCTGA ATGGATGATT CGACTGTCTA CCCAATGAAA CCAACTGTCT
ACCCTGTGAT ACCAATCATG CTAGTCTTTG TGCACACAGA ATANAAAACT GAAGGCCTCA
AACTCNAGCA TGCATCTAGA GGGCCGCATC ATGTAATTAG TTATGTNACG CTTACTTNAC
NCNCTNCCNN CACATNCGTN TAACCNAANN GGAANGANTT NNACNCCTNA NNNTANGNCN
TATTNNTTC
GiNum: 6981489
AccessionNum: NM_012876.1
GeneSymbol: Rps29
Description: Ribosomal protein S29

FileName: 089D.Seq
CloneName: 089
Seq: TTTNGGGNAN NGGTATTTTG GGGGGCCACG AGCGNGCCAA GGACCCCAGC CTTTCCGGNC
NGGANGACNA CTAGTAACGG GCGACAGNGC GCTGGAATNG GGCACGAGAA AACNATATAN
CAGNNTGAAC TNACTCGCTA ANATGANTAN GANACGGAAA TANGNCANNA NAGCGATNAN
NGCCACGCNN CAGNCNTTTT TTTTGNCCAN NGANNATGGN GGAAGCNGCA GNNGAANAAN
TANNNNAACA NAAAGCNNCN GCCAANNANN ATCCNNCCNN AGNAGNAANC CANCNCGNNA
CNNCCAANTN CCNANNGANN ANNGANACGC CAANNCCCAG NNGAACAANC ANNATNNCNA
GGNNNCANAN GCCNCAGNCN NGNAAGNCAN ANNACCCAGG ACTCGTNANC AAGNNAANNN

FileName: 090B.Seq
CloneName: 090
Seq: GGCACGAGGT CCCCGCGTGT GTGCGCCTTA TCTCAGCTGG TCTGCCCGAG ACCCCCTGAG
CGTGAACCTT AGTCCCCCGC GCGGCCCCAT TTCCACTCCG ACAAGATGAA AGAAACGATC
ATGAACCAGG AAAAACTCGC CAAACTGCAG GCACAAGTGC GCATTGGTGG GAAAGGAACT
GCTCGTAGAA AGAAGAAGGT GGTTCACAGA ACAGCCACAG CAGACGATAA AAAACTGCAG
TTCTCCTTAA AGAAGTTAGG GGTAAACAAT ATCTCTGGTA TTGAAGAGGT GAACATGTTT
ACAAACCAAG GAACAGTGAT CCATTTTAAC AACCCTAAAG TTCANGCGTN TTTGGCANNA
ACACCTTTNA CCATTACAGG NCANNCCNAG ACAANCANNT
GiNum: 27686914
AccessionNum: XM_215460.1
GeneSymbol: LOC294680
Description: basic transcription factor 3

FileName: 091M.Seq
CloneName: 091
Seq: GGCACGAGCT GAAGGCAAGA TGGGTCACCA GCAGCTCTAC TGGAGTCACC CGCGGAAGTT
CGGCCAGGGT TCTCGCTCTT GCCGCGTCTG CTCTAACCGC CNTTNTCTGA TCCGTNTTTT
NGGGCTGAAC ATGTGCCGAC AGTGCTTCCG TCAGTACGCG AAGGACATAG GCTTCATTAA
GTTGGACTAA GCGACCTGAA TGGATGATTC GACTGTCTAC CCAATGAAAC CAACTGTCTA
CCCTGTGATA CCAATCATGC TAGTCTTTGT GCACACAGAA TAAAAAACTG AAGGCCTCAA
AccessionNum: NM_012876.1
GeneSymbol: Rps29
Description: Ribosomal protein S29

FileName: 092B.Seq
CloneName: 092
Seq: GGCACGAGGC CCACAGGTGT ATTGTGCGGG CACTGAAGGA TCCAAATGCC TTTNTTTTTN
GACCATCTTC TTACTCTGAA GCCAGNCAAA TTTTTTTTTN NGCGAGCTTA TNCATGATNT
ATATGACCAT TTTCTGNGAG TGCTAAATCG GCANCATATG ACANGTNCTA TCANAATAAC
ANAGACTNNC ATAGATNCAC TTGGCTNGCC ACATGGAGCN AAANATGGAG AAANATGAGA
CTCCNCACCN GTAAGGGGAA TGGCAGAGGA ANACCNNGAG ACNTNTTTNG ATACANTGCA
NCAAAGAGCN ACAGATAGNA GCTGATGAAG ANGAANATNN AGCAGNAGAN
GiNum: 34856620
AccessionNum: XM_215794.2
GeneSymbol: LOC295975
Description: dendritic cell protein GA17

FileName: 093A.Seq
CloneName: 093
Seq: GGCACGAGGT GTGGCAGTAG GGTCGGAGTC GGGTGGAGCT AGTCAGGCAT GATCACGGAC
GTGCAGCTCG CCATCTTCGC CAACATGCTG GGCGTGTCGC TTTTCTTGCT TGTGGTCCTC
TATCACTACG TGGCAGTAAA TAACCCCAAG AAGCAGGAAT GAAAGTGGCG TTTTCTCCGC
CCCAGGCTTC CAGGACATAG TCTGAGGCAA GATGGAGGGT GTGAGGGGCC TTCACACTTC
ACTTCATCTC TCCTACCCAT CACANCATAC AAANCAACTA CACCTNNATT TTTCCAAACA
ACTTTTATTT NCTCAAANNN TTNCTTAACC TATNNAACAA NAANCTNNCC TNAAATANNN
ACANNATANN NNCTNCTTAT TTTCNANTNC TCNCCNTCNA ACANNCATNN AANAA
AccessionNum: AK003569.1
Description: P11F3 homolog ?

FileName: 094D.Seq
CloneName: 094
Seq: GGCACTGAGA TTTCACAGCC CAAGCAAAGN CACACTNNAG NAAACTCAAT CTNGAGGGTC
GGNNAATGNG CAGACCTCAC AGTACTGGCC GNCCGAGNTC CCTTTTTTTN AANGAAGTCT
AGGNGNGANA CACCCACTNG GGCAACTTNA NTATNNAACC ANNGGCTCNN NNNNNCCTNA
TNANTNTNTT AANCCTGANG TGNNAGACNG CCNCNACNCG TAAATCCANN GGACANTAGA
NNNNANAACN ATANNGANGA AGGANNCCNC TGNGGAGANA ACTGGGGAAN CNNCNCGNNN
CATGAAGNGA GCNACANCGC NTNNACCNTN AGNANNGNNA NANNCNCGAN ACGANGNNTG
NGGCGNANAA NAACNCGAAN CNNTAGTCNA CNCGNNAAGC NACNCNTGGG CAAACANNTC
GC

FileName: 095C.Seq
CloneName: 095
Seq: TTNNGGGGCC AGGNNGTTTT GGGGGGCCAA GAGCAAGCCT AAGGACCNCA GCCTTGCCGG
CCCGGGAGAC CCNCTAAGAA CGGACGNCAG ATGAGCNNGG GNTNGNGGCA CGAGGGCNTA
CAAGACGCCA GGANAACCGA TTCATGGGAN GANACNTCAC GNNACCCNCC AAGACCNCNC
NNCTAGGGAA GNNAGAAGCN NTNNATTCGN GNATTTTTTT TNTNANCGTG AAAGANNCNN
NGNGNCTNNA ANNCCNCNAC ANNCNNAANA CGNAACNANN AGCGGAGAGN CANCANAGNN
AGACGCNCAC NGCAAAGNAN ANNNCNACNG NNGGGCCANA ANNCTNACCC AGCTNGGACN
NCNACGNAGG CTNNTNANNG AGCNCCCNNN CNNTGNNAAG NCNGNNGGNC AANANACNNG
GAGGTAGGNN GCNAGGANNN ANCNNNCCCA ANGCCACNTA ACAGCGNANN NACNNTNNNG
AANANNNNAC NNGNANACNC GNAANNNCCN AACGNACCCC GGNCACTGAA AGACCCTATA
GCTNAGGGCC CGNNNACCAN TCCAANNCGA AAGGCNNNAC AG

FileName: 096A.Seq
CloneName: 096
Seq: GGCACGAGCC ATGACCACTA ACAGGAGCCC TATCAGCTCT TCTACTCACA TCCGGCTTAG
TAATATGATT CCATTACAAC TCCACAATTC TTNTTTTTTT AGGCCTCCTG ACAAACATCC
TAACTATATA TCAATGATGA CGAGATATCA TCCGTGAAGG AACATACCAN GGCCACCACA
CCCCTATTGT ACAAAAAGGC CTCCGATACG GAATANTCCT GTTTATTGTC TCCGAAGTAT
NCTTCTTTGC CGGATTTTTC TGAGCATTCT ATCATTCCAG CCTAGNTCCT ACCCACGACC
TAGGCGGATG NTGACCCCCC ANCAGGAAAT TACACCTTTA AATCCNCTAN GAAGGACCCC
TTTNCTAAAN ACATCA
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 097B.Seq
CloneName: 097
Seq: ACAAGNAGNA GGGGGCGANA GGGAGGGNAA NNNCAGNGGA NNNGNANNAG GGNGTTTTGG
GGGGNNAACG NGGNNTTTAG GGGGGGNANN AAGCNNCAAG CCNAAGGAAC CCCAACCAGN
TGCCGGCCCG GGNAGGACCC ACTGAGNNAN CGGCCGNCAG NCGANGCAGN CAAAACGGCA
CGAAGCAGAA ACATGCNANA GNANNACAGA CCCAGAAGGC GACAACAANC ACNGGCAACC
CAAANNCACG GACAAAAAGC AAACCCCAGC CCGAGAGAGG ANNNNNNTAC GAAGAAAANG
NAAAGAGACG GAGACAAGAA GGAAAAANGA NANCAACAGN ACAGAAACGG ANCGCAGCNN
CCCCCAAANN GAAGACCAAA CCAGCNANGN NANGANCANN GCANCNNANA GANANGAACC
GANAANAGNG GCNAAGCNCG GACGACCACA NCCGGGNAAC ACCNAAAGNA CGNNNGCNNN
AANGNACAGN ACGGNACACA ANACNGANAA AACANACNAN NNACCNACNN CCANACGNAG
NAAGNGGAGA AAANGAGGAA NCACAGNNNC NAAGCACACA AGCNNNAANC NNAANGANCC
G

FileName: 098B.Seq
CloneName: 098
Seq: GGCACGAGGG CCAAACCCAG CAGAAAGTGG CCGAGCTGGA GCCTCTGAAA GAGCAGCTCC
GAGGGGTCCA GGAACTTGCA GCCTCAAGCC AACAGAAAGC GGCCCTTCTT GGGGAGGAGT
TNTTTTTTTT NNNANNACTA TTNTTTNTGG NNATANCTNN ATCTATGC
GiNum: 21070933
AccessionNum: NM_139190.1
Description: KIAA1536 protein

FileName: 100A.Seq
CloneName: 100
Seq: GGCACGAGCG TGGTCTCTGG AGCTGATGAC TATACTGTTA AATTATCGGG ATATTCCAAA
CTCCAAAGAA ATTCTGGACA TTCAAAGAAC ATTCTGGACT ATGTGGAGGT GTGGCTGTGC
TAGCAAACTG AACCCAGACC TTTTTGTAAC AGGATCATAT GATCATACCG TGAAGATGTT
TGATGCCCGG ACAAATAAAA ATGTTCTCTG TGTGGAGCAT GGGCAGCCTG TGGAGAGTGT
CCTCCTTTTC CCCTCTGGAG GGCTTCTGGT GTCTGCAGGA GGCCGCTACG TTAAAGTCTG
GGACATGTTA AAAGGCGGGC AGCTGCTTGN TNCTTTGNAA AATCATCACA
GiNum: 34853460
AccessionNum: XM_226713.2
GeneSymbol: LOC310019
Description: similar to hypothetical protein FLJ12787

FileName: 101D.Seq
CloneName: 101
Seq: GGCACGAGAN CGGAACCCTC TACCTATTAT TTGGAGCCTG AGCAGGAATA GTAGGGACAG
CTTTAAGTAT TCTAATTCGA GCTGAACTAG GACAGCCAGG CGCACTCCTA GGAGATGACC
AAATCTATAA TGTCATCGTC ACAGCCCATG CATTCGTAAT AATTTTCTTT ATAGTAATAC
CTATAATAAT TGGAGGCTTC GGGAACTGAC TTGTACCACT AATAATTGGA GCCCCTGATA
TAGCATTCCC ACGAATAAAT AACATAAGCT TTTGACTGCT TCCTCCATCA TTTCTACTCC
TTTTAGCATC CTCCATAGTA GAAGCTGGAG CTGGAACAGG ATGAACAGTA TACCCCCCCT
TAGCCGGNAA CCTGCCCATG GCTGGGAGCA TCCGNAGATT
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 102A.Seq
CloneName: 102
Seq: GGCACGAGAA GGATTTTTGG CTCTAATAAA TGGACCACTG AACAACAGCA AAGCATTTCA
TGGAAATCTG CAGTAATCTA GTAAAAACTC GACGCAGNAG CTGTGGGCTG GTGGAAACGC
CTCTTTACGC TAAAGGAAGA AAAGCCTAAA ATGTACTTCA TGACCATGAT CATTTCTCTT
GCTGCGGTTG CTTGGGTGGG ACAGCAAGTC CACAACCTGC TTCTCACCTA CCTGATTGTG
ACTTTTGTGC TGATGCTTCC TGGATAAACC AACATGGAAT CATCTTGAAG TACATTGGAA
TGGCCAAAGG GGAGATAAAC AAGCTTCTCA AGCNAAAAGA AAANANAAAC
GiNum: 38454217
AccessionNum: NM_198737.1
GeneSymbol: LOC293551
Description: ADP-ribosylation-like factor 6-interacting protein

---

FileName: 103B.Seq
CloneName: 103
Seq: GGCACGAGGG AACTGCGGGC CCCTGTTGCT TTTCCCGCAG TTAACATGGC TGCGACTAGT
TATTCACCGA GACGACCGTG AGCAGAGGAA GGCAGGGCAC TGTGAGACTG CCCACTTCCG
GTCAGCGATG GCGCTTCAGC CGCGGTTCTG GAAATATCTG TCAGTTTGCA GGAACCTGGA
ATGTGGGTTC AAATCCCTCT CAACCAGTTC CACGCCGGCG GTGCAACCGG ATGTGGANAC
TANNGAAAAT GAANCNGNNA NCNNANANNN CAT
GiNum: 27729918
AccessionNum: XM_214751.1
GeneSymbol: LOC292244
Description: ribosomal protein L18

FileName: 105A.Seq
CloneName: 105
Seq: GGCACGAGCC TGGCTGTTAA AAAGTGGATT TAAGTGGTAT AAAAAAAGCT TTAAGAATGC
CAAGAAATCC TAAATCCTAC TACCCTGAGA GATAATTTTT TGTTTTAGTT TTTACATTTG
TTTGGTTTGT TTTGTTTTGT TGTTTTGTGT TGTTGAGAGA GGGTTTCTCT GTGTANCCCT
GNGTGCCCTG GAGCTCACTC TGNAAACTAN GNTGGNCTNA AACTCANANA TCTGNTGNCT
GNNCCTTNNA NANGCT
AccessionNum: AL646092.6

FileName: 106B.Seq
CloneName: 106
Seq: GGCACGAGTT TAACTATAGC CTTTACATTA TCTCTACTAG GTACTTTTAT ATTTCGCTCC
CACTTAATAT CTACTCTCCT CTGCCTAGNA AGGAATAATA CTATCACTAT TTGTCATAAC
TTCAACATCC ACATTAAACT CCAACTCCAT AATCTCCATA ACCATCCCAA TTACCATTCT
AGTTTTTGCA GCCTGCGAAG CAGCAGTAGG TTTAGCCTTA CTAGTANAAA TTTCAAATAC
TTACCGGAAC AGACTACGTA CCAAAACCTC AACCTTCTAC NATGNTAAAA ATNATNNTCC
CATANANCAT ACTCCNNCCN ANNACCCNGG ACTCTTNAAC CCCNANNACN NCNCTGNNCC
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 107B.Seq
CloneName: 107
Seq: GGCACGAGCG GACGAATTCG ATTCAACGGG GTTCCGGGCC AGGCTATGGA GCAGGTGAAT
GAGCTAAAGG AGAAGGGCAA TAAGGCCCTG AGTGCTGGGA ACATTGATGA TGCCTTACAG
TGCTNTTTTN AGGCAATTAA ACTAGATCCT CAGAACCATG TGCTCTATAG CAATCGCTCT
GCACCTATGC CCAAGAAAGG NNACTCCANA ANGCGNTGAA GGACCNGGTG CNAGAATNGT
GACCTTAACC TTACTTGGGC NANGGNTNTT TCANAAAANN NNNNNCCTTN GANTNCNAAA
GiNum: 40254729
AccessionNum: NM_138911.2
GeneSymbol: Stip1
Description: stress-induced phosphoprotein 1

FileName: 108B.Seq
CloneName: 108
Seq: GGCACCAGCA GGAAGGTGGA GAGACAGAAC TGACCTGAGT AAAGCTGCAG GAAATCATGC
TTCTGGAACA AGCCTCACAA CCTTTACTTC AACAGTCAAC ATTGGATCAG TTTATACCAT
ATAATNTTTT TTANACTTTA CTTCTCTTGA AGGTACAGTA ATAACTCTTC TCTTACTGAN
AAGANCCAAG CCTTTGGNGA ACCCNNTNAC AANGGCTANC NACTTAAATG ANAAGGNTNA
AATTNAAANA AAGGGAAACC NNTTNGGNGG ATTTNAAGAA NTNANNNAAN ACNANGNNAN
GiNum: 34858767
AccessionNum: XM_215825.2
GeneSymbol: LOC296178
Description: similar to 5730432L01Rik protein

FileName: 109BI.Seq
CloneName: 109
Seq: GGCACGAGGC TGGGACCCTG CTTCCCACAG CTGTGAGAAG AGCGTGGGTG AGGTCGGAAT
CCGNGCTATG GCTGCAGTGA CCTATGAGGA TGTGCACAGT GANNTTTACC CATGAANNTT
TNNGCNCCCG CTGGATCCNN TNCGAAAGAG CCNCTACNAA GATGCGACGN GGGAGNCCNA
CTGGATTNTN ACTGCNATAG GATACAAATT GGAAGACCCN GAACAGCCGA GGANCACCTC
TCACACAGCN CCTAGAANAC ANNGAAANGA CNTNGCATNC NGGCACTACT GGGNCTCAAA
CCCCGNGAAC CGNNAAACGG NANCTNGGAC AGAAGCACGG NACANCGTNG
GiNum: 38090583
AccessionNum: XM_125803.2
GeneSymbol: LOC216177
Description: similar to gonadotropin inducible ovarian transcription factor 1

FileName: 110D.Seq
CloneName: 110
Seq: CTNTTTTTAA AAANGGNCNA AACGNACNGG CCCGGGCNAA CCACTAGTAA CGGCCGCCAG
TGTTGCTGGG NANTCGGCAC GAGGNAGNAC TTTTGGAATA GAATCCTGTG AGGAAAGGTA
TTCCGGTAAG GGCTAGGCTT CCGATAATGA GACAAGATGA TGTGAATGGT ATTGCTTTTA
TCATATTGCC TATTTTTCGA ATNTTTTGNT CATTCGTTGA GGCTATGGAT GANTGATCCC
GANCATATGA ATNATATGGC TTTGAAGAAT GCATGGGTNC NAATGTNGAA GAAANCCANG
NNNGGGTGGG TANNCCTNAA GGGACTATTN TAANGGCTAN NNNGNTNGAN NTTNANAAAA
NTCNANNTTT TTNNAG
AccessionNum: AY172581.1
Description: Rattus norvegicus strain mitochondrion

FileName: 111A.Seq
CloneName: 111
Seq: GGCACGAGCA AAATGGCGGC GGCGGCGGCT ACGGCGGCTT CGGCGCCTCA GCAGCTCTCA
GCATGAGGAG CTTTTCTCTC AGCTCCGCCG TTTNTTTTTN TCTCCGGGTC CCGTGACAGA
GCAGCACTNA GGCCGGTCTA CCTCAANAAN CTGAAGAAGC NCAGNGAGGN AGANCAGNNA
NCAGCAGCAA CACCGGGCGG NGAGGCCGCG GCAACAAAGA CGCGAGAACT CGAGCATGCA
TANAGAGGGN CGCATCATGT NGGNNGNTAC GNACNCTNAA ANTCACANCC TACCNCCANA
TCCGCTCNAN CCGANNAAAG AACNANGNNA GACNANCNGN AAGNCTAGNN CCTNCNNANN
NNNCNANAGN CCATNAAACA C
GiNum: 34865681
AccessionNum: XM_216900.2
GeneSymbol: LOC299821
Description: similar to integral inner nuclear membrane protein

---

FileName: 112D.Seq
CloneName: 112
Seq: GGCACGAGCA CATGAGTTAT TACAATCATC TCCTCAATAG CCACACTATT TATTTTATTT
CAATTAAAAA TTTCTTCCCA AACCTTNCCT GCNNTTTTTT TGACCCAAAA CTATAGCTAC
AGCAAAAAAC GAATAACCCT TGAGAATCAN AATGAACGAA AATCTATNNG CCTCTTTCAT
TACCCCCACA ATAATAGGNC TACCAATNGN TGNAACCANT ATTATGNNCN NATCAATNNT
ATNCCCATCA TCAAAACGCC TAANCAGCAC CCGACCACNC TNATTTCAAC ACTGANAAAN
CAAACNCATC ACCCAANCAA CCNAAGNGAA NNCACACNCC AAAAGGANCG AANCTGAACC
CTANTANTTG NACNNCTAAA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 113A.Seq
CloneName: 113
Seq: GGCACGAGCT CATAGGGCCT GTAATCACTA TATCTAGCTC CAACTTACTC CTAATATGAG
TAGGATTGGA AATAAGCCTT TTAGCTATCA TCCCACTTCT ANNCAACAAA AAAAGCCCAC
GATCAACTGA AGCAGCAACA AAATATTTTC TAACCCAAGC TACAGCCTCA ATAATTATCC
TACTAGTCAT CATCCTCAAC TACAAACAAT CAGGAATATG AACCCTCCAA CAACAAACCA
ATAACATACT ACTCAACATA ATACTCATTT CACTAGCCAT AAAACTTGGA CTAGCCCCAT
TCCACTACTG ACTACCCGAA GTCACCCAAG GAATTCCCCT ACACATTGGA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 114D.Seq
CloneName: 114
Seq: GGCACGAGAT CTTCCAGTAA CTCGCCAAAA TGACGAACAC AAAAGGNAAA GAGGAGGGGT
ACTCGGTATA TGTTCTCTAG ACCTTTTAGG AAACATGGAG TCGTTCCTTT GGCCACATAC
ATGCTNNTTT TCCNGGAAGG GTGATATTGT AGACCTCAAG GGAATGGGCC CTGGTCAAAA
AGGAATGCCC CATAAGTGNT ACCCTGGNNA AACCNGAANA ACTACNANGN CACCCANCAT
GCCCNGGGCA TTATTGNGAA CCNCCAGTTN AANGGCANAN TNTNGGCCAN AAGATCAAGG
NCCGNNTNAG CCNTTNAGNC CTNNAGANNN NNNACNCTTN CTTAACNGGN NAANGGNAAN
CANCNNNNAA AANGGNGCCC NNNNAANGGN CC
AccessionNum: NM_053330.1
GeneSymbol: Rpl21
Description: ribosomal protein L21

FileName: 115D.Seq
CloneName: 115
Seq: GGCACGAGAG GGATCGTACT ATCTAACTCA TANNCTTGAC ATTGTACTTC ATGATACATA
CTATGTAGTA GCTCACTTCC ACTATGTCTT ATCTATAGGA GCNTTATTCG CCATCATAGC
NNGGCTTCGT CCACTGATTC CCACTATTCT CAGGCTATAC CCTAAATGAC ACATGAGCAA
AAGCCCACTT TGCCATTATA TTTGTAGGTG TAAACATAAC ATTTTTCCCT CAACACTTCC
TAGGATTAAG CGGGGATACC TCGTCGTTAC TCTGATTATC CAGATGCTTA CACCACATGA
AATACAGTCT CCTCTATAGG CTCATTCATC TCACTTACNG CCGTNCTTGT ATG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 116B.Seq
CloneName: 116
Seq: GGCACGAGCA TAAACCGGTG TAAAATCCCT TAAACATTTG CCTAAAACTT AAGGAGAGGG
CATCAAGCAC ATAATATAGC TCAAGGACGC CTTGCCTAGC CACACCCCCA CGGGACTCAG
NTTTTCAANN TTTTAAGCCA TGAACGAAAG NTTGGCTNAA CTATACCTTT TAAGGNTGGG
AAANTTTNGN GCCAANCACC CCGGGNATAC CAATTACCCC AACTNAATTT TTCNGNGTNA
AAAGTGGCCA CTNTTAAANT CATAATNNNA ANTTAAAANC CACCTTNTAT NNGNAAAANN
ATTGNTNGGG ACCTAANNCC NANANCNAAN GGNNNNNTAA NNANTNNTTT NANGNNCCNA
ANNNTNNAAC CCAANNGGGG NTNNAAANCC CCC
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 117A.Seq
CloneName: 117
Seq: GGCACGAGTA ATTGGAGGTA AGATTACACA TGCAAACATC CATAAACCGG TGTAAAATCC
CTTAAACATT TGCCTAAAAC TTAAGGAGAG GGCATCAAGC NCNTNATATA GCTCAAGACG
CCTTGCCTAG CCACACCCCC ACGGGACTCA GCAGTGATAA ATATTAAGCA ATGAACGAAA
GTTTGACTAA GCTATACCTC TCAGGGTTGG TAAATTTCGT GCCAGCCACC GCGGTCATAC
GATTAACCCA AACTAATTAT TTTCGGCGTA AAACGTGCCA ACTATAAATC TCATAATAGA
NTTAAAATCC AACTTATATG TGAAAAATTC TTGTTAGGAC CTAGCCCAT
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 118.Seq
CloneName: 118
Seq: GGCACGAGAA CAATTTGGTA GCAGTGTGTG CGCACAGCCA TCAGCTGATA GTGAGCAGCC
ATTCACTTGC CGCGAGCCGC GAAGCGGCCG CGCGGACGTC ACCACAGCCG TCAGCGTTTG
CCGCATCCTC AGACTCACTC TNTTACCAGC CGCAGTCACA GCTGCAGGAC CTCTCTGGAC
CAGCTCAGTC GCAGACTGCG CAACCACCAG ACCACTGCGG CAAACAAGCC CAGCTGAGCC
AAGCAATAGC GATGGCCGAC CCNGAGAAGC AGGGACCCGC TGAGAGCCGC NCCGAGGACG
GiNum: 34855949
AccessionNum: XM_342701.1
GeneSymbol: LOC362377
Description: nucleosome assembly protein 1-like 5 (Napl15-pending)

FileName: 119A.Seq
CloneName: 119
Seq: GGCACGAGGT CACGTTTCGT GCGTGCTCAT TCTGCCAAGA TGCCTGAGGA AACCCAGTAC
CCAAGACCAA CCAATGGAGG AAGAGGAGGT CGAAACCTTT GCCTTTCAGG CAGAAATTGC
CCAGTTAATG TCCTTGATCA TCAACACTTT CTACTCGAAC AAAGAGATCT TTCTGAGGGA
GCTCATTTCC AACTCCTCAG ACGCTCTGGA TAAGATCAGA TACGAGAGCT TGACCGACCC
TAGTAAACTG GACTCGGGGA AGGAGCTGCA CATTAATCTC ATTCCCAACA AGCAAGACCG
AACCCTCCCT ATTGTGGATA CTGGCATTGG AATGACCAGG CTGACTTGAT
GiNum: 28916694
AccessionNum: NM_175761.2
GeneSymbol: Hspca
Description: heat shock protein 86, alpha

FileName: 120B.Seq
CloneName: 120
Seq: GGCACGAGGT TGGTCAGACT AAAGAAGTAT TAGTTATTAA ATTAATAAGC CAAGGAACGA
TTGAAGAGTC CATGCTAAAA ATTAACCAAC AAAAGTTGAA GCTANAGCAA GACATTGTNT
ACAGTANATG AAGCTGATGA GGGAAGTATG CCAGGCGGCA TATAGCCACG CTACTGNAAA
CGTNTTTGGG CCNGNGAAAG GACAANTCAN AATTNCTGNN CANCANNCCN TATCAANTNN
GNGCANNNAT NGACANTCCN NACNNANNGA CCNNCCNGGA TNTNAANCCT CNNANCNNTC
GiNum: 34855955
AccessionNum: XM_231860.2
GeneSymbol: LOC312398
Description: similar to etl1 (M. muluscus) SMARCAD1 (H. sapiens)

FileName: 121D.Seq
CloneName: 121
Seq: GGCACGAGGT CGGAAGCAGG TTCTTGCCTG TGACATGGAC AGATGTAAAG AAAACTGNGT
CTCTAGGCCT GTCAAGTCCA CTGTTCCCTT CGGTCCGAAA CNAGTCTTGG TGACTGAGCA
GATTCCTTCT CAGCACCCAG GATCAGCGAG CAGTGGCCAG GCCCAGCGGG NCCTGTGNCC
CTNCAACTCC CAGCGTGTCC CTNCACAAGC ACAGAAACCT GTCGCANGTC AGAAGCCAGN
GCTCAAGCAG NTGNCGGATG CCAGCGGACC CNCGACCTNN TTNNNGGCTG AGTAACCCCC
GiNum: 23463274
AccessionNum: NM_153296.1
GeneSymbol: Stk6
Description: serine/threonine kinase 6

FileName: 122A.Seq
CloneName: 122
Seq: GGCACGAGGA AGATGGCGGT CGCCGTGAGA GCTTTGCAGG AGCAGCTAGA AAAGGCCAAA
GAGAGCCTCA AGAATGTGGA CGAGAATATC CGCAAGCTCA CCGTNCGGGA CCCGGAATGA
TGTGAGGCCC ATCCAAGCCA GATTGCTGGC CCTTTCTGGT CCTGGTGGAG GTAGAGGACG
AGGTAGTTTA TTGCTAAGGC GTGGATTCTC AGATAGTGGA GGAGGACCCC CAGCCAAACA
GAGAGACCTG GAAGGGGCAG TCAGTAGGCT GGGTGGAGAG CGTCGGACAA GAAGAGAATC
ACGCCAGGAA AGTGACCCAG AAGACGATGA TGTTAAAAAG CCAGCACTGC AATCTTCTG
GiNum: 34865261
AccessionNum: XM_343067.1
GeneSymbol: LOC362742
Description: similar to pinin

FileName: 123B.Seq
CloneName: 123
Seq: GGCACGAGCT CCTGCCTCAG TACCATGTGC TGGGTTTGTA GGTGTGTGCC AGCATGCCCA
AACATAATTT TGGGGGATGG AGAGATGACT CACTGATTAG AGCACTTGCT GCCTTTTTAG
AGGACCCAGG NTCAATTCCC AGCCCTACAT GTNAACCTAC AACTGNCTAT ANCTCCAATT
NCAAAGATCT GANGCCTTCT GGCCTCCAAG GGTACTATCN NACANGTGTG CATNAACATA
CATACATANA TGCATACANA CNGTACCNNC CTANAAACCA TACAGGCAGA ACATCCANNG
TNCATANNAA AAACNAACTA AACNNNANGC CANNTNNNAT AANNACNAAG
AccessionNum: AC099603.15
Description: Chromosomal DNA

FileName: 124A.Seq
CloneName: 124
Seq: GGCCGAGCGG CACGAGGCGA CAGAGGGAGG AAGAGAGGAG AAAAGAGATA GAAAGACGAG
AGGCAGCAAA ACAGGAGCTT GAAAGACAAC GGCGTTTAGA ATGGGAAAGA ATCCCGTCCG
ACAGGNNTTT TTTNANTCAA AAGGAATTGA GTANNAGGAN GGAAATTTGG CANGCTTGAA
TTCTNAAAAN AAAAGTCTTC ACCTTTGAAC NNGAANCCCN TGAATGGNAA ACATTTNCNN
ATNTTANGCC NACTNCNANA ANGTCCCNAT TNNAAAGCNA NCCCNAANAA TTGNGCTNNA
GiNum: 34882668
AccessionNum: XM_233945.2
GeneSymbol: LOC313934
Description: Similar to mouse KIAA1256 predicted coding sequence

---

FileName: 125C.Seq
CloneName: 125
Seq: GGCACGAGGA AAATCAAGGA GATGAGACCC AAGGTCAGCA GCCACCTCAA CGTCGGTATC
GCCGCAACTT CAATTACCGA CGCAGACGCC CAGAGCAACC CTATTTCACA AGATGGCAAA
GAGACAAAAG CAGCCGATCC ACCAGCTGAG AATTCGTCCG CTCCCGAGGC TGAGCAGGGC
GGGGCTGAGT AAATGCCGGC TTACCATCTC TACCATCATC CGGTTTGGTC ATCCAACGAG
AAGGTCCAAC TCGAGCATGC ATCTAGAGGG CCGCATCATG TAATTAGTTA TGTCCGCTTA
CATTCCGCCC TACNCCCACA TCCGCTTTAA CCGAAAAGGA AGGAGTTAGA
GiNum: 34870881
AccessionNum: XM_342898.1
GeneSymbol: LOC362579
Description: similar to YB-1

FileName: 126B.Seq
CloneName: 126
Seq: GGCACGAGCA TAACTAACCC CCCCCCCCCA TAAACTAAAA CATTTAACTC AAAAAGTATT
CGGAGAAAGA AATTTACTTA CCAGGAGCTA TAGAGAAAGT ACNTNTTNGG GAATGGTGAA
AGACTAATTT AAAGTAAAAA TAAGCAAAGA TTAAACCTTG TACCTTTTGC ATAATGAATT
AACTAGAAAA TCCTTAACAA AAAGAATTNG AGCTAAGAAC CCCGAAACCA AACGAGCTAC
CTAAAAACAA TTTCATGAAT CAACCCGTCT ATGTAGCAAA ATAGTGGGAA GATTTTTAGG
TANAGGTGAA AAGCCTATCG AGCTTGGTGA TAGCTGGNTG CCCAAAAAAG ATTTCAG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 127.Seq
CloneName: 127
Seq: GGCACGAGTC AGTGCCCTGC CGTTAGGTGA CAAGATGGTG AGGTTTGCCC ATGTACATTG
GTGGACCTTA ATGACACAAC TACCTTTTAA AGAATGTCAT CATGGGATTG GGGATTTAGC
TCAGTGGTAG AGCGCTTGCC TAGCAAGCAC AAGGCCCTGG GTTGGGTCCC CAGCTCCGAA
AAAAAGAAAA GGAAAAAAAA AAAAAAGAAT GTCATCATTG TGGAAAGAGC ATTTTGCTTT
TGTGTTGTTT CTCACTGTGA AGATGTAAGT GTGGCAGTGT GCACCACTGC AGAGTCTTTC
CCTGTCCTCA GAGGCTGTGC CTCTGCACAT TAGCCCAGCG CCTAGCCAAC GCTGCCCTTG
CACTGCTCTT CTTAGCC
GiNum: 7209570
AccessionNum: D83974.1
GeneSymbol: DAN
Description: DAN gene

FileName: 128A.Seq
CloneName: 128
Seq: GGCACGAGGA CTCTTTTCAA CTAACCACAA AGATATCCGG AACCCTCTAC CTATTATTTG
GAGCCTGAGC AGGAATAGTA GGGACAGCTT TAAGTATTTT AATTCGAGCT GAACTAGGAC
AGCCAGGCGC ACTCCTAGGA GATGACCAAA TCTATAATGT CATCGTCACA GCCCATGCAT
TCGTAATAAT TTTCTTTATA GTAATACCTA TAATAATTGG AGGCTTCGGG AACTGACTTG
TACCACTAAT AATTGGAGCC CCTGATATAG CATTCCCACG AATAAATAAC CTAAGCTTTT
GACTGCTTCC TCCATCATTT CTACTCCTTT TAGCATCCTN CATAGTANAA GCTGGAGCTG
GAACAGGATG AACAGTATAC NCCNNNTANN TNTANCATGC ATNTANAGGG CCNGANTNTG
NATTAGANAT
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 777A.Seq
CloneName: 777
Seq: GGCACGAGAA CCAACATAAC TAACCCCCCC CCCCCATAAA CTAAAACATT TAACTCAAAA
AGTATTGGAG AAAGAAATTT ACTTACCAGG AGCTATNGAG AAAGTACCGC AAGGGAATGG
TGAAAGANTA ATTTAAAGTA AAAATNAGCA AAGATTAAAC CTTGGACCTT TTGCATTATG
GATTAACTNG AAAATTCTTN ACNAAAAGAA TTTNAGCTNA GAACCCCGAA ACCNAACGAG
CTTCTTAAAC CTTTCTGAAT TACCCGGTAT TGTCCAATTN GGGGANATTT TTTGGTAAGG
AAAACTTTNG CTTNGGAATC TTGTGCCCAA AAAATTANTN ANTTAAGTTC TTNAANCCAT
TAATG
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrion genome

FileName: 777B.Seq
CloneName: 777
Seq: GGCACGAGTT ATCATGGAGA CCTCTGGAGC AGTCTGACCG GACTCGGTGA CGTGGGCTAC
TTGTAATTTC GGCGCTGGGT TGACTTTTCT TATTAAGCCT ACCCCTTTTC TGAAGGGAAT
TGTAGCGTTC CCAGAGTCCA TTACGTATTA AAGGATGCAG CTTCTTTCTT AGTTTTAATT
CGGCACTTAT GAATTTAAGT GAGGTGACNT GNCCCATTTG ATCTTNAAAA AAAAAATTCA
TNATGNNACC TTATTANACC TTNANTNTTT CTTTTGAACN ATTTTTNCNT TTTTTCTGGG
TTCNTTCANT GGNTTNGGAA AANATTTTGT GNAAAATTGG NNNGNGTTTA AAAAAAAAAA
AANNTTGGAT TAAACCCNAA ACTTTTTTAN CCTGGCG
AccessionNum: AK015237.1
Description: EST

FileName: 777C.Seq
CloneName: 777
Seq: GGCACGAGGA GCTGCCGCTG CTGCTGCTGT AGATTGACGG CCCGCGCTAG AGCTTCAGAG
AACCGGTTTA CTTTTGAGAC CTGACTACTC TGAGCCGACC TCATGGATGA ACTTCAGGAT
GTTCAGCTCA CAGAGATCAA ACCACTCCTG AATGATAAGG AACATGACAT TGAAACACCC
CATGGTATGG NCCACGTCAC CATNAGAGGC TTACCAAAGG AAACNCGACC TGGTATTCTG
ACATACCATG ACATTGGGCT TAACNTTAAN CCCTGGTTTA AACCGTTNTT NAACTTTNAG
GAANTCCNAG ATNCCCNNAT TTGNTNGNTG NNCNNGGATN CCCCCAAGCA CNGGAAGAAN
AACCCTCTTT CANNNGGGNT TANTNCCCCC NNTGG
AccessionNum: AF251054.1
GeneSymbol: NDR3
Description: N-myc downstream regulated 3

FileName: 777D.Seq
CloneName: 777
Seq: GGCACGAGCG GCACGAGCGA AGTCACCCAA GGAATTCCCC TACACATTGG ATTAATCTTA
CTAACATGAC AAAAAATTGC TCCACTATCA ATTCTATACC AATTTTATCA ACTCCTAAGC
CCAACTATTA CCACCATTCT TGCAATTTCA TCAAGTCTTT GTTGGCGCCT GAAGAGGACT
TNACCAGACC CAAACACGAA AAATCTTAGC ATATTCATTA ATTGGCCACA TNTGATGGAA
TTCCNGAAAT CTTTTATNTA ANCCTTAACT TAACCCTTTT TNACTTTACA ATTTNAANTC
TAATTANTGN GTCCAAATTT NNTTCCCTTT TAAACNAACT TTGGGACCAC CNTTTAANNA
AccessionNum: AY172581.1
Description: Rattus norvegicus mitochondrial genome

FileName: 777E.Seq
CloneName: 777
Seq: GGCACGAGCG GCACGAGGAA AACCGATATG GCGGCCACTC TGGGCAGCGG GGAGCGCTGG
ACCCAAGCTT ACATTGATGC AATTAGAAGA AACAAATACC CAGAAGACAA GCAACCTGAC
AGCCATGATC CCTGTGGTTG CTGTAACTGC ATGAAGGCAC AAAAGGAAAA AAAGTCTGAG
AACGAGTGGA GTCAGACCCG GCAGGGAGAT GGGAACGCCA CTTACACAGA AGAACAGCTA
CGTGGGGTAC AAAGAATCAA GAAGTGCAGA AATTACTATG ACATTCTGGG TGTTTCTACA
ATGCCAGCGA TGAAGAGCTT AAGAAAGCAT ACAAGAAACT CGCCCTGAAG TTTCACCCCG
ACAAGACTGT GCTCCTGGAC ACAGAT
GiNum: 34878666
AccessionNum: XM_214587.2
GeneSymbol: LOC291677
Description: DNAJ PROTEIN HOMOLOG 1

FileName: 777F.Seq
CloneName: 777
Seq: GGCACGAGGC AGGCGGGTCA GCGGACGGGC GGAGGGCGGC CGGGGCAGCG CGCAGGGGGC
ATGGCGGNTG CGGGACTGCG GGCGGCAGCT ANGCNCTGGC TGCTGNGCGG AGGCCANGGC
GGACCGCGNG CCGCNNGGNN TTTTTNTTNC TGACCTGGNN GNGGNCCNCC AGANNCAAGG
NANCCACTGA CNCAANACCC CNAGCTNGGG GNNNGCACNC CCGAAACANA NNNNAANACN
CCCTNNNANC CANACNACCA CAAAAAANCC NNACNGACNG CNNCNNAANA NCTCNTGCNN
CNCNNANNNA AGACAANCCN CNNCANCAAG GAAACGNATT CANNACAAAC NAACANNAAC
GiNum: 34876976
AccessionNum: XM_214258.2
GeneSymbol: LOC290558
Description: similar to hypothetical protein FLJ12442

FileName: 777G.Seq
CloneName: 777
Seq: GGCACGAGTG ATTTTAGTAC TTCAGATATT CAAGTTTTTT ATTTAGTCCT TTGCAATTGT
CAAAAATACT TATTTTGTGT TTTATACTGC TATTTAATTT TTGTTTTTAA TTCTTTTAAA
TTCTGCTTCT AATAGAATGA TGCCAATGGG TGGAATGATG CCACCTGGAC CTGGAATACC
ACCTCTGATG CCGGGTATGC CACCAGGTAT GCCCCCACCT GTTCCACGTC CTGGAATTCC
TCCAATGACT CAAGCACAGG CTGTTTCAGC ACCAGGTATT CTCAATCGAC CACCTGCACC
GACAGCAGCA GTACCCGCTC CACAGCCTCC AGTTACTAAG CCTCTTTTCC CAGTGCTGGA
CAGGTAAGGN GAAATTCTTG AAAAGGAGTG TACATTGATG
GiNum: 34872894
AccessionNum: XM_221231.1
GeneSymbol: LOC303763
Description: zinc finger protein 207

FileName: 777H.Seq
CloneName: 777
Seq: GGCACGAGCT CTCTTAAGGT AGCCAAATGC CTCGTCATCT AATTAGTGAC GCGCATGAAT
GGATGAACGA GATTCCCACT GTCCCTACCT ACTATCCAGC GAAACCACAG CCAAGGGAAC
GGGCTTGGCG GAATCAGCGG NTGAAAGAAG ACCTGTTGAG CTTGACTCTA GTCTGGCACG
GTGAAGAGAC ATGAGAGGTG TAGAATAAGT GGGAGGCCCC CGGCGCCCCC CCGTTCCCCG
CGAGGGGTCG GGGCGGGGTC CGCCGGCCTN CGGGCCGCCG GTGAAATACC ACTACTCTCA
AccessionNum: V01270.1
GeneSymbol: 28S rRNA
Description: 28S ribosomal RNA

FileName: 777I.Seq
CloneName: 777
Seq: GGCACGAGGN TCTCCTCCGC GTCGGCGGTT CCCCCGCCGG GTGCGCCCCC CGGGCCGCGG
AAAACCCGCG CGGNGCCTNG CCANANGNCG GNGCNTTTTT TTTCGACGNA NAACTGGCGC
NGACCANGNG AATNCAGACT GNCCAANNAA AANAAACCAC CGNGGGCGNA GNACGAGGGC
CCCAGCTGNA TNGGCATGAC GANGGGNAGC GCNNAGACCA GAGCCNACAG AGGGNAGAAN
CTCANAGACT GCNCAAGNCT GNGNAACCAG GAAGGNCCNN GANAGAANAG NAANNACNAG
AccessionNum: V01270.1
GeneSymbol: 28S rRNA
Description: 28S ribosomal RNA

FileName: 777J.Seq
CloneName: 777
Seq: GGCACGAGGT AAGATGGAAG ATGAGGAGGT CGCTGAGAGC TGGGAGGAGG CGGCAGACAG
CGGGCAGGAA ATCCAAATCT CCTCCCAAAG TGCCCATTGT GATTCAAGAC GATAGCCTTC
CCACGGGGCC CCCTCCACAN TTCCGCATCC TCAAGAGGCC CACCAGCAAC GGTGTGGTCA
GCAGCCCCAA CTCCACCAGC AGGCCAGCCC TTCCTGTCAA GTCCCTAGCA CAGCGGGAGG
CAGAGTACGC AGAGGCTCGG AGACGGATCC TAGGCAGTGC CAGCCCTGAG GAGGAGCAGG
AGAAACCCAT CCTCGACAGG CCAACCAGGA TCTCCCAACC CNAAGACAGC AGGCAGCCCA
GTAATGTNAT CAGACAGCCG TTGGGTCCTG ACGGGTCACA AGGCTTCAAC ANCGCNATAA
ATGCAGCC
AccessionNum: AB097045.1
GeneSymbol: PM21
Description: putative MAPK activating protein