Université de Montréal

PURIFIED PRE- SERTOLI CELLS EXPRESS GENES INVOLVED IN CELL PROLIFERATION AND CELL SIGNALLING DURING A CRITICAL WINDOW IN MALE SEX DETERMINATION

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PURIFIED PRE- SERTOLI CELLS EXPRESS GENES INVOLVED IN CELL PROLIFERATION AND CELL SIGNALLING DURING A CRITICAL WINDOW IN MALE SEX DETERMINATION

présenté par

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a été évalué par un jury composé des personnes suivantes

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SUMMARY

Sex can be defined as a sum of characteristics caused by a network of interacting genes and proteins that give rise to sexual phenotypes within an individual. Sertoli cells drive testis formation and therefore, sex determination in the male. While some of this pathway has been elucidated, a better understanding of the RNA environment within the pre-Sertoli cell will help clarify the mechanisms by which these cells drive the differentiation process. A mouse model expressing Red Fluorescent Protein (RFP) under the control of a hybrid mouse/pig *Sry* promoter (HybSRYp-RFP) was used to purify e12.0 pre-Sertoli cells. RNA was extracted and hybridized onto a micro-array (Affymetrix Mouse Genome 430 2.0) to compare the transcriptomes of stage-matched pre-Sertoli cell populations vs. whole female genital ridges. Genes identified as 2.5 fold overexpressed in the male notably consisted of cell signaling and extracellular components. This data represents the earliest microarray expression analysis of purified pre-Sertoli cells available to date. The expression of *Sox10*, *Wnt6*, *Fgf18*, *Fgf13* and *Stc2* were characterized by in situ hybridization in the male and female gonads between e11.5 and e13.5. *Sox10, Fgf18, Fgf13* and *Stc2* were detected in the testis cords while *Wnt6* was found in both the male and female gonad. Of these five genes *Stc2*, *Sox10* and *Wnt6* are present just after sex determination and may have a role to play in sex determination. On the other hand *Fgf18* and *Fgf13* are likely involved in the process of sex differentiation. Closer examination of the microarray data provides evidence for the activation of several intercellular signaling pathways and a new model for Sry is proposed.

RÉSUMÉ

Le sexe se définit comme la somme d'une série de caractéristiques émergeant des interactions entre certains gènes et protéines, engendrant le phénotype sexuel chez un individu. Les cellules de Sertoli dirigent la formation des testicules et, du coup, la détermination du sexe chez le mâle. Bien qu'une partie de cette voie ait été élucidée, une meilleure compréhension de l'environnement d'ARNm dans les cellules pré-Sertoli permettra de clarifier les mécanismes par lesquels ces cellules dirigent le processus de différentiation. Une souris exprimant la protéine fluorescente rouge (RFP) à partir d'un promoteur *Sry* hybride souris/porc (HybSRYp-RFP) fut utilisée pour purifier des cellules pré-Sertoli à e12,0. L'ARN extrait et hybridé sur micro-array (Affymetrix Mouse Genome 430 2.0) permit de comparer les transcriptomes de populations de cellules pré-Sertoli de stages spécifiques à des crêtes génitales femelles entières. Plusieurs gènes impliqués dans la signalisation cellulaire ou dans la formation de composantes extracellulaires comptent parmi ceux identifiés comme étant 2,5 fois plus exprimés chez le mâle. Ces données représentent l'analyse d'expression par micro-array de cellules pré-Sertoli purifiées la plus précoce réalisée à ce jour. L'expression de *Sox10*, *Wnt6*, *Fgf18*, *Fgf13* et *Stc2* fut étudiée par hybridisation in situ dans les gonades mâles et femelles entre e11,5 et e14,5. *Sox10*, *Fgf18*, *Fgf13* et *Stc2* furent détectés dans les cordons testiculaires, alors que *Wnt6* fut identifié chez les deux sexes. *Sox10*, *Wnt6* et *Stc2*, présents immédiatement après la détermination du sexe, pourraient jouer un rôle dans celle-ci, alors que *Fgf18* et *Fgf13* sont probablement impliqués dans la différentiation du sexe. Un examen plus poussé du microarray propose l'activation de plusieurs voies de signalisation intracellulaires. Finalement, un nouveau modèle est proposé pour *Sry*.

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List of Abbreviations

%	Percent
µg	Microgram
µl	Microlitre
µM	Micromolar
°C	Degrees Celcius
Aldh1a2	Aldehyde dehydrogenase 1 family, member A2
AHC	Adrenal hypoplasia congenita
AMH	Anti Müllerian Hormone
ARX	Aristalles-Related Homeobox gene
ASE	Autosomal signal elements
BCIP	5-bromo-4-chloro-3-indoyl phosphate
BMP	Bone morphogenic protein
bp	Base pair
BPES	Blepharophimosis-Ptosis-Epicanthus-inversus-Syndrome
BrdU	5'-bromo-2'-deoxyuridine
Ca	Calcium
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CD	Campomelic dysplasia
CDS	Complementary sex determination
cDNA	Complementary deoxyribonucleic acid
Cyp	Cytochrome
Da	Daughterless
Dax1	Dosage sensitive sex reversal-Adrenal hypoplasia expression critical region on the X chromosome
Dct	Dopachrome tautomerase
DEPC	Diethylpyrocarbonate
DHH	Desert Hedgehog
DIG	Digoxygenin

Dmc1	Disrupted meiotic cDNA1
DMRT1	Doublesex and mab-3 related transcription factor 1
DNA	Deoxyribonucleic acid
Dpc	Days post-coitum
Dpn	Deadpan
Dpy	Dosage compensation dumpy
Dscr1	Down syndrome critical region homolog 1
e or E	Embryonic day
Emc	Extramacrochaetae
Ems	Empty spiracle
Emx2	Empty Spiracles 2
ESD	Environmental sex determination
FACs	Fluorescent assisted cell sorting
FGF	Fibroblast growth factor
FGFr	Fibroblast growth factor receptor
Figα	Factor in the germline alpha
FOG	Friend of GATA
Foxl2	Forkhead box L2
Fst	Follistatin
GATA	Transcription factors which bind to the concensus sequence WGATAR
GFP	Green fluorescent protein
Gli3	Gli-Kruppel family member 3
Gro	Groucho
Grg6	Groucho related gene 6
GSD	Genetic sex determination
Her-1	Hermaphrodite-1
HDAC	Histone deacetylase
HGF	Hepatocyte growth factor
HMG	High mobility group
Hsd17b3	17β-hydroxysteroid dehydrogenase type 3

ic	Intercellular
Igfr	Insulin like growth factor 1 receptor
Inhb	Inhibin
Insl3	Insulin-like peptide 3
Ir	Insulin receptor
Irr	Insulin receptor-related receptor
Ix	Intersex
Kap-1	Krab-associating protein 1
Kb	kilobase
KO	Knockout
Krab-O	Kruppel-associated box only gene
LacZ	Lactose operon
Lef1	Lymphoid enhancer factor 1
LH	Luteinizing hormone
LHX	LIM homeobox domain
LIM	Transcription factor homeobox domain family
M33	Chromobox homolog 2
MAP	Mitogen activated protein
MAPK	Mitogen activated protein kinase
Mb	Megabase
MIS	Müllerian inhibiting substance
mRNA	Messenger ribonucleic acid
Msl-2	Male specific lethal-2
NLS	Nuclear localization signal
NR	Nuclear receptor
Nt	Neurotropin
Odd1	Odd-skipped related 1 gene
ORF	Open reading frame
P	Postnatal day
Pax	Paired box
PCR	Polymerase chain reaction

PGD2	Prostaglandin D2
Pdgf	Platelet-derived growth factor
Pdgfr	Platelet-derived growth factor receptor
PFA	Paraformaldehyde
Pfoxic	Promoter FOXL2 inverse complementary
PGC	Primordial germ cell
pH	relative hydrogen proton (H+) concentration
PI3K	Phosphatidylinositol 3-kinase
Pis	Polled/intersex syndrome
PKA	Protein kinase A
PLC	phospholipase C
Pod1	Podocyte-expressed 1
PSR	Paternal-Sex-Ratio
Ptch	Patched receptor
RA	Retinoic acid
RAR	Retinoic acid receptor
RARE	Retinoic acid-responsive element
RFP	Red fluorescent protein
RNA	Ribonucleic acid
RNAse	Ribonuclease
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase- Polymerase chain reaction
Run	Runt *gene*
Scp3	synaptonemal complex protein 3
Sdc	sex and dosage compensation
Sf1	Steroidogenic factor 1
SIS	Sisterless
SHP	Small Heterodimer Protein
sl-CDS	single locus-complimentary sex determination
Smad	Mothers against DPP homolog 3
SOX	SRY-related HMG box

SRY	Sex-determining region-Y chromosome
SSC	Side chain cleavage
SSH	Suppression subtractive hybridization
StAR	Steroidogenic acute regulatory protein
Stra8	Stimulated by retinoic acid 8
Sxl	Sex lethal
Tcf	T-cell transcription factor
TDF	Testis determining factor
TGF	Transforming growth factor
Tra	Transformer
Trk	Tyrosine kinase receptor family
ts	Tail somites
TSD	Temperature-dependent sex determination
WARG	Wilms' tumor-anirida-genitourinary anomalies mental retardation syndrome
WISH	Whole mount *in situ* hybridization
WNT	Wingless-Type MMTV Integration Site Family, Member
WT1	Wilms' tumor gene 1
Xol1	XO lethal protein 1
XSE	X signal element
YFP	Yellow fluorescent protein
Vnn1	Vannin

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Thank you, Stéphanie Bolduc-Beaulieu.

Sincerely,

Aron Cory

This Memoire is dedicated to my father, Dr. Neil Cory, for his good advice.

"Just carry on Aron. Just carry on."

I will.

INTRODUCTION

Hypothesis and Objective

The main objective of Dr. David W Silverside's laboratory has been to study sex determination and differentiation in mammals. We hypothesize that the pre-Sertoli cells drive male sex differentiation and that the genes involved are largely undefined. To further characterize genes differentially expressed by pre-Sertoli cells at the beginning of sex differentiation we have made use of a mouse model expressing Red Fluorescent Protein (RFP) under the control of a hybrid mouse/pig *Sry* promoter (HybSRYp-RFP) (Boyer et al., 2006). This mouse line was used to isolate and purify pre-Sertoli cells after the peak of *Sry* expression but before histological testis cord formation. RNA from purified e12.0 pre-Sertoli cells or stage matched whole female genital ridges was hybridized onto microarrays to study the transcriptomes of pre-Sertoli cells.

Literature Review

1.1) Definition of Sex

Throughout the animal kingdom the existence of two sexes, which contain cells that undergo meiosis and reproduce sexually, is almost universal. The word "sex" comes from the Latin word *sexus*, meaning division (from secare, to cut or separate). Ironically, sex (being male or female) is not a clear-cut biological entity. In several species sex is immaterial, with animals changing from male to female several times throughout their lives or being hermaphrodites, containing both sexes within one individual (Dimijian, 2005). Even within species with obvious male or female groups, masculinity and femininity will vary among individuals in a population. This means that populations are composed of individuals differing along a spectrum of peculiarities of structure and function that define males or females. Sex could be better defined as a sum of characteristics caused by a network of interacting genes and proteins that give rise to sexual phenotypes within an individual.

1.2) Definition of Sex Determining System

A sex determining system is a biological system that determines the development of sexual characteristics in an organism. Sexual reproduction requires a balance of two sexes in a population in order to function efficiently. If a male and a female are required for reproduction, having all male or all female offspring in the population can ultimately lead to extinction (Miller et al., 2004). In order to avoid this unfortunate quirk several sex determining systems have arisen to balance the ratio of males and females in a population. These strategies can be divided into two main categories: environmental sex determination (ESD) and genetic sex determination (GSD).

1.2.1) Environmental Sex Determination (ESD)

Several species of fish and reptiles rely on environmental clues to determine the sex of individuals. The sex of offspring can be determined by pH, environmental temperature or even the existing male/female ratio in a population (Haqq and Donahoe, 1998). For example, the marine turtle *L. olivacea* is classified as having temperature-dependent sex determination (TSD). Turtle eggs incubated at temperatures of 26°C will all develop as males while eggs incubated at temperatures of 33°C will produce females (Moreno-Mendoza et al., 2001). Environmental sex determination is taken one step further in some fish species. In *C. citrinellum* sexual identity is conferred to an individual through social signals such as size and dominance (Francis and Barlow, 1993).

1.2.2) Genetic Sex Determination (GSD)

In birds, mammals and several types of insects sex is determined at conception by one or more genes. Much like ESD there are several strategies contained within this category. Within Hymenoptera insects, which include ants, wasps and honeybees, the sex of the individual is determined by the total chromosome number. *Drosophilae melanogaster* and *Caenorhabditis elegans* measure relative amounts of inherited sex chromosomes. Within birds, inheritance of sex Z or W chromosomes defines sexual identity. Within humans, and mammals in

general, sex is thought to be assigned at fertilization with the inheritance of either an X or Y chromosome. In general these animals are born either male or female and develop testis or ovaries to produce sperm or ovum respectively. While populations often follow these defined mechanisms it is not the rule in every case.

1.2.3) Honeybee and Complementary Sex Determination (CDS)

The reason for sexual reproduction in general has been explained by several theories. The two main and opposing theories are Muller's ratchet (Muller, 1964) and the Red Queen (Ridley, 1993). Muller's ratchet basically states that sex acts as a way to purge harmful mutations and genetic disease from the gene pool while the Red Queen states that sexual reproduction is a means to increase genetic diversity to offset evolving parasites and a changing environment. An excellent example of these two theories working in tandem can be seen in the insect order Hymenoptera. This species comprises over 200,000 species of ants, bees, wasps and sawflies (Bull, 1983; White, 1973) All members have haplodiploid sex determination: diploid individuals derived from fertilized eggs develop as females and haploid individual derived from unfertilized eggs develop as males.

One mode of sexual determination in Hymenoptera is termed single locus complimentary sex determination (sl-CDS). In 2003 it was determined that the primary gene regulating sexual development was CDS (Beye et al., 2003). Honeybees heterozygous at this allele develop as females while hemizygous haploid individuals are male. Occasionally, crosses that produce homozygote individuals for the sex determining genes can result in diploid males as well. Within honeybee populations homozygous diploid males are usually sterile and in most cases are killed and eaten by the workers (van Wilgenburg et al., 2006). This negative selection against homozygosity in diploid individuals ensures that neutral or beneficially rare alleles have a higher transmission rate as postulated in the Red Queen theory. At the same time haploid males with detrimental mutations will die because a functional gene does not mask the mutated gene. As stated in Muller's Ratchet hypothesis this gene will not be passed on and it will be purged from the gene pool.

1.2.4) X:A sex determination and pathway evolution

Two well-studied organisms highlight how different sex determining pathways can develop and differentiate. The nematode *Caenorhabditis elegans* and the fly *Drosophilae melanogaster* display sex determining mechanisms based on a ratio of sex chromosomes to autosomes (reviewed by Cline and Meyer, 1996). In both animals sex is determined by a set of dosage sensitive genes on the X chromosomes. These X signal elements (XSEs) transmit information relative to the amount of X chromosomes to autosomes in order to determine sex. In this manner a double dose of XSEs (two X chromosomes) will specify a female fate while a single dose (one X chromosome) specifies a male fate. In *C. elegans* the primary target of XSEs is the repression of XO lethal -1 (xol-1), while in *D. melanogaster* XSEs activate *sex lethal* (sxl). Both *xol-1* and *sxl* have dual roles: first to control gene dosage compensation and second to initiate sexual differentiation cascades through the gene *transformer*. Even though both proteins have similar functions the functional strategies differ greatly.

1.2.4.1) *C. elegans*

C. elegans uses a sex determining system that measures the ratio of X chromosomes to autosomes. The sexual determination mechanism of *C. elegans* has been studied in great detail (reviewed by Stothard and Pilgrim, 2003; Cline and Meyer, 1996). Worms with an X:A ratio of 0.75 or above will be hermaphrodites, producing both sperm and eggs, while worms with an X:A ratio of 0.67 or below will be males, producing only sperm (Madl and Herman, 1979). The major factor influenced by this ratio is the expression of the *transformer-1* gene (TRA-1). The suppression of this gene leads to the development of sperm and male phenotypes whereas the expression of this gene causes the development of oocytes and female sexual characteristics. Rather than being a short cascade, the X:A ratio does not directly control the expression of TRA-1.

The initial signals in this cascade consist of repressive XSEs and up regulating autosomal signal elements (ASE). The balance of repressive XSEs to positive ASEs determines if xol-1 is active in sufficient amounts to promote male development

(Powell et al., 2005). If xol-1 is inhibited (X:A=1) a group of sex and dosage compensation (*sdc-1*, *sdc-2* and *sdc-3*) and dosage compensation dumpy (*dpy*) genes are transcribed. The SDC proteins are thought to act together as complexes with DPY proteins to hypo-activate both X chromosomes and suppress the expression of hermaphrodite-1 (*her-1*). In the absence of *her-1* the intracellular tail of TRA-2 is cleaved by TRA-3. This creates an intracellular TRA-2 (TRA-2ic) ligand that binds and inhibits the FEM proteins. Inactive FEM allows TRA-1 to repress genes such as *egl-1* and *mab-3* that, in turn, promote male development.

In the presence of xol-1 (X:A=0.5), SDC-2 protein production is suppressed allowing the activation of *her*-1. When active, *her*-1 produces a ligand that binds and inhibits the cleavage of the transmembrane protein TRA-2. TRA-2ic production is suppressed thus FEMs are not inhibited. Within the male nematode FEM proteins suppress TRA-1 action that allows *egl-1* and *mab-1* to be expressed. For a more in depth review the reader is directed to a review by Goodwin and Ellis (2002) and Cline and Meyer (1996).

1.2.4.2) *D. melanogaster*

D. melanogaster also uses an X:A sex determining system. Again the major downstream gene affected by this ratio is *Tra*. However rather than using negative feedback loops *D. melanogaster* achieves *Tra* activation through titration and gene splicing (reviewed by Schütt and Nöthiger, 2000; Cline and Meyer, 1996).

The initial control of *Sxl* requires maternal factors, XSEs and ASEs. Maternal factors are those products passed from the mother to the embryo via the oocyte and include the product of genes such as *daughterless* (*da*), *hermaphrodite* (*her*), *groucho* (*gro*) and *extramacrochaetae* (*emc*). The known XSEs are three *sisterless* (*sisA, sisB* and *sisC*) genes and the gene *runt* (*run*) while the only known ASE is *deadpan* (*dpn*). In this system DPN works to prevent SIS proteins from forming heterodimers with DA. If there are more SIS proteins than DPN proteins, as in females, a DA-SIS heterodimer is formed which binds and activates an establishment promoter of *sxl* (sxl_{pe}) which in turn causes the production of a functional SXL protein. In males DPN successfully binds enough SIS proteins so that this promoter site is not activated.

In the absence of SXL the gene *male specific lethal-2* (*msl-2*) is expressed and works with three other *msl* genes to hyper activate the single X chromosome in the male. Within the female there is also some evidence that SXL directly represses the expression of several genes including *msl-2*. Regardless of the protein involved X chromosome gene dosage is balanced in males and females.

Slightly after the titration step *sxl* is activated in both sexes through a maintenance promoter. This promoter transcribes a non-functional mRNA, which needs posttranscriptional splicing to occur in order to produce a functional protein. In the female, the functional SXL produced earlier, by activation of sxl_{pe}, will promote the proper splicing of *sxl* mRNA leading to more functional SXL protein. These functional SXL proteins will regulate the proper production of more SXL proteins. In males the absence of functional SXL will lead to a non-functional SXL protein and *sxl* gene expression will be lost.

In *D. melanogaster, tra* is active in both sexes and produces a pre-mRNA that again needs to be spliced. In females, the functional SXL protein will bind to and help splice *tra* mRNA in order to give a functional protein. TRA along with TRA2 will bind to a sub optimal 3' site on the *doublesex* (*dsx*) gene promoter which causes a female form of DSX protein to be produced, DSXF. Along with *her* and *intersex* (*ix*), DSXF works to up-regulate female specific genes while repressing genes involved in male differentiation. In the absence of SXL, and therefore functional TRA, a different 3' binding site activates *dsx* and leads to the production of a male form of DSX (DSXM or *doublesex* male protein). This gene promotes the activation of male genes and represses female genes.

1.2.4.3) Networks

The SXL protein is well conserved between several species of fly including *Musca*, *Chrysomya*, *Megaselia*, *Ceratitis* and *Drosophila* but SXL does not seem to determine sex in any other species other than *Drosophila.* On the other hand, homologues of DSXM and DSXF do appear to follow a sex specific expression pattern in all of these species (Schütt and Nöthiger, 2000). Several experiments in *Drosophila* could possibly illustrate that this genetic network can be rearranged to

give several different sex determining systems (reviewed by Cline and Meyer, 1996; Schütt and Nöthiger, 2000).

XX embryos from *da* mutant mothers do not activate SXL and therefore die resulting in 100% male offspring from these mothers (Parkhurst et al., 1990). If the production of DA became dependent on nutritional cues this would allow the mother to preferentially produce male offspring in certain environmental conditions and female offspring in others. When msl-2 is ectopically expressed in heterozygotic msl-1 knockouts in Drosophila, male flies will not readjust X chromosome gene dosage in response to ectopically expressed *sxl.* Therefore *msl-2* becomes the genetic switch for dosage compensation freeing the fly from sex specific lethality. In this case a functional *sxl* product will act as a dominant female determining factor regardless of the X:A ratio. There are also several examples in the literature showing circumvention of the *sxl* sex determination within Drosophila due to interruption or improper expression of *tra-1* or *tra-2*. These range from the gain of function causing male to female sex reversal (McKeon et al., 1988), loss of function causing female to male sex reversal (Sturtevant, 1945) and even a temperature sensitive allele that causes female to male sex reversal when larvae are raised in temperatures at or above 29°C (Belote and Baker, 1982). While none of these examples are shown to be actual sex determining mechanisms in nature they do demonstrate that sex is not a result of one gene but of genes working together as a network. Furthermore genes that function in a certain way in one network may have a very different role in another network. This is something that should be kept in mind when studying the vertebrate sex determining systems.

1.2.4.4) Wilkins' Theory

The information gathered from the study of sexual determination in *Caenorhabditis elegans* has led to the theory that sex determining pathways evolve in a reverse stepwise fashion driven by frequency-dependent selection for the minority sex (Wilkins, 1995). The theory assumes that a primordial sex determination pathway would have been very simple, depending on one or two genes that in turn responded to environmental cues. These environmentally responsive elements would have

allowed for an optimal sex ratio to be achieved in the population. Either through diseases, which skew sex determination, such as PSR or *Wolbachia* (reviewed by Normark, 2002; Werren and Stouthamer, 2003), or because of mutations in key genes this balanced sex ratio, would have become skewed to favour one sex (i.e. females). In an attempt to re-equilibrate the sex ratio, alleles that promote the minority sex (males) would have been under positive selection pressure.

One strategy may be to suppress the gene causing the unbalanced phenotype, as seen in *Caenorhabditis elegans*. A separate strategy may be not to suppress the disease but mask it by increasing gene expression, as seen in *Drosophila melanogaster*. If these alleles aggressively promoted the minority sex they would themselves push the population towards the sex originally suppressed by the disease (male) causing positive selection for genes promoting the new minority sex (female). In either case this seesaw sex ratio would continue until an equilibrium mechanism is eventually achieved or the species goes extinct. The stepwise evolution of superimposed sex determining mechanisms may be a major confounding factor in unraveling the genetic cascade behind vertebrate sex determination systems and sex differentiation.

1.2.5) Sex Chromosomes

In birds, mammals and several other species sex is genetically determined at the time of fertilization through the inheritance of sex chromosomes. The sex chromosomes are distinct from autosomes in that they differ in size, number, staining characteristics and gene content when the two sexes are compared. Ohno's law asserts that heteromorphic sex chromosomes originated from an ancestral autosomal chromosome, which obtained a mutation that conferred a sexual advantage (Ohno, 1967). It has been hypothesized that chromosomal rearrangements, such as inversions of specific sex related genes and operons, suppressed recombination and fostered the accumulation of sex specific genes within the sex chromosomes. The differences in size often observed between the sex chromosomes have been caused by subsequent deletions. This degeneration has been demonstrated in the W chromosome of

Neognathan birds and the Y chromosome of mammals (Ansari et al., 1988; Pigozzi and Solari, 1997).

1.2.5.1) ZW/ZZ sex chromosomes

The ZW/ZZ sex determination system is found in birds and some insects. In the ZW system females are the heterogametic sex (ZW) while the males are the homogametic sex (ZZ). *Bombyx mori*, the commercial silk worm, is known to have a dominant female determining factor residing on the W chromosome and therefore the inheritance of the W chromosome drives female development (Goldsmith et al., 2005). On the other hand, within *Lepidoptera* (butterflies and moths) examples of ZO, ZZW and ZZWW females have been found suggesting that the W chromosome is not essential for female development in some species. Within birds the presence of a female determining gene on the W chromosome has not been found. Currently the most accepted hypothesis stipulates that a double dose of an unknown gene on the Z chromosome is needed for male development (reviewed by Clinton and Haines, 2001).

1.2.5.2) XY/XX sex chromosomes

Most mammals use the XX/XY sex determination system. In the XX/XY system sex is conferred through the inheritance of either homogametic chromosomes (XX) creating females or heterogametic chromosomes (XY) creating males. The existence of Klineffelter's males, in which two or more X chromosomes and one Y chromosome are inherited, show that it is the presence of the Y chromosome and not the dosage of the X chromosome that determines the entry into the male developmental pathway. In 1990 the Sex –Determining Region of the Y chromosome (*SRY*) gene was identified as a dominant gene found on the Y chromosome, which drives testes determination (Sinclair et al., 1990).

1.2.5.3) Platypus

Comparative mapping of genes from the vertebrate Z and X chromosomes showed that the two share no homology, implying that they are derived from different

autosomes (Nanda et al., 1999; Shetty et al., 1999). According to a review by Gruetzner et al. (2006) the ZW/ZZ and XY/XX sex chromosomes may be related. This review points to studies of the platypus, which contain ten sex chromosomes, five X and five Y. These sex chromosomes construct multivalent chains in male meiosis creating $X_1X_2X_3X_4X_5$ gametes and $Y_1Y_2Y_3Y_4Y_5$ gametes. Gene mapping experiments have revealed that X_5 at one end contains homologues of bird Z chromosome genes and the X_1 shows homologues of mammalian X chromosome genes. These observations have lead to several theories which suggest that both sex determination system may have evolved and diverged from a common source.

2) VERTEBRATE SEX DETERMINATION

2.1) Common Goals in Vertebrate Sex Determination

As mentioned earlier reptiles, bird and mammals have differing sex determination strategies including ESD, ZZ/ZW chromosomal and XX/XY chromosomal sex determination respectively. Despite these differing strategies there is a close anatomical relationship between the development of the genital ridge and the excretory system during early ontogeny of all vertebrates. In reptiles, birds and mammals, a mesodermal layer ventral to the somites differentiates into structures involved in excretion and reproduction. Gonadal organogenesis begins with the thickening of the coelomic epithelium on the medial aspect of the mesonephros in which the germ cells will eventually settle (Merchant-Larois et al., 1984). At this point it is impossible to morphologically distinguish males from females. The embryo and the genital ridge are said to be bi-potential, possessing structures and cell types that can differentiate into male or female phenotypes (Ito et al., 2006). In response to signals from the ovary or testis, the female reproductive tract system develops primarily from the paramesonephros (Müllerian) duct, and the male reproductive tract forms from the pronephros (Wolffian) duct, respectively (Grobstein, 1955). There are differences among species in how closely connected the structures are in regard to sharing ducts for secretion, but amongst teleost fish, reptiles, birds and mammals the mature testes contain Sertoli and Leydig cells in addition to germ cells, and the ovaries consist of thecal and granulosa cells surrounding the ovum (reviewed by

Blum, 1984). There is evidence of some conservation of genes involved in sexual differentiation among all of these creatures. While sex determination of fish, birds and reptiles has been and is currently being studied the largest amount of information has been found in mammals.

3) MAMMALIAN SEX DETERMINATION

3.1) Alfred Jost and the Testis Determining Factor

The studies done in the 1940's by Alfred Jost were the first to point towards a Testis Determining Factor and the Female Default Pathway in mammals. Jost showed evidence that rabbits castrated *in utero* before the time of sex determination developed female reproductive systems (Jost, 1947). Through this study Jost showed that the testicle in mammals drives development of the male reproductive tract. This study also became the biological basis behind the theory of the female default pathway. The female default pathway stipulated ovaries and female reproductive organs would develop only in the absence of a Y chromosome male determining gene(s). This in turn led to the hypothesis of the Testis Determining Factor (TDF), a dominant gene that actively drives testis development. This gene remained hypothetical until 1990, when Sex –Determining Region of the Y chromosome (*Sry*) was identified (Sinclair et al., 1990).

3.2) Testicles Drive Internal Change

Early in embryonic development it is impossible to distinguish male embryos from female embryos. In effect the embryo is bi-potential containing both Müllerian ducts as well as Wolffian ducts. Two hormones secreted by the testicle drive male development of the Wolffian ducts. Sertoli cells produce the hormone Müllerian Inhibiting Substance (MIS) also known as Anti-Müllerian Hormone (AMH) and Leydig cells produce testosterone. MIS is responsible for the regression of the Müllerian ducts while testosterone maintains and differentiates the Wolffian ducts as well as the external male genitalia (reviewed by Viger et al., 2005). Due to the action of testosterone and its derivatives the Wolffian ducts differentiate into the epididymis, vas deference and seminal vesicle of the male reproductive tract. As well,

testosterone helps to develop the penis and scrotum. In the absence of MIS and testosterone the Müllerian ducts are maintained while the Wolffian ducts degenerate. The effector causing the differentiation of the Müllerian ducts is not well known. It has been suggested that the estrogens produced by the placenta, the fetal ovaries or even that from maternal circulation plays a role in Müllerian duct differentiation (Langmand and Sadler, 1996). As well, the morphogen Wnt4 has been implicated in the development of the Müllerian ducts and the repression of testosterone production (Vaino et al., 1999). In either case, the Müllerian ducts contribute a large portion of the female reproductive tract, differentiating into the oviduct, uterus, cervix and upper part of the vagina. While these processes are themselves accompanied by several other changes throughout the body they are all dependent upon the determination of sex and the subsequent interaction of the primordial germ cells, support cells, steroidogenic cells and connective tissue cells.

3.3) An Indifferent Embryo and Bi-potential Gonads.

During vertebrate embryogenesis, the urogenital system derives from the intermediate mesoderm of the gastrula. The male reproductive tract forms from the pronephros (Wolffian) duct and the female reproductive tract system develops primarily from the paramesonephrotic (Müllerian) duct. In the mouse, the Wolffian duct is first formed from the intermediate mesoderm by embryonic day 9 (e9). Before the Wolffian duct reaches the cloaca, the mesonephros forms. A thickening of the coelomic epithelium on the ventral medial surface of the mesonephros is the first indication of the genital ridge (Grobstein, 1955; Brennan and Chapel, 2004; Viger et al., 2005). At or near the same time, germ cells migrate from the hindgut to begin to populate the genital ridge (McLaren, 2000). Shortly after the arrival of the germ cells in the genital ridge at e10.5, the cells of the coelomic epithelium of the genital ridge begin to actively divide and invade the underlying mesenchyme.

The Müllerian duct starts to form by invagination of the surface epithelium of the anterior mesonephros around e11.5 in the developing urogenital ridge. This epithelial invagination extends caudally along the Wolffian duct, laterally and then medially towards the cloaca (Gruenwald, 1941). Thus regardless of their genetic sex,

the embryos have both male and female reproductive tract precursors, before morphological signs of sexual differentiation occur.

In the mouse, around e12.0, the epithelial cells of the genital ridge multiply to form primitive sex cords. The primitive sex cords actively surround the germ cells while still staying in contact with the surface epithelium. At this stage it is still morphologically impossible to distinguish male from female gonads (Langmand and Sadler, 1996). The gonads now contain the four main precursor cell types needed to form either the testicle or ovary: The primordial germ cells (PGC), the support cells, the steroidogenic cells and the connective tissue cells. The PGCs migrate into the gonad surrounded by the bi-potential support cells (Karl and Capel, 1998). The mesenchymal cells will eventually differentiate into the connective cells and steroidogenic cells (Buehr et al., 1993; Martineau et al., 1997; Ito et al., 2006). Within the male these cell types will differentiate into pro-spermatogonia, Sertoli cells, peritubular myoid cells, endothelial cells and Leydig cells. Within the female they form ovocytes, granulosa cells, stromal cells and theca cells.

4) CELLS AND STRUCTURES OF THE GONAD

4.1) Germ Cells

Primordial germ cells (PGCs) are first detectable at e7.0 as a group of approximately 50 cells clustered at the posterior of the primitive streak in the extra-embryonic region. As the embryo develops to the pre-somite stage, the germ cells become a distinct group of cells distributed along the base of the vitelline vesicle (Hogan et al., 1994).

By e8.0 the PGCs begin to actively migrate through the hindgut to the genital ridge (Hogan et al., 1994). Around e10, germ cells begin to enter the genital ridge (McLaren, 2000) and continue to do so until e11.0 – e11.5 (Hogan et al,. 1994). Those that do not enter the genital ridge by this time are lost and eventually eliminated (Molyneaux et al., 2001). During this migration and colonization of the genital ridge, the germ cells will divide mitotically once every 16 hours resulting in close to 25 000 PGCs colonizing each gonad by e13.0. Within the male genital ridge the PGCs will be requisitioned to the testis cords which consist of pre-Sertoli cells

surrounded by peritubular myoid cells. Through interactions with Sertoli cells the PGCs will stop dividing at phase G1 of the mitotic cell cycle at about e12.5 (McLaren, 2000). Though they are essential for fertility they play no role in the development of the testis (McLaren, 2000; Adams and McLaren, 2002). Within the female genital ridge the PGCs enter meiosis in an anterior-to-posterior wave and are theorized to drive differentiation of the ovary (McLaren, 1984; Yao et al., 2003; Bullejos and Koopman, 2004).

4.2) The Ovary

In the absence of Sry, the bi-potential gonads enter the female differentiation pathway. In the mouse, the first recognizable morphological difference in the ovary, the entrance of germ cells into meiosis, is seen at e13.5 (Nordqvist, 1995). In the ovaries, the primitive sexual cords segment themselves into irregular cellular masses containing germ cells. At this point germ cells are termed gonocytes. These gonocyte nests are situated in the medullar region of the ovary and are later replaced by vascular stroma (Langmand and Sadler, 1996). In contrast to the testicle, the superficial epithelium of the ovary thickens and continues to proliferate finally creating a second generation of sexual cords, the cortical sex cords. These cords will penetrate into the underlying mesenchyme without separating from the coelomic epithelium (Langmand and Sadler, 1996). The cortical cords themselves segment into cellular masses containing one or more gonocytes that subsequently transform into ovogonia and drive the differentiation and organization of the follicular cells and structures (Langmand and Sadler, 1996).

In the absence of Sertoli cells, gonocytyes enter meiosis at e13.5 and stop development at prophase I. Most of these cells will recommence their meiotic division at the beginning of the ovarian cycle at puberty (McLaren, 2000). The meiotic germ cells antagonize certain pro-testicular events. Meiotic gonocytes are essential to follicular formation as cell migration and testicular cord formation can be induced in XX gonads before gonocytes enter meiosis but not afterwards. Once meiosis begins XX gonads loose their plasticity and are committed to an ovarian pathway (Tilmann and Capel, 1999; Yao et al., 2003). Gonocytes in the ovary enter

into meiosis in an anterior to posterior wave, parallel to genetic markers that define follicular cells suggesting an influence of meiotic germ cells on the support cell population (Bullejos and Koopman, 2004).

Little biologic or genetic information is available to explain ovarian development. The PGCs are needed for the differentiation of the follicular cells. In the absence of PGCs follicles fail to develop in the ovary (Huang et al., 1993). One gene expressed by the ovocytes, *Figα*, is necessary for the initial stages of folliculogenesis; the recruitment of the granulosa cells around the primordial follicle (Soyal et al., 2000). At the level of the somatic cells, *Foxl2* is necessary for the differentiation of the granulosa cells. Although *Foxl2* is not essential for ovary formation, granulosa cells of *Foxl2* KO mice are unable to become squamous cuboid cells and there is an arrest of folliculogenesis (Schmidt et al., 2004; Uda et al., 2004). For a long time *Dax1* was considered to be the ovary determining factor but the creation of a *Dax1* KO mouse demonstrated it was not essential to ovary formation (Yu et al., 1998a). A second gene, *Wnt4* has been shown to restrict migration of endothelial and steroidogenic cells in the XX gonad and also inhibits the formation of the coelomic vesicle in male gonads when expressed ectopically. One last gene, Follistatin (*Fst*), is necessary to prevent XY specific vascularization and also permits survival of meiotic germ cells in the ovary (Yao et al., 2004).

4.3) The Testicle

There is still no visible difference between the male and female gonads by day 11.0 even though *Sry* starts being expressed around e10.5. Not until e12.5, two days after the initial expression of *Sry*, do the testicular cords become evident in the male. By e13.5 the male gonad has doubled its size compared to the ovary of the same age and is morphologically far more complex. Four processes rely directly or indirectly on the expression of *Sry* and are specific to male testicular development: 1) cellular proliferation and formation of the testicular cords; 2) cellular migration; 3) testis specific vascularization; and 4) differentiation of the Leydig cells.

4.3.1) Cell Proliferation and Differentiation of Sertoli Cells

Quantitative studies on the mammalian embryo have shown that faster growth of the male gonad compared with that of the female can be detected before any histological differences are apparent (Lindh, 1961; Mittwoch et al., 1969). This has led to the suggestion that testicular differentiation is dependent on an accelerated rate of cell proliferation (Mittwoch et al., 1969; Schmahl and Capel, 2003; Hunt and Mittwoch, 1987). During the early phase of proliferation both Sertoli cells and interstitial cells (which include Fibroblast and Leydig cells) are thought to originate from a population of cells expressing Steroidogenic factor 1 in the coelomic epithelium (Karl and Capel, 1998). Schmahl et al. (2000) investigated *Sry*'s role in the initiation of gonad size increase using 5'-bromo-2'-deoxyuridine (BrdU) incorporation into dividing cells. They reported that cell proliferation in the coelomic epithelium increases in the XY gonad by e11.2 and these cells migrate into the gonad. Later studies showed that as these cells migrate, Steroidogenic factor 1 (*Sf1*) expression is eventually lost from the coelomic epithelium (Sekido et al., 2004). Sekido et al. (2004) theorized that after the coelomic epithelial cells migrate into the gonad, there is a decision to become either interstitial or supporting cells based on the expression level of *Sry*. Relative levels of this gene in these cells may determine their fate as Sertoli cells or interstitial cells.

Pre-Sertoli cells are considered to be the organizers of testis differentiation. Early studies by Burgoyne et al. (1988) using XX/XY chimeric mice showed that over 80% of the Sertoli cells are XY, whereas other lineages are composed more evenly of XX and XY cells. This shows a high bias for the Y chromosome and *Sry* in Sertoli cells, but the incorporation of roughly 10% XX containing cells indicate the existence of a paracrine signaling system that is able to recruit cells lacking *Sry* expression to a Sertoli cell fate (Palmer and Burgoyne, 1991).

4.3.2) Migrating Cells

In 1993, Buehr, Gu and McLaren performed an *in virto* tissue incubation experiment, which placed a membrane between the mesonephros and male gonad. While this filter allowed cell signals to pass through, cell migration was effectively

stopped. This filter effectively blocked the formation of the testis cords demonstrating the importance of migrating cells for the formation of the testicle. Later studies found that *Sry* initiates these migration signals. Studies with XY gonads that do not express *Sry* showed no cell migration, while XX gonads expressing *Sry* do migrate (Capel et al., 1999). Several morphogens have been shown to aid in this migration process including TGFβs, NT3 and HGF (Cupp et al., 2000; 2003; Ricci et al., 1999, 2002; Ross et al., 2003). The only inhibitor of migration seems to be caused by meiotic germ cells (Tilmann and Capel, 1999). The role of meiotic germ cells in blocking cell migration is shown in PGC ablated XX genital ridges. Studied have shown that cell migration can be observed in these genital ridges (Yao et al., 2003).

Migration of mesonephric cells occurs in the XY gonad between e11.5-e16.5 (Martineau et al., 1997). In 1997, Martineau et al. characterized the cells migrating into the testis cords and concluded through morphology that they consist of peritubular myoid cells, endothelial cells and perivascular cells. In 2001, Nishino et al., managed to separate and characterize the cells migrating into the testis. The migrating cells contained three cell forms: a sharp cell form, a cluster-forming cell and a round cell form. Cell Culture studies showed that the cluster-forming cells readily differentiated into the round cell form and expressed 3β-HSD. From these observations it was predicted that the sharp cell form were precursor cells for peritubular myoid cells and the other two cell types corresponded to Leydig cell precusors. Brennan et al. (2003) used a marker to label the coelomic epithelium of the gonads between e11.5 – e12.0 and found that many of the migrating cells were *Sf1* negative (85%) while only 3% exhibited high levels of *Sf1* expression. They concluded that this would not represent a major source for the fetal Leydig cell lineage. While the origins of fetal Leydig cells is still under debate the importance of migrating cells to the testicular vasculature is not.

4.3.3) Vasculature

As with other processes in the embryo, initially the vasculature of the gonad is identical in both sexes. Differences in the male vasculature begin to arise around

e11.0 after the expression of *Sry* and the initiation of cell migration into the testes. Within the ovary vasculature will be created using cells already present in the organ. On the other hand, vasculature of the testes is dependent upon migration of cells from the mesonephros. In fact, the coelomic vessel, a large blood vessel situated on the surface of the testicle, is comprised almost exclusively of epithelial cells that have migrated into the testis. The early growth of the coelomic vessel is important for the masculinization of the embryo. Rather than simply bringing nutrients to a rapidly growing organ the vessel is also indispensable for delivering testosterone and other masculinization signals to the rest of the embryo (Brennan and Capel, 2004).

4.3.4) Leydig cells

In the mouse, the Leydig cells appear in the interstitial tissue around e12.5 through the differentiation of mesenchymal-like stem cells (Byskov, 1986). While adult Leydig cells do produce testosterone, fetal Leydig cells lack the enzyme 17β-hydroxysteroid dehydrogenase type 3 (HSD17β3) and therefore secrete androstenedione which is in turn converted to testosterone by the pre-Sertoli cells in the testis cords which express HSD17β3 (O'Shaughnessy et al., 2000). This testosterone ensures the masculinization of embryo. Several androgens as well as Insulin-like growth factor 3 (Insl3) are secreted by fetal Leydig cells that are important for the decent of the testis in some mammals (Kitamura et al., 2001).

The origins of fetal Leydig cells remain uncertain but it has been proposed that the steroid-secreting cells of the gonad and adrenal gland share a common progenitor. Both of these cell populations are thought to arise from *Sf1* expressing cells found at the cranial end of the mesonephros (Hatano et al., 1996). Several studies have suggested that Leydig cells arise from the migratory cell population (Nishino et al., 2001; Merchant-Larios and Moreno-Mendoza, 1998) but other studies dispute these origins. Studies by Merchant-Larios et al. (1993) have shown that if the mesonephros is the origin of fetal Leydig cells they must enter the gonad before e11.5 as removing the mesonephros after that time does not effect fetal Leydig development. Another study using the Desert Hedgehog inhibitor forskolin raised the

possibility that the Leydig cell population does not originate from the migratory cell population (Yao and Capel, 2002). This study showed that while migration of mesonephric cells was blocked by forskolin, fetal Leydig cell differentiation could be ectopically activated. The authors predicted that fetal Leydig cells originate from other sources such as the coelomic epithelium of the gonad or that fetal Leydig cell precursors migrate into the gonad before e11.25.

5) GENES IN MAMMALIAN SEX DETERMINATION

5.1) Methods used to Identify Genes in Sex Determination and Differentiation

Studying human patients or transgenic mouse knockout models displaying sex inversion or gonadal irregularities have permitted the discovery of several genes important in sex determination. These include transcription factors (*Sry, Sf1, Wt1, Pod1, Lhx9, Gata4, M33, Sox8* and *Sox9*) and cell signaling molecules (*Wnt4, TFGβs, Fgf9, Dhh, Pdgf, PDG2, Ir, Irr*) (reviewed by McLaren, 2000; Brennan and Capel, 2004; Ross and Capel, 2005). Several laboratories have employed subtractive screens or microarrays to search for sexually dimorphic gene expression between whole male and female embryonic mouse gonads (Bowles et al., 2000a; Grimmond et al., 2000; Wertz and Herrmann, 2000; Menke and Page, 2002; Smith et al., 2003; McClive et al., 2003; Small et al., 2005). Recently, a few groups have developed mice expressing fluorescent protein driven by various lengths of the *Sf1* or *Sry* promoter which have been used to compare gene expression of cell types in the genital ridge around the time of sex determination (Boyer et al., 2004; Nef et al., 2005; Beverdam and Koopman, 2006; Bouma et al., 2006). We will now review sex determination genes starting with transcription factors.

5.2) Transcription Factors

Transcription factors are proteins that regulate the activation of transcription in the eukaryotic nucleus. This is accomplished either through direct binding to specific promoter and enhancer sequence elements in the DNA or by creating complexes with other DNA bound proteins. The regulation of gene expression by

transcription factors is a highly complex process that often involves several transcription factors and specific DNA sequences, DNA structure and higher chromatin order.

5.2.1) SRY Function and Expression

With few exceptions, *Sry* is the testis-determining factor in mammals. The demonstration in 1991 that the addition of *Sry*, as a transgene, could cause XX mice to develop into males confirmed the involvement of this gene in male sex determination (Koopman et al., 1991). Further support for *Sry* being the sex-determining factor in humans came after the identification of several human XY sex reversals involving mutations of this gene (Schafer and Goodfellow, 1996). Since the discovery of *Sry*, over fifteen years ago, the mechanism of its action has been studied but remains largely unknown and somewhat debated.

Regardless of its action, *Sry* is transiently activated around e10.0 in the supporting cell lineage (Sekido et al., 2004; Albrecht and Eicher, 2001; Wilhelm et al., 2005; Hacker et al., 1995; Bullejo and Koopman, 2001). *Sry* is first seen in a small population in the center of the genital ridge. Either due to paracrine signaling or cell proliferation the number of cells expressing *Sry* increases, expanding from the center towards the poles until the most of the Sertoli cells express *Sry* (Bullejos and Koopman, 2001; Wilhelm et al., 2005). Within the mouse this will be followed by the reduction and loss of *Sry* expression by e12.5. In this regard, the mouse may be the exception as other reports note continued expression in the pig, sheep and human (Daneau et al., 1996; Payen et al., 1996; Hanley et al., 2000).

5.2.1.1) *Sry* the Transcription Factor

Structurally *Sry* is a single, or in some marsupials a double, exon gene containing a conserved DNA binding high mobility group (HMG) box (O'Neill and O' Neill, 1999). Several genes, including *Sry*-like HMG-box protein 9 (*Sox9*), display possible *Sry* binding sites (Sekido et al., 2004). *Sox9* has been shown to co-localize with *Sry* in the nucleus of Sertoli cell precursors as early as e11.5 (Sekido et al., 2004) consistent with the hypothesis that *Sox9* is a direct target of *Sry*. Though

several other putative SRY binding sites have been found *in vitro*, features that characterize active SRY binding sites *in vivo* are unknown. This may be further frustrated as *Sry* may have very few direct targets *in vivo*. It has been suggested that the male determining cascade may grow through the action of one or more secondary genes (Canning and Lovell-Badge, 2002).

5.2.1.2) Sry the RNA Splicing Factor

One study done by Ohe et al. (2002) has suggested a role for Sry as an RNA splicing factor. A splicing factor is a class of molecules usually, RNA or proteins, which modify mRNA post transcriptionally. When Sox6 was immunodepleted from HeLa nuclear extracts there was a significant reduction in splicing activity at the first step of the splicing reaction. When recombinant GST-SOX6, GST-SRY or GST-SOX9 HMG were added to SOX6 depleted HeLa cell nuclear extracts splicing activity was restored. Furthermore this study showed evidence that both SOX6 and SRY are functionally co-localized with splicing factors SC-35 in the nucleus of HeLa and NT2/D1 human embryonic carcinoma cells.

One hypothesis published by Lalli et al. (2003) has suggested that Sry may influence sex determination through sex specific splicing of mRNA similar to the action of *sxl* in *Drosophila.* Within this hypothesis similarities between SRY HMG domain and the RNA binding domain of hepatitis delta small antigen is pointed out by Veretnik and Gribskov (1999). As well this study mentions unpublished data that confirms SRY and SOX6 can bind RNA.

5.2.1.3) *Sry* and Higher Chromatin Order

Sry has been theorized to act as a chromatin remodeling protein. *In vitro* identification and characterization of the Kruppel-associated box only (*Krab-O*) protein as an *Sry*-interacting protein has provided experimental evidence supporting a model for *Sry* functioning as a chromatin-remodeling agent. Sry may recruit the *Krab-Kap1* (*Krab*-associating protein 1) complex as a chromatin modulator (Oh and Lau, 2006). It was shown that the mouse *Sry-Krab-Kap1* complexes with heterochromatin protein 1 (*Hp1*), suggesting that mouse *Sry* could use the *Krab-*

Kap1-Hp1 organized transcriptional regulatory complex to regulate downstream target genes through histone modification (Oh et al., 2005). At least one study debates the role of *Sry* as a chromatin-remodeling agent. Mizukami et al. (2004) incubated e11.5 genital ridges in Trichostatin A, a histone deacetylase inhibitor, at several different concentrations for three days to see the effect of inhibiting chromatin remodeling during sexual determination. This study found that Trichostatin A affects the development of germ cells, but it did not affect sex determination (Mizukami et al., 2004).

5.2.1.4) Repressor of a Repressor

The "Z" gene hypothesis postulates that an as yet unidentified gene is expressed in the XX gonad that represses the testis pathway and allows ovarian development to occur (McElreavey et al., 1993). Alternatively this hypothesis states that *Sry*, the male determining gene acts as an inhibitor of an inhibitor by repressing this gene. When mutated or inactivated the "Z" gene would result in the masculinization of the XX gonad with female to male sex reversal. Support for this theory is seen in the XX sex reversal in goats with Polled/intersex syndrome (*Pis*) (Vaiman et al., 1996). In accordance with the double-inhibition model, the *Pis* mutation affects primarily the support cells of the gonads. Deletions in the *Pis* region effected the transcription of the protein *Foxl2* and two non-coding genes, *Pisrti* and *Pfoxic* (Pailhoux et al., 2001; Pannetier et al., 2005). Though studies on *Pisrti* have shown it is unlikely to be involved in sex determination both *Foxl2* and *Pfoxic* are thought to play a role in the female pathway, as will be discussed below (Loffler et al., 2005; Pannetier et al., 2003).

5.2.1.5) Odsex Mice

Information involving Odsex (Ods) mice lends credence to the idea that *Sry* antagonizes a repressor protein. When mice carry a 150-kb deletion and transgene insertional mutation, approximately 1 Mb upstream of *Sox9*, XX mice develop as sterile XX males in the absence *Sry*. It is thought that this section of DNA represents a long-range, gonad specific repressor regulatory element that down regulates *Sox9* in

the ovary (Bishop et al., 2000) This was brought into question when data collected later by the same group supported the idea that the *Dct* promoter in the transgene could be modifying *Sox9* expression over a distance of 980 kb in *Ods* mice (Qin et al., 2004).

5.2.2) SRY-related HMG box 9 (Sox9)

The most studied of the Sox genes in sex determination is Sox9. During gonadogenesis of the mouse, *Sox9* is initially expressed in both sexes, with expression decreasing in the developing ovary and strongly increasing in the developing testis, concomitant with the peak of *Sry* expression at e11.5. As mentioned earlier *Sry* disappears after e12.5 but *Sox9* on the other hand is continuously expressed (Morais da Silva et al., 1996; Kent et al., 1996). Regulation of *Sox9* is thought to be initiated by Sry, but new data shows that continued expression is dependent on Wt1 action (Canning and Lovell-Badge, 2002; Gao et al., 2006). *In vivo* and *in vitro* studies have shown Sox9 is essential for the expression of several genes including *Amh*, *Sf1*, and *Vnn1* (de Santa Barbara et al., 1998; Arango et al., 1999; Shen and Ingraham, 2002).

The genital ridge is sensitive to the level of *Sox9* expression. In mice and humans, extra copies of the *Sox9* gene have been associated with XX sex reversal (Vidal et al., 2001; Huang et al., 1999). Even blocking nuclear export of *Sox9* in normally functioning XX genital ridges with leptomycin B can activate *Amh* and cause sex reversal (Gasca et al., 2002). On the other hand, haploinsufficiency (the loss of one of two functioning alleles) of *Sox9*, in humans, causes a sever bone disease, campomelic dysplasia (CD), that is associated with female development of XY individuals in 75% of the cases (Houston et al., 1983). While many of the genetic lesions that cause this disease have been identified in the open reading frame of *Sox9*, several breakpoints found between 50kb to 950kb upstream of *Sox9* have also been associated with CD XY sex reversal cases (Pfeifer et al., 1999). One study has shown that mice have a higher tolerance to *Sox9* haploinsufficiency compared to humans, as they do not develop abnormal phenotypes. The authors attribute this to the overlapping function of *Sox8* in mice (Chaboissier et al., 2004). On the farthest end of

the spectrum, tissue specific knockouts of *Sox9* in the gonads of mice do not show any signs of male differentiation. These mice lack expression of testis-specific markers, such as AMH and steroidogenic side chain cleavage (*Scc*) genes, and instead express the female-specific markers *Wnt 4, Bmp2*, *Fst* and *Foxl2* even in the presence of *Sry* expression (Barrionuevo et al., 2006; Chaboissier et al., 2004). Furthermore Sox9 may need to be expressed in a threshold number of cells within the testes. If proliferation of the coelomic epithelium is inhibited during an eight-hour period around e11.0, *Sox9* expression will be blocked and testis cords will fail to develop (Schmahl and Capel, 2003).

5.2.2.1) Redundancy of E box Sox Family and Sry

Sry, *Sox8, Sox9* and *Sox10* are all present in the genital ridge during sex determination in a testis specific manner (Sock et al., 2001; Chaboisser et al., 2004; Barrionuevo et al., 2006; Beverdam and Koopman, 2006; Nef et al., 2005). Members of the E Sox proteins, Sox8, Sox9 and Sox10, are thought to have evolved from a common ancestry (Bowles et al., 2000b; Wegner, 1999) and studies have shown significant functional redundancy between them (Bylund et al., 2003; Cheung and Briscoe, 2003; Graham et al., 2003; Taylor and LaBonne, 2005; Kellerer et al., 2006). *Sox8* and *Sox9* may be directly up regulated by Sry (Sekido et al., 2004) to influence male sex determination and Sertoli cell differentiation (Barrionuevo et al., 2006; Chaboisser et al., 2004; Sock et al., 2001; Takada and Koopman, 2003). *Sox9* has also been shown to completely replace the function of *Sry* in Ods mice (Qin and Bishop, 2005) and the HMG motif of *Sox9* can functionally substitute for *Sry* when expressed as an *Sry/Sox9* transgene (Bergstrom et al., 2000). Several studies also show that Sox8 and Sox10 have limited functional redundancy (Kellerer et al., 2006; Maka et al., 2005; Stolt et al., 2004) and ectopic expression of Sox9 will induce endogenous Sox10 expression in some cell types (Chueng and Briscoe, 2003). While Sox8 and Sox10 have not been shown to be essential to sex determination they may aid in tipping the balance towards male determination through their redundant action (Chaboissier et al., 2004).

Before the expression of SRY, loss-of-function mutations in a number of other transcription factors result in the degeneration of gonads before sex determination, suggesting that these proteins are required for the specification and maintenance of the gonadal expression. *M33, Emx2, Odd1, Pod1, Lhx1, Lhx9, Wt1*, and *Sf1* have been shown to play important roles both in the development of other organs and in several stages of gonadal differentiation. Mutations in these genes cause gonadal dysgenesis (or agenesis), and embryos develop a female phenotype regardless of chromosomal sex.

5.2.3) Chromobox homolog 2 (M33)

Polycomb genes in *Drosophila* maintain the repressed state of certain regulated genes by mediating changes in higher-order chromatin structure. Knockout mice for *M33*, a mouse homologue of Polycomb, were created and many exhibited male-to-female sex reversal though development was delayed in both XX and XY gonads. It was theorized that *M33* caused sex reversals by interfering with steps upstream of *Sry* (Katoh-Fukui et al., 1998).

5.2.4) Empty Spiracles 2 (Emx2)

The gene *Empty Spiracles 2* (*Emx2*) is the mammalian homologue of *Drosophila* gene empty spiracles (*ems*). *Emx2* null (*-/-*) mouse embryos appear normal until e11.5, when abnormal degeneration of the Wolffian ducts commences and the ceolomic epithelium fails to thicken where the gonadal ridge should form. By e13.5, Müllerian ducts also fail to develop. Finally, in *Emx2* mutant mice, the kidneys, ureters, gonads and genital tracts are completely absent, while the bladder and adrenal glands remain (Miyamoto et al., 1997).

Emx2 is first expressed in the urogenital system around e9.0 in the pronephric primordium. This is thought to be the earliest stage of urogenital differentiation from the intermediate mesoderm. *Emx2* is subsequently expressed in the mesonephros and the coelomic epithelium covering the genital ridges by e10.0 Expression is seen within the bi-potential gonad by e11.0 and persists in both XX and XY gonads after sex determination (Pellegrini et al., 1997). The Emx2 promoter has binding sites for

Gli3, *Tcf* and *Smad* proteins that are responsive to *Bmp* and *Wnt* signaling pathways (Theil et al., 2002).

5.2.5) Odd-skipped related 1 (Odd1)

Odd1 is first transcribed in the nascent intermediate mesoderm shortly after its migration from the primitive streak at the midgastrulation stage (e7.5). Knock out mice suggest that Odd1 is essential for the differentiation of the intermediate mesoderm. Homologous *Odd1* null mice exhibit complete agenesis of the adrenal glands, metanephric kidneys and gonads via massive apoptosis by e10.5 (Wang et al., 2005). Odd1 is proposed to be a regulator of several genes including *Lhx1*, *Pax2*, and *Wt1* (Wang et al., 2005).

5.2.6) Podocyte-Expressed 1 (Pod1)

Using a lacZ reporter knocked into the Podocyte-Expressed 1 (Pod1/Tcf21/capsulin) gene, putative expression patterns have been deduced for e11.5 and e12.5 mouse embryos. At e11.5, *Pod1* expression is observed in both XY and XX urogenital ridges localized primarily to the ceolomic epithelium of the gonad, and to the boundary region between the gonad and mesonephros. This same expression pattern was seen in e12.5 embryos with slightly increased expression in the male. Homozygote embryos, which are functional knockouts of this gene displayed an XY sex reversal, being phenotypically female. By e18.5 testes not only failed to descend but most were fused to the adrenals. Testes were significantly smaller and had an irregular shape. Ovaries on the left side were also often fused to the adrenals and had an irregular shape. Evidence also showed that *Sf1* was up regulated leading to ectopic steroidogenic cells in both the male and female genital ridges (Cui et al., 2004).

5.2.7) LIM homeobox gene 1 (Lim1)

LIM homeobox gene 1 (*Lim1* or *Lhx1*) is expressed in the intermediate mesoderm and lacZ mouse knockin models have shown expression as early as e7.5. Later on *Lim1* expression is also detected in the fetal gonad (Nagamine et al., 1999;

Birk et al., 2000; Bouchard et al., 2002). Expression is later restricted to the nephric duct system of the mesonephros and arises cell-autonomously in the developing Müllerian ducts (Kobayashi et al., 2004; Tsang et al., 2000). Mutations in *Lim1* result in a loss both of kidneys and gonads. It is shown that *Lim1* activity is essential for tissue differentiation, possibly by activating *Pax2*, *Hoxb6* genes and/or Wnt4 signaling activity (Tsang et al., 2000; Kobayashi et al., 2004).

5.2.8) LIM homeobox gene 9 (Lhx9)

LIM homeobox gene 9 (*Lhx9*) is one of the first genes present in the urogenital ridge. *Gata4* and *Lhx9* are co-expressed in a small area of the coelomic epithelium on the ventromedial aspect of the mesonephros specifically indicating the area of the presumptive genital ridge (McCoard et al., 2001). Mice lacking *Lhx9* were found to have a female phenotype and lacked gonads as well as sex steroid production (Birk et al., 2000; Wilhelm and Englert, 2002). One study done by Wilhelm et al. (2002) has shown the importance of Lhx9 binding to the *Sf1* promoter for proper the regulation of this nuclear receptor.

5.2.9) Wilm's tumor 1 (Wt1)

Wilm's tumor 1 (WT1) is known to be present in the genital ridge before and after sex determination in most species studied (Kent et al., 1995). Homozygous knockout of this gene in mice leads to an neonatal lethal phenotype as the ureteric buds in the kidney fail to develop (Kreidberg et al., 1993). Wt1 is also required for the survival and proliferation of cells in the genital ridge. In Wt1-/- mice the genital ridge is present at e10.5 but fails to thicken and gonads are undetectable by e14.5 (Kreidberg et al., 1993). Also, conditional knockouts of Wt1 after the time of testis determination show Wt1 may be an important regulator of the Sox genes. Male AMH-Cre/Wt1-floxed mice display testicular cord formation and a normal Leydig cell count but display persistent Müllerian duct syndrome and a complete lack of Sox9 and Sox8 expression (Gao et al., 2006).

In mammals, *Wt1* encodes a zinc finger protein with 24 different isoforms, and at least two of these are important in gonadogenesis. Alternative splicing of

Wilm's Tumor 1 can result in the inclusion or exclusion of a lysine-threonine-serine at the end of the third zinc finger, creating a +KTS isoform or a –KTS, isoform respectively. Both isoforms of *Wt1* must be present, at proper levels, for gonad formation as was shown by Hammes et al. in 2001. Knockouts of +KTS isoform resulted in mice with Fraiser Syndrome phenotypes (defective kidneys and male sex reversal) while knockouts of the –KTS isoform resulted in underdeveloped gonads and small kidneys. The WT1-KTS isoforme has been shown to bind and transactivate the *Sry*, *Dax1* and *Sf1* promoters in mammals (Kim et al., 1999; Hossain and Sanders, 2001; Wilhelm and Englert, 2002).

5.2.10) The NR5A proteins

5.2.10.1) Steroidogenic Factor 1 (Sf1)

Steroidogenic Factor 1 (*Sf1/NR5A1/AdBp4*) has been described as the master control gene of steroidogenic pathways and affects every level of the hypothalamus-pituitary-steroidogenic organ axis (reviewed by Parker and Schimmer, 1997). SF1 has been shown to be the key mediator of cytochrome P450 steroid hydroxylases, Müllerian inhibiting substance, lutenizing hormone and follicle stimulating hormone all of which are essential in sex determination and differentiation (reviewed by Achermann et al., 2001; Parker and Schimmer, 1997). Within the urogenital ridge cells expressing Sf1 early in development will differentiate into adrenal cells, Sertoli cells (in the male) and granulosa cells (in the female) (Albrecht and Eicher, 2001; reviewed by Brennan and Capel, 2004). *Sf1* may regulate survival and/or proliferation of bi-potential gonads as null mutations of *Sf1* result in gonad and adrenal dysgenesis.

Sf1 is a nuclear receptor that preferentially binds to the Sf1 response element (AAGGTCA) in the promoter region of several genes. Studies have shown phospholipids may act as ligands for Sf1 though there is debate as to where different phospholipids elect different responses *in vivo* (reviewed by Forman, 2005; Urs et al., 2006). Protein-protein interactions can also mediate the action of this transcription factor. Interactions between GATA4-SF1, WT1-SF1 and DAX1-SF1 are known to be important during the regulation of Anti Müllerian Hormone (Amh) (Tremblay and

Viger, 2001; Nachtigal et al., 1998). Also, β-catenin may aid Sf1 in the recruitment of other proteins when it is bound to steroid enzyme promoters (Jordan et al., 2003).

Mutations in SF1 have been reported in at least three patients, two individuals with a 46,XY genotype, apparent sex reversal and adrenal failure (Achermann et al., 1999; Achermann et al., 2002) and a 46,XX genotype individual with primary adrenal failure and a normal female reproductive tract (Biason-Lauber and Schoenle, 2000). SF1 null mice exhibit similar symptoms to those displayed by humans. Homozygous knockout mice die by postnatal day 8 most likely due to adrenal cortical insufficiency. These mice lack both adrenals and gonads and present with female reproductive systems (Luo et al., 2000). Haploinusfficiency in humans is thought to cause gonadal agenesis but allow normal adrenal function (Correa et al., 2004; Mallet et al., 2004), while SF1 halpoinsufficient mice exhibit adrenal insufficiency and small testes (Bland et al., 2000). Studies on haploisufficient mice and men show the exquisite sensitivity of Sf1 dosage effects during development.

SF1 expression in the mouse is first detected at embryonic day 9 (e9) in the urogenital ridge. Sf1 transcription continues in the male gonad there after. Expression disappears between day e13.5 and e16.5 in the female and reappears after day e18.5 until birth (Ikeda et al., 1994). In the male SF1 regulates expression of MIS within Sertoli cells and later controls steroid production within Leydig cells (Parker and Schimmer, 1997; Shen et al., 1994). SF1 also appears in adrenal cortical cells, hypothalamus and pituitary (Ingraham et al., 1994; Ikeda et al., 1994). Within the adult ovary SF1 is expressed in the granulosa and thecal cells at the beginning of folliculogenesis (Takayama et al., 1995).

5.2.10.2) Liver Receptor Homolog-1 (*LRH1*; *NR5A2*)

The liver receptor homolog-1 (*LRH1*; *NR5A2*) and steroidogenic factor-1 (*SF1*; *NR5A1*) are two members of the Ftz-F1 subfamily of nuclear receptors. *Lrh1* is expressed in tissues derived from endoderm, including intestine, liver and exocrine pancreas, as well as in the ovary. In these tissues, *Lrh1* plays a role in cholesterol metabolism and steroid synthesis. While some data does point toward a □xpressio

role of these two proteins (Siriannia et al., 2002) the severe phenotype encountered with *Sf1* knockout mice indicates that *Lrh1* cannot compensate for the *Sf1* during the sexual determination and development. In addition, preliminary studies demonstrate that mice homozygous for a germline mutation in *Lrh1* die before e7.5 therefore conditional knock-out will be needed to understand the true role of *Lrh1* in sex determination and dofferentation (Labelle-Dumais et al., 2006)

5.2.11) NR0B family

Dax-1 and SHP are two proteins wich make up the NR0B family of nuclear receptors. These two proteins are unique as they contain a N-terminal domain consisting of alinine/glycine-rich repeats of a novel 65-70 amino acid motif. Unlike other members of the nuclear receptor family these two protieins contain no DNA binding domain but instead contain a LXXL motif known as nuclear receptor boxes. Through these nuclear receptor boxes it is though that the NR0B family of proteins can bind to AF-2 domains of other nuclear receptors. The majority of information collected to date points toward a transcriptionally repressive function for these proteins that is achieved through these direct protein-protein interactions (reviewed by Båvner et al., 2005).

5.2.11.1) Dosage sensitive sex reversal-Adrenal hypoplasia critical region on the X chromosome (DAX1/NR0B1)

Dax1 is X linked in mice and humans (Zanaria et al., 1994; Swain et al., 1998) and autosomal in marsupials (Pask et al., 1997). Much like SF1, Dax1 is expressed at every level of the hypothamalic-pituatary-adrenal-gonadal axis (HPAG) but rather than being an activator of genes Dax1 mainly acts to repress gene function. Within the testis Dax1 is strongly expressed within the Sertoli cells until e12.5 after which expression is lost. It is later expressed within the Leydig cell population between e13.5 to e17.5. Expression in the ovary of Dax1 is seen between e 12.5 to e14.5 (Ikeda et al., 2001; Parker and Schimmer, 1997).

Dax1 knockouts in mice and mutations in humans present with similar phenotypes. Aplasia of the gonads and adrenals accompanied by defects in the

pituitary and hypothalamus are described in both adrenal hypoplasia □xpression (AHC) patients and knockout mice (Parker and Schimmer, 1997). Experiments by Meeks et al. (2003a) have shown that Dax-1 null mice have defects in the testis cords due to decreases in the peritubular myoid cell population. This ultimately leads to open and incompletely formed testis cords as well as ectopic Leydig cells (Meeks et al., 2003b). Cell specific Dax1 rescue showed that expression is needed in both Sertoli cells and Leydig cells as rescue of only one cell type will still result in abnormal testis morphology (Jeffs et al., 2001a; Meeks et al., 2003c).

Dax-1 has been proposed to be a male differentiation inhibitor *in vivo* (Swain et al., 1998), acting as a co-repressor in the female gonad by forming a heterodimer with *Sf1*. This *Sf1-Dax1* heterodimer works by repressing downstream genes in the testis determination cascade such as *Sox9* expression, Amh production and steroid production. *Sry* is theorized to change the conformation of the Sf1-Dax1 heterodimer to an inactive form therefore allowing the expression of these genes (Swain et al., 1998; Muscatelli et al., 1994; Zanaria et al., 1994). Data supporting the role of *DAX1* as a female determining gene is seen in XY female patients with a duplicated 160kb region of Xp21 that contains *Dax1* (Bardoni et al., 1994) and over expression of Dax1 in mice with hypoactive Sry (Swain et al., 1998). Even though some data does show that Dax1 is antagonistic to Sf1 action *in vivo* (Tremblay and Viger, 2001; Nachtigal et al., 1998), knockout studies in mice indicate that *Dax1* is not required for normal ovarian development (Yu et al., 1998b). On the other hand, *Dax1* is critical for testicular development. As mentioned above, null mutation of *Dax1* cause severe testicular dysgenesis (Jeffs et al., 2001a; 2001b; Meeks et al., 2003a, 2003b) also when the *Dax1* null mouse is crossed to a Mus domesticus poschiavinus, Y pos strain (which carries a hypomorphic *Sry* allele), a complete male to female sex reversal arises (Meeks et al., 2003a, 2003b). This evidence suggests that *Dax1* may actually work slightly down stream of *Sry* to promote testis development.

5.2.11.2) Small Heterodimer Protein (SHP)

Another member of the Nr0b family is SHP. *In vitro* studies have demonstrated that SHP inertacts with approximately half of all mammalian nuclear receptor proteins but

it has a preferential interaction with LRH1. While the LXXLL motif contained in the SHP protein has potentially 36 different NR targets the preferential binding of LHR1 does hint towards protein specification. The majority of research into SHP has focused on its' role in the liver and pancrease but recently in vitro work has displayed this proteins ability to form heterodimers with DAX1. The significance of this protein has yet to be shown in sex determination (reviewed by Båvner et al., 2005).

5.2.12) GATA Proteins

In mammals, the GATA family is composed of 6 members (GATA 1-6) (Lowery and Atchley, 2000). All of these GATA factors contain two highly conserved zinc finger domains that bind with high affinity to the DNA consensus domain WGATAR and closely related sequences (Lowery and Atchley, 2000; Molkentin, 2000). Since the GATA family members are involved in the development of several systems and organs, knockouts of GATA 1, 2, 3, 4, and 6 result in embryonic mortality (Lowery and Atchley, 2000; Ohneda and Yamamoto, 2002; Cantor and Orkin, 2002; Patient and McGhee, 2002; Molkentin, 2000; Molkentin et al., 2000; Morrisey et al; 1996). Within the developing gonad Gata 1, 2, 4, 5 and 6 have been characterized to different degrees (Robert et al., 2002). Of these five GATA proteins GATA4 is known to play a major role in male sex determination.

Gata1 has been described in the developing gonad of the mouse but not the human. Immunohistochemical analysis shows GATA1 expression in Sertoli cells between e13 to e14.5 (Ito et al., 1993). Due to the localization of GATA-1 to the cytoplasm it is not thought to be active as a transcription factor during male gonadogenesis. GATA2 has been detected in the germ cells of the mouse ovary between e11.5 and e14.5 and may play a role in female germ cell differentiation (Siggers et al., 2002). GATA5 is expressed within the genital ridge as well as the epithelial cells lining the urogenital sinus. While knockout GATA5 mice do survive and are fertile, they do show abnormalities including malpositioning of the urogenital sinus, vagina and urethra but display no phenotype within males (Molkentin et al., 2000). Gata6 is found within the Sertoli cells at e19 in mice (Robert et al., 2002; Ketola et al., 1999). In the human fetus GATA6 is expressed in Sertoli and Leydig

cells between weeks 16 to 40 of development (Ketola et al., 2003). Little functional data is known about GATA 6 within these cells.

5.2.12.1) GATA4

Gata4 testicular localization has been studied in several species including mice and humans. In the mouse, *Gata4* is first observed in the bi-potential gonad by e10.5 and persists in both Sertoli and Leydig cells throughout development and into adulthood (Viger et al., 1998; Anttonen et al., 2002; Ketola et al., 2002; Ketola et al., 1999). Immunohistochemical studies of mouse XX gonads show GATA4 expressed at e11.5 (Viger et al., 1998) but studies disagree as to whether GATA4 is then down regulated at e13.5-14.5 or persists thereafter (Anttonen et al., 2002). In humans GATA4 is found in the 12-week-old fetal testis, with peak expression during Sertoli cell differentiation, and is continuously expressed in both the Sertoli and Leydig cells throughout adulthood (Ketola et al., 2000). GATA4 expression in the ovary is not as clear. Within humans GATA4 is observed in fetal granulosa cells between 13 and 33 weeks of gestation (Vaskivou et al., 2001).

5.2.12.2) GATA4 Function

The role of *Gata4* in sex determination has not been studied directly because embryos with null mutations of *Gata4* die between e7.0 and e9.5 due to cardiac defects (Molkentin et al., 1997). Cell culture studies as well as *in silico* searches have revealed that GATA4 is potentially important in steroidogenesis. WGATAR sequences are found in the promoters of *StAR*, *Cyp11A*, *Cyp17*, *Cyp19* and *HSD3B2* that respond to GATA factors *in vitro* (reviewed by Viger et al., 2005). *Gata4* is also thought to up-regulate *Dmrt1*, a gene crucial to male fertility (Lei and Heickert, 2004).

5.2.12.3) GATA4 and Friend of GATA (FOG)

Friend of GATA (FOG) 1 and FOG2 proteins are multitype zinc finger proteins that interact with the N- terminal zinc finger domains of GATA factors to modify their function depending on the cellular environment (Fox et al., 1998;

Holmes et al., 1999). *In vitro*, both FOG1 and 2 are expressed within primary Sertoli cell lines prepared from neonate rats (Robert et al., 2002) and expression of FOG2 is thought to repress GATA/SF1 synergism in gonadal cells. *In vivo*, *Gata4* and *Fog2* are expressed in somatic cells of the genital ridge around e11.5 (Heikinheimo et al., 1997; Viger et al., 1998; Ketola et al., 2002; Tevosian et al., 2002). Two separate mouse lines show the importance of FOG-2-GATA4 interaction to male development. Mice null for *Fog2* or containing a GATA-4 knock-in allele (V217G) that abolishes GATA-4/FOG-2 interaction, lack testis cords. Both lines show a marked decrease in *Sry* expression after e11.5. As well, *Sox9*, *Amh* and *Dhh* are absent and *Wnt4* is up regulated in the XY gonad (Tevosian et al., 2002).

5.2.13) Forkhead Transcription Factor Foxl2 (Foxl2)

Foxl2 is expressed in an ovary specific manner across several species lines at the time of sexual differentiation suggesting an important role in ovarian development (Loffler et al., 2003). The blepharophimosis-ptosis-epicanthus-inversus-syndrome (BPES) in human with mutations in the *Foxl2* gene suggests a role in germ cell survival, which are important in female sex determination. Knockout studies in mice showed that the interruption of *Foxl2* does not initially effect ovary formation but does inhibit differentiation of granulosa and theca cells later on (Uda et al., 2004). Recently, Ottolenghi et al. (2005) found that *Foxl2* expression in granulosa cells is required to repress the testis determination pathway genes in the postnatal ovary. Though sex reversal was not observed in knockout mice this theory was supported by the observed activation of testis-determining genes such as *Sox9, Wt1, Gata4, Dhh, Sf1, Dmrt1, Fgf9* and *Fgfr2*; all were unregulated in *Foxl2–/–* ovaries postnatally (Ottolenghi et al., 2005). Also the discovery of *Pfoxic* transcripts created by the bi-directional activation of the *Foxl2* promoter has lead to the hypothesis of a self-regulating system of *Foxl2* at the level of mRNA (Pannetier et al., 2005).

5.2.14) Aristalles-Related Homeobox Gene (ARX)

Mutations within the homeobox domain of Arx have demonstrated a role in testis development of both mice and men. Mutations within this gene are linked to

several forms of X-linked mental retardation including X-linked lissencephaly with abnormal genitalia in humans. Humans and mice with *Arx* mutations have testis of smaller total size but larger seminipherous tubules and a small number of Leydig cells. *Arx* expression is restricted to fibroblast cells, endothelial cells and peritubular myoid cells but mutations of this gene effect the development of fetal Leydig cells. This suggests a role for this gene activating signaling molecules these cell types that are important to fetal Leydig cell development (Kitamura et al., 2002). Within this study several putative targets for Arx in other tissues were identified including target genes such as *Lhx9* and *Wnt8b* (Kitamura et al., 2002).

5.3) Extra-cellular signals

5.3.1) Retinoic acid (RA)

It is widely believed that fetal germ cells are intrinsically programmed to enter meiosis and initiate oogenesis, unless specifically prevented from doing so by a putative "meiosis-inhibiting factor". Recently data has shown that retinoic acid (RA), the active form of vitamin A, initiates meiosis in germ cells and the "meiosis inhibiting factor" in Sertoli cells may be the gene *Cyp26b1* (Bowles et al., 2006; Vernet et al., 2006). RA concentrations, controlled by a balance of synthesis and degradation, are known to regulate the development of many organ systems (Marletaz et al., 2006). *Cyp26b1* encodes a P450 cytochrome enzyme that degrades RA (White et al., 2000). Micro array analysis of *Sf1* expressing cells at the time of sex determination showed that *Cyp26b1* is initially expressed in gonads of both sexes, but became male-specific by e12.5 (Beverdam and Koopman, 2006) *In situ* hybridization revealed that *Cyp26b1* is expressed in the testis cords at e12.5 and e13.5 (Bowles et al., 2006). Bowles et al. (2006) found that when cultured on a layer of RA-sensitive *lacZ* reporter cells, mesonephroi from e11.5 and e12.5 embryos stimulated abundant *lacZ* gene activity. Furthermore, *Aldh1a2,* a major enzyme regulating RA synthesis, is robustly expressed in the mesonephoroi of both sexes during the time of sex determination (Bowles et al., 2006). In order to test the theory that RA induces meiosis, fetal mouse testes were cultured in all-*trans* RA. All-*trans* RA is an isoform

of RA that is less easily degraded by P450 cytochrome enzymes. This culture environment induced the pre-meiotic marker *Stra8* and expression of *Scp3* and *Dmc1*, as measured by quantitative RT-PCR. This was recapitulated when testes were cultured in the cytochrome P450 inhibitor ketoconazole and by *in vivo* analysis of *Cyp26b1*-null mouse embryos. Conversely, culturing of gonads in the RAR antagonist AGN193109 arrested meiosis in both sexes (Bowles et al., 2006).

5.3.2) Prostaglandin (PDG2)

Stimulation of ovarian cells with PDG2 can induce *Sox9* and *Amh* production (Malki et al., 2005; Wilhelm et al., 2005). It was also shown that when gonad and mesonephric cell aggregates were treated with BWA868C, an inhibitor of the PDG2 receptor DP, Sertoli cell recruitment was completely abolished (Wilhelm et al., 2005). Conversely, when BWA868C treated Sertoli cells were treated with increasing concentrations of PDG2 the production of *Sox9* and *Amh* were increased in a complimentary fashion indicating a positive feedback mechanism (Wilhelm et al., 2005; Adams and McLaren, 2002). Evidence from Palmer and Burgoyne (1991) would suggest that this paracrine system must achieve a threshold number of cells producing PDG2 since chimeric gonads composed of less than 30% XY cells will develop as ovaries.

5.3.3) Desert Hedgehog (DHH)

Desert hedgehog (*Dhh*) expression is initiated in Sertoli cell precursors shortly after the activation of *Sry* (Bitgood et al., 1996) *Ptch1*, the receptor for Dhh, is expressed around the mesonephric tubules at the anterior end of the mesonephros. By e12.0, interstitial cells near the anterior end of the gonad begin to express *Ptch1* under the positive regulation of *Dhh*. Expression of *Ptch1* gradually extends toward both anterior and posterior ends of the gonad (Yao et al., 2002). Dhh signaling is known to be necessary for fetal Leydig cell development and may be regulated by *Sry* (Yao et al., 2002). Based on *in vitro* experiments using cyclopamine to block hedgehog signals, Yao et al. (2002) concluded that *Dhh/Ptch1* signaling specifies Leydig cell fate by early up-regulation of *Sf1*. This was theorized because Leydig cell markers

were completely missing from the treated group but there was no recorded difference in proliferation or apoptosis between cell populations.

5.3.4) Wingless-Type MMTV Integration Site Family, Member 4 (WNT4)

Genes of the *Wnt* family play roles in cell proliferation, migration and tissue patterning through, at least, two different pathways. The best-described pathway is the "canonical" pathway in which Wnts bind to the Frizzled receptor family and a low-density lipoprotein family receptor. The binding of Wnts to these receptors disrupts a β catenin degradation complex consisting of glycogen synthase kinase 3β, casein kinase-2, adenomatous polyposis coli and axin. This disruption results in increased levels of β catenin in the cell eventually resulting in β catenin entering the nucleus where it interacts with DNA binding molecules such as the T cell factor/lymphocyte enhancer factor family (reviewed by Logan and Nusse, 2004). The "noncanonical" pathway works through the Frizzled receptor family and certain thyrosine kinase receptors to initate the release of internal calcium stores. This calcium signaling system has several effects including the antagonism of the canonical Wnt pathway (reviewed by Veeman et al., 2003). One of the most well characterized Wnts in the sex determination pathway is *WNT4*.

Leydig and adrenocortical cells are thought to be derived from an *Sf1* positive cell population on the cranial aspect of the ceolomic epithelium. *Wnt4* is thought to act early on to separate these two cell lineages. *Wnt4* null mice display altered adrenal cortex function and exhibit ectopic adrenal cells on both XX and XY gonads (Heikkila et al., 2002; Jeays-Ward et al., 2003). Furthermore *Wnt4* null female mice have no Müllerian ducts but retain the Wolffian ducts (Vainio et al., 1999). Improper expression of WNT4 in humans and mice has been linked to problems in testis differentiation (Jeay-Ward et al., 2003; Jordan et al., 2001) Over expression of *Wnt4* in mice will cause a reduction in StAR protein expression leading to a reduction of steroid and androgen production, as well as a marked decrease in germ cells and a disorganized vasculature of the testis (Jordan et al., 2003). Within humans, one XY female with a duplication of the Wnt4 locus has been reported (Jordan et al., 2001).

Wnt4 is known to be an upstream regulator of *Dax-1* and *Fst* in the female determination pathway (Mizusaki et al., 2003). *Wnt4* or *Fst* may act as repressors of the male pathway, as the coelomic vessel, a testis-specific vessel, appear on the surface of the *Wnt4* or *Fst* null ovaries (Jeays-Ward et al., 2003; Yao et al., 2004).

5.3.4.2) FGF/WNT balance

Fgf9 and Wnt4 are known to be important in sex determination. One model describes *Wnt4* and *Fgf9* genes acting antagonistically to each other (Kim et al., 2006). Up regulation of Fgf9 in relation to Wnt4 drives male differentiation by increasing the expression of Sox9, while higher relative expression of Wnt4 is thought to block Sox9 and Fgf9 production. The loss of Fgf9 does result in a female phenotype and down regulation of Sox9 but the loss of Wnt4 results in only a slight peak of Sox9 expression in XX gonads that is quickly abolished and does not result in a complete sex reversal (Kim et al., 2006).

5.3.5) Fibroblast Gowth Factor (FGF)

The *Fibroblast growth factor* (*Fgf*) family is composed of 22 members. Signaling is mediated through membrane-spanning tyrosine kinase receptors encoded by four different genes, each of which can generate several different isoforms. *Fgfr* signaling is similar among the different *Fgf* receptor subtypes (Powers et al., 2000). Following ligand binding and dimerization, *Fgfr* activates several major signaling pathways. These pathways include the ras–mitogen activated protein (*MAP*) kinase (Kouhara et al., 1997), phosphoinositol 3-kinase (*PI3K*), and phospholipase C (*PLC*) pathways (Burgess et al., 1990; Cross et al., 2000) which influence cell growth, cell mobility / survival, and cell differentiation, respectively (Wennstrom et al., 1994; Yao and Cooper, 1995; Alimandi et al., 1997).

Fgf9 transcripts are detected within both XY and XX gonads at e11.5, as well as within the mesonephric duct and tubules of the adjacent mesonephroi of both sexes, indicating that the sex-specific phenotype is not determined by sex-specific expression of *Fgf9* at this stage. Later in gonad development (e12.5) *Fgf9* expression is down regulated in the XX gonad and restricted to the testis cords of the XY gonad

(Schmahl et al., 2004). At e11.5 immunohistochemical analysis shows widespread expression of Fgfr1, Fgfr3 and Fgfr4 in germ cells and somatic cells throughout both XY and XX gonads. *Fgfr2* is expressed weakly at e11.0 and is strongly expressed at e11.2 in both XX and XY gonads. After e11.2 sexually dimorphic nuclear localization of Fgfr2 occurred in the male but not in the female gonad; this internalization coincides with an arrest of cell proliferation and migration away from the coelomic epithelium of the *Sf1* expressing cell line (Schmahl et al., 2004). Fgfr3 was shown to display a similar nuclear localization to Fgfr2 at e14.5 in Sertoli cells (the only stage investigated) (Willerton et al., 2004). As a final note, it has recently been shown that migratory germ cells express Fgfr1-IIIc and Fgfr2-IIIb (Takeuchi et al., 2005).

XY mice with homozygous deletions *Fgf9* fail to develop testis cord structures. As a consequence, most *Fgf9*-/- XY mice develop as sex-reversed females (Colvin et al., 2001). Supporting cells in male mice with *Fgf9* knockouts are reduced in number and rest in the coelomic epithelium much like an ovary (Colvin et al., 2001; Schmahl et al., 2004). Furthermore, Fgf9 null mice fail to express Sertoli cell markers such as Sox9 and Amh but instead exhibit markers of ovarian development including Fst and Bmp2 (Colvin et al., 2001; Schmahl et al., 2004; DiNapoli et al., 2006).

As well as being a proliferation factor, *Fgf9*, *Fgf2* and several Fgf receptors work together to promote testis cord organization. Fgf2 was found to be a peritubular myoid cell survival and mitogenic factor (Hoeben et al. 1999; Colvin et al., 2001; El Ramy et al., 2005; Willerton et al., 2004). When Sertoli cells were incubated with FGF9 *in vitro* they displayed a differentiated epithelial phenotype, with cells in cord-like aggregations (Willerton et al., 2004). El Ramy et al., (2005) hypothesized that *Fgf2* and *Fgf9* mediate mesenchymal–epithelial interactions between peritubular myoid cells and Sertoli cells by selectively regulating the expression pattern of specific proteinases and inhibitors that modify the basement membrane around the testis cords. *Fgf9* can also induce migration of mesonephric cells into the ovary (Colvin et al., 2001).

FGF9 has recently been identified as essential in male germ cell survival. Fgf9 -/- mice are seen to have increased germ cell death within the male gonad while female gonads are unaffected (DiNapoli et al., 2006). The effect of Fgf9-/- on Sertoli cell function and proliferation was rescued in organ culture supplemented with fetal bovine serum but most male germ cells were lost. As previously mentioned Fgf9-/- XY genital ridges express ovarian marker genes and by e14.5, the remaining germ cells in these XY genital ridges enter meiosis synchronously with ovarian germ cells (DiNapoli et al., 2006). The author hypothesizes that these germ cells have escaped or did not develop a dependence on Fgf9.

Fgf9 can bind and activate the four major FGFRs, although it has greatest specificity for the FGFR1c, FGFR2c, FGFR3b and FGFR3c isoforms (Ornitz et al., 1996). Null mutations in *Fgfr1* are lethal between e6.5 and e9.5, before gonad formation (Deng et al., 1994) but, chimeric mice indicate that the testis develops normally even with 90% *Fgfr1-/-* cells (Deng et al., 1997). As well, mice with null mutations for either *Fgfr3* or *Fgfr4* are at least partially fertile (Colvin et al., 1996; Weinstein et al., 1998). Homozygous null mutations for *Fgfr2* result in embryonic lethality at e10.5 (Arman et al., 1999), such that the reproductive function of this receptor has not been assessed. The redundancy between these receptors may explain why no single Fgf receptor has yet been shown to be essential in sex determination.

5.3.6) Platelet Derived Growth Factors (PDGF)

Actions of platelet derived growth factors (*Pdgf*) are mediated through *MAP* kinase, *PI3K*, and *PLC-*γ pathways (Wennstrom et al., 1994; Lubinus et al. 1994; Yao and Cooper, 1995; Alimandi et al., 1997). *Pdgf* and its' α and β receptor subunits are expressed after e11.5 to at least e13.5 in the genital ridge with dimorphic sexual expression of *Pdgfr*α after e12.5 in the male (Ricci et al., 2004; Brennan et al., 2003). *Pdgfr*α is expressed in the mesonephric mesenchyme and the coelomic epithelium of both sexes at e11.5 and is up regulated in the interstitial cells of the testis by e12.5 (Brennan et al., 2003).

Pdgfs have also been implicated in the migration of cells from the mesonephrose into the testicle (Uzumcu et al. 2002a; Puglianiello et al. 2004; Ricci et al. 2004; Smith et al., 2005). The population of interstitial cells in *Pdgfr-/-* mice is reduced by ~60% compared with wild-type levels (Brennan et al., 2003). Uzumcu et al. (2002a) showed that inhibition of *Pdgf* actions after e11.5 does not inhibit cord formation but does alter normal cord development and morphology. Furthermore, the addition of *Pdgf-aa*, *Pdgf-bb*, or *Pdgf-ab in vitro* will recruit cells into the XX gonad. Expression of *Pdgfrα* was also up regulated in treated XX gonads, but *Pdgf* treatment did not up-regulate other male specific markers such as *Sox9* or *Scc* (Brennan et al., 2003). *In vitro* experiments show that *Pdgf* must work with other factors.

Pdgf isoforms seem to have specific functions in the testis. *Pdgf-bb* will reorganize disassociated testicular cells into large cell aggregates but not into testicular cords (Ricci et al., 2004). Also, Pdgfrα expression was important in the gonad and not the migrating cells suggesting *Pdgf* activates a secondary migratory signal (Brennan et al., 2003). *Pdgfrα* is also known to play a role in Leydig cell differentiation. Brennan et al. showed that in *Pdgfr-/-* XY mouse gonads Leydig cell markers express at low levels, indicating that fetal Leydig cell differentiation was severely impaired. However, *Scc* expression is unaltered in adrenals of *Pdgfr-/-* embryos indicating a separate signal for adrenal cell differentiation. Furthermore, the expression of Patched 1 (*Ptch1*) was reduced in *Pdgfr-/-* cells, even though the ligand Desert Hedgehog (*Dhh*) was expressed normally, indicating *Ptch1* is downstream of *Pdgf-a* .

5.3.7) Neurotropins (NT)

In non-neuronal cells, neurotropins are known to induce both *PI3K* and *MAP* kinase signaling pathways leading to cell migration (Sawada et al., 2000). Neurotropins act through the *Trk* family of receptor kinases to mediate cell survival and growth (Kaplan and Miller, 2000; Patapoutian and Reichardt, 2001). The specific *Trk* receptor for nerve growth factor3 (*Nt3*) is *TrkC* (Friedman and Greene, 1999). *Nt3* and *TrkC* are expressed in embryonic Sertoli cells and migrating mesonephric

cells, respectively, during the time of cord formation (Cupp et al., 2000; Levine et al., 2000). Cupp et al. (2000; 2003) demonstrated that when rat e13 gonads were cultured in the presence of a specific *TrkC* inhibitor (AG879), Sertoli cell number is not affected but *Sox9* expression is reduced and testis cords fail to develop. The proposed function of Nt3 in the genital ridge is a peritubular myoid cell migration signal and interruption of this signal effects testicular cord formation because peritubular myoid cells fail to enter the gonad. It was also shown that Nts induce mesonephric cell migration into the ovary (Cupp et al., 2003).

5.3.8) Hepatocyte growth factor (HGF)

The actions of *Hgf* are through its specific tyrosine kinase receptor c-met (Bottaro et al., 1991). *Hgf* influences cell migration, cell proliferation, and morphogenesis (Stuart et al., 2000). The *Hgf* receptor, *c-met*, is present in the interstitial compartment and in the peritubular myoid cells of the embryonic mouse testis at the time of the cord formation (Ricci et al., 1999, 2002). HGF is proposed to play a role in testis morphogenesis by directing cell migration from the mesonephros as well as regulating polarized laminin deposition around the testis cords (Ricci et al 2002).

5.3.9) Insulin receptor family

Insulin receptor (Ir), Insulin growth factor 1 receptor (Igf1) and Insulin related receptor (Irr) are essential for the proper growth and development in mice. Knockouts of Ir and Igf1r, separately or in combination, are of below average size and die shortly after birth due to ketoacidosis or respiratory failure (Accili et al., 1996; Joshi et al., 1996; Liu et al., 1996; Louvi et al., 1997). Irr is the sole receptor that has no abnormal phenotype when globally knocked out (Kitamura et al., 2001). While most studies on these receptors have been used in diabetes research, the insulin receptor family is also needed for proper testis development as they promote early growth, testis cord organization and testes decent. Whereas some *Ir, Igf1r* mutant embryos had partially descended gonads, mutant male mice lacking *Ir*, *Irr*, *Igf1r*, *Ir* and *Irr*, or *Igf1r* and *Irr* appeared to develop normally with respect to the male reproductive system. The

author attributed the lack of phenotype in the gonads to a possible functional redundancy. When all of these receptors (*Ir/Irr/Igfr*) are mutated there were a reduced number of cells and reduced *Sry* and *Sox*9 expression much like that seen in *Sox9* knockouts. These knockout mice display expression of *Wnt4* and *Figα* as well as several other ovarian marker genes in the XY gonad at e12.5 (Nef et al., 2003).

One of the targets of insulin signaling is the activation of Akt (pro-kinase B). In response to insulin, Akt interacts with Smad2 and 3, DNA binding proteins important in TGF-β signaling. Akt inhibits Smad3 activation by TGF-β in order to decrease TGF-β/Smad3 mediated transcription and TGF-β-induced apoptosis (Conery et al., 2004; Remy et al., 2004). While Akt-Smad interactions have not been investigated with respect to sex determination an indirect association between Akt activation and sex determination has been shown.

5.3.10) MAPK and PI3K transduction pathways

A signal transduction pathway common to all the above growth factors (*Pdgfs*, *Fgfs*, *Hgfs* and *Nts*) is *PI3K* (Uzumcu et al., 2002b). Puglianielllo et al. (2004), using a *MAPK* inhibitor (U0126) and a *PI3K* inhibitor (Ly294002) *in vitro*, showed that mesonephric cell motility and growth induced by exposure to *Pdgf-bb* involve *MAP* kinase and *PI3K* pathways. Uzumcu et al. (2002b) showed the *PI3K* inhibitor LY294002 acted by dramatically reducing the phosphorylation of *Akt*, which in turn reduced cell migration and blocked cord formation during early stages of testis development but not after the 19-20 tail somite stage was reached. On the other hand, this paper also showed that inhibition of the *MAP* kinase-signaling pathway with PD98059 had no significant effect on cord formation or cell migration after e13.5. However the researches also point out that LY294002 inhibits casein kinase-2, a participant in *Wnt* signaling pathways, which may leave this study open to further interpretation (Davies et al., 2000; Song et al. 2000).

5.3.11) Anti-Müllerian Hormone (AMH)

Anti-Müllerian Hormone (AMH), also known as Müllerian Inhibiting Substance (MIS), is a glycoprotein belonging to the TGFβ super family of proteins. In the mouse, Amh is produced in Sertoli cells around e12.5 and expression is maintained until birth (Münsterberg and Lovell-Badge, 1991; Shen et al., 1994). AMH is thought to bind and act specifically through AMHRII and further stabilized by AMHRI (BMPRI) (Mishna et al., 1996). Expression of AMHRII is found within the mesenchymal cell surrounding the male Müllerian ducts, Sertoli and granulosa cells (in embryos and adults) as well as adult Leydig cell (Mishina et al., 1996; Racine et al., 1998).

When both AMH and its receptors are expressed they regulate the regression of the Müllerian ducts but when mutated or absent cause persistent Müllerian duct syndrome in men (Behringer et al., 1994; Josso et al., 1997; Jamin et al., 2002). Also studies have shown AMH is an important regulator of adult Leydig cell proliferation. Strong over expression of AMH in transgenic mice leads to incomplete fetal virilization and decreased serum testosterone in the adult (Behringer et al., 1990). Conversely, AMH-deficient mice exhibit Leydig cell hyperplasia in adulthood (Mishina et al., 1996).

Sertoli cells express AMH by e12.5 through a well-defined process (reviewed by Viger et al., 2005). A combination of genes including *Sf1*, *Sox9* or *Sox8*, *Gata4*, *Fog2*, *Wt1* and *Dax-1* are important for correct spatiotemporal expression of *Amh*. Within the first 180bp of the *Amh* promoter the binding sites for *Sox9*, *Sf1* and *Gata4* have been shown to be essential (Arango et al., 1999; Giuili et al., 1997; de Santa Barbara et al., 1998; Viger et al., 1998). In addition to showing that *Sf1* and *Sox9* (or *Sox8*) can activate the *Amh* promoter singly, several studied have shown the importance of synergism between *Sf1* and *Sox9* and *Gata4* in proper regulation through time and space (Schepers et al., 2003; de Santa Barbara et al., 1998; Tremblay and Viger, 2003).

Two studies have found that another function of *Amh* is the induction of mesonephric cell migration and testis cord formation (Behringer et al., 1990; Ross et

al., 2003). In 1990, Behringer et al. described the formation of testis cord like structures in rat ovaries cultured in the presence of MIS. This process was further investigated 13 years later by Ross et al. (2003) who described the development of a male-specific vascular pattern in cultures of XX gonads when treated with *Amh*. Studies have demonstrated that while AMH is the only ligand accepted by AMHRII, AMH is not the only TGFβ family member to signal through the AMH type I receptor. Many of the TGFβ proteins including bone morphogenic proteins and activins are able to induce mesonephric cell migration and ectopic coelomic vessel formation in the XX gonad in culture (Ross et al., 2003; Yao et al., 2006).

5.3.12) Inhibin β (INHBb) and Follistatin (FST)

FST binds to certain members of the TGFβ family of proteins and prevents them from activating their receptors (Chang et al., 2002). FST has the highest affinity for activins, and the inhibitory effect of FST on activins have been established *in vitro* and *in vivo* (reviewed by Welt et al., 2002). FST is expressed in the XX gonad starting at e11.5 (Menke and Page, 2002; Yao et al., 2004). Similar to Wnt4 -/- mice, FST-/- mice display ectopic expression of the coelomic vessel (Yao et al., 2004; Jeays-Ward et al., 2003). Fst and Wnt4 are thought to inhibit coelomic vessel formation by inhibiting activin B (the homodimer product of *Inhb*) or repressing inhibin beta mRNA expression respectively. In Wnt4 and FST null mice expression of Inhibin β is thought to facilitate coelomic vessel formation because Inhbb-/-, Wnt4-/- and Inhbb-/-, FST-/- XX gonads develop without a coelomic vessel (Yao et al., 2006). Over expression of FST in transgenic mice causes hypogonadism. Within adult testes of these transgenic lines there was some degree of Leydig cell hyperplasia, seminiferous tubular degeneration and an arrest of spermatogenesis leading to infertility (Guo et al., 1998).

5.4.1) Activation of *SRY*

From e10.5 to e12.5 the XX and XY genital ridges are morphologically indistinguishable from each other in the mouse though, in the male, decisions have

already been made. In XY mouse embryos, the Y-linked gene *Sry* is transiently activated around e10.0 in the *Sf1* cell lineage (Sekido et al., 2004; Wilhelm et al., 2005; Albrecht and Eicher, 2001; Hacker et al., 1995; Bullejos and Koopman, 2001). Studies suggest that both the XX and XY genital ridges contain the necessary factors to activate *Sry*, though there is some debate as to what factors are involved (Koopman et al., 1991; Albrecht and Eicher, 2001; Danean et al., 2002). It is thought that *Sf1* positively regulates *SRY* transcription (de Santa Barbara et al., 2001; Pilon et al., 2003). It is also reported that both *Wt1* and *Lhx9* will trans-activate *Sry* (Hossain and Saunders, 2001; Shimamura et al., 1997; Hammes et al., 2001).

As mentioned earlier, *SRY* up-regulation is augmented by *Gata4* and Friend-of-GATA-2 (*Fog2*). *Gata4* and *Fog2* are expressed in somatic cells of the genital ridge around e11.5 and further increase the expression levels of *Sry* (Heikinheimo et al., 1997; Viger et al., 1998; Ketola et al., 2000; Tevosian et al., 2002).

Interestingly, *Sox9* is shown to both up regulated and down regulate Sry (Daneau et al., 2002; Charboisser et al 2004). *In vitro* studies using the pig SRY promoter have shown that the pig *SOX9* cDNA can activate this promoter 9 fold and that when a putative Sox9 binding site (-205bp upstream) is mutated reporter gene activation is reduced by 70% (Daneau et al., 2002). On the other hand, conditional Sox9 knockout mice show an increased expression of *Sry* when compared to wildtype mice (Charboisser et al 2004).

5.4.2) Importin β and Calmodulin

SRY and SOX9 each contain two nuclear localization signals (NLS) that enable binding to the nucleocytoplasmic transport proteins importin β and calmodulin. Mutations within importin β or calmodulin, or the mutation of the Sox9 or Sry NLS, have been implicated in XY sex reversal in humans (Harley et al., 2003; Sim et al., 2005). Acetylation/deacetylation of the NLS by histone acetyltransferase p300 and HDAC3, respectively, are essential for the function of Sry and Sox9 (Thevenet et al., 2004; Furumatsu et al., 2005). *In vitro* studies have shown that the acetylation of the C-terminal NLS is essential for importin β binding and nuclear localization of Sry

and Sox9 while deacetylation by HDAC3 causes localization of these proteins to the cytoplasm (Malki et al., 2005, Forwood et al., 2001). Another nuclear import molecule, calmodulin, displays a Ca^{2+} dependent binding to SRY and SOX proteins that seem to be essential for their proper functioning (Harley et al., 1996). Both the mutation of the N-terminus NLS or the inhibition of Ca^{2+} binding by calmodulin is known to interrupt this process (Sim et al., 2005; Argentaro et al., 2003). Studies of XY females show that both of these nuclear import processes must function properly in order for male sex determination to occur (Sim et al., 2005).

5.4.3) Prostaglandin, Sry, Sox9, Importin β and Calmodulin Work Together

Recently, a model for cell-cell prostaglandin D2 (PGD2) signaling mediated up-regulation of *Sox9* in pre-Sertoli cells has been proposed. This theory involves Sry or Sox9 up-regulating PGD2 synthesis and secretion. Working through its DP receptor PDG2 in turn up-regulates *Sox9* in an autocrine and paracrine manner (Wilhelm et al., 2005; Malki et al., 2005). The PGD2 signaling pathway stimulates adenylcyclase-coupled DP1 receptor. The activation of cAMP-dependent protein kinase A (PKA) induces phosphorylation of Sox9 at two points on the protein. This phosphorylation enhances binding of Sox9 to the nucleocytoplasmic transport protein importin β, a protein that has been implicated in sex reversal in humans (Harley et al., 2003). Binding of Sox9 to importin β brings Sox9 into the nucleus and therefore closer to its binding targets (Malki et al., 2005). Though the pathway is not as well defined there is some evidence that calmodulin may act in a similar manner (Argentaro et al., 2003; Sim et al., 2005).

6) MATERIALS AND METHODS

6.1) Mouse Line, Cell Collection, Cell Purification

The cloning, generation and characterization of the HybSRYp-RFP transgenic mouse has been previously described (Boyer et al., 2006). Mice were housed and handled according to national and international standards. The institution Ethics Committee on use of animals in research approved the procedures used involving animals. Female mice were superovulated using standard protocols of gonadotropin injections (Nogy et al., 2003). Two days before mating female mice were injected with 0.1ml of PMSG, this was followed by a further injection of HCG on the day in which males were placed in the same cage as the females. The following day males were removed and females were checked for the presence of vaginal plugs. Noon on the first day of vaginal plug detection was designated as embryonic day 0.5 (e0.5) when staging embryonic development. Embryos were harvested at e12.0 and the genital ridges were dissected. Genital ridges were sexed via expression of RFP, as described previously (Boyer et al., 2006), and mesonephori were also removed. Male and female genital ridges were pooled separately and digested for 30 min at 37°C in M2 media (Sigma, St. Louis, MO) supplemented with collagenase (50U ml^{-1}) (Invitrogen, Canadian Life Technologies, Burlington, ON, Canada) and dispase (2.4U ml^{-1}) (Invitrogen). For the male genital ridge, fluorescent and non fluorescent cells were separated and collected via FACs (FACSTAR-Plus; FACs services, IRCM, Montréal, Canada). In order to insure samples were treated similarly female genital ridges were also subjected to FACs procedures.

6.2) Generation of Micro Array List

25 000 fluorescent pre-Sertoli cells were collected and compared against 25 000 ovarian cells from the same transgenic line. Total RNA was isolated from both samples using the Rneasy Microkit (Quiagen, Mississaguga, ON, Canada) according to the manufacturer's instructions. A two cycle RT-PCR amplification of about 10 μg RNA was performed per sample prior to probe synthesis in order to achieve sufficient amounts of the probe. Probe hybridization on microarrays was performed at the

Génome Québec Innovation Center at McGill University using GeneChip® Mouse Genome 430 2.0 arrays (Affymetrix). This experiment in its entirety was repeated in quadruplet. The methods used to further analyze this list and the criteria used to select relevant genes for further analysis by *in situ* hybridization is discussed within the article.

6.3) Generation of Riboprobes

Plasmids containing cDNA of the relevant candidate genes were created by RT-PCR of mRNA extracted from e12.0 male genital ridges. RT-PCR reaction was subjected to a hemi-nested PCR to purify the selected gene; the list of primers used is presented in the appendix. The PCR product was ligated into a pGemt vector (Promega, Madison, WI) and confirmed by sequence analysis. Sequence reactions were preformed on cDNA via the dideoxy sequencing method (Big Dye Terminator 3.1; ABI Prism, Applied BioSystem, PE, Branchburg, NJ) using oligos designed to target the SP6 or T7 domain of the pGEMT plasmid. Sequence reactions were analyzed on an ABI Prism 310 sequencer (Applied BioSystems) and nucleic acid sequences were analyzed by BLAST (Basic Local Alignment Search Tool; NCBI) against the mouse genome. A DNA sequence was considered confirmed when homology was greater than 95%. After confirmation, plasmids were linearized using restriction enzymes and then used as templates to generate digoxigenin-labeled antisense riboprobes. Riboprobes were made using a DIG RNA Labeling Kit (SP6/T7) according to the manufacturer's instructions (Roche Applied Science, Penzbreg, Germany).

6.4) Whole mount *in situ* Hybridization (WISH)

Whole mount in situ hybridization (WISH) was carried out as described below; unless otherwise mentioned all procedures were performed at room temperature. A list of solutions can be found in the Supplementary Data. Expression profiles were analyzed at e11.5, e12.5 and e13.5 using at least four pairs of embryos of each sex. Gonads were dissected in PBS-DEPC, fixed over night in 4% paraformaldehyde at 4°C, washed in PBS, and then dehydrated in graded methanol

solutions and stored at -20°C in 100% methanol for no more that one week. At this point these gonads are considered samples and will be referred to as such from here on. Samples were then rehydrated and bleached in a 6% H_2O_2 solution. Samples were washed twice with PBT-DEPC and digested lightly for 5 to 10 minute, depending on sizes, in a 10 µg/mL PK/PBT solution. Samples were then washed twice with 2 mg/mL solution of glycine/PBT for five minutes and then twice with PBT-DEPC. After this samples were postfixed in 0.2% glutaraldehyde / 4% PFA PBS-DEPC solution for 20 minutes. Two more washes in PBT-DEPC were followed by one wash (at room tepurature) and 1 hour incubation (at 68°C) in hybridization buffer. Hybridization buffer was replaced by RNA probe solutions (1µg digoxigenin-labeled RNA probe/mL of hybridization buffer) and incubated at 68°C overnight.

The following day the samples were washed three times with solution 1 and three times with solution 2. Each wash was performed at 68°C and lasted 30 minutes. This was followed by three washes of TBST at room temperature. Samples were then placed in 2 mL of blocking solution for two hours. Samples were then incubated in 1 mL of AB solution at 4°C overnight.

The following day samples were washed rapidly three times with TBS-levamisole followed by five 45 minute washes in the same solution. Two washes in NTM-Levamisole preceded the revealing stage that consisted of incubating samples in Revealing Solution at 34°C overnight. Samples were cleared with two washes of TBST followed by two washes in CMFET and a three hour wash in CMFET:glycerol (1:1). Embryos were then stored in CMFET:glycerol (1:4) at 4°C. A Leica MZ FLIII stereomicroscope was used to visualize the genital ridge.

Presumptive Pre- Sertoli Cells Express Genes Involved in Cell Proliferation and Cell Signalling During a Critical Window in Early Testis Differentiation.

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Running Head: pre-Sertoli cell transcriptome study

ABSTRACT

In mammals, the pre-Sertoli cell of the male genital ridge is the first cell type to display sex specific differentiation and gene expression. The genetic cascade driving the differentiation of pre-Sertoli cells and testis formation is beginning to be unravelled, but many questions remain; a better understanding of the transcriptome of pre-Sertoli cells immediately after sex determination is now essential. A mouse model expressing Red Fluorescent Protein under the control of a hybrid mouse/pig *SRY* promoter (Hyb*SRY*p-RFP) was used to purify cells from embryonic day 12.0 (e12.0) male genital ridges. The transcriptomes of of these cells was compared to age matched whole female genital ridge cells using Affymetrix Mouse Genome 430 2.0 microarrays. The expression of genes considered markers for pre-Sertoli cells, including *Sox9*, *Mis*, *Dhh* and *Fgf9* were identified within the Hyb*SRY*p-RFP expressing cell population, while markers for germ cells (*Oct4*, *SSEA-1*) and endothelial cells (*Ntrk3*) were not. In a general fashion, genes identified as 2.5 fold over expressed in Hyb*SRY*p-RFP expressing cells coded notably for cell signalling and extra cellular proteins. The expression of *Sox10*, *Stc2*, *Fgf18*, *Fgf13* and *Wnt6* were further characterized via whole mount *in situ* hybridization (WISH) on male and female genital ridges between e11.5 and e14.5. *Sox10*, *Fgf18*, *Fgf13* and *Stc2* gene expression was detected within the male genital ridges while *Wnt6* was found diffusely within both the male and female genital ridges. These data represent the earliest comprehensive microarray expression analysis of purified presumptive pre-Sertoli cells available to date.

INTRODUCTION

In the mammalian male embryo, the first molecular signal of sex determination is the expression of the Y-linked gene *SRY* within a sub-population of somatic cells of the indifferent male genital ridge (Koopman et al., 1990; Daneau et al., 1996; Hanley et al. 2000). In the mouse, the transient expression of *Sry* between embryonic day 10 (e10.0) and e12.5 drives the initial differentiation of pre-Sertoli cells that would otherwise follow a "female default pathway" becoming granulosa cells (Albrecht and Eicher, 2001; Sekido et al., 2004). Following male sex determination, pre-Sertoli cells proliferate, polarize and aggregate around the germ cells to define the testes cords, which by exclusion define the interstitial spaces between the cords (Tilmann and Capel, 1999; Schmahl et al., 2000). Migration of cells into the gonad from the mesonephros (Merchant-Larios and Moreno-Mendoza, 1998; Nishino et al., 2001) or the coelomic epithelium (Schmahl et al., 2000; Karl and Capel, 1998) is subsequently induced by signals emanating from the pre-Sertoli cells (Martineau et al., 1997; Capel et al., 1999). Peritubular myoid cells surround the testes cords and cooperate with pre-Sertoli cells to deposit the basal lamina and further define the testis cords. Signalling molecules produced by pre-Sertoli cells promote the differentiation of somatic cells found outside the cords into fetal Leydig cells, thus ultimately influencing the production of testosterone (Yao et al., 2002). Endothelial cells associate to form the coelomic vessel which promotes efficient export of testosterone.

Classically, many of the genes found to be involved in sex determination and differentiation have been identified by studying human patients displaying sex discordance syndromes or from mouse gene knockout models, and include *SF1*, *LHX9*, *GATA4*, *SOX8*, *SOX9*, *FGF9*, *IR*, *IRR*, *PDG2*, *AMH*, *DHH*, *PDGF* and *WNT4* (reviewed by: Brennan and Capel, 2004; Ross and Capel, 2005; Viger et al., 2005; Wilhelm and Koopman, 2006). Recently, several large scale transcriptome experiments have been performed to study genital ridge gene expression. Several laboratories have employed subtractive screens or microarray analysis to search for

sexually dimorphic gene expression between male and female whole embryonic mouse gonads (Bowles et al., 2000; Wertz and Herrmann, 2000; Menke and Page, 2002; Smith et al., 2003; McClive et al., 2003; Small et al., 2005; Grimmond et al., 2000). More recently, transgenic mouse models expressing fluorescent proteins driven by various lengths of *SRY* or *Sf1* promoters have been developed and used to compare gene expression of specific cell types within the genital ridge around the time of sex determination and differentiation (Boyer et al., 2004; Nef et al., 2005; Beverdam and Koopman, 2006; Bouma et al., 2006).

To further characterize genes differentially expressed by pre-Sertoli cells at the beginning of sex differentiation we have made use of a mouse model expressing Red Fluorescent Protein (RFP) under the control of a hybrid mouse/pig *Sry* promoter (Hyb*SRY*p-RFP) (Boyer et al., 2006). This mouse line was used to isolate and purify presumptive pre-Sertoli cells after the peak of *Sry* expression but before histological testis cord formation. RNA from purified e12.0 presumptive pre-Sertoli cells or stage matched whole female genital ridges were hybridized onto micro-arrays to study the transcriptomes of the presumptive pre-Sertoli cell. The expression patterns of selected genes were further characterized by whole mount *in situ* hybridization (WISH). To our knowledge this represents the earliest comprehensive transcriptome analysis of purified genital ridge somatic cells consistent with a pre-Sertoli cell phenotype reported to date.

MATERIALS AND METHODS

Mouse Line, Cell Collection, Cell Purification

The generation and preliminary characterization of the Hyb*SRY*p-RFP transgenic mouse has been previously reported (Boyer et al., 2006). In the current studies, mice were housed and handled according to national standards, and all experimental protocols involving animals were approved by the local institutional ethics committee on the use of animals in research. For staging embryonic development, noon on the first day of vaginal plug detection was designated as embryonic day 0.5 (e0.5). Embryos were harvested early on the morning of e12.0 and the genital ridges were dissected. Male genital ridges from embryos staged between tail somite (ts) 20 to 25 were collected (average = ts23); generally male genital ridges displaying visible testis cord formation were excluded from the data set. Female genital ridges were taken from embryos that displayed no genital ridge fluorescence at a comparable ts stage, generally from ts23 to ts25. Genital ridges were sexed via expression of RFP as described previously (Boyer et al., 2006), and the mesonephros was removed via dissection. Male and female genital ridges were pooled separately and digested for 30 min at 37°C in M2 media (Sigma, St. Louis, MO) supplemented with collagenase (50 U ml^{-1}) (Invitrogen, Canadian Life Technologies, Burlington, ON, Canada) and dispase (2.4 U ml^{-1}) (Invitrogen). For the male genital ridge, fluorescent and non fluorescent cells were separated and collected via fluorescence activated cell sorting (FACS) using a FACSTAR-Plus machine (FACS services, IRCM, Montreal, Canada). In order to insure uniformity in sample treatment, female genital ridges were also subjected to FACS separation procedures.

Generation of Micro Array List

A total of 21 FACS procedures were performed in two separate experiments. For the first experiment this involved dissecting 218 male embryos to give 436 genital ridges. An equivalent number of embryos were dissected for the second experiment, although the exact number was not counted. Thus in total the number of male embryos dissected was in excess of 400, and the number of total embryos processed (male and female) was in excess of 800. Based on 15 male genital ridges (displaying 2.15% fluorescent cells), a total of 366,367 cells (fluorescent plus non fluorescent) were recovered, representing about 25,000 total cells per genital ridge. Within this total cell population, 2.15% of cells were fluorescent, representing about 500 fluorescent cells per genital ridge. Based on tail somite counting of 64 male embryos used in the experiments, the average tail somite age was found to be ts=23.28, +/- 1.29 (SEM). Based on 20 FACS separations, where the mesonephros had been dissected to give just the genital ridge, the percent fluorescent cells present within the male genital ridge cell sample was 1.69%, +/- 0.92% (SEM). Therefore, for the overall experiment, the average genital ridge dissected was aged at about ts23, with a fluorescent cell content of about 2%.

For a single experiment, approximately 25,000 fluorescent pre-Sertoli cells were collected and compared to approximately 25,000 ovarian cells from the same transgenic line. Total RNA was isolated from both samples using the RNeasy Microkit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's instructions. Ten ng of total RNA was used for probe synthesis involving a two cycle amplification protocol. For each sample group (presumptive pre-Sertoli cells, female genital ridge cells), two probes were generated and hybridized to two replicates of the Affymetrix GeneChip® Mouse Genome 430 2.0 microarrays. This experimental protocol was performed twice, providing a total of 4 replicates per sample group. Probe generation, hybridization and microarray reading was preformed at the Genome Quebec Innovation Center at McGill University.

For precise staging of male genital ridges to determine the onset of Hyb*SRY*p-RFP transgene expression (see Figure 1), a genetic sexing method was employed.

This involved mating the Hyb*SRY*p-RFP transgene into a transgenic mouse line where a Gata4p-GFP transgene is integrated onto the X chromosome (Mazaud Guittot et al., 2006). When a hemizygote Gata4p-GFP positive male mouse is mated to a homozygote Hyb*SRY*p-RFP female mouse, all resulting embryos will be Hyb*SRY*p-RFP positive. All female embryos will be Gata4p-GFP positive and can be easily identified via GFP tissue fluorescence at e11.5 to e12.0 (and in fact as early as e8.5). All male embryos will be negative for GFP tissue fluorescence but will be transgenic for the Hyb*SRY*p-RFP transgene.

RT-PCR, Whole Mount *In Situ* Hybridization

To validate expression of selected genes identified via microarray analysis, RT-PCR amplifications were performed on mRNA derived from FACs purified e12.0 pre-Sertoli cells as described above. Primer oligomers and predicted amplification lengths are presented in Table 1. Amplified cDNA sequences were ligated into a pGem-T plasmid vector (Promega, Madison, WI), and sequences were verified via dideoxy sequencing techniques. Whole mount *in situ* hybridization (WISH) was carried out as previously described (Pilon et al., 2006). Briefly, gonads from e11.5, e12.5 and e13.5 embryos were dissected in PBS-DEPC, fixed overnight in 4% paraformaldehyde at 4°C, washed in PBS, and then dehydrated in graded methanol solutions and stored at -20°C in 100% methanol for no more that one week. To generate the WISH probe, plasmids were linearized, the cDNA portion was purified and then used as a template to generate digoxigenin-labeled antisense riboprobes. WISH expression profiles were generated and analysed using at least four pairs of embryos for each sex and each time point. As a positive control for the WISH protocol, a *Sox9* probe was generated using mouse *Sox9* cDNA sequences (generously provided by Richard Behringer). A Leica MZ FLIII stereomicroscope was used to visualize the genital ridge.

RESULTS

Onset of Genital Ridge Transgene Expression

The Hyb*SRY*p-RFP transgene results in RFP expression within cells that will become pre-Sertoli cells (Boyer et al., 2006). Fluorescence is first seen at e12.0, tail somite 20 (ts20) (Figure 1), which is slightly later than the peak of *Sry* expression at ts16 (Bullejos and Koopman, 2001; Mazaud Guittot et al., 2006). At this time, a small number of cells (generally from 10 to 20) of the genital ridge express readily visible fluorescence. These cells are located within the mesenchyme of the central part of the XY genital ridge, and are not associated with either the anterior or posterior poles of the genital ridge nor with the coelomic epithelium. Very quickly the number of fluorescent cells increases such that by ts23, they are distributed in a salt and pepper pattern throughout the mesenchyme of the genital ridge (Figure 1). Based on dissections and FACS purifications used for the microarray experiments, the number of fluorescent cells now averages about 500 per genital ridge, accounting for about 2% of the total number of cells per genital ridge.

Microarray Results

After normalization, the four data sets from the Hyb*SRY*p-RFP cells and the four data sets from the XX genital ridge cells were summed and averaged to give mean test (Hyb*SRY*p-RFP cells) and mean control (XX genital ridge) sample values. Mean control sample values were then divided by mean test sample values to determine the fold difference of expression between Hyb*SRY*p-RFP cells and XX genital ridge cells for each gene investigated. A total of 1,953 probe sets, representing 4.3% of a possible 45,101 probe sets, showed 2.5 fold or higher expression within either the XX genital ridge or Hyb*SRY*p-RFP expressing cells: 994 probe sets (51%) were specifically over-expressed in the Hyb*SRY*p-RFP expressing cells, while 959

probe sets (49%) were over-expressed in the XX genital ridge cells. Using Online Mendelian Inheritance in Man (OMIM: http://www.ncbi.nih.gov/entrez) and SOURCE (http://source.stanford.edu/cgi-bin/source/sourcesearch) databases each probe set over-expressed in the Hyb*SRY*p-RFP cell population was categorized according to biological function; these general results are summarized in Table 2. The top 100 Hyb*SRY*p-RFP expressing cell over-expressed probe sets (at e12.0) are presented in Table 3, while the complete list of over-expressed probe sets including both Hyb*SRY*p-RFP expressing cells and female genital ridge cells is available as supplementary data. A general comparison of our Hyb*SRY*p-RFP expressing cell transcriptome data with lists generated from *Sf1*promoter-GFP expressing cells of the male genital ridge at e11.5 or e12.5 (Nef et al., 2005; Beverdam and Koopman, 2006) is presented in Figure 2.

Gene Expression Profiling: RT-PCR and WISH

To confirm that genes identified by the microarray analysis are expressed in Hyb*SRY*p-RFP expressing cells, further expression analysis was performed for selected genes. A total of 15 genes were selected for further study based principally on their role in cell proliferation, cell survival or cell signaling within other tissues. The expression of these genes was confirmed by RT-PCR performed on mRNA derived from Hyb*SRY*p-RFP expressing cells purified from e12.0 male genital ridges (Figure 3). The validity of the amplified bands was further confirmed by DNA sequencing.

WISH expression experiments were performed on male and female genital ridges at e11.5, e12.5, and e13.5 for five genes, including *SRY-related HMG box gene10* (*Sox10*), *Stanniocalcin2* (*Stc2*), *Fibroblast growth factor 18* (*Fgf18*), *Fibroblast growth factor 13* (*Fgf13*), and *Wingless-type mmtv integration site family, member 6* (*Wnt6*). Genital ridge expression of *Sox10* was first detected at e11.5 and was found exclusively within the male genital ridge (Figure 4, A). Expression continued between e12.5 and e13.5 within the male genital ridge (Figure 4, B-C) as well as within the mesonephros after e12.5. WISH results for *Stc2* revealed a strong

signal within the male genital ridge at all stages studied (Figure 4, D-F). For *Fgf18* a weak WISH signal was detected in the testis shortly after e12.5; by e13.5 strong expression within the testis cords and mesonephros was evident in the male (Figure 4, G-I). *Fgf13* expression was first detected at e12.5 in the mesonephroi of both sexes (Figure 4, J-K). By e13.5, *Fgf13* expression was detected within the testis cords (Figure 4, L) and was no longer detectable in the mesonephroi of either sex. At the detection level of the WISH procedure, *Wnt6* expression was detected within the genital ridges of both sexes at e11.5 and e12.5 (Figure 4, M-N). By e13.5, *Wnt6* expression was slightly more associated with the testicular cords than with other tissues (Figure 4, O).

DISCUSSION

In mammals, the development of the male genital ridge is driven by pre-Sertoli cells which constitute the first cell line to commit to the male differentiation pathway and which are known to recruit other cell types to the male pathway through various signaling mechanisms (Martineau et al., 1997; Capel et al., 1999; Adams and McLaren, 2002; Malki et al., 2005). Characterization of the specific subset of genes differentially expressed in pre-Sertoli cells compared with the female genital ridge immediately after the moment of testicular sex determination should provide further understanding of the molecular mechanisms involved in these processes. To pursue this goal we have made use of a previously described transgenic mouse line expressing RFP under the control of a hybrid mouse/pig *SRY* promoter (Boyer et al., 2006). This model displays male genital ridge specific expression of fluorescence at e12.5 (Boyer et al., 2006). Via mating experiments, at e13.5, these Hyb*SRY*p-RFP positive cells are found within the testis cords and represent the same cells that are marked by a human *SRY* promoter-YFP transgene that themselves display a pre-Sertoli cell expression pattern via histology and RT-PCR analysis (Boyer et al., 2006). Furthermore, again at e13.5, Hyb*SRY*p-RFP positive cells represent a distinct cell population compared to germ cells as represented by *Oct4* promoter-GFP expressing cells (Boyer et al., 2006). Thus, at least by e13.5, it can be concluded that Hyb*SRY*p-RFP expressing cells are pre-Sertoli cells, since they are male genital ridge specific, found within the testis cords, distinct from germ cells, and co-mark cells showing pre-Sertoli cell marker gene expression. Working on the hypothesis that Hyb*SRY*p-RFP positive cells mark presumptive pre-Sertoli cells at earlier time points and may thus be useful in deciphering molecular mechanisms of mammalian sex determination and differentiation, we compared the transcriptomes of these cells at e12.0 to age matched female genital ridges using GeneChip® Mouse Genome 430 2.0 arrays (Affymetrix).

Fluorescent Hyb*SRY*p-RFP cells express pre-Sertoli cells markers but not germ cell markers

As predicted by our previous work (Boyer et al., 2006), our microarray transcriptome analysis of genital ridge cells expressing the Hyb*SRY*p-RFP transgene identified RFP positive cells as consistant with being pre-Sertoli cells. Within this cell population we identified genes known to be important for testes development or as pre-Sertoli cell markers, including *Sox9*, *Amh/Mis*, *Dhh*, *Sf1/Nr5a1*, *Gata4*, *Gata6*, *Dmrt1, Fgf9*, and *Ptgds* (Kent et al., 1996; Morais da Silva et al., 1996; Bitgood et al., 1996; Ikeda et al., 1994; Ketola et al., 1999; Tevosian et al., 2002; Colvin et al., 2001; Adams and McLaren, 2002; Wilhelm et al., 2005; Malki et al., 2005). Notably, Hyb*SRY*p-RFP expressing cells did not express markers for germ cells, such as *Oct4* and *Ssea-1*, or markers for endothelial cells, such as *Ntrk3*. In addition, genetic markers for ovarian development of somatic cells were found over-expressed in the ovarian data set, including *Fst* and *Bmp2*.

Global analysis of presumptive pre-Sertoli cell transcriptome data

A total of 1953 probe sets showed 2.5 fold or higher expression within either ovarian cells or presumptive pre-Sertoli cells at e12.0. Of the 994 probe sets that were over expressed within pre-Sertoli cells, 660 (61.1%) represented genes of know function based on OMIM and SOURCE databases (Table 2), leaving 334 probe sets (38.9%) representing gene sets of unknown function. The functional groups most represented are genes coding for cell signalling molecules followed by genes coding for molecules involved in tissue and extracellular organization. This is not unexpected as pre-Sertoli cells are known to drive the differentiation of the testis immediately after sex determination. Furthermore, these findings confirm observations made by Small et al. (2005) who observed a predominance of cell signalling transcripts within the male genital ridge shortly after sex determination. Genes coding for molecules involved in metabolism and nutrient transport were also up regulated in the presumptive pre-Sertoli cells most likely reflecting the increase in cell proliferation within this cell population.

RNA transcripts of DNA binding and RNA associated molecules made up only 4% of over expressed genes in presumptive pre-Sertoli cell population; this is in spite of the fact that most genes effecting testes determination identified to date (via human clinical cases and by mouse gene knockout models) code for transcription factors (Fleming and Vilain, 2005). This relative paucity of transcription factors may reflect quantitative differences in expression level requirements between transcription factors and signalling or structural molecules as well as the current detection limits of solid phase hybridization technologies. An alternative explanation is that only a relatively small number of transcription factors are actually required to drive testes determination. Thus it may be that conceptual advances in our understanding of the molecular genetics of testes determination will involve determining how an already identified set of transcription factors interact with each other and with their target genes, rather than identifying additional transcription factors previously not associated with sex determination. Furthermore, as the Affimetrix GeneChip® Mouse Genome 430 2.0 array does not survey the non coding RNA transcriptome, addressing the possibility of additional control of mammalian sex determination at the post-transcriptional level must await further technical refinements (Lalli et al. 2003).

Comparisons of genital ridge cell transcriptomes based on *SRY*, *Sf1* promoter models

Recently, two research groups have described transcriptome data generated by comparing purified *Sf1* promoter-GFP positive cells from the somatic cell population of male and female genital ridges at the time of sex determination and differentiation (Nef et al. 2005; Beverdam and Koopman, 2006). At e11.5, this represented approximately 5000 (Nef et al., 2005) and 4200 (Beverdam and Koopman, 2006) GFP expressing cells per genital ridge. Both of these studies used GeneChip® Mouse Genome 430 2.0 arrays (Affymetrix), with a replicate size of n=3 for both male and female genital ridge derived cells. In comparison, our *SRY* promoter RFP

mouse model expressed RFP only within the male genital ridge, and only after about e12.0 (ts21). At ts 23, this represented on average about 500 cells per male genital ridge. Characterization of the Hyb*SRY*p-RFP transgenic mouse line has shown that within the genital ridge RFP expression is restricted to presumptive pre-Sertoli cells at all points studied (Boyer et al., 2006; this study). Thus we propose that at e12.0 (ts23), our *SRY* promoter based model results in at least a 10 fold purification of presumptive pre-Sertoli cells compared to *Sf1* promoter based models, and should provide a valuable insight into the pre-Sertoli cell transcriptome in the time period immediately after sex determination.

In spite of these methodological differences, 91 genes were found in common to all three studies at the time points between e11.5 and e12.5, providing strong evidence that these 91 genes are important to pre-Sertoli cell physiology and early testis differentiation. Taken separately, over 45% of the microarray list reported by Beverdam and Koopman (2006), and over 31% of the microarray list reported by Nef et al., (2005) are also identified in our list (Figure 2).

Bouma et al. (2006) have recently described a micro-array study based on comparing the expression of isolated *Sry* promoter-GFP expressing cells from XY and XX gonads of e13.5 mice. Because this time period is well after histological differentiation of testes cords, it is more relevant to studies of later gonad differentiation rather than testes determination and early differentiation.

Genital ridge expression of selected genes via WISH

One of the consequences of *Sry* expression is a dramatic increase in cell number and relative size of the testis compared to the ovary (Schmahl et al., 2000). Studies that block cell proliferation at e11.0 have established the essential role of this increased cell proliferation in testis cord development and the male genital pathway in general (Schmahl and Capel, 2003). This increase in cell proliferation can also be blocked if cell signalling is interrupted (Uzumcu et al., 2002). From the list of genes

generated by the microarray, 5 genes were selected for further genital ridge expression profiling via WISH, based on their involvement in cell proliferation, cell signalling or cell survival. These genes included *Sox10* (13.2 fold increase in expression within presumptive pre-Sertoli cells compared to ovarian cells via microarray analysis), *Stc2* (23.5 fold increase), *Fgf18* (3.3 fold increase), *Fgf13* (8.9 fold increase) and *Wnt6* (2.6 fold increase). As a positive control for the WISH protocol, a probe for *Sox9* expression was included, which is known to have a pre-Sertoli cell expression pattern in the male genital ridge immediately after sex determination. In our microarray analysis, *Sox9* showed a 15 fold enhancement of expression within presumptive pre-Sertoli cells compared to age matched ovarian cells.

SOX10, SOX9 and SOX8 are all members of the E group of SOX transcription factor proteins, and are thought to have evolved from a common ancestral gene (Bowles et al., 2000b; Wegner, 1999). Indeed, studies have shown significant functional redundancy between these factors (Bylund et al., 2003; Cheung and Briscoe, 2003; Graham et al., 2003; Taylor and LaBonne, 2005; Kellerer et al., 2006). Within the male genital ridge, *Sox8* and *Sox9* gene expression are up-regulated as a consequence of *Sry* expression (Sekido et al., 2004) and influence male sex determination and pre-Sertoli cell differentiation (Barrionuevo et al., 2006; Chaboisser et al., 2004; Sock et al., 2001; Takada and Koopman, 2003). Several studies have shown that *Sox8* and *Sox10* can have limited functional redundancy (Kellerer et al., 2006; Maka et al., 2005; Stolt et al., 2004) and furthermore that ectopic expression of *Sox9* will induce endogenous *Sox10* expression in some cell types (Chueng and Briscoe, 2003). In our present study the detection of *Sox10* in pre-Sertoli cells of the male genital ridge between e11.5 to e13.5 suggests a potential involvement in sex determination and differentiation (Figure 4, A-C). Studies of naturally occurring mutations in mice and men, as well as transgenic knockout mice, reveal a role for *SOX10* predominantly in the neural crest cell populations and their derivatives. In humans, haploinsufficiency of *SOX10* can result in Hirschprung disease defined by an absence of enteric ganglia in the myenteric and submucosal

plexus along variable lengths of the distal gut (Gabriel et al., 2002). In homozygous *Sox10* knockout mice, melanocytes, peripheral glia and the enteric nervous system are missing (Kellerer et al., 2006). WISH analysis of *Sox10* confirms expression within the male genital ridge compared to the female genital ridge at e1.5, e12.5 and e13.5, but does not suggest a strictly pre-Sertoli cell expression pattern. Although no anomalies in sex determination or differentiation are reported for *SOX10* mutations, its expression within the male genital ridge during a critical period of early sex differentiation suggest that it may influence the action of other molecules such as SOX9, as is seen in the case of Sox8 (Chaboisser et al., 2004).

The *Stc2* gene codes for a protein believed to play a role in calcium homeostasis. STC2 is an antagonist of FSH-stimulated progesterone production in granulosa cells (Luo et al., 2005) and inhibits the sodium-phosphate transporters, NaPi-3, in the kidney (Ishibashi et al., 1998). In addition, STC2 is involved in cell survival during oxidative stress and hypoxia (Ito et al., 2004). Transgenic mice over-expressing STC2 display dramatic growth retardation in almost every organ and tissue type with the exception of the testes which are identical in weight to wild-type male mice at birth (Gagliardi et al., 2005). Via genital ridge WISH analysis, *Stc2* shows expression within the male but not the female genital ridge from e11.5 to e13.5 (Figure 4, D-F), suggesting a potential role for STC2 in testes formation. It is noteworthy that this expression is not confined to a strictly pre-Sertoli cell expression pattern, and also that expression is seen within the mesonephros at later time periods. Interestingly, it is known that Sry and Sox9 associate with the nuclear transport protein calmodulin in a calcium dependent manner (Harley et al., 1996); it is attractive to speculate that the genital ridge expression of *Stc2* may increase calcium concentrations within the testicular environment thereby facilitating calmodulin mediated nuclear localization of Sry and Sox proteins. Further expression studies are warranted.

FGF9 and FGF18 are secreted growth factors that can both signal through the Fgfr2 and Fgfr3 transmembrane receptor proteins. Both *Fgf9* and *Fgf18* display male

specific expression in the gonads during testes differentiation (Xu et al., 2000; Schmahl et al., 2004; Willerton et al., 2004). FGF9 is important in male sex determination and differentiation (Colvin et al., 2001; Schmahl et al., 2004); *Fgf9* null mice have reduced Sertoli cell populations and exhibit markers of ovarian development including *Fst* and *Bmp2* (DiNapoli et al., 2006). *Fgf18* is important in cell proliferation and differentiation in various tissues (Hu et al., 1998; Ohbayashi et al., 2002; Whitsett et al., 2002). We detect *Fgf18* expression via WISH in developing testis cords by e13.5, and also within the male mesonephros (Figure 4, G-I). Lack of detectable genital ridge expression at earlier time points via WISH may reflect the relative low resolution of this method compared to other methods such as RT-PCR and microarray analysis. Because of its expression pattern within the male genital ridge, we can speculate that FGF18 may have a supporting role to play in pre-Sertoli cell proliferation. An FGF18 knockout mouse line has been described, but to date this model has not been characterized with respect to the genital ridge (Ohbayashi et al., 2002).

Fgf13, also called Fibroblast growth factor homologous factor 2 (Fhf2) is known to be expressed within the brain and muscle (Gecz et al., 1999). Defects in *FGF13* in humans result in hypogonadism, severe mental defects, epilepsy and hypometabolism (Borjeson et al., 1962). Fgf13 serves as a cofactor recruiting mitogen-activated protein kinase (MAPK) to the scaffold protein IB2 (Schoorlemmer and Goldfarb, 2001). Extracellular signals including FGFs (Kouhara et al., 1997), neurotropins (Patapoutian and Reichardt, 2001; Sawada et al., 2000), HGF (Stuart et al., 2000) and PDGFs (Lubinus et al., 1994) stimulate several cellular responses involving the activation of MAPK, to coordinate proliferation, differentiation, migration and mitosis (Tibbles and Woodgett, 1999). We now show that *Fgf13* expression is detected by WISH within the testis cords of the male genital ridge by at least e13.5 (Figure 4, J-L). It is reasonable to suggest that FGF13 may contribute to signal transduction within pre-Sertoli cells of the developing male genital ridge.

Wnt6 is is a Wnt family signalling protein known to have a role in somite formation by regulating epithelialisation (Schmidt et al., 2004). Within the kidney, Wnt6 plays a role in tubulogenesis (Itäranta et al., 2002), and can have both redundant and antagonistic functions with Wnt4 (Itäranta et al., 2002). Within the developing ovary, the proper development of the Müllerian ducts and the repression of testosterone production depend upon Wnt4 expression (Vaino et al., 1999; Jeays-Ward et al., 2003). In our microarray study *Wnt6* is expressed at a higher level in pre-Sertoli cells compared to the XX genital ridge cells. However, via WISH, *Wnt6* expression was detected in both the male and female gonads at approximately equal levels at all time points studied (Figure 4, M-O). This discrepancy between microarray and WISH results could reflect the relative resolution of these two techniques. A more detailed expression study of *Wnt6* within the developing male and female genital ridges is now warranted.

In summary, we have combined fluorescent marking of cell populations in transgenic models with differential expression screening to study gene expression in presumptive pre-Sertoli cells of the mammalian genital ridge. Within the 994 genes found over expressed the largest functional groups identified were for cell signaling molecules followed by extracellular matrix molecules and cell structure component molecules. Using *in situ* hybridization, we have identified new candidate genes for involvement in testes differentiation and pre-Sertoli cell function, including *Sox10, Stc2, Fgf18,* and *Fgf13.* The exact nature of this involvement must await further studies.

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Table 1. List of selected genes and respective primer oligomers used to confirm expression in pre-Sertoli cell transcriptome via RT-PCR amplification. Length of amplified band is given in base pairs (bp).

Gene	Primers (sense, antisense)	PCR length (bp)
Bag3	5'-AGCATCCAGGTGTGCTGAAAGTGG	465
	5'-ACACTAGGGAGCCACCAGGTTGC	
Bag4	5'- CAAGTCCAGTATAGTGCTGAGCCT	406
	5'- TGGCCAAATCAGTCACAGGCTCC	
Bcl2	5'- TGACTTCTCTCGTCGCTACCGTC	404
	5'- CCCAGGTATGCACCCAGAGTGAT	
Ctgf	5'- AAGCTTACGCCATGTCTCCGTACATCTTC	1000
	5'- GAATTCATGCTCGCCTCCGTCGCAGGT	
Fgf13	5'- GAATTCATGGCGGCGGCTATCGCCAGCTCGCT	730
	5'- AAGCTTCTACGTTGATTCGTTGTGGCTCATG	
Fgf18	5'- AAGCTTCCTCTGATGTAGTTCTCTGGGG	600
	5'- GAATTCATGTGGACTTCCGCATCCACGTG	
Gdnf	5'- GAATTCATGGGTCTCCTGCATGGGATTCG	750
	5'- AAGCTTCAGATACATCCACACCGTTTAG	
Inhb	5'- TGTGTGGAAGGAGGAAGCTGAGC	470
	5'- GAATTCTACCTGAAACTGCTCCCCTATGTC	
Mitf	5'- GTTGTTGGTAAAGGTGATGGTACCG	233
	5'- GGCACCTGCTGCTCAGAGTACAG	
Nr5a2	5'- GAATTCGGACAATCTTCCTGGTTACTGGAG	425
	5'- AAGCTTAGGCTCTTTTGGCATGCAGC	
Nr0b2	5'- GAATTCAAGATCCTGCTAGAGGAAGCCAG	419
	5'- AAGCTTGGTCACCTCAGCAAAAGCATGTC	
Sox10	5'- GAATTCATGAACGCCTTCATGGTGTGGG	426
	5'- AAGCTTCTGTCTTTGGGGTGGTTGGAG	
Stc2	5'- GAATTCATGTGTGCGGAGCGGCTGGGCCA	900
	5'- AAGCTTCATTTCACCTCCGGATGTCGGA	
Trap1a	5'- CTGGATAGCCAGGCAAAGCAAGC	469
	5'- CTGCATGCCTAAGGTGAGAAGCC	
Wnt6	5'- AAGCTTCAGAGGCACAGGCTGAGTTCC	400
	5'- GAATTCTCTGCGCACCTGCTGGCAGAA	

Table 2. Biological classification of 994 genes over expressed (2.5x) in e12.0 pre-Sertoli cells.

Genes are classified based on OMIM and SOURCE databases.

Biological Function	Number of Genes	%
Unknown	334*	38.9
Cell signalling	168	19.6
Extracellular matrix/cell adhesion	91	10.6
Metabolism & Apoptosis	87	10.1
Nutrient Transport	45	5.2
DNA binding & RNA associated	34	4.0
Protein modification	25	2.9
Immune function	25	2.9
Miscellaneous	49	5.7

*Redundant robe sets were not eliminated.

Table 3. Top 100 probe sets over-expressed (2.5 fold or greater) in pre-Sertoli cells of the male genital ridge at e12.0. Redundant probe sets for a given gene are included. Genes known to be expressed within the male genital ridge at the time of testis determination or early testis differentiation are in bold. The full list of over-expressed probe sets is available as supplementary data.

Probe	Title	Gene	Genbank	Cytoband	Fold
1460734_at	procollagen type IX alpha 3	Col9a3	BG074456	2 108.0 cM	587
1460693_a_at	procollagen type IX alpha 3	Col9a3	BG074456	2 108.0 cM	260
1426438_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked	Ddx3y	AA210261	Y 2.07 cM	228
1443287_at	Mus musculus 2 days pregnant adult female ovary cDNA RIKEN full-length enriched library clone:E330020H17 product:unknown EST full insert sequence		BB555669	2 E5	165
1419080_at	**glial cell line derived neurotrophic factor**	**Gdnf**	NM_010275	15 A1	147
1423859_a_at	**prostaglandin D2 synthase (brain)**	**Ptgds**	**AB006361**	2 12.9 cM	143
1418744_s_at	RIKEN cDNA 2410011K10 gene	2410011K10Rik	NM_021344	5 64.0 cM	132
1434959_at	**desert hedgehog**	**Dhh**	**AV367068**	15 57.4 cM	124
1438654_x_at	monocyte to macrophage differentiation-associated 2	Mmd2	AV269411	5 G2	121
1453486_a_at	per-hexamer repeat gene 5	Phxr5	BI133839	7 E3	114
1417210_at	eukaryotic translation initiation factor 2 subunit 3 structural gene Y-linked	Eif2s3y	NM_012011	Y 2.12 cM	111
1418813_at	serine (or cysteine) proteinase inhibitor clade A member 5	Serpina5	NM_008785	12 E	95.1
1456076_at	testis specific beta defensin-like	Tdl	AV209128	2 H1	93.9
1423860_at	**prostaglandin D2 synthase (brain)**	**Ptgds**	**AB006361**	2 12.9 cM	55.7
1424534_at	monocyte to macrophage differentiation-associated 2	Mmd2	BC025064	5 G2	54.2
1416645_a_at	albumin 1	Alb1	NM_007423	5 50.0 cM	50.3
1418252_at	peptidyl arginine deiminase type II	Padi2	NM_008812	4 71.0 cM	48.7
1451712_at	RIKEN cDNA 8030411F24 gene	8030411F24Rik	AF440735	2 G3	43.6
1434873_a_at	potassium channel tetramerisation domain containing 11	Kctd11	BB115902	11 B3	39.5
1434528_at	alanine and arginine rich domain containing protein	Aard	AV256613	15 C	35.5
1450567_a_at	procollagen type II alpha 1	Col2a1	NM_031163	15 54.5 cM	34.9
1441213_at	cDNA sequence BC021891	BC021891	AV370141	8 E2	33.8
1448906_at	cadherin 16	Cdh16	NM_007663	8 50.0 cM	32.7
1449031_at	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	Cited1	U65091	X 40.1 cM	32.0
1436528_at	expressed sequence AI842353	AI842353	AI842353	19 C3	31.4
1423407_a_at	fibulin 2	Fbln2	BF228318	6 37.2 cM	30.8
1429768_at	dystrobrevin alpha	Dtna	BB355121	18 18.0 cM	30.2
1417924_at	p21 (CDKN1A)-activated kinase 3	Pak3	BQ174935	X F2	28.7
1456069_at	dystrobrevin alpha	Dtna	BM117918	18 18.0 cM	28.1
1416411_at	glutathione S-transferase mu 2	Gstm2	NM_008183	3 F2.3	26.5
1434653_at	PTK2 protein tyrosine kinase 2 beta	Ptk2b	AV026976	14 28.0 cM	26.9
1422072_a_at	glutathione S-transferase mu 6	Gstm6	NM_008184	3 F2.3	26.2
1455436_at	Mus musculus adult male hippocampus cDNA RIKEN full-length enriched library clone:2900052J15 product:unclassifiable full insert sequence		BM114282		26.2
1435486_at	p21 (CDKN1A)-activated kinase 3	Pak3	BQ175796	X F2	26.2

1433532_a_at	myelin basic protein	Mbp	AI323506	18 55.0 cM	25.9
1417109_at	lipocalin 7	Lcn7	BC005738	4 D2.2	25.8
1450673_at	procollagen type IX alpha 2	Col9a2	NM_007741	4 53.0 cM	25.5
1436879_x_at	albumin 1	Alb1	AV124668	5 50.0 cM	25.2
1435693_at	cDNA sequence BC012256	BC012256	AV378589	2 F1	24.8
1448220_at	RIKEN cDNA 2200008D09 gene	2200008D09Rik	NM_025583	8 E1	24.5
1438718_at	**fibroblast growth factor 9**	**Fgf9**	**AW046863**	14 21.0 cM	24.2
1447845_s_at	**vanin 1**	**Vnn1**	**AV360029**	10 A1-B2	24.2
1449484_at	stanniocalcin 2	Stc2	AF031035	11 A4	23.5
1452077_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked	Ddx3y	AA210261	Y 2.07 cM	22.8
1428571_at	procollagen type IX alpha 1	Col9a1	AK004383	1 15.0 cM	22.4
1424477_at	cDNA sequence BC019731	BC019731	BC019731	5 G2	21.8
1443823_s_at	ATPase Na+/K+ transporting alpha 2 polypeptide	Atp1a2	AV325919	1 94.2 cM	21.2
1449533_at	RIKEN cDNA 1810057C19 gene	1810057C19Rik	NM_026433	11 C	21.0
1419100_at	serine (or cysteine) proteinase inhibitor clade A member 3N	Serpina3n	NM_009252	12 E	21.0
1449392_at	hydroxysteroid (17-beta) dehydrogenase 1	Hsd17b1	NM_010475	11 60.25 cM	20.7
1423271_at	gap junction membrane channel protein beta 2	Gjb2	AV239646	14 21.0 cM	20.6
1415935_at	SPARC related modular calcium binding 2	Smoc2	NM_022315		20.5
1435126_at	dual specificity phosphatase-like 15	Dusp15	BB174864	2 H1	20.4
1419072_at	RIKEN cDNA 0610005A07 gene	0610005A07Rik	NM_026672	3 F2.3	19.9
1421114_a_at	dermatan sulphate proteoglycan 3	Dspg3	NM_007884	10 55.0 cM	19.8
1440813_s_at	plexin B3	Plxnb3	AI451018	X A7.1	19.7
1416632_at	malic enzyme supernatant	Mod1	BC011081	9 48.0 cM	19.7
1448871_at	mitogen activated protein kinase 13	Mapk13	NM_011950	17 A3-B	18.7
1416666_at	serine (or cysteine) proteinase inhibitor clade E member 2	Serpine2	NM_009255	1 48.6 cM	18.3
1418370_at	troponin C cardiac/slow skeletal	Tncc	NM_009393	14 10.0 cM	18.3
1434421_at	RIKEN cDNA B930052A04 gene, Immunoglobulin superfamily containing leucine-rich repeat 2	Islr2, B930052A04Rik	BB344549	9 B	17.8
1443322_at	Mus musculus transcribed sequences		AV328597		17.7
1416468_at	aldehyde dehydrogenase family 1 subfamily A1	Aldh1a1	NM_013467	19 12.0 cM	17.4
1438068_at	Mus musculus adult male olfactory brain cDNA RIKEN full-length enriched library clone:6430501N10 product:unknown EST full insert sequence		BB251859		17.3
1439604_at	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 16	Adamts16	AV319357	13 B3	17.1
1424930_s_at	hypothetical protein MGC27770	MGC27770	BC016600	15 E1	17.0
1448330_at	glutathione S-transferase mu 1	Gstm1	NM_010358	3 F2.3	16.9
1433787_at	Mus musculus similar to retinoblastoma-binding protein 6 isoform 2; proliferation potential-related protein; RB-binding Q-protein 1 (LOC243998) mRNA		AI841091	7 B3	16.9
1422127_at	**desert hedgehog**	**Dhh**	**NM_007857**	15 57.4 cM	16.8
1416225_at	alcohol dehydrogenase 1 (class I)	Adh1	BC013477	3 71.2 cM	16.6
1417923_at	p21 (CDKN1A)-activated kinase 3	Pak3	BQ174935	X F2	16.3
1418743_a_at	RIKEN cDNA 2410011K10 gene	2410011K10Rik	NM_021344	5 64.0 cM	16.1
1453063_at	clathrin light polypeptide (Lcb)	Cltb	AK009844	13 B1	16.0
1431362_a_at	SPARC related modular calcium binding 2	Smoc2	AK006809		15.7
1418486_at	**vanin 1**	**Vnn1**	**NM_011704**	10 A1-B2	15.4
1455080_at	protein phosphatase 1 regulatory (inhibitor) subunit 16B	Ppp1r16b	BB375209	2 H2	15.2
1424950_at	**SRY-box containing gene 9**	**Sox9**	**BC024958**	11 69.5 cM	15.1
1456823_at	Mus musculus similar to chromosome 14 open reading frame 50 (LOC210762) mRNA		AV274491	12 C3	14.8

1417150_at	solute carrier family 6 (neurotransmitter transporter serotonin) member 4	Slc6a4	NM_010484	11 42.0 cM	14.7
1418412_at	tumor protein D52-like 1	Tpd52l1	NM_009413	10 A4	14.7
1433607_at	cerebellin 4 precursor protein	Cbln4	AV343573	2 H3	14.6
1448723_at	retinol dehydrogenase 7	Rdh7	NM_017473	10 D3	14.4
1444038_at	Mus musculus transcribed sequences		BI076720		14.4
1448955_s_at	Ca<2+>dependent activator protein for secretion	Cadps	NM_012061	14 A1	14.3
1436295_at	hypocretin (orexin) receptor 1	Hcrtr1	BB803143	4 D2.2	14.2
1425985_s_at	mannan-binding lectin serine protease 1	Masp1	AB049755	16 B2-B3	14.2
1424903_at	selected mouse cDNA on the Y	Smcy	AF127244	Y 2.03 cM	13.8
1434195_at	RIKEN cDNA 6030424L22 gene	6030424L22Rik	BB042892	9 E3.1	13.7
1438408_at	RIKEN cDNA 5730467H21 gene	5730467H21Rik	BB131927	5 E3	13.7
1449396_at	amine oxidase copper containing 3	Aoc3	NM_009675	11 B2-B5	13.6
1431400_a_at	growth arrest specific 7	Gas7	AK017394	11 B3	13.5
1417381_at	complement component 1 q subcomponent alpha polypeptide	C1qa	NM_007572	4 66.1 cM	13.5
1451689_a_at	SRY-box containing gene 10	Sox10	BC018551	15 46.6 cM	13.2
1418237_s_at	procollagen type XVIII alpha 1	Col18a1	NM_009929	10 41.3 cM	13.2
1448507_at	RIKEN cDNA 4931430I01 gene	4931430I01Rik	BC019531	1 D	13.1
1434494_at	zygote arrest 1	Zar1	BG071693	4 C5	13.1
1437318_at	p21 (CDKN1A)-activated kinase 3	Pak3	BB468082	X F2	13.0
1417160_s_at	extracellular proteinase inhibitor	Expi	NM_007969	11 C	13.0
1422823_at	epidermal growth factor receptor pathway substrate 8	Eps8	NM_007945	6 66.0 cM	12.9
1415883_a_at	elastase 3B pancreatic	Ela3b	NM_026419	4 D3	12.9

FIGURES:

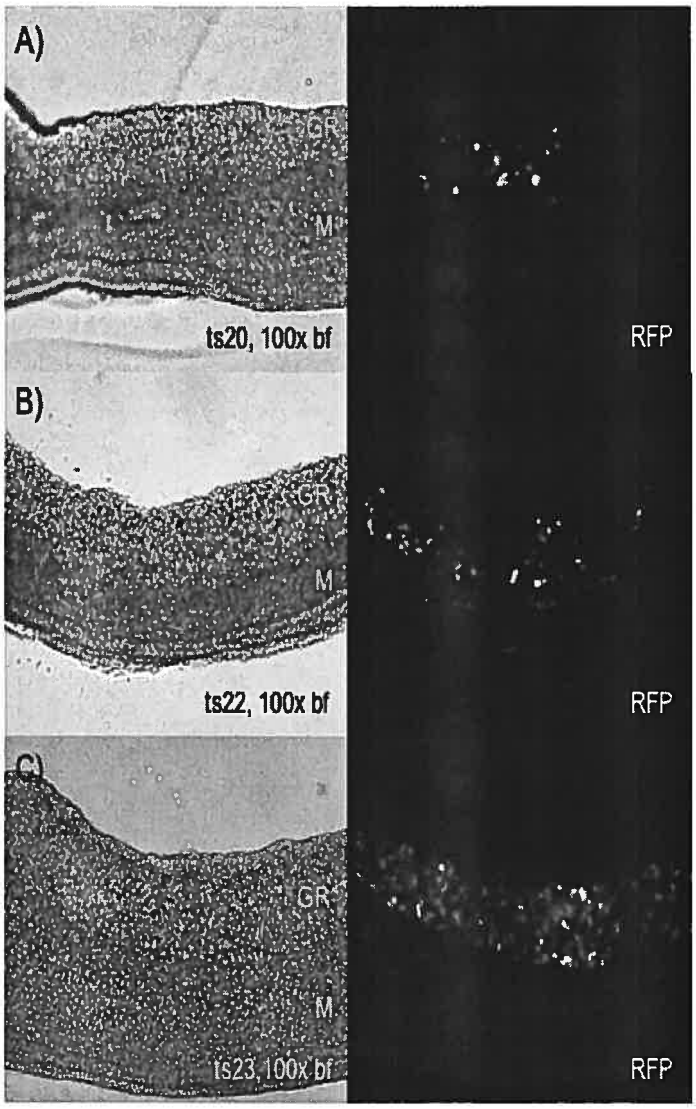

Figure 1. Onset of cell fluorescence within the male genital ridge via the HybSRY promoter-RFP transgene. Male genital ridges (GR) plus mesonephros (M) were dissected from e12.0 embryos, and then aged more precisely by counting tail somites. Left panel represents bright field image at 100x magnification; right panel represents the same image using filters for red fluorescence protein (RFP). (A) At tail somite 20 (ts20), a few cells within the genital ridge display readily detectable fluorescence. These cells were found generally in the mid portion of the genital ridge within the mesenchyme and not associated with the coelomic epithelium, and were generally dispersed in a "salt and pepper" pattern. (B) By ts22, considerably more cells of the

genital ridge display fluorescence; these cells remain dispersed and are not as yet organised. (C) By ts23 there is a marked increase in the number of cells within the genital ridge that display fluorescence. On average, about 500 cells per genital ridge display fluorescence, representing about 1-3% of genital ridge cell content. Distribution of fluorescent cells is throughout the genital ridge.

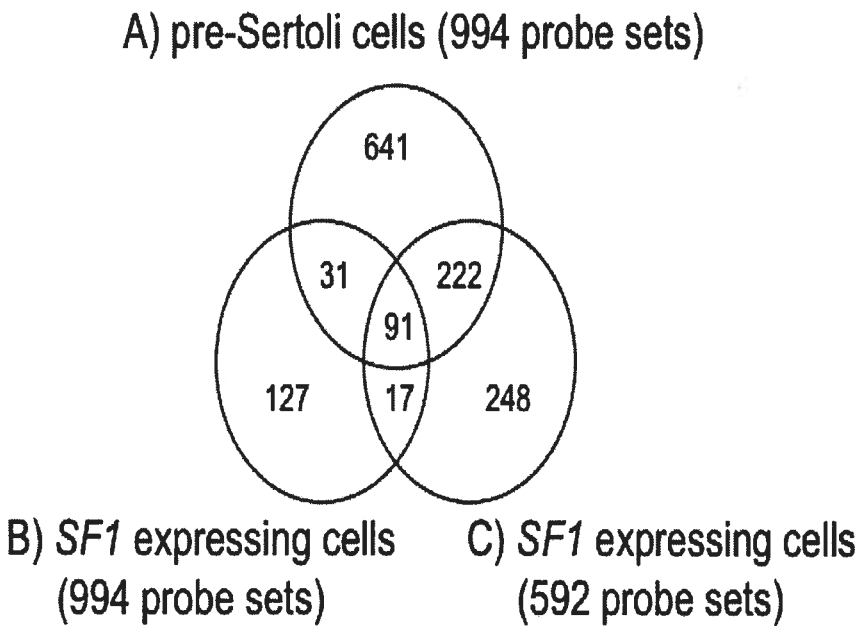

Figure 2. General comparison of transcriptome data generated from specific cell populations of the male genital ridge. (A) pre-Sertoli cells at e12.0 (present work). (B) Male genital ridge cells expressing *Sf1* at e11.5 (Beverdam and Koopman 2006). (C) Male genital ridge cells expressing *Sf1* at e12.5 (Nef et al., 2006).

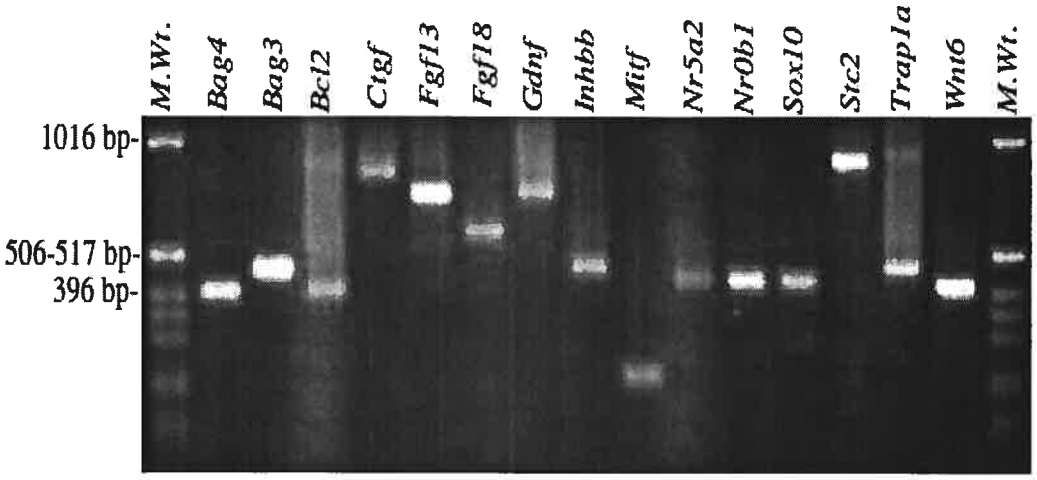

Figure 3. Examples of PCR amplified cDNAs used to validate gene expression within pre-Sertoli cell transcriptome (see Table 1), size fractionated on a 1% agarose gel. Amplified cDNA fragments were confirmed via sequencing, and selected fragments were used to generate probes for whole mount *in situ* hybridization (WISH) analysis (see Figure 4). Molecular weight (M.Wt.) standards are 1 Kb ladder (Invitrogen Canada, Burlington).

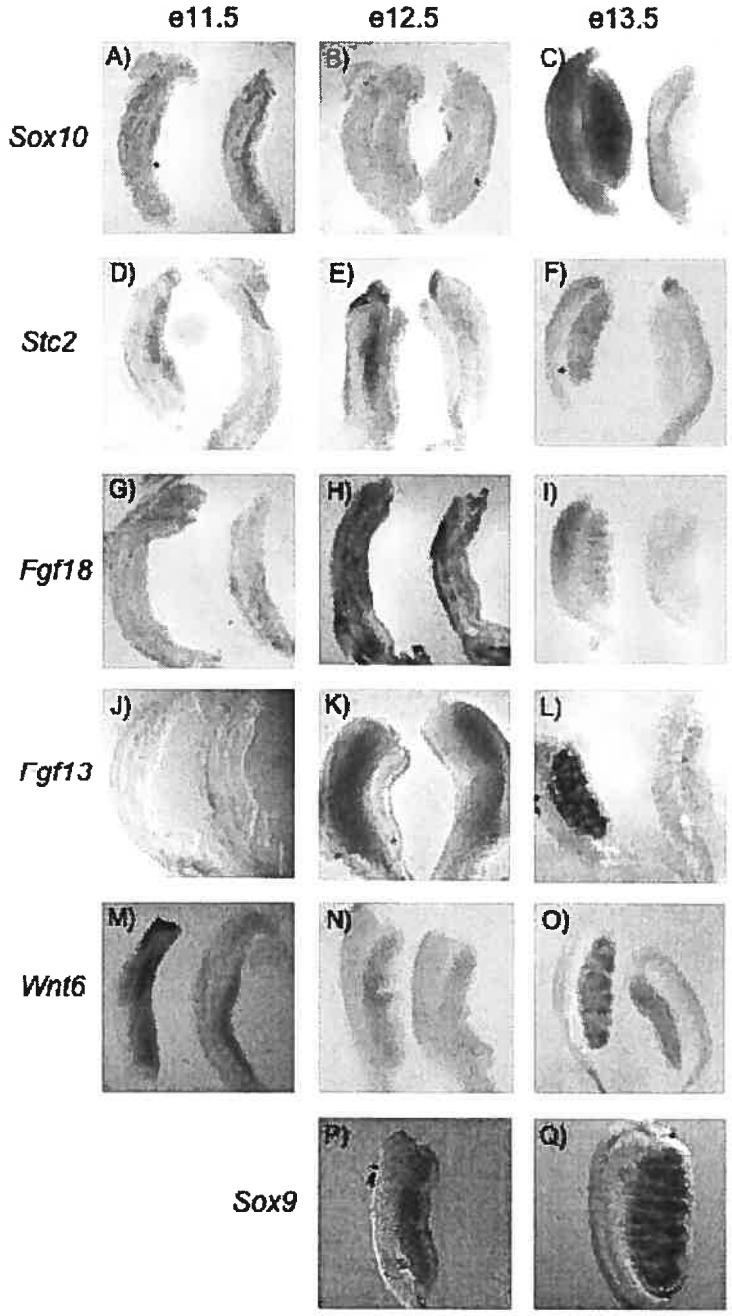

Figure 4. WISH expression patterns for *Sox10*, *Stc2*, *Fgf18*, *Fgf13*, and *Wnt6* genes in mouse fetal gonads at different developmental time points. Testes are presented on the left and ovaries are presented on the right. (A, B, C) Expression pattern of *Sox10* from e11.5, e12.5 and e13.5 respectively. Generalized expression is seen within the testes at all time periods. (D, E, F) *Stc2* gene expression for e11.5, e12.5 and e13.5 respectively. *In situ* hybridisation signal is seen within the testicle at all time periods

studied, with some staining of the mesonephros of both sexes at e12.5 and e13.5. (G, H, I) *Fgf18* gene expression at e11.5, e12.5 and e13.5 respectively. Expression is not detected in e11.5 gonads, while slight expression is detected within the testis as well as the mesonephros of both sexes at e12.5. By e13.5, strong expression is seen within the testicular cords and male mesonephros. (J, K, L) *Fgf13* gene expression for e11.5, e12.5 and e13.5 respectively. *Fgf13* expression is first detected within the mesonephros of both sexes at e12.5. By e13.5 the signal is restricted to the testis. (M, N, O) *Wnt6* gene expression at e11.5, e12.5 and e13.5 respectively. At e11.5 *Wnt6* expression is seen in both male and female gonads and is evident in all stages studied. Within the testes expression is restricted to the testes cords by e13.5 while remaining diffuse within the ovary. As a positive control for the WISH protocol, male genital ridges were hybridized with a probe for *Sox9* at e12.5 and e13.5 (P, Q).

8) CONCLUSION

8.1) Contributions to Sex Determination and Differentiation.

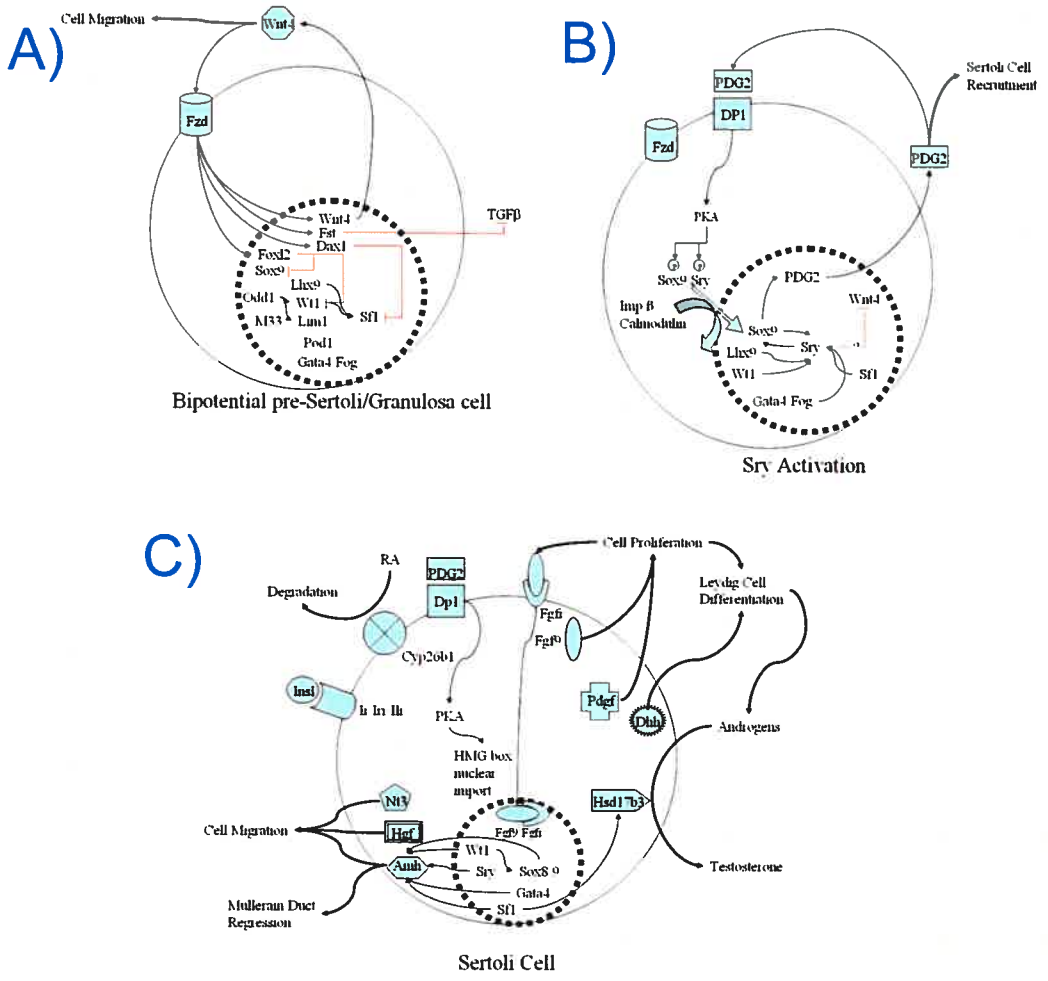

Figure 5. Putative mammalian Sertoli cell determination model. A) Largely cell autotnomous actions brought about by nuclear transcription factors specify the pre-Sertoli/granulosa cell with external Wnt4 specifying cell migration patterns. B) Expression of Sry is initiated by a combination of nuclear transcription factors leading to the production of PDG2. This initiates a positive feedback loop increasing the concentration of Sox9 and Sry in the nucleous, PDG2 also initiates the nuclear localization of Sox9 in other cells recruiting them to a Sertoli cell fate. C) The fully differentiated Sertoli cell initiates the production of several paracrine and endocrine products which in turn drive testis differentiation.

A putative schematic of some of the possible gene and protein interactions that have been discussed in the literature is presented in Figure 5.

The contribution of this thesis to the study of sex determination and differentiation is the identification of genes up regulated in the pre-Sertoli cells compared to the XX genital ridge shortly after the time of *Sry* expression. This study represents the earliest putative pre-Sertoli cell microarray done within the genital ridge to date. By FAC sorting cells from the HybSRYp-RFP transgenic mouse genital ridge at tail somite 20 we have isolated the earliest pre-Sertoli cells thus far. The limitations imposed by RFP being a protein that needs approximately 24 hours from gene expression to signal preclude us from sorting an earlier cell population. In order to have sorted cells at an earlier date we would need to use a GFP marker that has a shorter translation time (about 6-8 hours). On the other hand this 24 hour time lag may mean that we have isolated the original pre-Sertoli "stem cells" originating from the coelomic epithelium (see 4.3.1). This would correspond to the salt and pepper phenotype observed in the genital ridge as the original cells expressing RFP mRNA would have 24 hours to migrate from the coelomic epithelium and enter the gonad Subsequent cell divisions of these original "stem cells" would give them an appearance of random placement in the genital ridge.

A putative model of the five genes studied in this microarray study and their placement in the male sex determination and differentiation cascade is shown in figure 6. Several of these genes including *Wnt6*, *Fgf18* and *Fgf13* are most likely involved in sex differentiation and not sex determination. Wnt6 and Fgf18 may be involved in a redundant system of checks and balances working antagonistically to each other similar to the theory of Fgf9 vs. Wnt4 balance proposed by Kim et al. (2006). Fgf13 is perhaps involved in the regulation and modification of MAPK signaling pathways stimulated by such proteins as Hgf, Pdgf and neurotropins. Stc2 and Sox10 display an expression pattern more consistent with a direct role in sex determination. Sox10 may influence the concentration of E box Sox proteins in the nucleus directly. On the other hand, Stc2 may influence the nuclear localization of Sry and E box Sox proteins by increasing calcium concentrations thereby increasing

the effectiveness of calmodulin mediated transport of these proteins to the nucleus. While all of these hypotheses seem attractive more studies will need to be done to determine their authenticity.

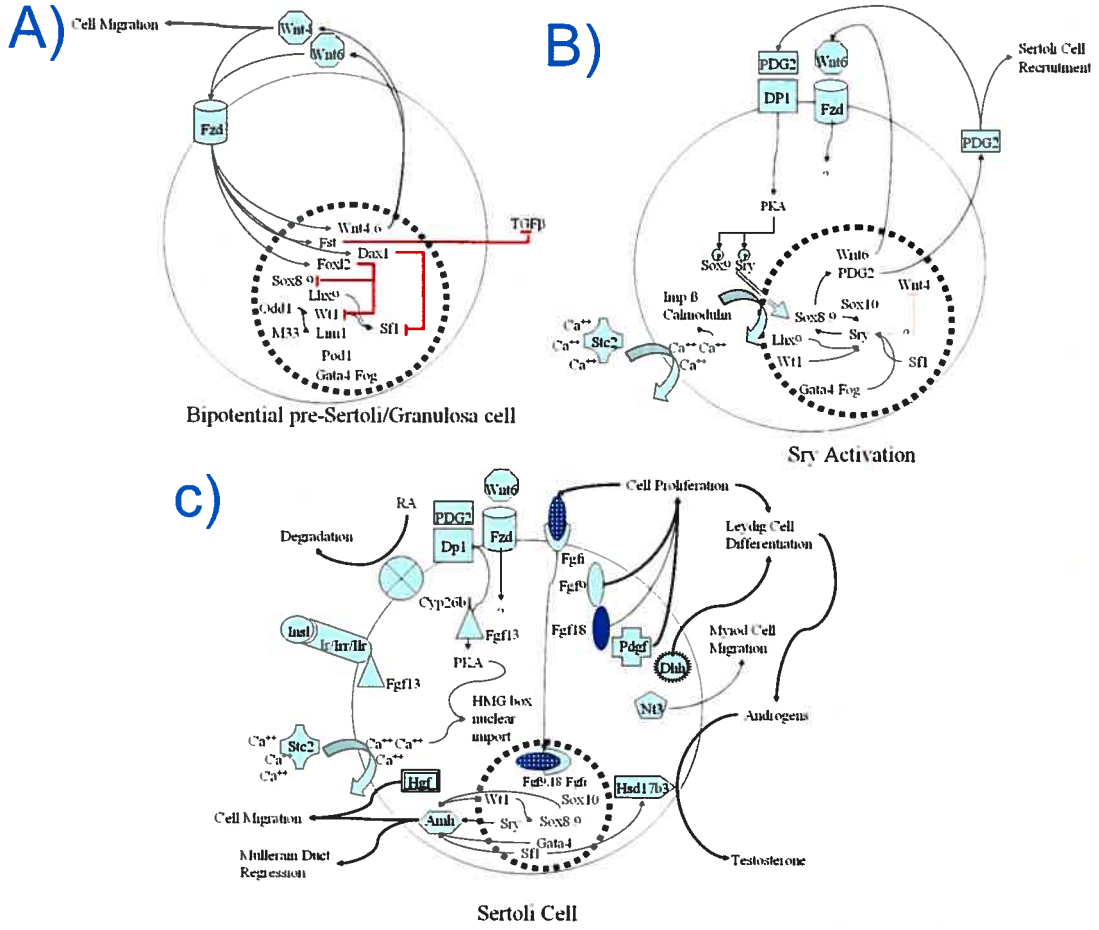

Figure 6. Putative mammalian Sertoli cell determination model. A) Wnt6 is produced and participates in roles similar to Wnt4. B) Stc2 and Sox10 help with male sex determination by increasing the efficiency of calmodulin and increasing the amount of Sox related genes in the nucleous. C) Fgf13 works to modifiy the intercellular signaling, while Fgf18 influences cell growth within the same pathway as Fgf9.

8.1.2) Other Interpretations of the Microarray Data

One way that we can infer whether a given pathway is activated in one cell population as compared to another is by looking for its' "footprint," those genes that

would be activated by the pathway. While few of the probes associated with core proteins in signaling pathways are represented in our data many of the modifiers of these pathways are. The reason that evidence for the core proteins are missing may be due to the comparative nature of this microarray. mRNA that is expressed at similar levels between the ovarian cells and the pre-Sertoli cells would cancel each other out and therefore not be represented in the microarray. Several themes are seen within the data collected from the microarray that have not been mentioned in the paper. Within the genes overexpressed there is evidence for the activation of several cell signaling categories including probes associated with Wnt, Rho/Rac/Ras, G-protein, insulin, MAPK and calcium signaling.

8.1.3) Sry as a Canonical Transcription Factor

One interpretation of this microarray data may help to answer the central question in sex determination, the exact role of SRY. Sry is thought to recognize and bind to the consensus sequence AACAAT, this consensus sequence is also shared by Lef-1 and Tcf-1, two transcription factors that are direct targets of the WNT pathway. These two transcription factors are known to act in protein complexes both as enhancers and repressors of transcription depending on the relative amounts of β-catenin. In the resting cell these transcription factors are complexed with the Groucho protein (GRO) causing them to be transcriptionally repressive. In the presence of canonical Wnt signaling, β-catenin concentrations are increased in the cell, leading it to enter the nucleus (see 5.3.4). When in the nucleus β-catenin will replace GRO, switching this repressive complex into a transcriptionally active one (reviewed by Logan and Nusse, 2004).

Wnt4, Wnt5 and Wnt11 are known as the Wnt5a subclass. These Wnt molecules can activate a noncanonical pathway that is antagonistic to the canonical pathway at two levels. Upon stimulation of Frizzled by these proteins, a G-protein complex phosphorilates Dishevelled making it unable to participate in the canonical pathway (reviewed by Veeman et al., 2003). Furthermore, the stimulated G-protein complex works to release internal calcium stores and initated a cascades involving

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) that leads to the phosphorilation of Tcf1 and exportation of this protein out of the nucleous preventing it from binding to DNA (Ishitani et al., 2003). If Wnt4 is acting in a non-canonical fashion in the pre-Sertoli cell it may work to repress the Tcf/Lef/β-catenin complex and therefore the expression of downstream genes (reviewed by Veman et al., 2003).

A putative role for Sry action may be to compete for these binding sites and therefore negate the effect of this repressive complex or simply circumvent the repressive noncanonical pathway and allow the expression of Wnt target genes. SRY acting similarly to a canonical transcription factor is an attractive theory in light of the Wnt6 expression pattern described. While Wnt4 is seen during the same time period as Wnt6 it may be suppressing the action of Wnt6 through the non-canonical pathway. Within the kidney, Wnt4 has both redundant and antagonistic functions with Wnt6 therefore it would not be unprecedented to see these two Wnts acting antagonistically in the testes (Itäranta et al., 2002). Secondly, the presence of mRNA species including those for CaMKII, Down syndrome critical region homolog 1 (Dscr1) and groucho related gene 6 (Grg6/Tle6) in the pre-Sertoli cell microarray suggest an active non-cannonical pathway. Thirdly, the presence of Wnt5a mRNA is seen as four fold overexpressed in the ovarian tissue compared to pre-Sertoli cell mRNA. The Wnt5a activation of the noncanonical pathway may be why ovaries of Wnt4 knockout mice do not become totally sex reversed.

Activation of genes such as the E box Sox genes may in turn repress the non-canonical pathway allowing the canonical pathway to function and favor the formation of the Tcf/Lef/β-catenin complex which may or may not be able to replace the action of Sry. Initial experiments using a promoter containing 2 Lef-1 binding sites that drives a lucifrase reporter show both pig SRY and mouse Lef-1/β-catenin can activate the expression of the reporter gene in a similar manner (unpublished data). This lends some credence to the theory that Sry works by circumventing the noncanonical Wnt pathway. If the noncanonical pathway is preventing TCF/LEF from entering the nucleus, SRY is well situated to interact with these binding sites.

While this is promising, further study is needed to identify whether SRY can outcompete TCF/LEF/GRO complexes for these same binding sites.

8.2) Analysis of the Microarray Technique

Within the last two years the study of sex determination has entered an era of microarray data. This new technology has allowed us to peer more closely at the genes expressed at different time points throughout sex determination and differentiation. These studies have revealed several new mechanisms involved in germ cell differentiation (see 5.3.1) and sex determination (see 5.4.2) as well as at least one theory of checks and balances (see 5.3.4.2). While microarray analysis is a powerful tool it is still in its infancy and as with most children it has some problems that it must grow out of and a lot of potential to grow into.

8.2.1) Limitations of the Microarrays

While microarrays do provided a useful tool for researchers there are specific limitations some of which are immediately evident and others that are less evident. The sensitivity of the microarray is a major limiting factor. The best example of this is with the probe for *Sry*. Within our study, as well as the experiment by Beverdam and Koopman (2006), *Sry* was not found when comparing cells from the male to the female genital ridge. Within our study *Sry* may not have been present as the mRNA is diminishing in the genital ridge by ts23 but Beverdam and Koopman (2006) should have found it at e11.5. As mentioned *Sry* is arguably the most important gene effecting male sex determination therefore the inability of the microarray to detect *Sry* as overexpressed in the male genital ridge when compared to the female is concerning. This glaring omission begs the question as to what else has been missed? Also the amount of RNA needed to perform a single hybridization forces researchers to either limit the specificity of their sample by including more than one cell type or, as in this study, resort to pooling of cells from several samples. This limits the amount of specificity by increasing the likelihood of having including unwanted cell types in the sample.

A 2nd limitation to the microarray is that it limits the amount of information to RNA species that are already known. The design of the microchip is such that the manufacturer is limited to RNA species that are already discovered. Other screening types such as the SSH are not limited in this regard allowing the discovery of new gene expression and perhaps new splice variants. By claiming, as the Affymetrix GeneChip® Mouse Genome 430.20 microarray does, that this microchip has probes for the completely expressed genome it may hamper discovery of other RNA species or splice variants.

A 3rd technical limitation of this microarray is the use of the poly A tail to create the probes. The use of the poly A tail to amplify and create the cDNA probes biases the results in two ways: 1) it ensures that only mRNA is converted to a readable probe and 2) it has a limited capability to identify splice variants if the splicing event occurs on the 5 prime end of the mRNA. This can cause several problems: 1) non-coding RNA that may be regulating cell processes are entirely ignored and 2) splice variants may be just as easily overlooked due to these technical limitations. This has resently been addressed by the newer human, rat and mouse microarrays. Through the use of random primer amplification and whole RNA transcript probes the Affymetrix Mouse Exon 1.0 ST array can now avoid these problems.

The 4th limitation of microarrays has to do with the limited amount of general knowledge in genetics. Within the microarray used by this study a large portion of the probes on the microchip correspond to RNA species with no functional data. Examples of these probes include the 334 genes classified into the unknown category in Table 2 of the article. While these four areas are major limiting factors of the microarray they represent technological problems that will no doubt be dealt with in the near future.

8.2.2) Potential of the Microarray

The limitations of any tool are those imposed by its intended function and by the imagination of its user. In this a microarray is no different. This tool has an amazing potential in the study of sex determination and differentiation.

The gathering of massive amounts of information on tissues and cell lines is the most obvious function of microarrays. By comparing transcriptomes across cell lines we will have a better understanding of how these cells function. Indeed this has been demonstrated in the present study. By comparing pre-Sertoli cells to the ovary we have identified a large number of genes expressed by pre-Sertoli cells. Other comparisons that could be done with this model system could be the comparison of pre-Sertoli cells to other cells in the male genital ridge, much like the study done by Boyer et al. (2004). This could potentially highlight potential interactions between pre-Sertoli cells and other cells in the genital ridge environment. Also by comparing this cell line to other cell lines such as the neural crest or Leydig cells we may be able to determine the interactions between developing organ systems and different origins of cells.

By comparing similar cells across time (eg. one developmental stage to the next) we will be able to develop a better understanding of how cells and tissues differentiate. With our model system the comparison of RFP pre-Sertoli cells from one day to the next would give greater insight into how Sertoli cells differentiate over time. The coupling of this microarray data to protein data would also allow us greater insight into the working of the same cell on several levels. By comparing mRNA data to the proteins that are present at similar time points we would be able to determine how much of sex determination and differentiation is regulated at the RNA-protein translation level.

Perhaps the greatest function of microarray technology is that it can allow a researcher to have a holistic approach to understanding gene interactions. By comparing tissues or cell lines from mice subjected to certain treatments (eg. gene knockout, replacement, mutation, ect.) to normal mice a researcher would have a much better understanding of how the treatment alters gene expression. Indeed, some research in sex determination has started to realize this potential as seen in the recent work by Coveney et al. (2006).

8.3) Defining Sex

The word "sex" comes from the Latin word *sexus*, to cut or separate, but sex is not a clear-cut biological entity. Even within species with obvious male or female groups, individuals can differ along a spectrum of peculiarities of structure and function that define males or females. The reason for this is the genetic complexity behind these peculiarities. While this genetic cascade has been difficult to unravel the microarray is a powerful tool that may help us to define sex.

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Appendix

Probename	Mean T/Mean C	Gene	Cytoband	Title
1460734_at	586,6933	Col9a3	2 108.0 cM	procollagen type IX alpha 3
1460693_a_at	259,9531	Col9a3	2 108.0 cM	procollagen type IX alpha 3
1426438_at	227,9422	Ddx3y	Y 2.07 cM	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked
1443287_at	164,7935		2 E5	Mus musculus 2 days pregnant adult female ovary cDNA RIKEN full-length enriched library clone:E330020H17 product:unknown EST full insert sequence
1419080_at	146,8949	Gdnf	15 A1	glial cell line derived neurotrophic factor
1423859_a_at	142,7645	Ptgds	2 12.9 cM	prostaglandin D2 synthase (brain)
1418744_s_at	131,8033	2410011K10Rik	5 64.0 cM	RIKEN cDNA 2410011K10 gene
1434959_at	123,7884	Dhh	15 57.4 cM	desert hedgehog
1438654_x_at	120,996	Mmd2	5 G2	monocyte to macrophage differentiation-associated 2
1453486_a_at	113,5104	Phxr5	7 E3	per-hexamer repeat gene 5
1417210_at	110,635	Eif2s3y	Y 2.12 cM	eukaryotic translation initiation factor 2 subunit 3 structural gene Y-linked
1418813_at	95,05861	Serpina5	12 E	serine (or cysteine) proteinase inhibitor clade A member 5
1456076_at	93,92593	Tdl	2 H1	testis specific beta defensin-like
1423860_at	55,69662	Ptgds	2 12.9 cM	prostaglandin D2 synthase (brain)
1424534_at	54,2311	Mmd2	5 G2	monocyte to macrophage differentiation-associated 2
1416645_a_at	50,27015	Alb1	5 50.0 cM	albumin 1
1418252_at	48,6934	Padi2	4 71.0 cM	peptidyl arginine deiminase type II
1451712_at	43,56691	8030411F24Rik	2 G3	RIKEN cDNA 8030411F24 gene
1434873_a_at	39,52498	Kctd11	11 B3	potassium channel tetramerisation domain containing 11
1434528_at	35,50753	Aard	15 C	alanine and arginine rich domain containing protein
1450567_a_at	34,91833	Col2a1	15 54.5 cM	procollagen type II alpha 1
1441213_at	33,80502	BC021891	8 E2	cDNA sequence BC021891
1448906_at	32,64817	Cdh16	8 50.0 cM	cadherin 16
1449031_at	32,02401	Cited1	X 40.1 cM	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
1436528_at	31,40535	AI842353	19 C3	expressed sequence AI842353
1423407_a_at	30,82195	Fbln2	6 37.2 cM	fibulin 2
1429768_at	30,18566	Dtna	18 18.0 cM	dystrobrevin alpha
1417924_at	28,7291	Pak3	X F2	p21 (CDKN1A)-activated kinase 3
1456069_at	28,11944	Dtna	18 18.0 cM	dystrobrevin alpha
1416411_at	26,52878	Gstm2	3 F2.3	glutathione S-transferase mu 2
1434653_at	26,38299	Ptk2b	14 28.0 cM	PTK2 protein tyrosine kinase 2 beta
1422072_a_at	26,19306	Gstm6	3 F2.3	glutathione S-transferase mu 6
1455436_at	26,17879			Mus musculus adult male hippocampus cDNA RIKEN full-length enriched library clone:2900052J15 product:unclassifiable full insert sequence
1435486_at	26,15383	Pak3	X F2	p21 (CDKN1A)-activated kinase 3
1433532_a_at	25,86108	Mbp	18 55.0 cM	myelin basic protein

1417109_at	25,74703	Lcn7	4 D2.2 4 53.0	lipocalin 7
1450673_at	25,51294	Col9a2	cM 5 50.0	procollagen type IX alpha 2
1436879_x_at	25,23135	Alb1	cM	albumin 1
1435693_at	24,82407	BC012256	2 F1	cDNA sequence BC012256
1448220_at	24,51484	2200008D09Rik	8 E1 14 21.0	RIKEN cDNA 2200008D09 gene
1438718_at	24,17502	Fgf9	cM	fibroblast growth factor 9
1447845_s_at	24,15176	Vnn1	10 A1-B2	vanin 1
1449484_at	23,52219	Stc2	11 A4 Y 2.07	stanniocalcin 2
1452077_at	22,82726	Ddx3y	cM 1 15.0	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked
1428571_at	22,4166	Col9a1	cM	procollagen type IX alpha 1
1424477_at	21,75098	BC019731	5 G2 1 94.2	cDNA sequence BC019731
1443823_s_at	21,16184	Atp1a2	cM	ATPase Na+/K+ transporting alpha 2 polypeptide
1449533_at	20,95882	1810057C19Rik	11 C	RIKEN cDNA 1810057C19 gene
1419100_at	20,95479	Serpina3n	12 E 11 60.25	serine (or cysteine) proteinase inhibitor clade A member 3N
1449392_at	20,68206	Hsd17b1	cM 14 21.0	hydroxysteroid (17-beta) dehydrogenase 1
1423271_at	20,55737	Gjb2	cM	gap junction membrane channel protein beta 2
1415935_at	20,52003	Smoc2		SPARC related modular calcium binding 2
1435126_at	20,40138	Dusp15	2 H1	dual specificity phosphatase-like 15
1419072_at	19,8722	0610005A07Rik	3 F2.3 10 55.0	RIKEN cDNA 0610005A07 gene
1421114_a_at	19,77151	Dspg3	cM	dermatan sulphate proteoglycan 3
1440813_s_at	19,72487	Plxnb3	X A7.1 9 48.0	plexin B3
1416632_at	19,6741	Mod1	cM	malic enzyme supernatant
1448871_at	18,70329	Mapk13	17 A3-B 1 48.6	mitogen activated protein kinase 13
1416666_at	18,2955	Serpine2	cM 14 10.0	serine (or cysteine) proteinase inhibitor clade E member 2
1418370_at	18,28054	Tncc	cM	troponin C cardiac/slow skeletal
1434421_at	17,81277	Islr2, B930052A04Rik	9 B	RIKEN cDNA B930052A04 gene, Immunoglobulin superfamily containing leucine-rich repeat 2
1443322_at	17,6791		19 12.0	Mus musculus transcribed sequences
1416468_at	17,44414	Aldh1a1	cM	aldehyde dehydrogenase family 1 subfamily A1
1438068_at	17,24624			Mus musculus adult male olfactory brain cDNA RIKEN full-length enriched library clone:6430501N10 product:unknown EST full insert sequence
1439604_at	17,06847	Adamts16	13 B3	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 16
1424930_s_at	17,01866	MGC27770	15 E1	hypothetical protein MGC27770
1448330_at	16,93596	Gstm1	3 F2.3	glutathione S-transferase mu 1
1433787_at	16,87683		7 B3 15 57.4	Mus musculus similar to retinoblastoma-binding protein 6 isoform 2; proliferation potential-related protein; RB-binding Q-protein 1 (LOC243998) mRNA
1422127_at	16,84316	Dhh	cM 3 71.2	desert hedgehog
1416225_at	16,62522	Adh1	cM	alcohol dehydrogenase 1 (class I)
1417923_at	16,24576	Pak3	X F2 5 64.0	p21 (CDKN1A)-activated kinase 3
1418743_a_at	16,04998	2410011K10Rik	cM	RIKEN cDNA 2410011K10 gene
1453063_at	15,94582	Cltb	13 B1	clathrin light polypeptide (Lcb)
1431362_a_at	15,68041	Smoc2		SPARC related modular calcium binding 2

1418486_at	15,3653	Vnn1	10 A1-B2	vanin 1
1455080_at	15,15691	Ppp1r16b	2 H2	protein phosphatase 1 regulatory (inhibitor) subunit 16B
1424950_at	15,05233	Sox9	11 69.5 cM	SRY-box containing gene 9
1456823_at	14,76314		12 C3	Mus musculus similar to chromosome 14 open reading frame 50 (LOC210762) mRNA
1417150_at	14,74083	Slc6a4	11 42.0 cM	solute carrier family 6 (neurotransmitter transporter serotonin) member 4
1418412_at	14,73842	Tpd52l1	10 A4	tumor protein D52-like 1
1433607_at	14,60704	Cbln4	2 H3	cerebellin 4 precursor protein
1448723_at	14,44166	Rdh7	10 D3	retinol dehydrogenase 7
1444038_at	14,37036			Mus musculus transcribed sequences
1448955_s_at	14,26373	Cadps	14 A1	Ca<2+>dependent activator protein for secretion
1436295_at	14,24464	Hcrtr1	4 D2.2	hypocretin (orexin) receptor 1
1425985_s_at	14,17039	Masp1	16 B2-B3	mannan-binding lectin serine protease 1
1424903_at	13,8165	Smcy	Y 2.03 cM	selected mouse cDNA on the Y
1434195_at	13,66993	6030424L22Rik	9 E3.1	RIKEN cDNA 6030424L22 gene
1438408_at	13,65961	5730467H21Rik	5 E3	RIKEN cDNA 5730467H21 gene
1449396_at	13,58548	Aoc3	11 B2-B5	amine oxidase copper containing 3
1431400_a_at	13,45449	Gas7	11 B3	growth arrest specific 7
1417381_at	13,45312	C1qa	4 66.1 cM	complement component 1 q subcomponent alpha polypeptide
1451689_a_at	13,23076	Sox10	15 46.6 cM	SRY-box containing gene 10
1418237_s_at	13,20298	Col18a1	10 41.3 cM	procollagen type XVIII alpha 1
1448507_at	13,08535	4931430I01Rik	1 D	RIKEN cDNA 4931430I01 gene
1434494_at	13,06718	Zar1	4 C5	zygote arrest 1
1437318_at	12,98205	Pak3	X F2	p21 (CDKN1A)-activated kinase 3
1417160_s_at	12,96539	Expi	11 C	extracellular proteinase inhibitor
1422823_at	12,9391	Eps8	6 66.0 cM	epidermal growth factor receptor pathway substrate 8
1415883_a_at	12,89801	Ela3b	4 D3	elastase 3B pancreatic
1425627_x_at	12,85009	Gstm1	3 F2.3	glutathione S-transferase mu 1
1452227_at	12,71123	2310045A20Rik	5 C1	RIKEN cDNA 2310045A20 gene
1422824_s_at	12,63219	Eps8	6 66.0 cM	epidermal growth factor receptor pathway substrate 8
1453345_at	12,5821	Npal1, 3830408G10Rik	5 C3.2	RIKEN cDNA 3830408G10 gene, NIPA-like domain containing 1
1437661_at	12,56472		16 A1	Mus musculus similar to MGC45438 protein (LOC239691) mRNA
1426955_at	12,53046	Col18a1	10 41.3 cM	procollagen type XVIII alpha 1
1423569_at	12,5255	Gatm	2 E5	glycine amidinotransferase (L-arginine:glycine amidinotransferase)
1417494_a_at	12,45274	Cp	9 55.0 cM	ceruloplasmin
1448918_at	12,33831	Slco3a1	7 C	solute carrier organic anion transporter family member 3a1
1433454_at	12,3258	AW539457	2 E2	expressed sequence AW539457
1422478_a_at	12,1798	Acas2	2 H1	acetyl-Coenzyme A synthetase 2 (ADP forming)
1451763_at	12,14916	Cnga1	5 41.0 cM	cyclic nucleotide gated channel alpha 1
1426439_at	12,12803	Ddx3y	Y 2.07 cM	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked
1419413_at	11,99332	Ccl17	8 45.0 cM	chemokine (C-C motif) ligand 17
1434252_at	11,93742	C630016B22Rik	10 C2	RIKEN cDNA C630016B22 gene
1456321_at	11,90134	Npal1, 3830408G10Rik	5 C3.2	RIKEN cDNA 3830408G10 gene

1428975_at	11,80512	1700017I11Rik	13 A5	RIKEN cDNA 1700017I11 gene
1420388_at	11,75736	Prss12	3 G1	protease serine 12 neurotrypsin (motopsin)
1416416_x_at	11,69137	Gstm1	3 F2.3	glutathione S-transferase mu 1
1430307_a_at	11,67876	Mod1	9 48.0 cM	malic enzyme supernatant
1456228_x_at	11,56311	Mbp	18 55.0 cM	myelin basic protein
1435994_at	11,51555			Mus musculus adult male corpus striatum cDNA RIKEN full-length enriched library clone:C030012J07 product:unknown EST full insert sequence
1428547_at	11,45887	Nt5e	9 E3.1	5' nucleotidase ecto
1436013_at	11,33301	C230098I05	7 F3	hypothetical protein C230098I05
1421602_at	11,28789	Shbg	11 35.0 cM	sex hormone binding globulin
1427529_at	11,28302	Fzd9	5 75.0 cM	frizzled homolog 9 (Drosophila)
1449873_at	11,25627	Oxct2a	4 D2.2	3-oxoacid CoA transferase 2A
1454622_at	11,24309	Slc38a5, C81234	X A1.1	Solute carrier family 38, member 5, expressed sequence C81234
1442019_at	11,1968			Mus musculus adult male corpora quadrigemina cDNA RIKEN full-length enriched library clone:B230343A10 product:unknown EST full insert sequence
1425546_a_at	11,14018	Trf	9 56.0 cM	transferrin
1428717_at	11,13691	2810019K23Rik	6 B3	RIKEN cDNA 2810019K23 gene
1435012_x_at	11,06471	Ela3b	4 D3	elastase 3B pancreatic
1422670_at	11,03725	Arhn	11 C-D	ras homolog N (RhoN)
1434945_at	11,03134	A330042H22	8 C5	hypothetical protein A330042H22
1455664_at	10,96932	Rtn4rl1	11 B5	reticulon 4 receptor-like 1
1438602_s_at	10,95908	Masp1	16 B2-B3	mannan-binding lectin serine protease 1
1421556_at	10,95888	Serpina3a	12 E	serine (or cysteine) proteinase inhibitor clade A member 3A
1427386_at	10,93807	Arhgef16	4 E2	Rho guanine nucleotide exchange factor (GEF) 16
1448453_at	10,81437	Hsd3b1	3 49.1 cM	hydroxysteroid dehydrogenase-1 delta<5>-3-beta
1425559_a_at	10,68073	Sah	7 F1	SA rat hypertension-associated homolog
1453191_at	10,64532	Col27a1	4 B3	procollagen type XXVII alpha 1
1422013_at	10,57516	Clecsf6	6 F2	C-type (calcium dependent carbohydrate recognition domain) lectin superfamily member 6
1422695_at	10,56227	Ttyh1	7 A1	tweety homolog 1 (Drosophila)
1449158_at	10,53441	Kcnk2	1 H6	potassium channel subfamily K member 2
1416523_at	10,52237	Rnase1	14 18.5 cM	ribonuclease RNase A family 1 (pancreatic)
1449401_at	10,47071	C1qg	4 66.1 cM	complement component 1 q subcomponent gamma polypeptide
1439566_at	10,3424	C730021L23	6 B3	hypothetical protein C730021L23
1450754_at	10,26687	Cacna2d2	9 60.0 cM	calcium channel voltage-dependent alpha 2/delta subunit 2
1429682_at	10,15546	4930431B09Rik	3 F2.2	RIKEN cDNA 4930431B09 gene
1417384_at	9,969489	Entpd5	12 39.0 cM	ectonucleoside triphosphate diphosphohydrolase 5
1417495_x_at	9,914971	Cp	9 55.0 cM	ceruloplasmin
1437726_x_at	9,824385	C1qb	4 66.1 cM	complement component 1 q subcomponent beta polypeptide
1435554_at	9,820449	C630016B22Rik	10 C2	RIKEN cDNA C630016B22 gene
1449164_at	9,7593	Cd68	11 39.0 cM	CD68 antigen
1417063_at	9,729437	C1qb	4 66.1 cM	complement component 1 q subcomponent beta polypeptide
1417343_at	9,709277	Fxyd6	9 A5.2	FXYD domain-containing ion transport regulator 6

1436870_s_at	9,697076	AU041783	19 D2	expressed sequence AU041783
1417577_at	9,673335	Trpc3	3 18.2 cM	transient receptor potential cation channel subfamily C member 3
1435793_at	9,648543	4632417K02	9 C	hypothetical protein 4632417K02
1449285_at	9,636237	Cst9	2 G3	cystatin 9
1452001_at	9,63204	Nfe2	15 61.7 cM	nuclear factor erythroid derived 2
1449172_a_at	9,609737	Lin7b	7 B3	lin 7 homolog b (C. elegans)
1418028_at	9,536512	Dct	14 58.0 cM	dopachrome tautomerase
1427168_a_at	9,504552	Col14a1	15 D	procollagen type XIV alpha 1
1448660_at	9,4703	Arhgdig	17 A3.3	Rho GDP dissociation inhibitor (GDI) gamma
1427537_at	9,370694	Eppk1	15 D3	epiplakin 1
1423523_at	9,272878	Aass	6 4.5 cM	aminoadipate-semialdehyde synthase
1418551_at	9,24533	Mybpc3		myosin binding protein C cardiac
1455136_at	9,193327	Atp1a2	1 94.2 cM	ATPase Na+/K+ transporting alpha 2 polypeptide
1455794_at	9,101688	D130058I21Rik	11 B4	RIKEN cDNA D130058I21 gene
1429276_at	9,057213		9 F3	Mus musculus similar to mKIAA0342 protein (LOC235639) mRNA
1419599_s_at	9,046317	Ms4a11	19 A	membrane-spanning 4-domains subfamily A member 11
1419811_at	9,032575			Mus musculus transcribed sequence with strong similarity to protein sp:P51830 (M.musculus) CYA9_MOUSE Adenylate cyclase type IX (ATP pyrophosphate-lyase) (Adenylyl cyclase) (Adenylyl cyclase type 10) (ACTP10)
1419503_at	8,993855	Stc2	11 A4	stanniocalcin 2
1439240_x_at	8,992973	Lin7b	7 B3	lin 7 homolog b (C. elegans)
1460459_at	8,978847	0610010I15Rik	9 C	RIKEN cDNA 0610010I15 gene
1435343_at	8,905704	Dock10	1 C5	dedicator of cytokinesis 10
1418497_at	8,863267	Fgf13	X 18.0 cM	fibroblast growth factor 13
1418626_a_at	8,807479	Clu	14 28.0 cM	clusterin
1440889_at	8,740523	BC006965	11 E2	cDNA sequence BC006965
1418492_at	8,723312	Prdc	1 H3	protein related to DAN and cerberus
1420697_at	8,701158	Slc15a3	19 A	solute carrier family 15 member 3
1434874_x_at	8,687607	Kctd11	11 B3	potassium channel tetramerisation domain containing 11
1426959_at	8,656646	Bdh	16 A1	3-hydroxybutyrate dehydrogenase (heart mitochondrial)
1435830_a_at	8,646791	5430435G22Rik	1 E4	RIKEN cDNA 5430435G22 gene
1417673_at	8,632111	Grb14	2 C1.3	growth factor receptor bound protein 14
1429831_at	8,567814	Pik3ap1	19 C3	phosphoinositide-3-kinase adaptor protein 1
1440200_at	8,56658			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130042N08 product:inferred: KIAA1276 protein {Homo sapiens} full insert sequence
1415936_at	8,564687	Bcar3	3 G1	breast cancer anti-estrogen resistance 3
1445897_s_at	8,506356	2010008K16Rik	11 D	RIKEN cDNA 2010008K16 gene
1419289_a_at	8,409311	Syngr1	15 E1	synaptogyrin 1
1422645_at	8,40251	Hfe	13 15.0 cM	hemochromatosis
1454795_at	8,372552	D430044D16Rik	2 C1.3	RIKEN cDNA D430044D16 gene
1437326_x_at	8,337014	Ela3b	4 D3	elastase 3B pancreatic
1434510_at	8,323867	Papss2	19 32.0 cM	3'-phosphoadenosine 5'-phosphosulfate synthase 2
1428455_at	8,322022	Col14a1	15 D	procollagen type XIV alpha 1
1434202_a_at	8,279081	MGC58343	14 A1	hypothetical protein MGC58343
1440156_s_at	8,238098			Mus musculus transcribed sequences
1460336_at	8,214194	Ppargc1	5 C1	peroxisome proliferative activated receptor gamma coactivator 1

1454909_at	8,19829	B230378H13Rik	8 A2	RIKEN cDNA B230378H13 gene
1424393_s_at	8,183462	Adhfe1	1 A2	alcohol dehydrogenase iron containing 1
1460601_at	8,126008	Myrip	9 F4	myosin VIIA and Rab interacting protein
1441380_at	8,092921	2810439F02Rik	18 A1	RIKEN cDNA 2810439F02 gene
1421671_at	8,091856	Hsd17b3	2 67.1 cM	hydroxysteroid (17-beta) dehydrogenase 3
1425248_a_at	8,078045	Tyro3	2 E2	TYRO3 protein tyrosine kinase 3
1433453_a_at	7,968385	AW539457	4 A1	expressed sequence AW539457
1436279_at	7,964459	Slc26a7	10 70.0 cM	solute carrier family 26 member 7
1422523_at	7,946197	Si	11 69.5 cM	silver
1428835_at	7,943419	2400004E04Rik	7 B3	RIKEN cDNA 2400004E04 gene
1417212_at	7,93126	9530058B02Rik	17 A3.3	RIKEN cDNA 9530058B02 gene
1450020_at	7,890772	Cx3cr1	9 F4	chemokine (C-X3-C) receptor 1
1427477_at	7,785297	LOC214531	9 A5.2	mosaic serine protease-like
1451538_at	7,748915	Sox9	11 69.5 cM	SRY-box containing gene 9
1424392_at	7,717159	Adhfe1	1 A2	alcohol dehydrogenase iron containing 1
1448110_at	7,706679	Sema4a	3 F1	sema domain immunoglobulin domain (Ig) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 4A
1418654_at	7,690658	Hao3	3 F2.2	hydroxyacid oxidase (glycolate oxidase) 3
1421989_s_at	7,667973	Papss2	19 32.0 cM	3'-phosphoadenosine 5'-phosphosulfate synthase 2
1457270_at	7,648746			Mus musculus adult male corpora quadrigemina cDNA RIKEN full-length enriched library clone:B230343A10 product:unknown EST full insert sequence
1418051_at	7,640173	Ephb6	6 B1	Eph receptor B6
1460011_at	7,622035	Cyp26b1	6 C3	cytochrome P450 family 26 subfamily b polypeptide 1
1425214_at	7,590178	P2ry6	7 E1	pyrimidinergic receptor P2Y G-protein coupled 6
1422818_at	7,572	Nedd9	13 A4	neural precursor cell expressed developmentally down-regulated gene 9
1448975_s_at	7,532744	Ren1	1 69.9 cM	renin 1 structural
1436996_x_at	7,514908			
1433579_at	7,489162	9130011B11Rik	12 C3	RIKEN cDNA 9130011B11 gene
1437766_at	7,47191			
1419489_at	7,416168	AW049604	15 E3	expressed sequence AW049604
1428336_at	7,412545	Agpat4	17 A1	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase delta)
1428804_at	7,40396	4933428A15Rik	8 B3.1	RIKEN cDNA 4933428A15 gene
1451019_at	7,40234	Ctsf	19 A 4 60.0 cM	cathepsin F
1449854_at	7,336724	Nr0b2		nuclear receptor subfamily 0 group B member 2
1452301_at	7,325997	1700001N19Rik	19 A	RIKEN cDNA 1700001N19 gene
1423729_a_at	7,241177	2500002L14Rik	6 C1	RIKEN cDNA 2500002L14 gene
1417275_at	7,166371	Mal	2 F1	myelin and lymphocyte protein T-cell differentiation protein
1427214_at	7,098153	Agmat	4 E1	agmatine ureohydrolase (agmatinase)
1448475_at	7,071025	2810002E22Rik	3 F2.2	RIKEN cDNA 2810002E22 gene
1419482_at	7,027275	C3ar1	6 F1	complement component 3a receptor 1
1418586_at	7,006979	Adcy9	16 2.0 cM	adenylate cyclase 9
1436092_at	6,994358			Mus musculus transcribed sequence with moderate similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1435495_at	6,99371	Mybph	1 E4	myosin binding protein H
1418030_at	6,950911	Slco3a1	7 C	solute carrier organic anion transporter family member 3a1
1437132_x_at	6,950011	Nedd9	13 A4	neural precursor cell expressed developmentally down-regulated

				gene 9
1433763_at	6,933158	Entpd5	12 39.0 cM	ectonucleoside triphosphate diphosphohydrolase 5
1431339_a_at	6,919895	D4Wsu27e	4 69.8 cM	DNA segment Chr 4 Wayne State University 27 expressed
1448239_at	6,907719	Hmox1	8 35.0 cM	heme oxygenase (decycling) 1
1458298_at	6,897265			Mus musculus transcribed sequences
1436945_x_at	6,89056	Stim1	7 50.0 cM	stromal interaction molecule 1
1425807_at	6,888899	BC021891	8 E2	cDNA sequence BC021891
1450988_at	6,770716	Gpr49	10 D2	G protein-coupled receptor 49
1447707_s_at	6,759869			phosphodiesterase 2A, cGMP-stimulated,Pde2a,---
1428891_at	6,674429	9130213B05Rik	5 E2	RIKEN cDNA 9130213B05 gene
1455274_at	6,673308			Mus musculus transcribed sequences
1421385_a_at	6,612845	Myo7a	7 48.1 cM	myosin VIIa
1426598_at	6,570302	Uty	Y 2.06 cM	ubiquitously transcribed tetratricopeptide repeat gene Y chromosome
1450792_at	6,563086	Tyrobp	7 B	TYRO protein tyrosine kinase binding protein
1438934_x_at	6,557739	Sema4a	3 F1	sema domain immunoglobulin domain (Ig) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 4A
1426189_at	6,557467	Dusp15	2 H1	dual specificity phosphatase-like 15
1427231_at	6,545025	Robo1	16 A1	roundabout homolog 1 (Drosophila)
1453794_at	6,532451	Fer1l4	2 H1	fer-1-like 4 (C. elegans)
1435148_at	6,530531	Atp1b2	11 40.0 cM	ATPase Na+/K+ transporting beta 2 polypeptide
1452980_at	6,486511			Mus musculus 16 days neonate cerebellum cDNA RIKEN full-length enriched library clone:9630053J03 product:unknown EST full insert sequence
1416942_at	6,463901	Arts1	13 C1	type 1 tumor necrosis factor receptor shedding aminopeptidase regulator
1419223_a_at	6,456411	Dtna	18 18.0 cM	dystrobrevin alpha
1420795_at	6,446916	Fgf9	14 21.0 cM	fibroblast growth factor 9
1417757_at	6,433117	Unc13a		unc-13 homolog A (C. elegans)
1424479_at	6,429855	Cst8	2 86.0 cM	cystatin 8 (cystatin-related epididymal spermatogenic)
1425887_at	6,404624	4930511J11Rik	16 A1	RIKEN cDNA 4930511J11 gene
1457825_x_at	6,381359			
1418057_at	6,373151	Tiam1	16 61.8 cM	T-cell lymphoma invasion and metastasis 1
1421694_a_at	6,370251	Cspg2	13 55.0 cM	chondroitin sulfate proteoglycan 2
1439015_at	6,359183	Gfra1	19 D2-D3	glial cell line derived neurotrophic factor family receptor alpha 1
1428184_at	6,325524	3110035E14Rik	1 A2	RIKEN cDNA 3110035E14 gene
1433720_s_at	6,314702	1620401E04Rik	10 C1	RIKEN cDNA 1620401E04 gene
1432197_at	6,313968			Mus musculus adult male tongue cDNA RIKEN full-length enriched library clone:2310039G09 product:hypothetical Vacuolar sorting protein 9 (VPS9) domain containing protein full insert sequence
1432332_a_at	6,306442	D7Rp2e	7 15.0 cM	DNA segment Chr 7 Roswell Park 2 complex expressed
1416023_at	6,296651	Fabp3	4 61.0 cM	fatty acid binding protein 3 muscle and heart
1447808_s_at	6,284619	Slc15a2	16 A1	solute carrier family 15 (H+/peptide transporter) member 2
1430128_a_at	6,283152	Dp1l1	10 C1	deleted in polyposis 1-like 1
1416953_at	6,279613	Ctgf	10 17.0 cM	connective tissue growth factor
1448320_at	6,273475	Stim1	7 50.0	stromal interaction molecule 1

			cM	
1419873_s_at	6,27			
1434776_at	6,249704	Sema5a	15 19.7 cM	sema domain seven thrombospondin repeats (type 1 and type 1-like) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 5A
1426784_at	6,23964	2210023F24Rik	11 E2	RIKEN cDNA 2210023F24 gene
1435033_at	6,197056	9330140K16Rik	1 B	RIKEN cDNA 9330140K16 gene
1438074_at	6,155394	2210010C17Rik	7 A2	RIKEN cDNA 2210010C17 gene
1419486_at	6,140197	Foxc1	13 20.0 cM	forkhead box C1
1451935_a_at	6,128531	Spint2	7 A3	serine protease inhibitor Kunitz type 2
1450285_at	6,123199	Ube1y1	Y 2.02 cM	ubiquitin-activating enzyme E1 Chr Y 1
1451236_at	6,1019	Rerg	6 G1	RAS-like estrogen-regulated growth-inhibitor
1431763_a_at	6,081062	Ctrl	8 53.0 cM	chymotrypsin-like
1421833_at	6,063904	Pip5k1a	19 C1	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha
1439500_at	6,063575	2810019K23Rik	6 B3	RIKEN cDNA 2810019K23 gene
1424277_at	6,057608	1110020L19Rik	X A7.1	RIKEN cDNA 1110020L19 gene
1448415_a_at	6,041848	Sema3b	9 60.3 cM	sema domain immunoglobulin domain (Ig) short basic domain secreted (semaphorin) 3B
1449984_at	6,032181	Cxcl2	5 51.0 cM	chemokine (C-X-C motif) ligand 2
1442291_at	6,008824	Edg4	8 33.5 cM	endothelial differentiation lysophosphatidic acid G-protein-coupled receptor 4
1420944_at	6,005875	Zfp185	X 28.95 cM	zinc finger protein 185
1450606_at	5,982313	Pnmt	11 D	phenylethanolamine-N-methyltransferase
1427076_at	5,96326	Mpeg1	19 A	macrophage expressed gene 1
1434606_at	5,96234	Erbb3	10 70.0 cM	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
1436552_at	5,953858		18 B3	Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:4930402I24 product:hypothetical protein full insert sequence
1428952_at	5,930475	1810041F13Rik	17 A3.3	RIKEN cDNA 1810041F13 gene
1435611_x_at	5,925198	Ela3b	4 D3	elastase 3B pancreatic
1460582_x_at	5,90613			Mus musculus transcribed sequences
1456509_at	5,905817	1110032F04Rik	3 E1	RIKEN cDNA 1110032F04 gene
1422467_at	5,896131	Ppt1	4 59.1 cM	palmitoyl-protein thioesterase 1
1436905_x_at	5,858395	Laptm5	4 D2.3	lysosomal-associated protein transmembrane 5
1433578_at	5,854346	E130304D01	5 C3.2	hypothetical protein E130304D01
1424754_at	5,846382	A430103C15Rik	19 A	RIKEN cDNA A430103C15 gene
1448021_at	5,843421			Mus musculus transcribed sequences
1449590_a_at	5,835643	Mras	9 E3.3	muscle and microspikes RAS
1430352_at	5,834163	8430417A20Rik	6 D1	RIKEN cDNA 8430417A20 gene
1417185_at	5,794759	Ly6a	15 42.7 cM	lymphocyte antigen 6 complex locus A
1424259_at	5,78506	2400010G15Rik	17 A3.3	RIKEN cDNA 2400010G15 gene
1416687_at	5,782431	Plod2	9 52.0 cM	procollagen lysine 2-oxoglutarate 5-dioxygenase 2
1418088_a_at	5,771204	Stx8	11 B3	syntaxin 8
1455833_at	5,765481	AU041783	19 D2	expressed sequence AU041783
1436868_at	5,722392	Rtn4rl1	11 B5	reticulon 4 receptor-like 1
1419872_at	5,719254			
1456010_x_at	5,716076	Hes5	4 81.5 cM	hairy and enhancer of split 5 (Drosophila)
1420919_at	5,704887	Sgk3	1 A2	serum/glucocorticoid regulated kinase 3

1436532_at	5,685438	C730036H08	9 F3	hypothetical protein C730036H08
1456951_at	5,684549			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:4921513I01 product:unknown EST full insert sequence
1425258_at	5,680484	Cst11	2 G3	cystatin 11
1429549_at	5,663826	Col27a1	4 B3	procollagen type XXVII alpha 1
1447800_x_at	5,657882			
1452302_at	5,655762	Cln8	8 6.0 cM	ceroid-lipofuscinosis neuronal 8
1419627_s_at	5,655482	Clecsf10	6 F2	C-type (calcium dependent carbohydrate recognition domain) lectin superfamily member 10
1426399_at	5,647689	4932416A11Rik	RIKEN cDNA 4932416A11 gene	
1434537_at	5,644186			Mus musculus transcribed sequence with moderate similarity to protein pdb:1LBG (E. coli) B Chain B Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna Alpha Carbons Only
1429466_s_at	5,642734	0610008A10Rik	9 C	RIKEN cDNA 0610008A10 gene
1437341_x_at	5,619773	Cnp1	11 60.0 cM	cyclic nucleotide phosphodiesterase 1
1438968_x_at	5,616998	Spint2	7 A3	serine protease inhibitor Kunitz type 2
1450430_at	5,615987	Mrc1	2 5.0 cM	mannose receptor C type 1
1417707_at	5,601182	B230342M21Rik	5 G3	RIKEN cDNA B230342M21 gene
1423626_at	5,566185	Dst	1 16.5 cM	dystonin
1416646_at	5,526485	Alb1	5 50.0 cM	albumin 1
1418601_at	5,52577	Aldh1a7	19 B	aldehyde dehydrogenase family 1 subfamily A7
1426808_at	5,514894	Lgals3	14 B	lectin galactose binding soluble 3
1449455_at	5,50568	Hck	2 86.0 cM	hemopoietic cell kinase
1451765_a_at	5,494708	Entpd5	12 39.0 cM	ectonucleoside triphosphate diphosphohydrolase 5
1451822_a_at	5,492899	D11Moh48	11 56.3 cM	DNA segment Chr 11 KL Mohlke 48
1416806_at	5,47946	Fdxr	11 E2	ferredoxin reductase
1455297_at	5,470494		X F3	Mus musculus similar to Spindlin-like protein 2 (SPIN-2) (LOC278240) mRNA
1454849_x_at	5,467651	Clu	14 28.0 cM	clusterin
1416460_at	5,461626	Aldrl6	15 E3	aldehyde reductase (aldose reductase)-like 6
1426858_at	5,456906	Inhbb	1 64.1 cM	inhibin beta-B
1424615_at	5,453918	Arhg	7 E2	ras homolog gene family member G
1448617_at	5,439277	Cd53	3 50.5 cM	CD53 antigen
1416934_at	5,417936	Mtm1	X 27.8 cM	X-linked myotubular myopathy gene 1
1434366_x_at	5,414599	C1qb	4 66.1 cM	complement component 1 q subcomponent beta polypeptide
1437785_at	5,410841	8430403M15Rik	6 D1	RIKEN cDNA 8430403M15 gene
1418204_s_at	5,405915	Aif1	17 19.05 cM	allograft inflammatory factor 1
1440668_at	5,39681	C130057K09	7 D2	hypothetical protein C130057K09
1445186_at	5,388441	Stc2	11 A4	stanniocalcin 2
1451606_at	5,379731	A530016L24Rik	12 F1	RIKEN cDNA A530016L24 gene
1455721_at	5,373184	Gspt2	X C2	G1 to phase transition 2
1440823_x_at	5,372747	D130058I21Rik	11 B4	RIKEN cDNA D130058I21 gene
1426288_at	5,35929	6430526J12Rik	2 E1	RIKEN cDNA 6430526J12 gene
1435469_at	5,346134	BC030934	2 A3	cDNA sequence BC030934
1436990_s_at	5,340768	1620401E04Rik	10 C1	RIKEN cDNA 1620401E04 gene

1418980_a_at	5,336341	Cnp1	11 60.0 cM	cyclic nucleotide phosphodiesterase 1
1417382_at	5,328394	Entpd5	12 39.0 cM	ectonucleoside triphosphate diphosphohydrolase 5
1418791_at	5,325983	Sh3gl2	4 41.6 cM	SH3-domain GRB2-like 2
1434100_x_at	5,321325	A830037N07Rik	5 C1	RIKEN cDNA A830037N07 gene
1419561_at	5,256716	Ccl3	11 47.59 cM	chemokine (C-C motif) ligand 3
1420504_at	5,230284	Slc6a14	X A2	solute carrier family 6 (neurotransmitter transporter) member 14
1458347_s_at	5,225765	Tmprss2	16 C2	transmembrane protease serine 2
1447100_s_at	5,224757	5730508B09Rik	3 G2	RIKEN cDNA 5730508B09 gene
1435375_at	5,194609	9830126M18	15 B1	hypothetical protein 9830126M18
1426806_at	5,152878	5830411E10Rik	1 C1.1	RIKEN cDNA 5830411E10 gene
1448200_at	5,150013	Tcn2	11 3.0 cM	transcobalamin 2
1418265_s_at	5,135918	Irf2	8 B1.1	interferon regulatory factor 2
1448591_at	5,12135	Ctss	3 42.7 cM	cathepsin S
1429987_at	5,116357	AY007814	7 42.2 cM	hypothetical protein 12H19.01.T7
1460241_a_at	5,101084	Siat9	6 C1	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-23-sialyltransferase)
1444589_at	5,098563		17 A3.2	Mus musculus similar to KRAB zinc finger protein KR18 (LOC240038) mRNA
1459903_at	5,095515	Sema7a	9 A3.3-B	sema domain immunoglobulin domain (Ig) and GPI membrane anchor (semaphorin) 7A
1437885_at	5,087976			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030029J20 product:unknown EST full insert sequence
1424214_at	5,087644	9130213B05Rik	5 E2	RIKEN cDNA 9130213B05 gene
1460674_at	5,074181	2310021M12Rik	4 D3	RIKEN cDNA 2310021M12 gene
1419276_at	5,064653	Enpp1	10 19.0 cM	ectonucleotide pyrophosphatase/phosphodiesterase 1
1452919_a_at	5,048534	1700012G19Rik	17 A3.3	RIKEN cDNA 1700012G19 gene
1437689_x_at	5,022439	Clu	14 28.0 cM	clusterin
1418340_at	5,021888	Fcer1g	1 93.3 cM	Fc receptor IgE high affinity I gamma polypeptide
1425668_a_at	5,013681	Siat4c	9 A4	sialyltransferase 4C (beta-galactoside alpha-23-sialytransferase)
1436201_x_at	5,009609	Mbp	18 55.0 cM	myelin basic protein
1442798_x_at	5,004449			
1435842_at	5,001583			Mus musculus 18-day embryo whole body cDNA RIKEN full-length enriched library clone:1110038O08 product:unknown EST full insert sequence
1422903_at	5,001553	Ly86	13 A3.3	lymphocyte antigen 86
1417388_at	4,994806	Bex2	X 57.5 cM	brain expressed X-linked 2
1435246_at	4,961314	1700019B16Rik	1 A4	RIKEN cDNA 1700019B16 gene
1437117_at	4,949616	Centb1	11 B3	centaurin beta 1
1459151_x_at	4,943633	2010008K16Rik	11 D	RIKEN cDNA 2010008K16 gene
1424807_at	4,927805	Lama4	10 25.0 cM	laminin alpha 4
1435229_at	4,924008	A930008A22Rik	9 A5.1	RIKEN cDNA A930008A22 gene
1422468_at	4,901308	Ppt1	4 59.1 cM	palmitoyl-protein thioesterase 1
1448852_at	4,898808	Rgn	X A1.3	regucalcin
1423856_at	4,86639			
1451532_s_at	4,863209	Steap	5 3.0 cM	six transmembrane epithelial antigen of the prostate
1418398_a_at	4,848845	Phemx	7 69.0	pan hematopoietic expression

			cM	
1441850_x_at	4,834335			
1424544_at	4,825109	BC011468	15 D3	cDNA sequence BC011468
1421381_a_at	4,820376	Col9a1	1 15.0 cM	procollagen type IX alpha 1
1435402_at	4,809535	A930008A22Rik	9 A5.1	RIKEN cDNA A930008A22 gene
1440339_at	4,808871			Mus musculus 0 day neonate head cDNA RIKEN full-length enriched library clone:4833416E15 product:unknown EST full insert sequence
1423547_at	4,797555	Lyzs	10 66.0 cM	lysozyme
1453304_s_at	4,789942	Ly6e	15 41.7 cM	lymphocyte antigen 6 complex locus E
1450389_s_at	4,781546	Pip5k1a	19 C1	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha
1427331_at	4,76956	Adora1	1 E4	adenosine A1 receptor
1437058_at	4,769113	Egfl3	4 E2	EGF-like-domain multiple 3
1452202_at	4,761836	Pde2a	7 E1	phosphodiesterase 2A cGMP-stimulated
1433615_at	4,761788	B930062P21Rik	15 E3	RIKEN cDNA B930062P21 gene
1442315_at	4,757138	Foxd2	4 56.5 cM	forkhead box D2
1430269_at	4,751032	1110037P11Rik	3 F3	RIKEN cDNA 1110037P11 gene
1456020_at	4,741826	D430044G18Rik	18 E1	RIKEN cDNA D430044G18 gene
1455093_a_at	4,719144	Ahsg	16 15.0 cM	alpha-2-HS-glycoprotein
1448978_at	4,718969	Ngef	1 54.0 cM	neuronal guanine nucleotide exchange factor
1460235_at	4,703413	Scarb2	5 E3	scavenger receptor class B member 2
1435225_s_at	4,686248	Brpf3	17 A3.3	bromodomain and PHD finger containing 3
1431130_at	4,682375	2010110P09Rik	7 F2	RIKEN cDNA 2010110P09 gene
1435551_at	4,662877	FHOS2	18 A2	formin-family protein FHOS2
1419608_a_at	4,65493	Egln2	7 7.0 cM	EGL nine homolog 2 (C. elegans)
1436614_at	4,648337	A530081L18Rik	18 D3	RIKEN cDNA A530081L18 gene
1447885_x_at	4,633603			
1455269_a_at	4,63027	Coro1a	7 62.5 cM	coronin actin binding protein 1A
1423091_a_at	4,617743	Gpm6b	X F5	glycoprotein m6b
1431795_a_at	4,613045	Sema3b	9 60.3 cM	sema domain immunoglobulin domain (Ig) short basic domain secreted (semaphorin) 3B
1422167_at	4,612751	Sema5a	15 19.7 cM	sema domain seven thrombospondin repeats (type 1 and type 1-like) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 5A
1417706_at	4,589964	Naglu	11 D	alpha-N-acetylglucosaminidase (Sanfilippo disease IIIB)
1453386_at	4,58835	2200001D17Rik		RIKEN cDNA 2200001D17 gene
1427256_at	4,579571	Cspg2	13 55.0 cM	chondroitin sulfate proteoglycan 2
1448354_at	4,563633	G6pdx	X 30.02 cM	glucose-6-phosphate dehydrogenase X-linked
1436324_at	4,522178	4831403C07Rik	2 E5	RIKEN cDNA 4831403C07 gene
1426013_s_at	4,521045	2410005C22Rik	7 B3	RIKEN cDNA 2410005C22 gene
1418414_at	4,51618	Kcnh1	1 H6	potassium voltage-gated channel subfamily H (eag-related) member 1
1420786_a_at	4,506854	Mm.4711	Y 3.05 cM	RNA binding motif protein Y chromosome family 1 member A1
1416003_at	4,506023	Cldn11	3 12.6 cM	claudin 11
1443013_at	4,496368			Mus musculus transcribed sequences
1417120_at	4,487205	D4Wsu114e	4 76.4 cM	DNA segment Chr 4 Wayne State University 114 expressed
1454889_x_at	4,484892	C630016B22Rik	10 C2	RIKEN cDNA C630016B22 gene
1430579_at	4,460513	1500031A17Rik	3 A3	RIKEN cDNA 1500031A17 gene
1444468_at	4,459111	1700019B16Rik	1 A4	RIKEN cDNA 1700019B16 gene

1453317_a_at	4,455873	Khdrbs3	15 37.5 cM	KH domain containing RNA binding signal transduction associated 3
1448891_at	4,444732	Msr2	3 F1	macrophage scavenger receptor 2
1433945_at	4,444509	5730507A09Rik	7 B5	RIKEN cDNA 5730507A09 gene
1424614_at	4,439169	Arhg	7 E2	ras homolog gene family member G
1434092_at	4,421203	Nos3	5 9.0 cM	nitric oxide synthase 3 endothelial cell
1433559_at	4,419576	9330175B01Rik	15 D3	RIKEN cDNA 9330175B01 gene
1434387_at	4,41749	AI429612	17 A3.3	expressed sequence AI429612
1416328_a_at	4,407337	Atp6v0e	17 A3.3	ATPase H+ transporting V0 subunit
1439426_x_at	4,389594	Lyzs	10 66.0 cM	lysozyme
1436012_s_at	4,367199	D11Moh48	11 56.3 cM	DNA segment Chr 11 KL Mohlke 48
1457407_at	4,361492			Mus musculus transcribed sequences
1435672_at	4,356861			Mus musculus 18 days pregnant adult female placenta and extra embryonic tissue cDNA RIKEN full-length enriched library clone:3830612M24 product:unknown EST full insert sequence
1452707_at	4,354742	4631423F02Rik	1 D	RIKEN cDNA 4631423F02 gene
1442082_at	4,353961	C3ar1	6 F1	complement component 3a receptor 1
1419483_at	4,352928	C3ar1	6 F1	complement component 3a receptor 1
1438165_x_at	4,340268	Vat1	11 D	vesicle amine transport protein 1 homolog (T californica)
1419494_a_at	4,33913	Tpd52	3 A1-A2	tumor protein D52
1452257_at	4,337667	Bdh	16 A1	3-hydroxybutyrate dehydrogenase (heart mitochondrial)
1419400_at	4,334419	Mttp	3 66.2 cM	microsomal triglyceride transfer protein
1451204_at	4,327304	4933425F03Rik	14 D1	RIKEN cDNA 4933425F03 gene
1452232_at	4,323163	Galnt7	8 B2	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7
1443401_at	4,312833			Mus musculus 10 days neonate cerebellum cDNA RIKEN full-length enriched library clone:B930025K11 product:unclassifiable full insert sequence
1438097_at	4,312077	Rab20	8 10.0 cM	RAB20 member RAS oncogene family
1446741_at	4,300779			Mus musculus 13 days embryo heart cDNA RIKEN full-length enriched library clone:D330001M17 product:unclassifiable full insert sequence
1454742_at	4,274491	4732452O09Rik	5 E3	RIKEN cDNA 4732452O09 gene
1437259_at	4,253515	4932415O19	1 B	hypothetical protein 4932415O19
1426223_at	4,241721	2810439F02Rik	18 A1	RIKEN cDNA 2810439F02 gene
1435135_at	4,214852	B230106I24Rik	3 A3	RIKEN cDNA B230106I24 gene
1437458_x_at	4,205857	Clu	14 28.0 cM	clusterin
1436008_at	4,20199	Tpd52	3 A1-A2	tumor protein D52
1441937_s_at	4,194772			
1425733_a_at	4,193736	Eps8	6 66.0 cM	epidermal growth factor receptor pathway substrate 8
1423653_at	4,186778	Atp1a1	3 48.4 cM	ATPase Na+/K+ transporting alpha 1 polypeptide
1421118_a_at	4,185547	Gpr56	8 45.0 cM	G protein-coupled receptor 56
1418315_at	4,18497	Nr5a1	2 23.5 cM	nuclear receptor subfamily 5 group A member 1
1450380_at	4,181077	AU040950	13 A2	expressed sequence AU040950
1424617_at	4,180706	2010008K16Rik	11 D	RIKEN cDNA 2010008K16 gene
1436640_x_at	4,17825	Agpat4	17 A1	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase delta)
1428706_at	4,174208	3110013H01Rik	11 B2	RIKEN cDNA 3110013H01 gene
1425245_a_at	4,167985	Rgs11	17 A3.3	regulator of G-protein signaling 11
1447878_s_at	4,166851			
1434812_s_at	4,163664	A930013K19	5 B2	hypothetical protein A930013K19
1421834_at	4,163485	Pip5k1a	19 C1	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha
1447352_at	4,148599		7 F4	Mus musculus similar to DNA segment Chr 7 ERATO Doi 680 expressed (LOC384685) mRNA

1438561_x_at	4,147529			
1450713_at	4,142068	Cspg5	9 33.1	chondroitin sulfate proteoglycan 5
1417073_a_at	4,13037	Qk	17 5.9 cM	quaking
1421840_at	4,124895	Abca1	4 23.1 cM	ATP-binding cassette sub-family A (ABC1) member 1
1429590_at	4,121552	B230378H13Rik	8 A2	RIKEN cDNA B230378H13 gene
1424616_s_at	4,112895	Arhg	7 E2	ras homolog gene family member G
1436568_at	4,102524	Jam2	16 A1	junction adhesion molecule 2
1460626_at	4,102406	D5Ertd606e	5 52.0 cM	DNA segment Chr 5 ERATO Doi 606 expressed
1423175_s_at	4,099934	Pard6b	2 H3	par-6 (partitioning defective 6) homolog beta (C. elegans)
1451183_at	4,089867	1110055A02Rik	11 C	RIKEN cDNA 1110055A02 gene
1435175_at	4,088624	BC034090	1 G3	cDNA sequence BC034090
1428964_at	4,087272	Slc25a18	6 54.0 cM	solute carrier family 25 (mitochondrial carrier) member 18
1433485_x_at	4,082062	Gpr56	8 45.0 cM	G protein-coupled receptor 56
1424016_at	4,06919	2310007F21Rik	9 D	RIKEN cDNA 2310007F21 gene
1419490_at	4,068729	AW049604	15 E3	expressed sequence AW049604
1428788_at	4,067697	1700012G19Rik	17 A3.3	RIKEN cDNA 1700012G19 gene
1428867_at	4,04636	4933417E01Rik	7 A2	RIKEN cDNA 4933417E01 gene
1426195_a_at	4,033879	Cst3	2 84.0 cM	cystatin C
1417859_at	4,032503	Gas7	11 B3	growth arrest specific 7
1416205_at	4,005156	Glb1	9 66.0 cM	galactosidase beta 1
1432466_a_at	3,995319	Apoe	7 4.0 cM	apolipoprotein E
1460555_at	3,976955	6330500D04Rik	13 A3.1	RIKEN cDNA 6330500D04 gene
1417620_at	3,976031	Rac2	15 E1	RAS-related C3 botulinum substrate 2
1436183_at	3,968234	Zc3hav1	6 B1	zinc finger CCCH type antiviral 1
1419493_a_at	3,965894	Tpd52	3 A1-A2	tumor protein D52
1415884_at	3,954449	Ela3b	4 D3	elastase 3B pancreatic
1456022_at	3,953879			Mus musculus 2 days pregnant adult female ovary cDNA RIKEN full-length enriched library clone:E330014L20 product:unknown EST full insert sequence
1416589_at	3,953707	Sparc	11 29.9 cM	secreted acidic cysteine rich glycoprotein
1443847_x_at	3,943638			Mus musculus transcribed sequences
1433501_at	3,941741	Ctso	3 E3	cathepsin O
1418507_s_at	3,940933	Socs2	10 52.0 cM	suppressor of cytokine signaling 2
1417116_at	3,933282	Slc6a8	X A7.1	solute carrier family 6 (neurotransmitter transporter creatine) member 8
1425602_a_at	3,927723	2610011A08Rik	7 F3	RIKEN cDNA 2610011A08 gene
1438470_at	3,924306	Socs2	10 52.0 cM	suppressor of cytokine signaling 2
1425118_at	3,917354	BC026502	8 E1	cDNA sequence BC026502
1430318_at	3,914775	Sat2	11 B3	spermidine/spermine N1-acetyl transferase 2
1449620_s_at	3,899363			Mus musculus transcribed sequence with strong similarity to protein sp:P51830 (M.musculus) CYA9_MOUSE Adenylate cyclase type IX (ATP pyrophosphate-lyase) (Adenylyl cyclase) (Adenylyl cyclase type 10) (ACTP10)
1422124_a_at	3,896389	Ptprc	1 74.0 cM	protein tyrosine phosphatase receptor type C
1432591_at	3,886229			Mus musculus 0 day neonate head cDNA RIKEN full-length enriched library clone:4833440O21 product:unknown EST full insert sequence
1449106_at	3,871432	Gpx3	11 B3-B5	glutathione peroxidase 3
1455192_at	3,847038	A230078I05Rik	1 C4	RIKEN cDNA A230078I05 gene
1437478_s_at	3,836107	D4Wsu27e	4 69.8 cM	DNA segment Chr 4 Wayne State University 27 expressed
1435203_at	3,823632	Man2a2	7 D2	mannosidase 2 alpha 2
1423556_at	3,822397	Akr1b7	6 14.0 cM	aldo-keto reductase family 1 member B7
1437057_at	3,821289	Egfl3	4 E2	EGF-like-domain multiple 3

1455150_at	3,820138			Mus musculus 13 days embryo forelimb cDNA RIKEN full-length enriched library clone:5930438H13 product:unknown EST full insert sequence
1451804_a_at	3,815767	1110037D04Rik	13 A3.1	RIKEN cDNA 1110037D04 gene
1417601_at	3,80226	Rgs1	1 78.0 cM	regulator of G-protein signaling 1
1460694_s_at	3,800644	Svil	18 A1	supervillin
1416109_at	3,784074	Fuca	4 65.7 cM	fucosidase alpha-L- 1 tissue
1426880_at	3,783185	9430077C05Rik	2 A3	RIKEN cDNA 9430077C05 gene
1447842_x_at	3,779737			
1441930_x_at	3,776233			
1456210_at	3,760734			Mus musculus transcribed sequences
1454651_x_at	3,75892	Mbp	18 55.0 cM	myelin basic protein
1452861_at	3,755619	2010300C02Rik	1 B	RIKEN cDNA 2010300C02 gene
1427247_at	3,742179	D3Bwg0562e	3 55.0 cM	DNA segment Chr 3 Brigham & Women's Genetics 0562 expressed
1457118_at	3,735276	LOC271849	2 F1	rai-like protein RaLP
1417426_at	3,723658	Prg	10 B4	proteoglycan secretory granule
1459740_s_at	3,715899			
1434714_at	3,701674			Mus musculus transcribed sequence with weak similarity to protein ref:NP_055399.1 (H.sapiens) ERO1-like (S. cerevisiae); ERO1 (S. cerevisiae)-like [Homo sapiens]
1417836_at	3,701177	3110050F08Rik	4 C7	RIKEN cDNA 3110050F08 gene
1448620_at	3,700095	Fcgr2b	1 92.3 cM	Fc receptor IgG low affinity IIb
1449198_a_at	3,697907	Siat9	6 C1	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-23-sialyltransferase)
1428896_at	3,695952	Pdgfrl	8 A4	platelet-derived growth factor receptor-like
1448241_at	3,690693	Gm2a	11 29.0 cM	GM2 ganglioside activator protein
1451422_at	3,684344	Myo18a	11 B5	myosin XVIIIa
1436977_at	3,682756			Mus musculus transcribed sequences
1452203_at	3,67604	5830411E10Rik	1 C1.1	RIKEN cDNA 5830411E10 gene
1417701_at	3,672406	Ppp1r14c	10 A1	protein phosphatase 1 regulatory (inhibitor) subunit 14c
1456607_at	3,667862	5730538E15Rik	1 A2	RIKEN cDNA 5730538E15 gene
1424669_at	3,66643	1110013H04Rik	12 F1	RIKEN cDNA 1110013H04 gene
1455849_at	3,660198	Nav1	1 E4	neuron navigator 1
1436591_at	3,657681	BC023744	5 F	cDNA sequence BC023744
1441971_at	3,653507			Mus musculus transcribed sequences
1433833_at	3,647336	1600019O04Rik	3 A3	RIKEN cDNA 1600019O04 gene
1447326_s_at	3,646851	Zfp261	X 57.0 cM	zinc finger protein 261
1420394_s_at	3,63549	Gp49b	10 32.0 cM	glycoprotein 49 B
1427180_at	3,634341	Slc27a3	3 F1	solute carrier family 27 (fatty acid transporter) member 3
1416226_at	3,633754	Arpc1b	5 G2	actin related protein 2/3 complex subunit 1B
1433933_s_at	3,627428	Slco2b1	7 E1	solute carrier organic anion transporter family member 2b1
1449109_at	3,623966	Socs2	10 52.0 cM	suppressor of cytokine signaling 2
1439794_at	3,613656	Ntn4		netrin 4
1450036_at	3,613417	Sgk3	1 A2	serum/glucocorticoid regulated kinase 3
1448732_at	3,610401	Ctsb	14 28.0 cM	cathepsin B
1422247_a_at	3,600421	Uty	Y 2.06 cM	ubiquitously transcribed tetratricopeptide repeat gene Y chromosome
1421129_a_at	3,592288	Atp2a3	11 B4	ATPase Ca++ transporting ubiquitous
1446769_at	3,582661	2810439F02Rik	18 A1	RIKEN cDNA 2810439F02 gene
1433994_at	3,580291	4931406P16Rik	7 B1	RIKEN cDNA 4931406P16 gene
1455256_at	3,573434	1500031A17Rik	3 A3	RIKEN cDNA 1500031A17 gene

1429379_at	3,565393	Xlkd1	7 E3	extra cellular link domain-containing 1
1436223_at	3,563899			Mus musculus 0 day neonate kidney cDNA RIKEN full-length enriched library clone:D630049N15 product:unknown EST full insert sequence
1448894_at	3,562553	Akr1b8	6 13.0 cM	aldo-keto reductase family 1 member B8
1422327_s_at	3,558414	G6pd2	5 39.0 cM	glucose-6-phosphate dehydrogenase 2
1436545_at	3,556678	BC044798	19 A	cDNA sequence BC044798
1458464_at	3,5551	D030049F17	1 C1.1	hypothetical protein D030049F17
1422597_at	3,551799	Mmp15	8 45.5 cM	matrix metalloproteinase 15
1418195_at	3,549017	Galnt10	11 B1.3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10
1425942_a_at	3,546286	Gpm6b	X F5	glycoprotein m6b
1426658_x_at	3,543379	Phgdh	8 C1	3-phosphoglycerate dehydrogenase
1415803_at	3,539646	Cx3cl1	8 46.0 cM	chemokine (C-X3-C motif) ligand 1
1450062_a_at	3,529712	Maged1	X 34.6 cM	melanoma antigen family D 1
1426315_a_at	3,528172	6330416G13Rik	4 B3	RIKEN cDNA 6330416G13 gene
1454984_at	3,526388			Mus musculus 13 days embryo heart cDNA RIKEN full-length enriched library clone:D330042I16 product:unknown EST full insert sequence
1422479_at	3,526086	Acas2	2 H1	acetyl-Coenzyme A synthetase 2 (ADP forming)
1434216_a_at	3,52374	D7Rp2e	7 15.0 cM	DNA segment Chr 7 Roswell Park 2 complex expressed
1435064_a_at	3,518936	0610008J07Rik	X F5	RIKEN cDNA 0610008J07 gene
1437422_at	3,515055	Sema5a	15 19.7 cM	sema domain seven thrombospondin repeats (type 1 and type 1-like) transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 5A
1417932_at	3,514941	Il18	9 29.0 cM	interleukin 18
1448321_at	3,514859	Smoc1	12 C3	SPARC related modular calcium binding 1
1425525_a_at	3,510324	P2rx4	5 65.0 cM	purinergic receptor P2X ligand-gated ion channel 4
1459897_a_at	3,494971	Sbsn	7A3	suprabasin
1452094_at	3,48703	P4ha1	10 B4	procollagen-proline 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase) alpha 1 polypeptide
1425749_at	3,480926	Stxbp6	12 B1	syntaxin binding protein 6 (amisyn)
1459890_s_at	3,47746	1110008P14Rik	2 B	RIKEN cDNA 1110008P14 gene
1436756_x_at	3,470726	Hadhsc	3 G3	L-3-hydroxyacyl-Coenzyme A dehydrogenase short chain
1419971_s_at	3,468869	Slc35a5	16 29.9 cM	solute carrier family 35 member A5
1418826_at	3,468588	Ms4a6b	19 A	membrane-spanning 4-domains subfamily A member 6B
1416600_a_at	3,465254	Dscr1	16 62.0 cM	Down syndrome critical region homolog 1 (human)
1427878_at	3,460894	0610010O12Rik	18 B2	RIKEN cDNA 0610010O12 gene
1448530_at	3,455204	Gmpr	13 A5	guanosine monophosphate reductase
1434706_at	3,446716	5730538E15Rik	1 A2	RIKEN cDNA 5730538E15 gene
1437621_x_at	3,444354			Mus musculus transcribed sequence with strong similarity to protein sp:Q61753 (M.musculus) SERA_MOUSE D-3-phosphoglycerate dehydrogenase (3-PGDH) (A10)
1452924_at	3,440186	2310007D09Rik	2 H2	RIKEN cDNA 2310007D09 gene
1450627_at	3,437749	ank	15 14.4 cM	progressive ankylosis
1426260_a_at	3,432824	Ugt1a1	1 51.7 cM	UDP-glucuronosyltransferase 1 family member 1
1428958_at	3,432271	1700019B16Rik	1 A4	RIKEN cDNA 1700019B16 gene
1453473_a_at	3,431822	Tctex1	17 3.1 cM	t-complex testis expressed 1
1456195_x_at	3,431478	Itgb5	16 A1	integrin beta 5
1450872_s_at	3,417924	Lip1	19 C1	lysosomal acid lipase 1
1427352_at	3,417908	BC031593	15 F2	cDNA sequence BC031593
1431833_a_at	3,41164	Hmgcs2	3 48.0 cM	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2
1419646_a_at	3,405919	Mbp	18 55.0 cM	myelin basic protein

1451255_at	3,404424	Lisch7	7 A3	liver-specific bHLH-Zip transcription factor
1448275_at	3,403629	2810428F02Rik	10 D2	RIKEN cDNA 2810428F02 gene
1428116_a_at	3,395999	Tctex1	17 3.1 cM	t-complex testis expressed 1
1421034_a_at	3,395062	Il4ra	7 62.0 cM	interleukin 4 receptor alpha
1437872_at	3,392344	AI448472	5 A3	expressed sequence AI448472
1450241_a_at	3,39057	Evi2a	11 46.13 cM	ecotropic viral integration site 2a
1456165_at	3,386834	Crygc	1 32.0 cM	crystallin gamma C
1424652_at	3,386553	BC014699	6 C3	cDNA sequence BC014699
1454809_at	3,377275	9030406N13Rik	10 A4	RIKEN cDNA 9030406N13 gene
1456155_x_at	3,376926	Fuca	4 65.7 cM	fucosidase alpha-L- 1 tissue
1424863_a_at	3,373792	Hipk2	6 B	homeodomain interacting protein kinase 2
1453419_at	3,372943	Mras	9 E3.3	muscle and microspikes RAS
1423593_a_at	3,361054	Csf1r	18 30.0 cM	colony stimulating factor 1 receptor
1416637_at	3,357591	Slc4a2	5 14.0 cM	solute carrier family 4 (anion exchanger) member 2
1452864_at	3,356075	6530405F15Rik	3 D	RIKEN cDNA 6530405F15 gene
1415801_at	3,355628	Gja1	10 29.0 cM	gap junction membrane channel protein alpha 1
1440795_x_at	3,352777			
1460398_at	3,350678	9830141C09Rik	X F3	RIKEN cDNA 9830141C09 gene
1439018_at	3,348227	6330505N24	3 F1	hypothetical protein 6330505N24
1428789_at	3,347724	4921528G01Rik	1 H1	RIKEN cDNA 4921528G01 gene
1416382_at	3,343684	Ctsc	7 D3-E1.1	cathepsin C
1449248_at	3,335228	Clcn2	16 16.0 cM	chloride channel 2
1439630_x_at	3,33021	Sbsn	7A3	suprabasin
1460351_at	3,320218	S100a11	3 F2.1	S100 calcium binding protein A11 (calizzarin)
1427685_a_at	3,313077	Synj2	17 3.3 cM	synaptojanin 2
1426657_s_at	3,307236	Phgdh	8 C1	3-phosphoglycerate dehydrogenase
1417010_at	3,305451	Zfp238	1 H4	zinc finger protein 238
1456471_x_at	3,302906			Mus musculus transcribed sequence with strong similarity to protein sp:Q61753 (M.musculus) SERA_MOUSE D-3-phosphoglycerate dehydrogenase (3-PGDH) (A10)
1427476_a_at	3,291241	Trim32	4 C1	tripartite motif protein 32
1459818_x_at	3,289595	Zfp261	X 57.0 cM	zinc finger protein 261
1460428_at	3,286719	1100001D10Rik	5 F	RIKEN cDNA 1100001D10 gene
1429678_at	3,286523	5730508B09Rik	3 G2	RIKEN cDNA 5730508B09 gene
1452344_at	3,28508	Synj2	17 3.3 cM	synaptojanin 2
1449545_at	3,281476	Fgf18	11 A4	fibroblast growth factor 18
1433857_at	3,279529	Fath	8 25.0 cM	fat tumor suppressor homolog (Drosophila)
1423865_at	3,278099	AW547365	4 B2	expressed sequence AW547365
1438306_at	3,277995	3110001E11Rik	13 D1	RIKEN cDNA 3110001E11 gene
1418123_at	3,277769	Unc119	11 B5	unc-119 homolog (C. elegans)
1417591_at	3,277216	Ptges2	2 B	prostaglandin E synthase 2
1450647_at	3,276159	Hps3	3 12.5 cM	Hermansky-Pudlak syndrome 3 homolog (human)
1423285_at	3,274955	Coch	12 23.0 cM	coagulation factor C homolog (Limulus polyphemus)
1426735_at	3,263667	MGC63429	1 H5	similar to mitochondrial isoleucine tRNA synthetase
1416760_at	3,261823	Galntl1	12 C3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1
1435484_at	3,258574			Mus musculus 0 day neonate head cDNA RIKEN full-length enriched library clone:4831440M03 product:unknown EST full insert sequence
1423726_at	3,252567	Vat1	11 D	vesicle amine transport protein 1 homolog (T californica)
1428283_at	3,251119	Cyp2s1	7 A3	cytochrome P450 family 2 subfamily s polypeptide 1

1417490_at	3,244622	Ctsb	14 28.0 cM	cathepsin B
1455583_at	3,240569	Gne	4 B1	glucosamine
1434073_at	3,235296	3110031O14Rik	X E3	RIKEN cDNA 3110031O14 gene
1427475_a_at	3,232679	1110001A05Rik	3 H1	RIKEN cDNA 1110001A05 gene
1438980_x_at	3,229133			
1449084_s_at	3,228271	Sh3d19	3 F1	SH3 domain protein D19
1425702_a_at	3,219645	Enpp5	17 23.5 cM	ectonucleotide pyrophosphatase/phosphodiesterase 5
1438321_x_at	3,218521			
1416036_at	3,218374	Fkbp1a	2 G3	FK506 binding protein 1a
1428644_at	3,21758	Mgat5	1 E3	mannoside acetylglucosaminyltransferase 5
1453844_at	3,213547	Chit1	1 E4	chitinase 1 (chitotriosidase)
1418269_at	3,213256	Loxl3	6 34.76 cM	lysyl oxidase-like 3
1429637_at	3,209967	1110032E23Rik	3 E3	RIKEN cDNA 1110032E23 gene
1455397_at	3,205408	C630036E02Rik	17 A3.3	RIKEN cDNA C630036E02 gene
1454875_a_at	3,204743	Rbbp4		retinoblastoma binding protein 4
1417389_at	3,204499	Gpc1	1 D	glypican 1
1458351_s_at	3,203491	Klhl2	8 B3.1	kelch-like 2 Mayven (Drosophila)
1449408_at	3,199893	Jam2	16 A1	junction adhesion molecule 2
1448596_at	3,195488	Slc6a8	X A7.1	solute carrier family 6 (neurotransmitter transporter creatine) member 8
1421746_a_at	3,192865	Fbxo17	7 A3	F-box only protein 17
1423174_a_at	3,18997	Pard6b	2 H3	par-6 (partitioning defective 6) homolog beta (C. elegans)
1424037_at	3,188494	Itpka	2 E5	inositol 145-trisphosphate 3-kinase A
1454268_a_at	3,186444	Cyba		cytochrome b-245 alpha polypeptide
1454714_x_at	3,185267			Mus musculus transcribed sequence with strong similarity to protein sp:Q61753 (M.musculus) SERA_MOUSE D-3-phosphoglycerate dehydrogenase (3-PGDH) (A10)
1458706_at	3,180745			Mus musculus transcribed sequences
1426975_at	3,171199	4632413K17Rik	10 D3	RIKEN cDNA 4632413K17 gene
1434099_at	3,166741	A830037N07Rik	5 C1	RIKEN cDNA A830037N07 gene
1426655_a_at	3,16619	4930504E06Rik	3 F2.1	RIKEN cDNA 4930504E06 gene
1433560_at	3,165054	9330175B01Rik	15 D3	RIKEN cDNA 9330175B01 gene
1452146_a_at	3,163499	2900026G05Rik	19 C3	RIKEN cDNA 2900026G05 gene
1451071_a_at	3,163312	Atp1a1	3 48.4 cM	ATPase Na+/K+ transporting alpha 1 polypeptide
1417492_at	3,158475	Ctsb	14 28.0 cM	cathepsin B
1425029_a_at	3,156511	2810049G06Rik	12 A1.2	RIKEN cDNA 2810049G06 gene
1422574_at	3,153676	Mad4	5 20.0 cM	Max dimerization protein 4
1426648_at	3,153602	Mapkapk2	1 E4	MAP kinase-activated protein kinase 2
1419466_at	3,153384	Nkd2	13 C1	naked cuticle 2 homolog (Drosophila)
1452093_at	3,152183	2500001K11Rik	1 E2.3	RIKEN cDNA 2500001K11 gene
1423025_a_at	3,150735	Schip1	3 E1	schwannomin interacting protein 1
1448392_at	3,141144	Sparc	11 29.9 cM	secreted acidic cysteine rich glycoprotein
1430522_a_at	3,140101	Vamp5	5	vesicle-associated membrane protein 5
1423582_at	3,138076	Dmrt1	19 C2-C3	doublesex and mab-3 related transcription factor 1
1451148_at	3,127732	Pink1	4 D3	PTEN induced putative kinase 1
1452308_a_at	3,12371	Atp1a2	1 94.2 cM	ATPase Na+/K+ transporting alpha 2 polypeptide
1428758_at	3,123657	1810054O13Rik	7 B3	RIKEN cDNA 1810054O13 gene
1416340_a_at	3,123453	Man2b1	8 37.0 cM	mannosidase 2 alpha B1
1425626_at	3,118752	Gstm1	3 F2.3	glutathione S-transferase mu 1
1455547_at	3,11863	Scrg3	15 E1	scrapie responsive gene 3
1430291_at	3,116488			Mus musculus adult male corpora quadrigemina cDNA

				RIKEN full-length enriched library clone:B230210C03 product:unknown EST full insert sequence
1417534_at	3,114673	Itgb5	16 A1	integrin beta 5
1434504_at	3,111895	9630058O20	5 B1	hypothetical protein 9630058O20
1443779_s_at	3,111012			Mus musculus 2 days pregnant adult female ovary cDNA RIKEN full-length enriched library clone:E330009D23 product:unknown EST full insert sequence
1429292_a_at	3,107437	2310046K01Rik	2 H1	RIKEN cDNA 2310046K01 gene
1438650_x_at	3,106093	Gja1	10 29.0 cM	gap junction membrane channel protein alpha 1
1450875_at	3,104702	Gpr37	6 7.2 cM	G protein-coupled receptor 37
1429052_at	3,103341			Mus musculus 12 days embryo head cDNA RIKEN full-length enriched library clone:3000002J10 product:unknown EST full insert sequence
1416165_at	3,102453	1700093E07Rik	17 E1.1	RIKEN cDNA 1700093E07 gene
1418983_at	3,100283	Cipp	4 C6	channel-interacting PDZ domain protein
1422711_a_at	3,090695	Pnck	X 23.5 cM	pregnancy upregulated non-ubiquitously expressed CaM kinase
1423362_at	3,084257	Sort1	3 F3	sortilin 1
1419329_at	3,082769	Sh3d4	14 34.5 cM	SH3 domain protein 4
1425548_a_at	3,076596	Lst1	17 19.06 cM	leukocyte specific transcript 1
1436965_at	3,07658	Lpin3	2 H2	lipin 3
1434909_at	3,076422	Rragd	4 11.4 cM	Ras-related GTP binding D
1455301_at	3,075298			Mus musculus adult male colon cDNA RIKEN full-length enriched library clone:9030621G03 product:unknown EST full insert sequence
1424365_at	3,072698	1810037I17Rik	RIKEN cDNA	1810037I17 gene
1434275_at	3,072219	Nkd2	13 C1	naked cuticle 2 homolog (Drosophila)
1439994_at	3,072111			Mus musculus adult male corpora quadrigemina cDNA RIKEN full-length enriched library clone:B230306E21 product:unknown EST full insert sequence
1440684_at	3,061558	A330042H22	8 C5	hypothetical protein A330042H22
1425518_at	3,058025	5730402K07Rik	2 C3	RIKEN cDNA 5730402K07 gene
1448587_at	3,054165	Tbc1d10	11 A1	TBC1 domain family member 10
1428643_at	3,051426	2610024A01Rik	1 E3	RIKEN cDNA 2610024A01 gene
1416383_a_at	3,050286	Pcx	19 0.0 cM	pyruvate carboxylase
1448568_a_at	3,045498	Slc20a1	2 73.0 cM	solute carrier family 20 member 1
1420984_at	3,040759	Pctp	11 52.0 cM	phosphatidylcholine transfer protein
1448883_at	3,034371	Lgmn	12 E	legumain
1450642_at	3,033216			Mus musculus RIKEN cDNA 3110001I20 gene mRNA (cDNA clone IMAGE:6439419) partial cds
1448908_at	3,032441	Ppap2b	4 52.7 cM	phosphatidic acid phosphatase type 2B
1421194_at	3,031584	Itga4	2 46.0 cM	integrin alpha 4
1459741_x_at	3,023292			
1437982_x_at	3,009296	2900026G05Rik	19 C3	RIKEN cDNA 2900026G05 gene
1427131_s_at	3,004411	C330018J07Rik	16 A1	RIKEN cDNA C330018J07 gene
1450767_at	2,998677	Nedd9	13 A4	neural precursor cell expressed developmentally down-regulated gene 9
1428209_at	2,996935	Rex3	X 57.5 cM	reduced expression 3
1422432_at	2,99172	Dbi	1 E2.3	diazepam binding inhibitor
1435537_at	2,990747			Mus musculus 12 days embryo head cDNA RIKEN full-length enriched library clone:3000002J10 product: unknown EST full insert sequence
1448384_at	2,988317	BC003494	10 C1	cDNA sequence BC003494
1456616_a_at	2,986333	Bsg	10 42.4 cM	basigin
1434738_at	2,98537	A530046H20Rik	7 C	RIKEN cDNA A530046H20 gene
1421402_at	2,984081	Mta3	17 E4	metastasis associated 3

1454614_at	2,972491	1810013D10Rik	5	RIKEN cDNA 1810013D10 gene
1426575_at	2,970701	9530058O11Rik	19 C1	RIKEN cDNA 9530058O11 gene
1424670_s_at	2,967545	1110013H04Rik	12 F1	RIKEN cDNA 1110013H04 gene
1439882_at	2,967169			Mus musculus transcribed sequence with moderate similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse
1454978_at	2,967053	2900029G13Rik	5 G2	RIKEN cDNA 2900029G13 gene
1428794_at	2,964563	B230396K10Rik	11 B2	RIKEN cDNA B230396K10 gene
1416882_at	2,961403	Rgs10	7 F3	regulator of G-protein signalling 10
1429719_at	2,960752	Foxp4	17 C	forkhead box P4
1438437_a_at	2,958818			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030071E17 product:unknown EST full insert sequence
1427465_at	2,957326	Atp1a2	1 94.2 cM	ATPase Na+/K+ transporting alpha 2 polypeptide
1430623_s_at	2,956524	5830411E10Rik	1 C1.1	RIKEN cDNA 5830411E10 gene
1448737_at	2,955867	Tm4sf2	X A1.3-A2	transmembrane 4 superfamily member 2
1435867_at	2,953652			Mus musculus transcribed sequences
1416368_at	2,951448	Gsta4	9 E1	glutathione S-transferase alpha 4
1448433_a_at	2,948291	Pcolce	5 78.0 cM	procollagen C-proteinase enhancer protein
1441047_at	2,946296	9330175B01Rik	15 D3	RIKEN cDNA 9330175B01 gene
1426755_at	2,945164	5630400A09Rik	10 C1	RIKEN cDNA 5630400A09 gene
1451149_at	2,943665	Pgm2	4 45.8 cM	phosphoglucomutase 2
1417936_at	2,943352	Ccl9	11 47.4 cM	chemokine (C-C motif) ligand 9
1448929_at	2,938853	F13a	13 A3.3	coagulation factor XIII alpha subunit
1440353_at	2,93818	Ntf5	7 23.0 cM	neurotrophin 5
1447841_x_at	2,937487	Siat10	16 A1	sialyltransferase 10 (alpha-23-sialyltransferase VI)
1456500_at	2,933605	4632417K02	9 C	hypothetical protein 4632417K02
1435220_s_at	2,93312	2810404F18Rik	11 B1.3	RIKEN cDNA 2810404F18 gene
1456133_x_at	2,931348	Itgb5	16 A1	integrin beta 5
1429736_at	2,930341			Mus musculus 15 days embryo male testis cDNA RIKEN full-length enriched library clone:8030484F22 product:unknown EST full insert sequence
1450191_a_at	2,927381	Sox13	1 E4	SRY-box containing gene 13
1416686_at	2,926209	Plod2	9 52.0 cM	procollagen lysine 2-oxoglutarate 5-dioxygenase 2
1433735_a_at	2,924618	9630015D15Rik	4 A2	RIKEN cDNA 9630015D15 gene
1417324_at	2,924099	Mtssk	4 D1	microtubule associated testis specific serine/threonine protein kinase
1453481_at	2,921989			
1428296_at	2,921933			
1433885_at	2,920668	A630053O10	13 D1	hypothetical protein A630053O10
1460444_at	2,916958	Arrb1	7 50.0 cM	arrestin beta 1
1436309_at	2,916517	Neto2	8 C3	neuropilin (NRP) and tolloid (TLL)-like 2
1425249_a_at	2,914103	Tyro3	2 67.1 cM	TYRO3 protein tyrosine kinase 3
1448748_at	2,913961	Plek	11 6.5 cM	pleckstrin
1437046_x_at	2,913132	Anxa9	3 F2.1	annexin A9
1455214_at	2,912005			Mus musculus adult male medulla oblongata cDNA RIKEN full-length enriched library clone:6330417O12 product:microphthalmia-associated transcription factor full insert sequence
1439964_at	2,900611			Mus musculus transcribed sequences
1458802_at	2,894951	Krc	4 D2.2	kappa B and Rss recognition component
1452878_at	2,893902	Prkce	17 E4	protein kinase C epsilon
1416441_at	2,893601	1190003P12Rik	15 B3.1	RIKEN cDNA 1190003P12 gene
1435964_a_at	2,893208			Mus musculus transcribed sequence with strong similarity

				to protein ref:NP_057365.1 (H.sapiens) STE20-like kinase; STE2-like kinase [Homo sapiens]
1454713_s_at	2,893188	Hdc	2 E5-G	histidine decarboxylase
1418572_x_at	2,890553	Tnfrsf12a	17 A3.3	tumor necrosis factor receptor superfamily member 12a
1448810_at	2,890048	Gne	4 B1	glucosamine
1420372_at	2,887237	Sntb2	8 52.0 cM	syntrophin basic 2
1417533_a_at	2,882881	Itgb5	16 A1	integrin beta 5
1417040_a_at	2,882848	Bok	1 D	Bcl-2-related ovarian killer protein
1429418_at	2,882729	A530086E13Rik	13 B3	RIKEN cDNA A530086E13 gene
1420637_at	2,882539	Prps2	X 72.0 cM	phosphoribosyl pyrophosphate synthetase 2
1452655_at	2,882319	Zdhhc2	8 A4	zinc finger DHHC domain containing 2
1453003_at	2,881665	Sorl1		sortilin-related receptor LDLR class A repeats-containing
1422483_a_at	2,88163	Cycs	6 23.0 cM	cytochrome c somatic
1422728_at	2,875877	Inha	1 41.6 cM	inhibin alpha
1425026_at	2,872272	2010005O13Rik	1 24.1	RIKEN cDNA 2010005O13 gene
1421998_at	2,871115	Tor3a	1 G3	torsin family 3 member A
1448118_a_at	2,864126	Ctsd	4 50.0 cM	cathepsin D
1455291_s_at	2,86151	Znrf2	6	zinc finger/RING finger 2
1451031_at	2,859247	Sfrp4	13 7.0 cM	secreted frizzled-related sequence protein 4
1429160_at	2,856003	2810012L14Rik	5 G3	RIKEN cDNA 2810012L14 gene
1451912_a_at	2,855095	Fgfrl1		fibroblast growth factor receptor-like 1
1424175_at	2,854588	Tef	15 46.7 cM	thyrotroph embryonic factor
1445679_at	2,853299			Mus musculus transcribed sequence with moderate similarity to protein pir:I67760 (E. coli) I67760 transposase – Escherichia coli insertion sequence IS10
1452011_a_at	2,852452	Uxs1	1 C1.1	UDP-glucuronate decarboxylase 1
1427075_s_at	2,848975	5330414D10Rik	2 H4	RIKEN cDNA 5330414D10 gene
1453569_s_at	2,848303	Mark4	7 A2	MAP/microtubule affinity-regulating kinase 4
1434451_at	2,847089			Mus musculus transcribed sequence with strong similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1433495_at	2,844689	2810024B22Rik	8 B3.3	RIKEN cDNA 2810024B22 gene
1445597_s_at	2,842803	Hrasls3	19 A	HRAS like suppressor 3
1419003_at	2,842689	Bves	10 29.0 cM	blood vessel epicardial substance
1433520_at	2,83794	Scap	9 F3	Sreb cleavage-activating protein
1460081_at	2,832321	Syt7	19 A	synaptotagmin 7
1457275_at	2,830919	Dmn	7 C	desmuslin
1434845_at	2,826236			Mus musculus transcribed sequence with strong similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1415823_at	2,825917	Scd2	19 43.0 cM	stearoyl-Coenzyme A desaturase 2
1450984_at	2,821996	Tjp2		tight junction protein 2
1450744_at	2,821103	Ell2	13 C1	elongation factor RNA polymerase II 2
1428636_at	2,820362	4921538B17Rik	5 A1-h	RIKEN cDNA 4921538B17 gene
1426908_at	2,81756	Galnt7	8 B2	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7
1437462_x_at	2,81405	Mmp15	8 45.5 cM	matrix metalloproteinase 15
1435494_s_at	2,813015	5730453H04Rik	13 A3.3	RIKEN cDNA 5730453H04 gene
1429511_at	2,812494	4933402E13Rik	X A5	RIKEN cDNA 4933402E13 gene
1452592_at	2,809343	Mgst2	3 C	microsomal glutathione S-transferase 2
1418540_a_at	2,807611	Ptpre	7 67.6 cM	protein tyrosine phosphatase receptor type E
1435608_at	2,805842		11 A1	Mus musculus 13 days embryo heart cDNA RIKEN full-length enriched library clone:D330021C01 product:unknown EST full insert sequence

1426576_at	2,802685	9530058O11Rik	19 C1	RIKEN cDNA 9530058O11 gene
1428527_at	2,80246	Snx7	3 G1	sorting nexin 7
1435342_at	2,802413	D7Ertd764e	7 9.5 cM	DNA segment Chr 7 ERATO Doi 764 expressed
1436472_at	2,802349			
1448599_s_at	2,800563	D4Wsu114e	4 76.4 cM	DNA segment Chr 4 Wayne State University 114 expressed
1454728_s_at	2,796881	Atp8a1	5 C3.1	ATPase aminophospholipid transporter (APLT) class I type 8A member 1
1424886_at	2,796053	Ptprd	4 38.0 cM	protein tyrosine phosphatase receptor type D
1417441_at	2,789204	Jdp1	10 B4	J domain protein 1
1428657_at	2,785082	1110037N09Rik	13 A3.3	RIKEN cDNA 1110037N09 gene
1455750_at	2,784113	BC053994	2 G1	cDNA sequence BC053994
1455243_at	2,784058	Brpf3	17 A3.3	bromodomain and PHD finger containing 3
1454078_a_at	2,783777	Gcst	11 A1	galactosylceramide sulfotransferase
1435829_at	2,777996			Mus musculus transcribed sequence with weak similarity to protein pir:RGECDW (E. coli) RGECDW transcription activator of D-serine dehydratase – Escherichia coli
1423903_at	2,776806	D7Ertd458e	7 4.0 cM	DNA segment Chr 7 ERATO Doi 458 expressed
1423341_at	2,761704	Cspg4	9 B	chondroitin sulfate proteoglycan 4
1450955_s_at	2,761479	Sort1	3 F3	sortilin 1
1449325_at	2,760055	Fads2	19 A	fatty acid desaturase 2
1460740_at	2,756798	Cltb	13 B1	clathrin light polypeptide (Lcb)
1415837_at	2,756333	Klk6	7 23.0 cM	kallikrein 6
1416497_at	2,75374	Cai	6 18.0 cM	calcium binding protein intestinal
1420918_at	2,75321	Sgk3	1 A2	serum/glucocorticoid regulated kinase 3
1416222_at	2,753096	Nsdhl	X 28.87 cM	NAD(P) dependent steroid dehydrogenase-like
1436542_at	2,751974	Ptger1	8 38.0 cM	prostaglandin E receptor 1 (subtype EP1)
1460167_at	2,751956	Aldh7a1	18 29.0 cM	aldehyde dehydrogenase family 7 member A1
1435283_s_at	2,750292			Mus musculus transcribed sequence with moderate similarity to protein sp:Q15884 (H.sapiens) X123_HUMAN Putative protein X123
1457404_at	2,749447	AA408868	16 A1	expreexpressed sequence AA408868
1451731_at	2,746014	Abca3	17 A3.3	ATP-binding cassette sub-family A (ABC1) member 3
1423746_at	2,743512	Txndc5	13 A3.3	thioredoxin domain containing 5
1424386_at	2,741705	BC020184	18 B1	cDNA sequence BC020184
1415824_at	2,73982	Scd2	19 43.0 cM	stearoyl-Coenzyme A desaturase 2
1433754_at	2,739402	Mbnl2	14 E4	muscleblind-like 2
1419263_a_at	2,734801	Adrm1	2 H4	adhesion regulating molecule 1
1436848_x_at	2,733093	Impa1	3 A1	inositol (myo)-1(or 4)-monophosphatase 1
1428447_at	2,732168	5730496E24Rik	1 A4	RIKEN cDNA 5730496E24 gene
1428580_at	2,729983	Blvra	2 62.0 cM	biliverdin reductase A
1424358_at	2,729569	BC016265	14 11.2	cDNA sequence BC016265
1448188_at	2,725406	Ucp2	7 50.0 cM	uncoupling protein 2 mitochondrial
1455029_at	2,724609	Kif21a	15 55.1 cM	kinesin family member 21A
1416998_at	2,722856	Rrs1	1 8.0 cM	RRS1 ribosome biogenesis regulator homolog (S. cerevisiae)
1445031_at	2,721777			Mus musculus transcribed sequences
1439496_at	2,719214			Mus musculus adult male eyeball cDNA RIKEN full-length enriched library clone:7530422H18 product:unclassifiable full insert sequence
1416996_at	2,712851	Tbc1d8	1 B	TBC1 domain family member 8
1435493_at	2,711447	5730453H04Rik	13 A3.3	RIKEN cDNA 5730453H04 gene
1456584_x_at	2,710396			Mus musculus transcribed sequence with strong similarity to protein sp:Q61753 (M.musculus) SERA_MOUSE

				D-3-phosphoglycerate dehydrogenase (3-PGDH) (A10)
1439256_x_at	2,709534			Mus musculus transmembrane 7 superfamily member 1 (Tm7sf1) mRNA
1456014_s_at	2,707687	Tpt1h	19 A	tRNA splicing 2' phosphotransferase 1 homolog (S. cerevisiae)
1452783_at	2,704613	1600019O04Rik	3 A3	RIKEN cDNA 1600019O04 gene
1450674_at	2,700499	Cdk5	5 12.0 cM	cyclin-dependent kinase 5
1452872_at	2,699758	Ank3	10 38.0 cM	ankyrin 3 epithelial
1455048_at	2,69719	Igsf2	3 48.5 cM	immunoglobulin superfamily member 2
1426656_at	2,69508	4930504E06Rik	3 F2.1	RIKEN cDNA 4930504E06 gene
1451160_s_at	2,691758	D7Ertd458e	7 4.0 cM	DNA segment Chr 7 ERATO Doi 458 expressed
1435282_at	2,689807			Mus musculus transcribed sequence with moderate similarity to protein sp:Q15884 (H.sapiens) X123_HUMAN Putative protein X123
1455684_at	2,685444			Mus musculus 0 day neonate cerebellum cDNA RIKEN full-length enriched library clone:C230090M03 product:unknown EST full insert sequence
1417794_at	2,683291	Zfp261	X 57.0 cM	zinc finger protein 261
1420371_at	2,683155	Sntb2	8 52.0 cM	syntrophin basic 2
1460253_at	2,679362	Cklfsf7	9 F3	chemokine-like factor super family 7
1418927_a_at	2,678875	Habp4	13 B3	hyaluronic acid binding protein 4
1428433_at	2,677442			Mus musculus 18-day embryo whole body cDNA RIKEN full-length enriched library clone:1110014O20 product:unknown EST full insert sequence
1424250_a_at	2,672633	Arhgef3	14 9.0 cM	Rho guanine nucleotide exchange factor (GEF) 3
1448727_at	2,665435	Tle6	10 C1	transducin-like enhancer of split 6 homolog of Drosophila E(spl)
1419757_at	2,661633	Rdgb2	5 68.0 cM	retinal degeneration B2 homolog (Drosophila)
1449804_at	2,661204	Pnmt	11 D	phenylethanolamine-N-methyltransferase
1429273_at	2,661126	3110056H04Rik	9 A3	RIKEN cDNA 3110056H04 gene
1424711_at	2,656942	Tmem2	19 B	transmembrane protein 2
1455288_at	2,656919	1110036O03Rik	11 D	RIKEN cDNA 1110036O03 gene
1428465_at	2,653479	5033425B17Rik	7 A3	RIKEN cDNA 5033425B17 gene
1424468_s_at	2,647575	D330037A14Rik	9 A5.2	RIKEN cDNA D330037A14 gene
1460735_at	2,643331	Svil	18 A1	supervillin
1424653_at	2,638656	2700063A19Rik	10 B4	RIKEN cDNA 2700063A19 gene
1448263_a_at	2,637736	0610010E05Rik	18 E4	RIKEN cDNA 0610010E05 gene
1433571_at	2,636153	A130038L21Rik	13 C3	RIKEN cDNA A130038L21 gene
1423891_at	2,633322	Gstt3	10 C1	glutathione S-transferase theta 3
1427332_at	2,629075	Zdhhc5	2 D	zinc finger DHHC domain containing 5
1442340_x_at	2,628223	Cyr61	3 72.9 cM	cysteine rich protein 61
1454378_at	2,622282	Ywhaq		tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein theta polypeptide
1436209_at	2,62189	4732437J24Rik	4 E1	RIKEN cDNA 4732437J24 gene
1416505_at	2,620323	Nr4a1	15 F	nuclear receptor subfamily 4 group A member 1
1418480_at	2,620176	Cxcl7	5 E2	chemokine (C-X-C motif) ligand 7
1428544_at	2,619558	0610007L01Rik	5 G1.3	RIKEN cDNA 0610007L01 gene
1449575_a_at	2,617542	Gstp2	19 0.0 cM	glutathione S-transferase pi 2
1424852_at	2,615439	Mef2c	13 45.0 cM	myocyte enhancer factor 2C
1452385_at	2,614855	AA939927	3 G1	expressed sequence AA939927
1429233_at	2,606406	D5Ertd606e	5 52.0 cM	DNA segment Chr 5 ERATO Doi 606 expressed
1455345_at	2,605992	Phf15	11 B1.3	PHD finger protein 15
1419688_at	2,604731	Gpc6	14 E4	glypican 6
1423478_at	2,602617	Prkcb	7 60.0 cM	protein kinase C beta
1435251_at	2,602324	Snx13	12 A2	sorting nexin 13

1452654_at	2,601387	Zdhhc2	8 A4	zinc finger DHHC domain containing 2
1452124_at	2,598767	Ank3	10 38.0 cM	ankyrin 3 epithelial
1429713_at	2,591205	2610040F05Rik	5 G1.3	RIKEN cDNA 2610040F05 gene
1429812_at	2,590723	2610002D18Rik	4 D3	RIKEN cDNA 2610002D18 gene
1424474_a_at	2,58769	Camkk2	5 F	calcium/calmodulin-dependent protein kinase kinase 2 beta
1424726_at	2,587598	BC014685	6 C1	cDNA sequence BC014685
1452759_s_at	2,587063	Ppfibp1	6 G3	PTPRF interacting protein binding protein 1 (liprin beta 1)
1434600_at	2,586482	Tjp2		tight junction protein 2
1435345_at	2,584856	AL024097	2 B	expressed sequence AL024097
1423141_at	2,58438	Lip1	19 C1	lysosomal acid lipase 1
1420647_a_at	2,580217	Krt2-8	15 58.86 cM	keratin complex 2 basic gene 8
1437040_at	2,577132	4933417N20Rik	1 E4	RIKEN cDNA 4933417N20 gene
1416805_at	2,575235	1110032E23Rik	3 E3	RIKEN cDNA 1110032E23 gene
1448893_at	2,574917	Ncor2		nuclear receptor co-repressor 2
1434645_at	2,573651	C530008M17Rik	5 D	RIKEN cDNA C530008M17 gene
1452823_at	2,572955	Gstk1	6 B2.1	glutathione S-transferase kappa 1
1428510_at	2,572589	Lphn1	8 C2	latrophilin 1
1419708_at	2,569793	Wnt6	1 C4	wingless-related MMTV integration site 6
1428142_at	2,569106	Etv5	16 A1	ets variant gene 5
1460177_at	2,567923	0610010E05Rik	18 E4	RIKEN cDNA 0610010E05 gene
1426883_at	2,565132	AW491445	19 A	expressed sequence AW491445
1429065_at	2,565015	1700023M03Rik	10 C2	RIKEN cDNA 1700023M03 gene
1424440_at	2,564946	Mrps6	16 A1	mitochondrial ribosomal protein S6
1456475_s_at	2,564418	Prkar2b	12 A2	protein kinase cAMP dependent regulatory type II beta
1448842_at	2,56281	Cdo1	18 23.0 cM	cysteine dioxygenase 1 cytosolic
1426856_at	2,562408	2610207I16Rik	4 B3	RIKEN cDNA 2610207I16 gene
1418863_at	2,559029			Mus musculus 13 days embryo male testis cDNA RIKEN full-length enriched library clone:6030408M17 product:GATA binding protein 4 full insert sequence
1434768_at	2,557531	Cln2	7 50.0 cM	ceroid-lipofuscinosis neuronal 2
1433855_at	2,555116			Mus musculus transcribed sequence with strong similarity to protein ref:NP_065580.1 (M.musculus) hypothetical protein I54 [Mus musculus]
1423749_s_at	2,554734	Rangap1	15 43.3 cM	RAN GTPase activating protein 1
1438170_x_at	2,552304			
1424265_at	2,548361	Npl	1 G3	N-acetylneuraminate pyruvate lyase
1436499_at	2,54797	9530058O11Rik	19 C1	RIKEN cDNA 9530058O11 gene
1422491_a_at	2,547555	Bnip2	9 D	BCL2/adenovirus E1B 19kDa-interacting protein 1 NIP2
1448923_at	2,546691	Prkra	2 C3	protein kinase interferon inducible double stranded RNA dependent activator
1427339_at	2,543886	Slc30a2	4 D3	solute carrier family 30 (zinc transporter) member 2
1448107_x_at	2,543463	Klk6	7 23.0 cM	kallikrein 6
1422208_a_at	2,541114	Gnb5	9 41.0 cM	guanine nucleotide binding protein beta 5
1439858_at	2,541111			Mus musculus 12 days embryo spinal ganglion cDNA RIKEN full-length enriched library clone:D130031D15 product:unknown EST full insert sequence
1415988_at	2,536731	Hdlbp	1 55.3 cM	high density lipoprotein (HDL) binding protein
1420859_at	2,534565	Pkia	3 A1	protein kinase inhibitor alpha
1450264_a_at	2,534087	Chk	19 3.0 cM	choline kinase
1448404_at	2,530449	Scamp2	9 B	secretory carrier membrane protein 2
1448566_at	2,529856	Slc40a1	1 B	solute carrier family 40 (iron-regulated transporter) member 1
1448995_at	2,52978	Cxcl4	5 E2	chemokine (C-X-C motif) ligand 4

1417876_at	2,526664	Fcgr1	3 45.2 cM	Fc receptor IgG high affinity I
1452182_at	2,52554	Galnt2	8 E2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2
1435396_at	2,524816	Stxbp6	12 B1	syntaxin binding protein 6 (amisyn)
1459546_s_at	2,524644	Enpp1	10 19.0 cM	ectonucleotide pyrophosphatase/phosphodiesterase 1
1422943_a_at	2,52415	Hspb1	5 76.0 cM	heat shock protein 1
1416246_a_at	2,522474	Coro1a	7 62.5 cM	coronin actin binding protein 1A
1417301_at	2,521085	Fzd6	15 13.1 cM	frizzled homolog 6 (Drosophila)
1433486_at	2,520109	Clcn3	8 32.2 cM	chloride channel 3
1429055_at	2,518389	4930506M07Rik	19 D3	RIKEN cDNA 4930506M07 gene
1418095_at	2,517492	Smpx	X F4	small muscle protein X-linked
1457247_at	2,517333			Mus musculus transcribed sequences
1419551_s_at	2,517027			Mus musculus 6 days neonate head cDNA RIKEN full-length enriched library clone:5430411A13 product:serine/threonine kinase 39 STE20/SPS1 homolog (yeast) full insert sequence
1415832_at	2,513385	Agtr2	X 12.5 cM	angiotensin II receptor type 2
1428132_at	2,512019	1300002M12Rik	3 F2.1	RIKEN cDNA 1300002M12 gene
1426649_at	2,511922	Tmeff1	4 B1	transmembrane protein with EGF-like and two follistatin-like domains 1
1421315_s_at	2,505381	Cttn	13 31.0 cM	cortactin
1438945_x_at	2,501513	Gja1	10 29.0 cM	gap junction membrane channel protein alpha 1
1433908_a_at	2,501341			Mus musculus transcribed sequences
1422484_at	2,501105	Cycs	6 23.0 cM	cytochrome c somatic
1440985_at	-2,50244			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130079B08 product:unknown EST full insert sequence
1420731_a_at	-2,50427	Csrp2	10 D1	cysteine and glycine-rich protein 2
1426063_a_at	-2,50851	Gem	4 2.6 cM	GTP binding protein (gene overexpressed in skeletal muscle)
1437819_s_at	-2,50901	9530020G05Rik	6 B1	RIKEN cDNA 9530020G05 gene
1435598_at	-2,50921	Shc2	10 42.0 cM	src homology 2 domain-containing transforming protein C2
1436007_a_at	-2,515	6330575P11Rik	7 F1	RIKEN cDNA 6330575P11 gene
1420994_at	-2,51511	B3gnt5	16 A1	UDP-GlcNAc:betaGal beta-13-N-acetylglucosaminyltransferase 5
1449368_at	-2,51519	Dcn	10 55.0 cM	decorin
1452298_a_at	-2,51726		18	Mus musculus similar to myosin homolog brain - mouse (LOC383411) mRNA
1416130_at	-2,52253	Prnp	2 75.0 cM	prion protein
1448296_x_at	-2,52368	Tuba3	6 F3	tubulin alpha 3
1427299_at	-2,52368	Rps6ka3	X 65.7 cM	ribosomal protein S6 kinase polypeptide 3
1442977_at	-2,52411			Mus musculus transcribed sequences
1428797_at	-2,52453	2810004N23Rik	8 E2	RIKEN cDNA 2810004N23 gene
1437119_at	-2,52467	Ern1	11 E1	endoplasmic reticulum (ER) to nucleus signalling 1
1421374_a_at	-2,52757	Fxyd1	7 A3	FXYD domain-containing ion transport regulator 1
1427176_s_at	-2,52875	AI428936	7 A3	expressed sequence AI428936
1451260_at	-2,52889	Aldh1b1	4 B1	aldehyde dehydrogenase 1 family member B1
1419761_a_at	-2,52968	Gabpb1	2 71.0 cM	GA repeat binding protein beta 1
1448020_at	-2,53123	Rap1a	3 48.5 cM	RAS-related protein-1a
1438946_at	-2,53146	Pdgfra	5 42.0 cM	platelet derived growth factor receptor alpha polypeptide
1443260_at	-2,53207			Mus musculus transcribed sequences
1422786_at	-2,53345	Slc30a1	1 106.0 cM	solute carrier family 30 (zinc transporter) member 1
1452378_at	-2,53429	2210401K01Rik	RIKEN cDNA 2210401K01 gene	
1428440_at	-2,53531	Slc25a12	2 C2	solute carrier family 25 (mitochondrial carrier Aralar) member 12
1426363_x_at	-2,53569	5430437E11Rik	10 B4	RIKEN cDNA 5430437E11 gene

1437174_at	-2,5362	A330080J22Rik	9 E3.3	RIKEN cDNA A330080J22 gene
1458447_at	-2,53645	Lek1	1 H6	leucine glutamic acid lysine family 1 protein
1447775_x_at	-2,53679			
1452418_at	-2,53827			
1449407_at	-2,53991	Cdv1	5 65.0 cM	carnitine deficiency-associated gene expressed in ventricle 1
1435008_at	-2,54953	Slc9a6	X A5	solute carrier family 9 (sodium/hydrogen exchanger) isoform 6
1433670_at	-2,55091	Emp2	16 2.4 cM	epithelial membrane protein 2
1433955_at	-2,55437	Wdr9	16 A1	WD repeat domain 9
1429530_a_at	-2,55461	4122402O22Rik	16 A1	RIKEN cDNA 4122402O22 gene
1435303_at	-2,55732	4932409F03Rik	18 A1	RIKEN cDNA 4932409F03 gene
1432143_a_at	-2,55802	Hbp1	12 A2	high mobility group box transcription factor 1
1450716_at	-2,55847	Adamts1	16 53.4 cM	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 1
1448171_at	-2,5585	Siah2	3 35.0 cM	seven in absentia 2
1417656_at	-2,5587	Mybl2	2 93.0 cM	myeloblastosis oncogene-like 2
1451342_at	-2,56003	Spon1	7 F1	spondin 1 (f-spondin) extracellular matrix protein
1437889_x_at	-2,56131	Bgn	X 29.3 cM	biglycan
1422697_s_at	-2,56393	Jmj	13 27.0 cM	jumonji
1441328_at	-2,56503			Mus musculus adult male aorta and vein cDNA RIKEN full-length enriched library clone:A530080E04 product:unknown EST full insert sequence
1438071_at	-2,56631	Pms1	1 C1.1	postmeiotic segregation increased 1 (S. cerevisiae)
1448666_s_at	-2,56888	Tob2	15 E1	transducer of ERBB2 2
1437544_at	-2,57258	D3Ertd330e	3 H3	DNA segment Chr 3 ERATO Doi 330 expressed
1433985_at	-2,57666	Abi2	1 C2	abl-interactor 2
1435878_at	-2,57702	Stk38l	6 G3	serine/threonine kinase 38 like
1436210_at	-2,57703	C330018K18Rik	9 E3.3	RIKEN cDNA C330018K18 gene
1425101_a_at	-2,57934	Fkbp6	5 75.0 cM	FK506 binding protein 6
1423836_at	-2,58111	Zfp503	14 9.0 cM	zinc finger protein 503
1427672_a_at	-2,58186	Utx	X 5.5 cM	ubiquitously transcribed tetratricopeptide repeat gene X chromosome
1436926_at	-2,5827	Esrrb	12 41.0 cM	estrogen related receptor beta
1424116_x_at	-2,59196	Ppp5c	7 4.0 cM	protein phosphatase 5 catalytic subunit
1426766_at	-2,59242	6330403K07Rik	11 B4	RIKEN cDNA 6330403K07 gene
1449137_at	-2,59374	Pdha1	X 66.5 cM	pyruvate dehydrogenase E1 alpha 1
1429372_at	-2,59707	Sox11		SRY-box containing gene 11
1420725_at	-2,59754	Tmlhe		trimethyllysine hydroxylase epsilon
1449089_at	-2,60234	Nrip1	16 A1	nuclear receptor interacting protein 1
1453774_at	-2,60256	2810002O09Rik	X C2	RIKEN cDNA 2810002O09 gene
1425543_s_at	-2,60309	2810431N21Rik	6 G2	RIKEN cDNA 2810431N21 gene
1434671_at	-2,60321			Mus musculus adult male corpora quadrigemina cDNA RIKEN full-length enriched library clone:B230337E12 product:unclassifiable full insert sequence
1452330_a_at	-2,6058	1200013A08Rik	4 83.0 cM	RIKEN cDNA 1200013A08 gene
1460592_at	-2,60582	Epb4.1l1	2 88.0 cM	erythrocyte protein band 4.1-like 1
1428267_at	-2,60748	Dhx40	11 C	DEAH (Asp-Glu-Ala-His) box polypeptide 40
1448754_at	-2,60826	Rbp1	9 52.0 cM	retinol binding protein 1 cellular
1433977_at	-2,60955	Hs3st3b	11 33.0 cM	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B
1442497_at	-2,61085	4931400A14Rik	17 E1.3	RIKEN cDNA 4931400A14 gene
1446571_at	-2,61186			Mus musculus 13 days embryo heart cDNA RIKEN full-length enriched library clone:D330040F03 product:unclassifiable full insert sequence
1439885_at	-2,61311	Hoxc5	15 57.4 cM	homeo box C5

1456808_at	-2,6142			Mus musculus transcribed sequences
1451501_a_at	-2,61477	Ghr	15 4.6 cM	growth hormone receptor
1439652_at	-2,61531			Mus musculus 2 days neonate thymus thymic cells cDNA RIKEN full-length enriched library clone:C920011F17 product:unclassifiable full insert sequence
1448014_s_at	-2,61665	2810030C21Rik	4 C7	RIKEN cDNA 2810030C21 gene
1450172_at	-2,61665	Pknox1	17 B-C	Pbx/knotted 1 homeobox
1426799_at	-2,61793	D330025I23Rik	9 C	RIKEN cDNA D330025I23 gene
1423422_at	-2,61887	Asb4	6 0.6 cM	ankyrin repeat and SOCS box-containing protein 4
1433930_at	-2,62201	Hpse	5 E3	heparanase
1426208_x_at	-2,62257	Plagl1	10 15.0 cM	pleiomorphic adenoma gene-like 1
1423878_at	-2,62273	0610037F22Rik	18 B1	RIKEN cDNA 0610037F22 gene
1416034_at	-2,62351	Cd24a	10 26.0 cM	CD24a antigen
1427540_at	-2,62445	D10Ertd749e	10 38.0 cM	DNA segment Chr 10 ERATO Doi 749 expressed
1429564_at	-2,62597	5830443C21Rik	19 C2	RIKEN cDNA 5830443C21 gene
1454727_at	-2,62696	AI173486	18 E1	expressed sequence AI173486
1448745_s_at	-2,62778	Lor	3 42.1 cM	loricrin
1445427_at	-2,62901			Mus musculus transcribed sequences
1417756_a_at	-2,62971	Lsp1	7 69.0 cM	lymphocyte specific 1
1428146_s_at	-2,63147	Acaa2	18 45.0 cM	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
1424375_s_at	-2,63392	Ian1	6 B2.3	immune associated nucleotide 1
1418258_s_at	-2,63549	Arhgdia	11 E2	Rho GDP dissociation inhibitor (GDI) alpha
1415999_at	-2,63725	Hey1	3 2.4 cM	hairy/enhancer-of-split related with YRPW motif 1
1425918_at	-2,63915	Egln3	12 B3	EGL nine homolog 3 (C. elegans)
1446929_at	-2,63944			Mus musculus 12 days embryo spinal ganglion cDNA RIKEN full-length enriched library clone:D130062J21 product:hypothetical protein full insert sequence
1445191_at	-2,6409	4932702D22	2 E5	hypothetical protein 4932702D22
1448804_at	-2,64457	Cyp11a1	9 31.0 cM	cytochrome P450 family 11 subfamily a polypeptide 1
1440604_at	-2,65151			Mus musculus transcribed sequences
1419356_at	-2,65355	Klf7	1 C1-C3	Kruppel-like factor 7 (ubiquitous)
1431226_a_at	-2,65469	2810430J06Rik	5 B1	RIKEN cDNA 2810430J06 gene
1424191_a_at	-2,65535	5730578N08Rik	16 A1	RIKEN cDNA 5730578N08 gene
1417359_at	-2,66046	Mfap2	4 D3-E1	microfibrillar-associated protein 2
1448990_a_at	-2,66056	Myo1b	1 24.8 cM	myosin IB
1451998_at	-2,66153	4930485D02Rik	2 F3	RIKEN cDNA 4930485D02 gene
1429971_at	-2,66631	Txnrd2	16 11.2 cM	thioredoxin reductase 2
1454942_at	-2,67037	Niban	1 G2	niban protein
1429060_at	-2,67123	2210401K01Rik	RIKEN cDNA 2210401K01 gene	
1420385_at	-2,67233	Gna14	19 9.0 cM	guanine nucleotide binding protein alpha 14
1454703_x_at	-2,67316			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030060F23 product:Mus musculus U22 snoRNA host gene (UHG) gene complete sequence full insert sequence
1449645_s_at	-2,67365	Cct3	3 50.0 cM	chaperonin subunit 3 (gamma)
1428502_at	-2,67857	2010200J04Rik	10 C2	RIKEN cDNA 2010200J04 gene
1438788_at	-2,67858			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130043L18 product:unknown EST full insert sequence
1439095_at	-2,67987	2610019N13Rik	3 H4	RIKEN cDNA 2610019N13 gene
1417780_at	-2,68172	Lass4	8 A1.1	longevity assurance homolog 4 (S. cerevisiae)
1416689_at	-2,68406	Tuft1	3 F2.1	tuftelin 1
1449026_at	-2,68562	Ifnar1	16 63.2 cM	interferon (alpha and beta) receptor 1

1416710_at	-2,68725	9030603L14Rik	X E3	RIKEN cDNA 9030603L14 gene
1439373_x_at	-2,6891	Wnt5b	6 56.2 cM	wingless-related MMTV integration site 5B
1446550_at	-2,68938	Gspt1	16 3.8 cM	G1 to phase transition 1
1421916_at	-2,69001	Pdgfra	5 42.0 cM	platelet derived growth factor receptor alpha polypeptide
1425673_at	-2,69323			Mus musculus cDNA clone MGC:12142 IMAGE:3710749 complete cds
1416414_at	-2,69443	Emilin1	5 B1	elastin microfibril interface located protein 1
1437945_x_at	-2,69465	Nap1l1	10 D1	nucleosome assembly protein 1-like 1
1456046_at	-2,69542	C1qr1	2 84.0 cM	complement component 1 q subcomponent receptor 1
1436948_a_at	-2,69602	AW260253	X A3.1	expressed sequence AW260253
1428667_at	-2,7004	Maoa	X 5.2 cM	monoamine oxidase A
1417096_at	-2,70405	2810430M08Rik	1 H5	RIKEN cDNA 2810430M08 gene
1434428_at	-2,7063	D330028D13Rik	6 B2.3	RIKEN cDNA D330028D13 gene
1438816_at	-2,70791	6230412P20Rik	1 102.0 cM	RIKEN cDNA 6230412P20 gene
1443037_at	-2,70905			Mus musculus adult male hypothalamus cDNA RIKEN full-length enriched library clone:A230106P21 product:unknown EST full insert sequence
1422053_at	-2,71271	Inhba	13 10.0 cM	inhibin beta-A
1429310_at	-2,71463	Flrt3	2 F3	fibronectin leucine rich transmembrane protein 3
1419301_at	-2,71614	Fzd4	7 44.5 cM	frizzled homolog 4 (Drosophila)
1420565_at	-2,71849	Hoxa1	6 26.28 cM	homeo box A1
1434754_at	-2,71998		11 B5	Mus musculus LOC380710 (LOC380710) mRNA
1417403_at	-2,72167	Elovl6	3 G3	ELOVL family member 6 elongation of long chain fatty acids (yeast)
1418673_at	-2,72193	Snai2	16 9.4 cM	snail homolog 2 (Drosophila)
1446228_at	-2,72295	D19Wsu12e	19 24.5 cM	DNA segment Chr 19 Wayne State University 12 expressed
1438651_a_at	-2,72525			
1425114_at	-2,72755	C030034J04Rik	7 F2	RIKEN cDNA C030034J04 gene
1443846_x_at	-2,7289			Mus musculus transcribed sequence with weak similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1419706_a_at	-2,7324	Akap12	10 5.5 cM	A kinase (PRKA) anchor protein (gravin) 12
1419655_at	-2,73494	Tle3		transducin-like enhancer of split 3 homolog of Drosophila E(spl)
1428498_at	-2,73713	2610206B13Rik	14 E2.3	RIKEN cDNA 2610206B13 gene
1434129_s_at	-2,73899	AI447312	13 C3	expressed sequence AI447312
1438702_at	-2,74249	Map4k5	12 C2	mitogen-activated protein kinase kinase kinase kinase 5
1425536_at	-2,74266	Stx3	19 A	syntaxin 3
1416915_at	-2,74424	Msh6	17 47.0 cM	mutS homolog 6 (E. coli)
1428136_at	-2,74752	Sfrp1	8 9.5 cM	secreted frizzled-related sequence protein 1
1450068_at	-2,74807	Baz1b	5 G2	bromodomain adjacent to zinc finger domain 1B
1437507_at	-2,74858	C430014D17Rik	3 F1	RIKEN cDNA C430014D17 gene
1428640_at	-2,74986	Hsf2bp	17 A3.3	heat shock transcription factor 2 binding protein
1440830_at	-2,7501	8430401C09Rik	17 B3	RIKEN cDNA 8430401C09 gene
1447757_x_at	-2,75506	Inpp5f	7 F3	inositol polyphosphate-5-phosphatase F
1448862_at	-2,75679	Icam2	11 63.0 cM	intercellular adhesion molecule 2
1452682_at	-2,75786	4632404H22Rik	X A4	RIKEN cDNA 4632404H22 gene
1452283_at	-2,75993	AW123240	6	expressed sequence AW123240
1418917_at	-2,76052	Hebp2	10 A3	heme binding protein 2
1453007_at	-2,76076	3110082I17Rik	5 G2	RIKEN cDNA 3110082I17 gene
1431166_at	-2,76193	Chd1	17 7.2 cM	chromodomain helicase DNA binding protein 1
1425516_at	-2,76264	Ogt	X C3	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)

1440225_at	-2,76308	8430401C09Rik	17 B3	RIKEN cDNA 8430401C09 gene
1438620_x_at	-2,76316			
1444008_at	-2,76415	4933432H23Rik	15 D1	RIKEN cDNA 4933432H23 gene
1437658_a_at	-2,76556			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030060F23 product:Mus musculus U22 snoRNA host gene (UHG) gene complete sequence full insert sequence
1453285_at	-2,76589	2600017H02Rik	11 B3	RIKEN cDNA 2600017H02 gene
1438041_at	-2,76801	Pde7a	3 7.0 cM	phosphodiesterase 7A
1435302_at	-2,76923	4932409F03Rik	18 A1	RIKEN cDNA 4932409F03 gene
1455655_a_at	-2,76973	Tardbp	4 E2	TAR DNA binding protein
1441098_at	-2,77018		17 A1	Mus musculus similar to hypothetical protein FLJ40240 (LOC240023) mRNA
1428081_at	-2,77177	1810045K06Rik	4 E2	RIKEN cDNA 1810045K06 gene
1422587_at	-2,77382	C630002M10Rik	16 A1	RIKEN cDNA C630002M10 gene
1422452_at	-2,7744	Bag3	7 F3	Bcl2-associated athanogene 3
1429911_at	-2,78321	D030046N04Rik	8 A1.3	RIKEN cDNA D030046N04 gene
1427298_at	-2,78507	Npn1		neoplastic progression 1
1424186_at	-2,78548	2610001E17Rik	16 A1	RIKEN cDNA 2610001E17 gene
1449071_at	-2,78787	Myl7	11 0.75 cM	myosin light polypeptide 7 regulatory
1427084_a_at	-2,78867	Map4k5	12 C2	mitogen-activated protein kinase kinase kinase kinase 5
1450625_at	-2,79046	Col5a2	1 C1	procollagen type V alpha 2
1444693_at	-2,79163			Mus musculus transcribed sequences
1426530_a_at	-2,79262	1300013C10Rik	5 C3.1	RIKEN cDNA 1300013C10 gene
1439123_at	-2,79592			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130034A17 product: unknown EST full insert sequence
1428523_at	-2,79795	Lphn3	5 E1	latrophilin 3
1417823_at	-2,7982	Gcat	15 46.6 cM	glycine C-acetyltransferase (2-amino-3-ketobutyrate-coenzyme A ligase)
1450070_s_at	-2,80057	Pak1	7 46.5 cM	p21 (CDKN1A)-activated kinase 1
1457840_at	-2,80265	Plxna4	6 B1	plexin A4
1454011_a_at	-2,80536	Rpa2	4 D2.3	replication protein A2
1460229_at	-2,80619	Stag3	5 67.0 cM	stromal antigen 3
1444302_at	-2,80718	MGC59592	6 D3	Unknown (protein for MGC:59592)
1418100_at	-2,80722	A030009H04Rik	11 B3	RIKEN cDNA A030009H04 gene
1460015_at	-2,80873		2 A3	Mus musculus hypothetical LOC227631 (LOC227631) mRNA
1422671_s_at	-2,80962	Naalad2	9 A2	N-acetylated alpha-linked acidic dipeptidase 2
1450863_a_at	-2,81062	Dcamkl1	3 C	double cortin and calcium/calmodulin-dependent protein kinase-like 1
1434442_at	-2,81173	D5Ertd593e	5 52.0 cM	DNA segment Chr 5 ERATO Doi 593 expressed
1452840_at	-2,81441			Mus musculus adult male cerebellum cDNA RIKEN full-length enriched library clone:1500009L16 product:hypothetical protein full insert sequence
1448395_at	-2,8167	Sfrp1	8 9.5 cM	secreted frizzled-related sequence protein 1
1429982_at	-2,81764	4931417E21Rik	6 F2	RIKEN cDNA 4931417E21 gene
1440438_at	-2,81793			Mus musculus transcribed sequence with moderate similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1448152_at	-2,81994	Igf2	7 69.09 cM	insulin-like growth factor 2
1459713_s_at	-2,82114	AU040576	7 F5	expressed sequence AU040576
1437173_at	-2,82144	Edg3	13 A5	endothelial differentiation sphingolipid G-protein-coupled receptor 3
1452736_at	-2,82198	1700020M16Rik	14 32.5 cM	RIKEN cDNA 1700020M16 gene
1455900_x_at	-2,82338	Tgm2	2 89.0 cM	transglutaminase 2 C polypeptide
1422663_at	-2,82594	Orc1l	4 D	origin recognition complex subunit 1-like (S.cereviaiae)

1455169_at	-2,82642			Mus musculus 0 day neonate cerebellum cDNA RIKEN full-length enriched library clone:C230068K15 product:unknown EST full insert sequence
1441732_at	-2,82667			Mus musculus transcribed sequences
1430059_at	-2,82713			Mus musculus transcribed sequences
1428798_s_at	-2,82959	2810004N23Rik	8 E2	RIKEN cDNA 2810004N23 gene
1460329_at	-2,82979	B4galt6	18 A2	UDP-Gal:betaGlcNAc beta 14-galactosyltransferase polypeptide 6
1418260_at	-2,83326	Hunk	16 58.0 cM	hormonally upregulated Neu-associated kinase
1443939_at	-2,84074		4 D1	Mus musculus hypothetical LOC230628 (LOC230628) mRNA
1435123_at	-2,84395	AI852640	12 A1.1	expressed sequence AI852640
1439837_at	-2,84598	Tnrc15	1 D	trinucleotide repeat containing 15
1416405_at	-2,84845	Bgn	X 29.3 cM	biglycan
1436173_at	-2,85026	Dlc1	8 21.0 cM	deleted in liver cancer 1
1423393_at	-2,86089	Clic4	4 D3	chloride intracellular channel 4 (mitochondrial)
1419354_at	-2,86122	Klf7	1 C1-C3	Kruppel-like factor 7 (ubiquitous)
1450699_at	-2,86499	Selenbp1	3 50.8 cM	selenium binding protein 1
1416159_at	-2,86615	Nr2f2	7 33.0 cM	nuclear receptor subfamily 2 group F member 2
1421910_at	-2,86754	Tcf20	15 E	transcription factor 20
1419497_at	-2,86891	Cdkn1b	6 62.0 cM	cyclin-dependent kinase inhibitor 1B (P27)
1460163_at	-2,87527			Mus musculus transcribed sequences
1455609_at	-2,8784	C030025P15Rik	5 F	RIKEN cDNA C030025P15 gene
1451516_at	-2,88144	1810036J22Rik	15 F1	RIKEN cDNA 1810036J22 gene
1444777_at	-2,88342	Rai14	15 A2	retinoic acid induced 14
1440092_at	-2,88425			Mus musculus transcribed sequences
1455418_at	-2,8845			Mus musculus transcribed sequences
1459749_s_at	-2,88551	6030410K14	3 B	hypothetical protein 6030410K14
1433542_at	-2,88665	Inpp5f	7 F3	inositol polyphosphate-5-phosphatase F
1449519_at	-2,89013	Gadd45a	3 70.5 cM	growth arrest and DNA-damage-inducible 45 alpha
1452250_a_at	-2,89269	Col6a2	10 41.1 cM	procollagen type VI alpha 2
1437917_at	-2,89279			Mus musculus 13 days embryo stomach cDNA RIKEN full-length enriched library clone:D530037H12 product:unknown EST full insert sequence
1431085_a_at	-2,89364	Pcmt1	10 7.0 cM	protein-L-isoaspartate (D-aspartate) O-methyltransferase 1
1436970_a_at	-2,8948	Pdgfrb	18 30.0 cM	platelet derived growth factor receptor beta polypeptide
1447602_x_at	-2,89523	2010004N24Rik	2 H3	RIKEN cDNA 2010004N24 gene
1437605_at	-2,89644	Nphs2	1 G3	nephrosis 2 homolog podocin (human)
1444232_at	-2,89903			Mus musculus 16 days neonate heart cDNA RIKEN full-length enriched library clone:D830017E01 product:protein kinase cGMP-dependent type I full insert sequence
1428647_at	-2,89951	Pbx1	1 88.1 cM	pre B-cell leukemia transcription factor 1
1441547_at	-2,89959			Mus musculus 12 days embryo spinal ganglion cDNA RIKEN full-length enriched library clone:D130027H11 product:unknown EST full insert sequence
1436911_at	-2,90383	A230053O16Rik	2 H4	RIKEN cDNA A230053O16 gene
1431771_a_at	-2,90392	Irak1bp1	9 E2	interleukin-1 receptor-associated kinase 1 binding protein 1
1458665_at	-2,90482			Mus musculus transcribed sequence with strong similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1437466_at	-2,90633	Alcam	16 A1	activated leukocyte cell adhesion molecule
1459288_at	-2,90782			Mus musculus transcribed sequences
1447676_x_at	-2,90787			
1429268_at	-2,90905	2610318N02Rik	16 A1	RIKEN cDNA 2610318N02 gene
1425310_a_at	-2,91178	Col26a1	5 G2	collagen type XXVI alpha 1

1451703_s_at	-2,91248	Aprt	8 67.0 cM	adenine phosphoribosyl transferase
1450784_at	-2,91625	Reck	4 B1	reversion-inducing-cysteine-rich protein with kazal motifs
1448601_s_at	-2,91646	Msx1	5 21.0 cM	homeo box msh-like 1
1460056_at	-2,91688	1700064D17Rik	2	RIKEN cDNA 1700064D17 gene
1452942_at	-2,91879	4930438D12Rik	15 D1	RIKEN cDNA 4930438D12 gene
1457110_at	-2,92105			Mus musculus transcribed sequences
1421102_a_at	-2,92121	Vamp3	4 E2	vesicle-associated membrane protein 3
1423072_at	-2,92401	6720475J19Rik	19 A	RIKEN cDNA 6720475J19 gene
1436737_a_at	-2,92576	Sorbs1	19 36.5 cM	sorbin and SH3 domain containing 1
1423450_a_at	-2,92613	Hs3st1	5 22.0 cM	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
1454659_at	-2,92895	Dctd	8 B1.1	dCMP deaminase
1437673_at	-2,93018			Mus musculus transcribed sequences
1415865_s_at	-2,9318	Bpgm	6 A3.3	23-bisphosphoglycerate mutase
1427226_at	-2,93738	Epn2	11 B2	epsin 2
1417680_at	-2,94903	Kcna5	6 61.0 cM	potassium voltage-gated channel shaker-related subfamily member 5
1438884_at	-2,95261			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:4932442L11 product:hypothetical protein full insert sequence
1427474_s_at	-2,95448	Gstm3		glutathione S-transferase mu 3
1428145_at	-2,95497	Acaa2	18 45.0 cM	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)
1458218_s_at	-2,95539	Pde7a	3 7.0 cM	phosphodiesterase 7A
1417937_at	-2,95566	Dact1	12 C2	dapper homolog 1 antagonist of beta-catenin (xenopus)
1426146_a_at	-2,95803	Chpt1	10 C1	choline phosphotransferase 1
1440660_at	-2,95869			Mus musculus transcribed sequences
1454637_at	-2,95997	Klhl8	5 55.0 cM	kelch-like 8 (Drosophila)
1436790_a_at	-2,9625	Sox11		SRY-box containing gene 11
1426926_at	-2,96717	Plcg2	8 E1	phospholipase C gamma 2
1416346_at	-2,96908	Timm8a	X 51.0 cM	translocase of inner mitochondrial membrane 8 homolog a (yeast)
1427240_at	-2,96965	4931431C02Rik	9 A3	RIKEN cDNA 4931431C02 gene
1417311_at	-2,96971	Crip2	12 F1	cysteine rich protein 2
1443156_at	-2,97423			Mus musculus transcribed sequences
1448250_at	-2,97715	9030425E11Rik	9 A5.1	RIKEN cDNA 9030425E11 gene
1451177_at	-2,97851	2010306G19Rik	3 H3	RIKEN cDNA 2010306G19 gene
1434839_s_at	-2,97916	8030499H02Rik	3 A3	RIKEN cDNA 8030499H02 gene
1452761_a_at	-2,98146	6720477E09Rik	9 F3	RIKEN cDNA 6720477E09 gene
1450776_at	-2,98685	AU041707	8 A2	expressed sequence AU041707
1443897_at	-2,98742	Ddit3	10 D3	DNA-damage inducible transcript 3
1444378_at	-2,98921			Mus musculus transcribed sequences
1459722_at	-2,98993			Mus musculus transcribed sequences
1457783_at	-2,99082	Rab12	17 E1.1	RAB12 member RAS oncogene family
1452359_at	-2,99825	AA536743	5 C3.1	expressed sequence AA536743
1416077_at	-3,00122	Adm	7 50.5 cM	adrenomedullin
1427932_s_at	-3,00605			
1424451_at	-3,00606	MGC29978	9 F3	3-ketoacyl-CoA thiolase B
1455670_at	-3,0085			Mus musculus 15 days embryo head cDNA RIKEN full-length enriched library clone:D930003N06 product:unknown EST full insert sequence
1419033_at	-3,00953			Mus musculus transcribed sequence with weak similarity to protein pir:I58401 (M.musculus) I58401 protein-tyrosine kinase (EC 2.7.1.112) JAK3 - mouse
1425784_a_at	-3,01112	Olfm1	2 A3	olfactomedin 1

1443325_at	-3,01187			Mus musculus transcribed sequence with strong similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse
1435872_at	-3,01356	Pim1	17 16.4 cM	proviral integration site 1
1438244_at	-3,01458			Mus musculus transcribed sequences
1421498_a_at	-3,02047	2010204K13Rik	RIKEN cDNA 2010204K13 gene	
1436894_at	-3,02708	LOC381325	1 H2.3	LISCH7-like
1447643_x_at	-3,02947			
1433617_s_at	-3,03443	B4galt5	2 H3	UDP-Gal:betaGlcNAc beta 14-galactosyltransferase polypeptide 5
1436115_at	-3,03642		12 F1	Mus musculus similar to RAP2A member of RAS oncogene family; K-REV; RAP2 member of RAS oncogene family (K-rev) (LOC212539) mRNA
1457726_at	-3,03866	Rps15a	7 F1	ribosomal protein S15a
1450981_at	-3,04112	Cnn2	10 C1	calponin 2
1453375_at	-3,04153			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:4930422N03 product:unknown EST full insert sequence
1450976_at	-3,0529	Ndr1	15 D2	N-myc downstream regulated 1
1438878_at	-3,05428			Mus musculus adult male olfactory brain cDNA RIKEN full-length enriched library clone:6430537K16 product:unknown EST full insert sequence
1455220_at	-3,05853	Frat2	19 C3	frequently rearranged in advanced T-cell lymphomas 2
1428377_at	-3,06582	6330404E16Rik	10 C1	RIKEN cDNA 6330404E16 gene
1417110_at	-3,06664	Man1a	10 B3	mannosidase 1 alpha
1433733_a_at	-3,06761	Cry1	10 46.0 cM	cryptochrome 1 (photolyase-like)
1435644_at	-3,06893	G431001E03Rik	11 A4	RIKEN cDNA G431001E03 gene
1422642_at	-3,07163	Cdc42ep3	17 E3	CDC42 effector protein (Rho GTPase binding) 3
1421355_at	-3,07312	Tgm3	2 F1	transglutaminase 3 E polypeptide
1447526_at	-3,07449			Mus musculus transcribed sequences
1438855_x_at	-3,07456	Tnfaip2	12 56.0 cM	tumor necrosis factor alpha-induced protein 2
1455037_at	-3,07613	Plxna2		plexin A2
1435284_at	-3,0777			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130026I10 product:unknown EST full insert sequence
1429861_at	-3,07952	Pcdh9	14 E2.1	protocadherin 9
1418102_at	-3,08179	Hes1	16 27.0 cM	hairy and enhancer of split 1 (Drosophila)
1421072_at	-3,0848	Irx5	8 43.3 cM	Iroquois related homeobox 5 (Drosophila)
1416158_at	-3,10126	Nr2f2	7 33.0 cM	nuclear receptor subfamily 2 group F member 2
1455426_at	-3,10401	Epha3	16 A1	Eph receptor A3
1447934_at	-3,10487	9630033F20Rik	6 F3	RIKEN cDNA 9630033F20 gene
1439909_at	-3,10646	Evi3	18 A1	ecotropic viral integration site 3
1428393_at	-3,1152	Nrn1	13 A3.3	neuritin 1
1451191_at	-3,11636	Crabp2	2 54.0 cM	cellular retinoic acid binding protein II
1428220_at	-3,1169	5730419I09Rik	6 G3	RIKEN cDNA 5730419I09 gene
1429028_at	-3,11804			Mus musculus 11 days pregnant adult female ovary and uterus cDNA RIKEN full-length enriched library clone:5033414A21 product:inferred: human CLASP-4 {Homo sapiens} full insert sequence
1448889_at	-3,12196	Slc38a4	15 F1	solute carrier family 38 member 4
1460232_s_at	-3,12408	Hsd3b6	3 F2.2	hydroxysteroid dehydrogenase-6 delta<5>-3-beta
1449514_at	-3,12424	Gprk5	19 55.0 cM	G protein-coupled receptor kinase 5
1451189_at	-3,1258	2410003H12Rik	2 H3	RIKEN cDNA 2410003H12 gene
1434401_at	-3,13112	9930114B20Rik	1 E2.1	RIKEN cDNA 9930114B20 gene
1443870_at	-3,13403	A830021K08Rik	14 E4	RIKEN cDNA A830021K08 gene

1452889_at	-3,13733		7 F3	Mus musculus similar to phospholysine phosphohistidine inorganic pyrophosphate phosphata (LOC384677) mRNA
1454873_at	-3,13874	C130032F08	6 B2.3	hypothetical protein C130032F08
1433716_x_at	-3,14057			Mus musculus transcribed sequences
1435275_at	-3,14382	Coxvib2	5	cytochrome c oxidase subunit VIb testes-specific
1439628_x_at	-3,14503	Rab38	7 46.0 cM	Rab38 member of RAS oncogene family
1446489_at	-3,14615			
1423549_at	-3,15283	Slc1a4	11 10.92 cM	solute carrier family 1 (glutamate/neutral amino acid transporter) member 4
1438453_at	-3,15305	Rad51c	11 49.0 cM	Rad51 homolog c (S. cerevisiae)
1448594_at	-3,15555	Wisp1	15 38.5 cM	WNT1 inducible signaling pathway protein 1
1458739_at	-3,15646			Mus musculus transcribed sequences
1441771_at	-3,15685			Mus musculus transcribed sequences
1452151_at	-3,15761	BC021523	15 E2	cDNA sequence BC021523
1442676_at	-3,15897	Maoa	X 5.2 cM	monoamine oxidase A
1416129_at	-3,15945	1300002F13Rik	4 E2	RIKEN cDNA 1300002F13 gene
1439887_at	-3,16061			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130025B04 product: unknown EST full insert sequence
1444785_at	-3,16258			Mus musculus transcribed sequences
1440721_at	-3,16321			Mus musculus 13 days embryo forelimb cDNA RIKEN full-length enriched library clone:5930433N17 product:unknown EST full insert sequence
1418788_at	-3,16836	Tek	4 43.6 cM	endothelial-specific receptor tyrosine kinase
1425028_a_at	-3,17067	Tpm2	4 A5	tropomyosin 2 beta
1422834_at	-3,18055	Kcnd2	6 7.2 cM	potassium voltage-gated channel Shal-related family member 2
1429399_at	-3,18913	4930553F04Rik	18 A2	RIKEN cDNA 4930553F04 gene
1460602_at	-3,19316	Dlc1	8 21.0 cM	deleted in liver cancer 1
1455727_at	-3,19376	U2af1-rs2	X 68.5 cM	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 1 related sequence 2
1443302_at	-3,20942			Mus musculus 12 days embryo eyeball cDNA RIKEN full-length enriched library clone:D230045F22 product:unknown EST full insert sequence
1425503_at	-3,21158	Gcnt2	13 17.0 cM	glucosaminyltransferase I-branching enzyme
1440708_at	-3,21519	Myh9	15 43.3 cM	myosin heavy chain IX
1448593_at	-3,21905	Wisp1	15 38.5 cM	WNT1 inducible signaling pathway protein 1
1434848_at	-3,21968	Gpr27	6 D3	G protein-coupled receptor 27
1431146_a_at	-3,22601	Cpne8	15 E3	copine VIII
1437406_x_at	-3,22981	Igfbp4	11 D	insulin-like growth factor binding protein 4
1457445_at	-3,23331	Trps1	15 30.1 cM	trichorhinophalangeal syndrome I (human)
1437638_at	-3,24884	Srrm2	17 A3.3	serine/arginine repetitive matrix 2
1431295_a_at	-3,25239	Stx18	5 B3	syntaxin 18
1416871_at	-3,2534	Adam8	7 F3-F5	a disintegrin and metalloprotease domain 8
1435456_at	-3,25379	AI428795	5 F	expressed sequence AI428795
1459983_at	-3,25409	B430212C06Rik	18 E1	RIKEN cDNA B430212C06 gene
1430521_s_at	-3,25865	Cpne8	15 E3	copine VIII
1423581_at	-3,2591	Nmt2	2 A1	N-myristoyltransferase 2
1457042_at	-3,26027			Mus musculus transcribed sequences
1450117_at	-3,26047	Tcf3	6 30.4 cM	transcription factor 3
1418664_at	-3,2655	Mpdz	4 38.6 cM	multiple PDZ domain protein
1441620_at	-3,26729			Mus musculus 13 days embryo lung cDNA RIKEN full-length enriched library clone:D430001H03 product:unclassifiable full insert sequence
1456139_at	-3,26737			Mus musculus 16 days embryo head cDNA RIKEN

				full-length enriched library clone:C130073E08 product:unknown EST full insert sequence
1434325_x_at	-3,26772	Prkar1b	5 82.0 cM	protein kinase cAMP dependent regulatory type I beta
1431356_at	-3,2708			Mus musculus 11 days pregnant adult female ovary and uterus cDNA RIKEN full-length enriched library clone:5033406J01 product:unknown EST full insert sequence
1440739_at	-3,28442	Vegfc	8 B3	vascular endothelial growth factor C
1445758_at	-3,28881			Mus musculus transcribed sequences
1416492_at	-3,28895	Ccne1	7 16.0 cM	cyclin E1
1437685_x_at	-3,29363	Fmod	1 74.3 cM	fibromodulin
1423313_at	-3,29738	Pde7a	3 7.0 cM	phosphodiesterase 7A
1427522_at	-3,29902	A530023E23Rik	9 A5.3	RIKEN cDNA A530023E23 gene
1436791_at	-3,3017	Wnt5a	14 7.8 cM	wingless-related MMTV integration site 5A
1449145_a_at	-3,30614	Cav	6 A2	caveolin caveolae protein
1435084_at	-3,31023			Mus musculus adult male liver tumor cDNA RIKEN full-length enriched library clone:C730049O14 product:unknown EST full insert sequence
1450710_at	-3,3111	Jmj	13 27.0 cM	jumonji
1449379_at	-3,31457	Kdr	5 42.0 cM	kinase insert domain protein receptor
1458053_at	-3,31714	Abi2	1 C2	abl-interactor 2
1448323_a_at	-3,3172	Bgn	X 29.3 cM	biglycan
1424691_at	-3,31945	5930434B04Rik	2 A3	RIKEN cDNA 5930434B04 gene
1424986_s_at	-3,31955	Fbxw7	3 E3.3	F-box and WD-40 domain protein 7 archipelago homolog (Drosophila)
1424659_at	-3,31997	Slit2	5 B3	slit homolog 2 (Drosophila)
1418946_at	-3,32309	Siat4a	15 D2	sialyltransferase 4A (beta-galactoside alpha-23-sialytransferase)
1419738_a_at	-3,33094	Tpm2	4 A5	tropomyosin 2 beta
1443161_at	-3,33158			Mus musculus transcribed sequences
1455160_at	-3,3321			Mus musculus adult male diencephalon cDNA RIKEN full-length enriched library clone:9330165B11 product:unknown EST full insert sequence
1423260_at	-3,34204	Idb4	13 31.0 cM	inhibitor of DNA binding 4
1420719_at	-3,34621	Tex15	8 A3	testis expressed gene 15
1437405_a_at	-3,34804	Igfbp4	11 D	insulin-like growth factor binding protein 4
1452138_a_at	-3,3488	Ace2	X 70.5 cM	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2
1435256_at	-3,34957	1500005P14Rik	7 A3	RIKEN cDNA 1500005P14 gene
1428111_at	-3,35137	Slc38a4	15 F1	solute carrier family 38 member 4
1432331_a_at	-3,3556	Prrx2	2 19.0 cM	paired related homeobox 2
1451968_at	-3,36079	Xrcc5	1 42.0 cM	X-ray repair complementing defective repair in Chinese hamster cells 5
1451415_at	-3,3626	1810011O10Rik	8 A2	RIKEN cDNA 1810011O10 gene
1418314_a_at	-3,36334	A2bp1	16 7.2 cM	ataxin 2 binding protein 1
1423294_at	-3,36585			Mus musculus transcribed sequence with moderate similarity to protein sp:Q9UBF2 (H.sapiens) CPG2_HUMAN Coatomer gamma-2 subunit (Gamma-2 coat protein) (Gamma-2 COP)
1449439_at	-3,37679	Klf7	1 C1-C3	Kruppel-like factor 7 (ubiquitous)
1427883_a_at	-3,37759	Col3a1	1 21.1 cM	procollagen type III alpha 1
1450922_a_at	-3,3846	Tgfb2	1 101.5 cM	transforming growth factor beta 2
1433431_at	-3,3863	1810007A24Rik	19 D2	RIKEN cDNA 1810007A24 gene
1456683_at	-3,38775	5730555F13Rik	9 D	RIKEN cDNA 5730555F13 gene
1417580_s_at	-3,39002	Selenbp2	3 50.8 cM	selenium binding protein 2
1436799_at	-3,3929	D230005D02Rik	14 24.1	RIKEN cDNA D230005D02 gene
1441629_at	-3,39348			Mus musculus transcribed sequences
1415864_at	-3,39889	Bpgm	6 A3.3	23-bisphosphoglycerate mutase

1450079_at	-3,40008	Nrk	X 53.0 cM	Nik related kinase
1457277_at	-3,40301	BC038925	5 G2	cDNA sequence BC038925
1433662_s_at	-3,40425	Timp2	11 72.0 cM	tissue inhibitor of metalloproteinase 2
1448436_a_at	-3,40447	B430217B02Rik	11 B1.3	RIKEN cDNA B430217B02 gene
1452217_at	-3,40523	2310047C17Rik	19 A	RIKEN cDNA 2310047C17 gene
1456521_at	-3,40766			Mus musculus transcribed sequences
1421654_a_at	-3,42291	Lmna	3 42.6 cM	lamin A
1425514_at	-3,43278	Pik3r1	13 50.0 cM	phosphatidylinositol 3-kinase regulatory subunit polypeptide 1 (p85 alpha)
1435110_at	-3,43305	Unc5b	10 B4	unc-5 homolog B (C. elegans)
1418560_at	-3,4361	Pdha1	X 66.5 cM	pyruvate dehydrogenase E1 alpha 1
1456862_at	-3,44082	Six4	12 C3	sine oculis-related homeobox 4 homolog (Drosophila)
1429175_at	-3,44617	2810417M05Rik	17 E3	RIKEN cDNA 2810417M05 gene
1419123_a_at	-3,45223	Pdgfc	3 E3	platelet-derived growth factor C polypeptide
1448471_a_at	-3,45601	Tpbpb	13 B3	trophoblast specific protein beta
1435436_at	-3,45672			Mus musculus transcribed sequences
1432261_at	-3,45764	2310039D24Rik	8 C5	RIKEN cDNA 2310039D24 gene
1429154_at	-3,45986	1500009K05Rik	9 B	RIKEN cDNA 1500009K05 gene
1419355_at	-3,46445	Klf7	1 C1-C3	Kruppel-like factor 7 (ubiquitous)
1459512_at	-3,46481			Mus musculus transcribed sequences
1448194_a_at	-3,47171	H19	7 69.03 cM	H19 fetal liver mRNA
1450000_at	-3,47228	Rnh2	7 A1	ribonuclease/angiogenin inhibitor 2
1455665_at	-3,47429			Mus musculus transcribed sequences
1428026_at	-3,47504	Sdccag33l	2 H3	serologically defined colon cancer antigen 33 like
1416613_at	-3,47605	Cyp1b1	17 E3	cytochrome P450 family 1 subfamily b polypeptide 1
1448254_at	-3,48127	Ptn	6 13.5 cM	pleiotrophin
1418536_at	-3,48217	H2-Q7	17 19.19 cM	histocompatibility 2 Q region locus 7
1416617_at	-3,48954	Acas2l	2 G3	acetyl-Coenzyme A synthetase 2 (AMP forming)-like
1435603_at	-3,49273	SST3	1 D	secreted protein SST3
1425511_at	-3,49445	Mark1	1 H5	MAP/microtubule affinity-regulating kinase 1
1453448_at	-3,49478	Nfib	4 38.6 cM	nuclear factor I/B
1459133_at	-3,50075			Mus musculus transcribed sequences
1434283_at	-3,50225	Desrt	10 B5.2	developmentally and sexually retarded with transient immune abnormalities
1447812_x_at	-3,50313	1110055E19Rik	6 A3.3	RIKEN cDNA 1110055E19 gene
1454966_at	-3,50407	Itga8	2 A1	integrin alpha 8
1417130_s_at	-3,51055	Angptl4	17 B1	angiopoietin-like 4
1452792_at	-3,51401	2510025K24Rik	14 E4	RIKEN cDNA 2510025K24 gene
1417839_at	-3,51846	Cldn5	16 11.65 cM	claudin 5
1416579_a_at	-3,51998	Tacstd1	17 E4	tumor-associated calcium signal transducer 1
1422477_at	-3,52314	Cables1	18 A1	Cdk5 and Abl enzyme substrate 1
1417355_at	-3,53442	Peg3	7 6.5 cM	paternally expressed 3
1416673_at	-3,53479	Bace2	16 A1	beta-site APP-cleaving enzyme 2
1418402_at	-3,54012	Adam19	11 20.0 cM	a disintegrin and metalloproteinase domain 19 (meltrin beta)
1448785_at	-3,5429	Cbfa2t1h	4 4.4 cM	CBFA2T1 identified gene homolog (human)
1421992_a_at	-3,54475	Igfbp4	11 D	insulin-like growth factor binding protein 4
1423110_at	-3,5519	Col1a2	6 0.68 cM	procollagen type I alpha 2
1439065_x_at	-3,55368			Mus musculus transcribed sequence with weak similarity to protein ref:NP_443092.1 (H.sapiens) kruppel-like zinc finger protein [Homo sapiens]

1436562_at	-3,55432	6430573D20Rik	4 A5	RIKEN cDNA 6430573D20 gene
1456648_at	-3,55499			Mus musculus transcribed sequences
1436717_x_at	-3,55722	Hbb-y	7 49.95 cM	hemoglobin Y beta-like embryonic chain
1457483_at	-3,56254			Mus musculus transcribed sequences
1428434_at	-3,56341	2810028A01Rik	X A3.1	RIKEN cDNA 2810028A01 gene
1425767_a_at	-3,56411	Six4	12 C3	sine oculis-related homeobox 4 homolog (Drosophila)
1433525_at	-3,5691	Ednra		endothelin receptor type A
1451753_at	-3,57019	Plxna2		plexin A2
1419234_at	-3,57609	Helb	10 69.0 cM	helicase (DNA) B
1450752_at	-3,57793	Cyct	2 47.0 cM	cytochrome c testis
1454301_at	-3,58489			Mus musculus adult male hippocampus cDNA RIKEN full-length enriched library clone:2900073C17 product:unclassifiable full insert sequence
1455646_at	-3,58955			Mus musculus adult male small intestine cDNA RIKEN full-length enriched library clone:2010004M13 product:unknown EST full insert sequence
1437385_at	-3,59752			Mus musculus transcribed sequences
1436823_x_at	-3,60601	Hbb-y	7 49.95 cM	hemoglobin Y beta-like embryonic chain
1451105_at	-3,61122	B130052G07Rik	1 H6	RIKEN cDNA B130052G07 gene
1416345_at	-3,61281	Timm8a	X 51.0 cM	translocase of inner mitochondrial membrane 8 homolog a (yeast)
1460574_at	-3,62051	6030410K14	3 B	hypothetical protein 6030410K14
1452030_a_at	-3,62104	Hnrpr	4 D3	heterogeneous nuclear ribonucleoprotein R
1447551_x_at	-3,62257	Lphn3	5 E1	latrophilin 3
1456200_at	-3,62381	2410017C19Rik	10 B5.3	RIKEN cDNA 2410017C19 gene
1418435_at	-3,63349	Mkrn1	6 B1	makorin ring finger protein 1
1422537_a_at	-3,63893	Idb2	12 7.0 cM	inhibitor of DNA binding 2
1424759_at	-3,63914	2410003C09Rik	7 C	RIKEN cDNA 2410003C09 gene
1416625_at	-3,64317	Serping1	2 D	serine (or cysteine) proteinase inhibitor clade G member 1
1436853_a_at	-3,64411	Snca	6 29.0 cM	synuclein alpha
1426725_s_at	-3,65912	Ets1	9 15.0 cM	E26 avian leukemia oncogene 1 5' domain
1456917_at	-3,66298	D130059B05Rik	1 A2	RIKEN cDNA D130059B05 gene
1430520_at	-3,66345	Cpne8	15 E3	copine VIII
1428387_at	-3,66369	Facl3	1 C4	fatty acid Coenzyme A ligase long chain 3
1435468_at	-3,66803			Mus musculus transcribed sequence with moderate similarity to protein ref:NP_171608.1 (H.sapiens) hypothetical protein MGC4655 [Homo sapiens]
1423505_at	-3,67597	Tagln	9 27.0 cM	transgelin
1440990_at	-3,67853	4832420M10	1 H4	hypothetical protein 4832420M10
1437493_at	-3,68125			Mus musculus adult male xiphoid cartilage cDNA RIKEN full-length enriched library clone:5230400M03 product:unclassifiable full insert sequence
1420664_s_at	-3,68175	Procr	2 H1-3	protein C receptor endothelial
1457304_at	-3,68511			Mus musculus transcribed sequences
1455991_at	-3,69418			Mus musculus transcribed sequence with weak similarity to protein pir:S52790 (H.sapiens) S52790 kynurenine—oxoglutarate transaminase (EC 2.6.1.7) / glutamine—phenylpyruvate transaminase (EC 2.6.1.64) cytosolic [similarity] - human
1453384_at	-3,69511	4930467M19Rik	1	RIKEN cDNA 4930467M19 gene
1415777_at	-3,69783	Pnliprp1	19 29.0 cM	pancreatic lipase related protein 1
1420903_at	-3,70032	Siat7c	3 H3	sialyltransferase 7 ((alpha-N-acetylneuraminyl 23-betagalactosyl-13)-N-acetyl galactosaminide alpha-26-sialyltransferase) C
1440093_at	-3,70565			Mus musculus transcribed sequences
1440431_at	-3,70669			Mus musculus 2 days pregnant adult female oviduct cDNA RIKEN full-length enriched library clone:E230038I17

				product:unknown EST full insert sequence
1438214_at	-3,7162	AI115454	15 C	expressed sequence AI115454
1435525_at	-3,72133	2900008M13Rik	15 E1	RIKEN cDNA 2900008M13 gene
1433953_at	-3,72476	Zfp277	12 A3	zinc finger protein 277
1448783_at	-3,73527	Slc7a9	7 B1	solute carrier family 7 (cationic amino acid transporter y+ system) member 9
1445773_at	-3,73798			Mus musculus transcribed sequences
1426301_at	-3,73838	Alcam	16 A1	activated leukocyte cell adhesion molecule
1417901_a_at	-3,74641	Ica1	6 2.9 cM	islet cell autoantigen 1
1422437_at	-3,74679	Col5a2	1 C1	procollagen type V alpha 2
1424699_at	-3,75324	BC006583	6 A3.3	cDNA sequence BC006583
1416053_at	-3,7546	Lrrn1	6 E1	leucine rich repeat protein 1 neuronal
1421895_at	-3,75524	Eif2s3x	X 32.0 cM	eukaryotic translation initiation factor 2 subunit 3 structural gene X-linked
1458296_at	-3,75634			Mus musculus transcribed sequence with weak similarity to protein ref:NP_081764.1 (M.musculus) RIKEN cDNA 5730493B19 [Mus musculus]
1417649_at	-3,75816	Cdkn1c	7 69.49 cM	cyclin-dependent kinase inhibitor 1C (P57)
1416160_at	-3,76877	Nr2f2	7 33.0 cM	nuclear receptor subfamily 2 group F member 2
1450923_at	-3,78298	Tgfb2	1 101.5 cM	transforming growth factor beta 2
1423756_s_at	-3,78516	Igfbp4	11 D	insulin-like growth factor binding protein 4
1437584_at	-3,79589			Mus musculus transcribed sequences
1418589_a_at	-3,79882	Mlf1	3 31.0 cM	myeloid leukemia factor 1
1416431_at	-3,79909	2310057H16Rik	18 E1	RIKEN cDNA 2310057H16 gene
1450700_at	-3,80597	Cdc42ep3	17 E3	CDC42 effector protein (Rho GTPase binding) 3
1416029_at	-3,8116	Tieg1	15 B3.1	TGFB inducible early growth response 1
1455056_at	-3,82055		14 E2.3	Mus musculus 0 day neonate lung cDNA RIKEN full-length enriched library clone:E030010D13 product:inferred: unnamed protein product {Homo sapiens} full insert sequence
1422852_at	-3,82431	2810434I23Rik	9 B	RIKEN cDNA 2810434I23 gene
1444746_at	-3,82522			Mus musculus transcribed sequence with weak similarity to protein pir:I58401 (M.musculus) I58401 protein-tyrosine kinase (EC 2.7.1.112) JAK3 - mouse
1419126_at	-3,83727	Hoxd9	2 45.0 cM	homeo box D9
1436293_x_at	-3,83834	LOC381325	1 H2.3	LISCH7-like
1421612_a_at	-3,84642	4933432H23Rik	15 D1	RIKEN cDNA 4933432H23 gene
1442749_at	-3,84673	Braf	6 15.5 cM	Braf transforming gene
1417602_at	-3,85218	Per2	1 D	period homolog 2 (Drosophila)
1458205_at	-3,85562			Mus musculus 11 days embryo gonad cDNA RIKEN ull-length enriched library clone:7030414N10 product:unknown EST full insert sequence
1448977_at	-3,86734	Tcfap2c	2 H3-H4	transcription factor AP-2 gamma
1428922_at	-3,86817	1200009O22Rik	6 B3	RIKEN cDNA 1200009O22 gene
1449641_at	-3,86873	Adk	14 A2-B	adenosine kinase
1455692_x_at	-3,87802			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:1700122G02 product:unknown EST full insert sequence
1442999_at	-3,88361			Mus musculus 10 days neonate cerebellum cDNA RIKEN full-length enriched library Clone :B930036G03 product:unknown EST full insert sequence
1445268_at	-3,88366			Mus musculus similar to Copg2 protein (LOC382145) mRNA
1434194_at	-3,88565	Mtap2	1 33.7 cM	microtubule-associated protein 2
1437637_at	-3,88721	1110054G21Rik	5 A3	RIKEN cDNA 1110054G21 gene
1417837_at	-3,89248	Phlda2	7 69.5 cM	pleckstrin homology-like domain family A member 2
1449351_s_at	-3,89878	Pdgfc	3 E3	platelet-derived growth factor C polypeptide

1449848_at	-3,90647	Gna14	19 9.0 cM	guanine nucleotide binding protein alpha 14
1418091_at	-3,91781	D930018N21Rik	1 E2.3	RIKEN cDNA D930018N21 gene
1417111_at	-3,92287	Man1a	10 B3	mannosidase 1 alpha
1440662_at	-3,94423			Mus musculus adult male olfactory brain cDNA RIKEN full-length enriched library clone:6430521P21 product:unclassifiable full insert sequence
1424769_s_at	-3,95011	Cald1	6 11.5 cM	caldesmon 1
1449755_at	-3,96071			Mus musculus transcribed sequences
1437841_x_at	-3,96505	AI481750	15 E1	expressed sequence AI481750
1459838_s_at	-3,96703	6330404E16Rik	10 C1	RIKEN cDNA 6330404E16 gene
1450857_a_at	-3,97193	Col1a2	6 0.68 cM	procollagen type I alpha 2
1440150_at	-3,97529	Tgm3	2 F1	transglutaminase 3 E polypeptide
1424295_at	-3,9756	Dppa3	6 F2	developmental pluripotency-associated 3
1437112_at	-3,97666	Pld1	3 10.5 cM	phospholipase D1
1433789_at	-3,98077			Mus musculus 18 days pregnant adult female placenta and extra embryonic tissue cDNA RIKEN full-length enriched library clone:3830421G02 product:unknown EST full insert sequence
1434141_at	-3,99188	Gucy1a3	3 E3	guanylate cyclase 1 soluble alpha 3
1443219_at	-4,00198			Mus musculus transcribed sequences
1437784_at	-4,00228			Mus musculus transcribed sequence with strong similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
1418892_at	-4,00757	Arhj	12 C3	ras homolog gene family member J
1435595_at	-4,01297	1810011O10Rik	8 A2	RIKEN cDNA 1810011O10 gene
1455851_at	-4,02061	Bmp5	9 42.0 cM	bone morphogenetic protein 5
1449534_at	-4,02399	Sycp3	10 C	synaptonemal complex protein 3
1419240_at	-4,02492	Tex14	11 C	testis expressed gene 14
1436637_at	-4,04612			Mus musculus transcribed sequences
1443362_at	-4,06114	Zfp277	12 A3	zinc finger protein 277
1455493_at	-4,06191	Syne1	10 A1	synaptic nuclear envelope 1
1424770_at	-4,07198	Cald1	6 11.5 cM	caldesmon 1
1456505_at	-4,07825			Mus musculus 2 days neonate thymus thymic cells cDNA RIKEN full-length enriched library clone:E430027H06 product:unknown EST full insert sequence
1441248_at	-4,07986	Clcn3	8 32.2 cM	chloride channel 3
1419693_at	-4,09055	Colec12	18 A1	collectin sub-family member 12
1424067_at	-4,10014	Icam1	9 7.0 cM	intercellular adhesion molecule
1454631_at	-4,10118	Gtf2a1	12 D3	general transcription factor II A 1
1435458_at	-4,10548	Pim1	17 16.4 cM	proviral integration site 1
1434172_at	-4,11427			Mus musculus transcribed sequences
1417466_at	-4,11703	Rgs5	1 86.5 cM	regulator of G-protein signaling 5
1436600_at	-4,11705	C230068E13	8 C4	hypothetical protein C230068E13
1430435_at	-4,11893	Laf4	1 18.7 cM	lymphoid nuclear protein related to AF4
1433434_at	-4,12424	AW551984	9 A5.1	expressed sequence AW551984
1429159_at	-4,12748			Mus musculus adult male cecum cDNA RIKEN full-length enriched library clone:9130010F19 product:unknown EST full insert sequence
1437012_x_at	-4,13455	9330170P05Rik	15 F1	RIKEN cDNA 9330170P05 gene
1451817_at	-4,13814	C630029K18Rik	19 A	RIKEN cDNA C630029K18 gene
1456960_at	-4,13924			Mus musculus 2 days pregnant adult female ovary cDNA RIKEN full-length enriched library clone:E330016N04 product:unknown EST full insert sequence
1450773_at	-4,14462	Kcnd2	6 7.2 cM	potassium voltage-gated channel Shal-related family member 2

1454997_at	-4,15174	D430026P16Rik	10 D2	RIKEN cDNA D430026P16 gene
1444406_at	-4,15841			Mus musculus transcribed sequence with moderate similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse
1456891_at	-4,16094	A930010I20	3 F2.2	hypothetical protein A930010I20
1420425_at	-4,16344	Prdm1	10 27.0 cM	PR domain containing 1 with ZNF domain
1429013_at	-4,16878	5330432J06Rik	X F4	RIKEN cDNA 5330432J06 gene
1442760_x_at	-4,17191			Mus musculus transcribed sequences
1429654_at	-4,17548	Dppa2	16 A1	developmental pluripotency associated 2
1427170_at	-4,18181	2410072D24Rik	18 A1	RIKEN cDNA 2410072D24 gene
1456561_s_at	-4,19084			Mus musculus transcribed sequences
1460256_at	-4,19608	Car3	3 11.7 cM	carbonic anhydrase 3
1435095_at	-4,19966			Mus musculus adult male corpora quadrigemina cDNA RIKEN full-length enriched library clone:B230313A11 product:unknown EST full insert sequence
1422870_at	-4,20436	Hoxc4	15 57.4 cM	homeo box C4
1448818_at	-4,21044	Wnt5a	14 7.8 cM	wingless-related MMTV integration site 5A
1438966_x_at	-4,21477	Fmod	1 74.3 cM	fibromodulin
1417262_at	-4,21616	Ptgs2	1 76.2 cM	prostaglandin-endoperoxide synthase 2
1435154_at	-4,21776		7 A1	Mus musculus similar to solute carrier family 7 (cationic amino acid transporter y+ system) member 3 (LOC245128) mRNA
1440997_at	-4,22528	9930033H14Rik	11 E2	RIKEN cDNA 9930033H14 gene
1434982_at	-4,2282			Mus musculus transcribed sequence with weak similarity to protein pir:S07330 (M.musculus) S07330 keratin epidermal - mouse
1453469_at	-4,24003	Pnliprp1	19 29.0 cM	pancreatic lipase related protein 1
1426911_at	-4,24326	Dsc2	18 7.0 cM	desmocollin 2
1440014_at	-4,24332			Mus musculus transcribed sequences
1416454_s_at	-4,24705	Acta2	19 C1	actin alpha 2 smooth muscle aorta
1442903_at	-4,25372			Mus musculus adult male olfactory brain cDNA RIKEN full-length enriched library clone:6430566E03 product:unknown EST full insert sequence
1454903_at	-4,26135	Ngfr	11 55.6 cM	nerve growth factor receptor (TNFR superfamily member 16)
1455447_at	-4,26314	D430019H16Rik	12 E	RIKEN cDNA D430019H16 gene
1424890_at	-4,26686	Bnc	7 D2	basonuclin
1427308_at	-4,26875	C630028C02Rik	4 C6	RIKEN cDNA C630028C02 gene
1440874_at	-4,28918			Mus musculus transcribed sequences
1424638_at	-4,29065	Cdkn1a	17 15.23 cM	cyclin-dependent kinase inhibitor 1A (P21)
1423516_a_at	-4,29971			Mus musculus transcribed sequence with weak similarity to protein pir:G00043 (H.sapiens) G00043 osteonidogen - human
1452014_a_at	-4,30744	Igf1	10 48.0 cM	insulin-like growth factor 1
1429030_at	-4,31279	C1qtnf7	5 B3	C1q and tumor necrosis factor related protein 7
1423630_at	-4,32097	Cygb	11 E2	cytoglobin
1453286_at	-4,33391	Plxna2		plexin A2
1451675_a_at	-4,34489	Alas2	X 63.0 cM	aminolevulinic acid synthase 2 erythroid
1422028_a_at	-4,34492	Ets1	9 15.0 cM	E26 avian leukemia oncogene 1 5' domain
1417148_at	-4,34811	Pdgfrb	18 30.0 cM	platelet derived growth factor receptor beta polypeptide
1422771_at	-4,35304	Madh6	9 C	MAD homolog 6 (Drosophila)
1439933_at	-4,36888			Mus musculus 4 days neonate male adipose cDNA RIKEN full-length enriched library clone:B430316J06 product:unknown EST full insert sequence
1419606_a_at	-4,36896	Tnnt1	7 9.0 cM	troponin T1 skeletal slow

1432176_a_at	-4,3731	Eng	2 21.4 cM	endoglin
1417643_at	-4,37733	Tsga2	17 13.25 cM	testis specific gene A2
1449288_at	-4,39256	Gdf3	6 60.6 cM	growth differentiation factor 3
1454734_at	-4,39414	Lef1	3 61.6 cM	lymphoid enhancer binding factor 1
1452670_at	-4,40545	Myl9	2 H1	myosin light polypeptide 9 regulatory
1457377_at	-4,41184			Mus musculus transcribed sequences
1454286_at	-4,42394			Mus musculus 18-day embryo whole body cDNA RIKEN full-length enriched library clone:1110004M10 product:unclassifiable full insert sequence
1433768_at	-4,4311	2410003B16Rik	8 B3.1	RIKEN cDNA 2410003B16 gene
1427287_s_at	-4,44251	Itpr5	6 72.0 cM	inositol 145-triphosphate receptor 5
1432757_at	-4,45858			Mus musculus adult male hippocampus cDNA RIKEN full-length enriched library clone:2900011L18 product:unknown EST full insert sequence
1451466_at	-4,45917	D16Ertd472e	16 A1	DNA segment Chr 16 ERATO Doi 472 expressed
1437846_x_at	-4,46118	Bace2	16 A1	beta-site APP-cleaving enzyme 2
1448251_at	-4,46306	9030425E11Rik	9 A5.1	RIKEN cDNA 9030425E11 gene
1438531_at	-4,47523			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030072J05 product:unknown EST full insert sequence
1453599_at	-4,4828			Mus musculus 10 days embryo whole body cDNA RIKEN full-length enriched library clone:2610206G21 product:unknown EST full insert sequence
1441224_at	-4,48509			Mus musculus 0 day neonate cerebellum cDNA RIKEN full-length enriched library clone:C230088J21 product:unknown EST full insert sequence
1418649_at	-4,4969	Egln3	12 B3	EGL nine homolog 3 (C. elegans)
1426642_at	-4,49786	Fn1	1 36.1 cM	fibronectin 1
1434278_at	-4,50588			
1438820_at	-4,50857	LOC380905	14 C1	similar to Tudor domain containing protein 4
1460713_at	-4,5119		17 B3	Mus musculus mRNA similar to putative c-Myc-responsive (cDNA clone MGC:54855 IMAGE:5388297) complete cds
1428750_at	-4,51738			Mus musculus transcribed sequence with moderate similarity to protein ref:NP_006770.1 (H.sapiens) Cdc42 effector protein 2 [Homo sapiens]
1434539_at	-4,53063	Lrrn3	12 A3	leucine rich repeat protein 3 neuronal
1455280_at	-4,54285			Mus musculus 0 day neonate lung cDNA RIKEN full-length enriched library clone:E030022N22 product:unclassifiable full insert sequence
1437718_x_at	-4,55603	Fmod	1 74.3 cM	fibromodulin
1449077_at	-4,56259	Eraf		erythroid associated factor
1436125_at	-4,5629	D16Ertd472e	16 A1	DNA segment Chr 16 ERATO Doi 472 expressed
1424939_at	-4,56594	4933400N19Rik	6 A2	RIKEN cDNA 4933400N19 gene
1456258_at	-4,57343	Emx2	19 53.5 cM	empty spiracles homolog 2 (Drosophila)
1438512_at	-4,57879	LOC210321	7 D2	epididymal protein Av381126
1429274_at	-4,61202	2310010M24Rik	2 C1.1	RIKEN cDNA 2310010M24 gene
1454745_at	-4,62246	B130017I01Rik	3 G1	RIKEN cDNA B130017I01 gene
1454869_at	-4,62318	A230038L21Rik	X A3.3	RIKEN cDNA A230038L21 gene
1419519_at	-4,63848	Igf1	10 48.0 cM	insulin-like growth factor 1
1418131_at	-4,66063	Samhd1	2 H1	SAM domain and HD domain 1
1436937_at	-4,68634	6720477E09Rik	9 F3	RIKEN cDNA 6720477E09 gene
1436938_at	-4,69224	6720477E09Rik	9 F3	RIKEN cDNA 6720477E09 gene
1420119_s_at	-4,69878	Phf3	1 A5	PHD finger protein 3
1453238_s_at	-4,69915			
1438532_at	-4,70606		1 G1	Mus musculus similar to hemicentin; fibulin 6 (LOC240793) mRNA

1429897_a_at	-4,71801	D16Ertd472e	16 A1	DNA segment Chr 16 ERATO Doi 472 expressed
1457314_at	-4,72514		4 C6	Mus musculus similar to hypothetical protein FLJ10884 (LOC381591) mRNA
1435564_at	-4,72951	C230078M08Rik	17 A3.3	RIKEN cDNA C230078M08 gene
1418136_at	-4,74037	Tgfb1i1	7 F1-F3	transforming growth factor beta 1 induced transcript 1
1455170_at	-4,74852			Mus musculus 10 11 days embryo whole body cDNA RIKEN full-length enriched library clone:2810001G20 product:unknown EST full insert sequence
1422835_at	-4,75108	Kcnd2	6 7.2 cM	potassium voltage-gated channel Shal-related family member 2
1425103_at	-4,76066	Ace2	X 70.5 cM	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2
1436405_at	-4,76659	Dock4	12 A3	dedicator of cytokinesis 4
1426927_at	-4,7792	Ap3b2	7 38.0 cM	adaptor-related protein complex 3 beta 2 subunit
1424076_at	-4,78175	2610020H15Rik	11 C	RIKEN cDNA 2610020H15 gene
1436387_at	-4,79586			Mus musculus ES cells cDNA RIKEN full-length enriched library clone:C330006P03 product:unknown EST full insert sequence
1441116_at	-4,81624			Mus musculus transcribed sequences
1452163_at	-4,83345	Ets1	9 15.0 cM	E26 avian leukemia oncogene 1 5' domain
1424768_at	-4,83767	Cald1	6 11.5 cM	caldesmon 1
1441013_at	-4,83796			Mus musculus transcribed sequence with weak similarity to protein ref:NP_081764.1 (M.musculus) RIKEN cDNA 5730493B19 [Mus musculus]
1420813_at	-4,84134	4930570C03Rik	15 F1	RIKEN cDNA 4930570C03 gene
1438407_at	-4,84709	9330132E09Rik	1 E2.1	RIKEN cDNA 9330132E09 gene
1422926_at	-4,85312	Mc2r	18 37.0 cM	melanocortin 2 receptor
1423250_a_at	-4,86764	Tgfb2	1 101.5 cM	transforming growth factor beta 2
1429977_at	-4,86962			Mus musculus adult male colon cDNA RIKEN full-length enriched library clone:9030425L15 product:unknown EST full insert sequence
1420598_x_at	-4,87277	Defcr-rs2	8	defensin related cryptdin related sequence 2
1457146_at	-4,88342			Mus musculus transcribed sequences
1438682_at	-4,89591	C530050K14	13 D1	hypothetical protein C530050K14
1415856_at	-4,90467	Emb	13 D2.3	embigin
1452514_a_at	-4,90783	Kit	5 42.0 cM	kit oncogene
1420534_at	-4,91162	Gucy1a3	3 E3	guanylate cyclase 1 soluble alpha 3
1434678_at	-4,918			Mus musculus 0 day neonate lung cDNA RIKEN full-length enriched library clone:E030002K20 product:similar to MUSCLEBLIND-LIKE PROTEIN FLJ11316/DKFZP434 G2222/DJ842K24.1 [Homo sapiens] full insert sequence
1449347_a_at	-4,92016	Xlr4	X 29.0 cM	X-linked lymphocyte-regulated 4
1437250_at	-4,92024		1 C3	Mus musculus similar to FLJ10116 protein (LOC381269) mRNA
1441680_at	-4,92903			Mus musculus transcribed sequences
1428156_at	-4,93632			Mus musculus 15 days embryo male testis cDNA RIKEN full-length enriched library clone:8030491K24 product:unknown EST full insert sequence
1458057_at	-4,96635			Mus musculus transcribed sequences
1416049_at	-4,97603	Gldc	19 25.0 cM	glycine decarboxylase
1456768_a_at	-4,98119	Emilin3	14 B	elastin microfibril interfacer 3
1450078_at	-4,99086	Nrk	X 53.0 cM	Nik related kinase
1436419_a_at	-4,99216			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:1700122G02 product:unknown EST full insert sequence
1437401_at	-4,99547	Igf1	10 48.0 cM	insulin-like growth factor 1
1416529_at	-5,01236	Emp1	6 65.0 cM	epithelial membrane protein 1
1438737_at	-5,03471	Zic3	X 16.5 cM	zinc finger protein of the cerebellum 3
1427809_at	-5,04693			Mus musculus clone IMAGE:3708374 mRNA

1418380_at	-5,05728	Terf1	1 11.8 cM	telomeric repeat binding factor 1
1442322_at	-5,06713			Mus musculus transcribed sequences
1418681_at	-5,08582	4833435D08Rik	X F2	RIKEN cDNA 4833435D08 gene
1415944_at	-5,08676	Sdc1	12 1.0 cM	syndecan 1
1440037_at	-5,11056			Mus musculus transcribed sequences
1433902_at	-5,1268	Takrp	6 D2	T-cell activation kelch repeat protein
1415996_at	-5,12913	Txnip		thioredoxin interacting protein
1423259_at	-5,13753	Idb4	13 31.0 cM	inhibitor of DNA binding 4
1427025_at	-5,16107	D8Ertd531e	8 22.0 cM	DNA segment Chr 8 ERATO Doi 531 expressed
1448154_at	-5,16762	Ndr2	14 C1	N-myc downstream regulated 2
1436398_at	-5,18335			Mus musculus 12 days embryo eyeball cDNA RIKEN full-length enriched library clone:D230032A16 product: unknown EST full insert sequence
1435832_at	-5,19286	Lrrc4	6 A3.3	leucine rich repeat containing 4
1428861_at	-5,19586	4631422O05Rik	16	RIKEN cDNA 4631422O05 gene
1421881_a_at	-5,19977	Elavl2	4 42.6 cM	ELAV (embryonic lethal abnormal vision Drosophila)-like 2 (Hu antigen B)
1422655_at	-5,20111	Ptch2	4 56.5 cM	patched homolog 2
1446906_at	-5,22013			Mus musculus transcribed sequences
1416855_at	-5,2296	Gas1	13 37.0 cM	growth arrest specific 1
1448816_at	-5,24708	Ptgis	2 H3	prostaglandin I2 (prostacyclin) synthase
1415927_at	-5,25008	Actc1	2 64.0 cM	actin alpha cardiac
1440803_x_at	-5,25452	Tacr3	3 G3	tachykinin receptor 3
1423757_x_at	-5,25604	Igfbp4	11 D	insulin-like growth factor binding protein 4
1416840_at	-5,25849	3110038L01Rik	X A1.1	RIKEN cDNA 3110038L01 gene
1417411_at	-5,25883	1110020M21Rik	6 B3	RIKEN cDNA 1110020M21 gene
1434170_at	-5,26019	A230038L21Rik	X A3.3	RIKEN cDNA A230038L21 gene
1451264_at	-5,2685	4930488L10Rik	12 C2	RIKEN cDNA 4930488L10 gene
1444345_at	-5,2718			Mus musculus transcribed sequences
1436361_at	-5,28651	Vgll2	10 B3	vestigial like 2 homolog (Drosophila)
1419229_at	-5,28763	Ehox	X A3.1	ES cell derived homeobox containing gene
1456410_at	-5,29265			Mus musculus transcribed sequences
1455796_x_at	-5,30014	Olfm1	2 A3	olfactomedin 1
1450928_at	-5,30727	Idb4	13 31.0 cM	inhibitor of DNA binding 4
1443512_at	-5,32267			Mus musculus transcribed sequences
1420941_at	-5,33453	Rgs5	1 86.5 cM	regulator of G-protein signaling 5
1419402_at	-5,33833	Mns1	9 D	meiosis-specific nuclear structural protein 1
1429817_at	-5,37974	4933406N12Rik	3 C	RIKEN cDNA 4933406N12 gene
1437052_s_at	-5,38791	Slc2a3	6 59.0 cM	solute carrier family 2 (facilitated glucose transporter) member 3
1429743_at	-5,40189			Mus musculus 12 days embryo female mullerian duct includes surrounding region cDNA RIKEN full-length enriched library clone:6820416N07 product:unknown EST full insert sequence
1456084_x_at	-5,40889	Fmod	1 74.3 cM	fibromodulin
1435421_at	-5,4139	MGC58341	17 C	hypothetical protein MGC58341
1427302_at	-5,42926	Enpp3	10 A4	ectonucleotide pyrophosphatase/phosphodiesterase 3
1427228_at	-5,48002	2410003B16Rik	8 B3.1	RIKEN cDNA 2410003B16 gene
1417300_at	-5,49376	1110054A24Rik	4 D2.3	RIKEN cDNA 1110054A24 gene
1433707_at	-5,52734	Gabra4	5 C3.2	gamma-aminobutyric acid (GABA-A) receptor subunit alpha 4
1454799_at	-5,53168	4933408F15	5 E4	hypothetical protein 4933408F15
1452114_s_at	-5,54259	Igfbp5	1 36.1 cM	insulin-like growth factor binding protein 5
1428568_at	-5,54621	B230217C12Rik	RIKEN cDNA B230217C12 gene	

1429772_at	-5,54868	Plxna2		plexin A2
1434502_x_at	-5,56745	Slc4a1	11 62.0 cM	solute carrier family 4 (anion exchanger) member 1
1453219_a_at	-5,5686	D7Rp2e	7 15.0 cM	DNA segment Chr 7 Roswell Park 2 complex expressed
1424220_a_at	-5,57071	Porcn	X A1.1	porcupine homolog (Drosophila)
1437029_at	-5,57351	Tacr3	3 G3	tachykinin receptor 3
1423754_at	-5,59736	1110004C05Rik	7 F4	RIKEN cDNA 1110004C05 gene
1418762_at	-5,6171			Mus musculus transcribed sequences
1449319_at	-5,62941	Rspondin	4 D2.2	thrombospondin type 1 domain containing gene
1455898_x_at	-5,64207	Slc2a3	6 59.0 cM	solute carrier family 2 (facilitated glucose transporter) member 3
1417122_at	-5,68056	Vav3	3 G1	vav 3 oncogene
1425102_a_at	-5,71755	Ace2	X 70.5 cM	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2
1437218_at	-5,7577	Fn1	1 36.1 cM	fibronectin 1
1422121_at	-5,76396	Oprd1	4 64.8 cM	opioid receptor delta 1
1427919_at	-5,7691	1110039C07Rik	X E3	RIKEN cDNA 1110039C07 gene
1443913_at	-5,80175			
1447260_at	-5,81129			Mus musculus transcribed sequence with weak similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse
1419295_at	-5,83476	Oasis	2 E1	old astrocyte specifically induced substance
1422458_at	-5,8463	Tcl1	12 52.0 cM	T-cell lymphoma breakpoint 1
1428157_at	-5,86536			Mus musculus 15 days embryo male testis cDNA RIKEN full-length enriched library clone:8030491K24 product:unknown EST full insert sequence
1454881_s_at	-5,86818	Upk3b	5 G2	uroplakin 3B
1454926_at	-5,86924	4930544G21Rik	1 C5	RIKEN cDNA 4930544G21 gene
1421096_at	-5,90994	Trpc1	9 51.0 cM	transient receptor potential cation channel subfamily C member 1
1434413_at	-5,97459	Igf1	10 48.0 cM	insulin-like growth factor 1
1438672_at	-5,99029			Mus musculus transcribed sequences
1420797_at	-5,99882	Otog	7 28.0 cM	otogelin
1441317_x_at	-6,00381			
1456174_x_at	-6,01823	Ndr1	15 D2	N-myc downstream regulated 1
1455722_at	-6,06823			
1445965_at	-6,07606			Mus musculus transcribed sequences
1424167_a_at	-6,08693	Pmm1	15 E1	phosphomannomutase 1
1420760_s_at	-6,08817	Ndr1	15 D2	N-myc downstream regulated 1
1425163_at	-6,1205	LOC224833	17 C	hypothetical protein BC006605
1422890_at	-6,12625	Pcdh18	3 C	protocadherin 18
1450042_at	-6,13043	Arx	X C1	aristaless related homeobox gene (Drosophila)
1455367_at	-6,14051	Dnd1	18 B2	dead end homolog 1 (zebrafish)
1452092_at	-6,1823			Mus musculus transcribed sequences
1417945_at	-6,2132	Pou5f1	17 19.23 cM	POU domain class 5 transcription factor 1
1419414_at	-6,24884	Gng13	17 A3.3	guanine nucleotide binding protein 13 gamma
1453841_at	-6,26272			Mus musculus adult male tongue cDNA RIKEN full-length enriched library clone:2310050P20 product:unknown EST full insert sequence
1429887_at	-6,305			Mus musculus adult male tongue cDNA RIKEN full-length enriched library clone:2310005C01 product:unknown EST full insert sequence
1431865_a_at	-6,3082	4933405K07Rik	7 B3	RIKEN cDNA 4933405K07 gene
1424445_at	-6,34568	2010003F10Rik	11 B3	RIKEN cDNA 2010003F10 gene
1416114_at	-6,34997	Sparcl1	5 55.0 cM	SPARC-like 1 (mast9 hevin)
1454681_at	-6,35247	BC031468	4 A1	cDNA sequence BC031468

1456319_at	-6,35287			
1444797_at	-6,35562	LOC382231	X C3	hypothetical gene supported by AK033245
1448600_s_at	-6,39423	Vav3	3 G1	vav 3 oncogene
1456532_at	-6,4113	Pdgfd	9 A1	platelet-derived growth factor D polypeptide
1423635_at	-6,41154	Bmp2	2 76.1 cM	bone morphogenetic protein 2
1438989_s_at	-6,41167			Mus musculus 13 days embryo stomach cDNA RIKEN full-length enriched library clone:D530034D18 product:unknown EST full insert sequence
1452384_at	-6,43167	Enpp3	10 A4	ectonucleotide pyrophosphatase/phosphodiesterase 3
1428089_at	-6,43711	3200001I04Rik	14 E3	RIKEN cDNA 3200001I04 gene
1427242_at	-6,43808	Ddx4	13 67.0 cM	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4
1460591_at	-6,45202	Esr1	10 12.0 cM	estrogen receptor 1 (alpha)
1449876_at	-6,47747	Prkg1	19 C1	protein kinase cGMP-dependent type I
1417616_at	-6,48961	Siat7b	11 E2	sialyltransferase 7 ((alpha-N-acetylneuraminyl 23-beta-galactosyl-13)-N-acetyl galactosaminde alpha-26-sialyltransferase) B
1437752_at	-6,56624	Lin28	4 D3	lin-28 homolog (C. elegans)
1419324_at	-6,57348	Lhx9	1 G-H2	LIM homeobox protein 9
1440534_at	-6,57995			Mus musculus adult male hypothalamus cDNA RIKEN full-length enriched library clone:A230069K02 product:hypothetical protein full insert sequence
1429074_at	-6,59411	1700026D08Rik	7 D2	RIKEN cDNA 1700026D08 gene
1458238_at	-6,6193	Desrt	10 B5.2	developmentally and sexually retarded with transient immune abnormalities
1439260_a_at	-6,63068	Enpp3	10 A4	ectonucleotide pyrophosphatase/phosphodiesterase 3
1460226_at	-6,63996	Trap1a	X 58.0 cM	tumor rejection antigen P1A
1420533_at	-6,66916	Gucy1a3	3 E3	guanylate cyclase 1 soluble alpha 3
1447851_x_at	-6,67564	Atp10a	7 B5	ATPase class V type 10A
1425575_at	-6,67854	Epha3	16 A1	Eph receptor A3
1431403_a_at	-6,68185	5330432J06Rik	X F4	RIKEN cDNA 5330432J06 gene
1452757_s_at	-6,7097	Hba-a1	11 16.0 cM	hemoglobin alpha adult chain 1
1437113_s_at	-6,77265	Pld1	3 10.5 cM	phospholipase D1
1428685_at	-6,80103	4933406J07Rik	7 F4	RIKEN cDNA 4933406J07 gene
1418147_at	-6,87996	Tcfap2c	2 H3-H4	transcription factor AP-2 gamma
1416612_at	-6,91832	Cyp1b1	17 E3	cytochrome P450 family 1 subfamily b polypeptide 1
1438889_at	-6,97607			Mus musculus transcribed sequences
1433988_s_at	-6,99978			Mus musculus 0 day neonate cerebellum cDNA RIKEN full-length enriched library clone:C230098O21 product:unknown EST full insert sequence
1438200_at	-7,00071	Sulf1	1 A3	sulfatase 1
1452365_at	-7,01218	4732435N03Rik	8 B3.2	RIKEN cDNA 4732435N03 gene
1427320_at	-7,03671			Mus musculus 0 day neonate lung cDNA RIKEN full-length enriched library clone:E030030K01 product:coatomer protein complex subunit gamma 2 antisense 2 full insert sequence
1434025_at	-7,04236	Klf5	14 E2.1	Kruppel-like factor 5
1423327_at	-7,09187	4930517K11Rik	16 A1	RIKEN cDNA 4930517K11 gene
1421317_x_at	-7,11152	Myb	10 16.0 cM	myeloblastosis oncogene
1415857_at	-7,15393	Emb	13 D2.3	embigin
1436329_at	-7,17068	Egr3	14 D1	early growth response 3
1420720_at	-7,27717	Nptx2	5 G2	neuronal pentraxin 2
1420561_at	-7,33766	Trpc7	13 B1	transient receptor potential cation channel subfamily C member 7
1422058_at	-7,35644	Nodal	10 31.5 cM	nodal
1448494_at	-7,37981	Gas1	13 37.0 cM	growth arrest specific 1
1433919_at	-7,45463	Asb4	6 0.6 cM	ankyrin repeat and SOCS box-containing protein 4

1423429_at	-7,52201	Pem	X 12.7 cM	placentae and embryos oncofetal gene
1434237_at	-7,53441	Upk3b	5 G2	uroplakin 3B
1455599_at	-7,57592			Mus musculus adult male liver tumor cDNA RIKEN full-length enriched library clone:C730009F09 product:unknown EST full insert sequence
1449991_at	-7,59271	Cd244	1 93.0 cM	CD244 natural killer cell receptor 2B4
1425233_at	-7,62731	2210407C18Rik	11 B1.3	RIKEN cDNA 2210407C18 gene
1425317_x_at	-7,77358	Stk31	6 B2.3	serine threonine kinase 31
1416715_at	-7,84651	Gjb3	4 58.0 cM	gap junction membrane channel protein beta 3
1429330_at	-7,88161	Gabra4	5 C3.2	gamma-aminobutyric acid (GABA-A) receptor subunit alpha 4
1422567_at	-8,04276	Niban	1 G2	niban protein
1449434_at	-8,0575	Car3	3 11.7 cM	carbonic anhydrase 3
1438237_at	-8,07842			Mus musculus transcribed sequences
1422836_at	-8,09203	Mbnl3	X A4	muscleblind-like 3 (Drosophila)
1423424_at	-8,09816	Zic3	X 16.5 cM	zinc finger protein of the cerebellum 3
1449559_at	-8,11116	Msx2	13 32.0 cM	homeo box msh-like 2
1441527_at	-8,1353			Mus musculus transcribed sequences
1416464_at	-8,31074	Slc4a1	11 62.0 cM	solute carrier family 4 (anion exchanger) member 1
1417482_at	-8,3244	Tex19	11 E2	testis expressed gene 19
1457026_at	-8,35006	Liph	16 A1	lipase member H
1428361_x_at	-8,3572	Hba-a1	11 16.0 cM	hemoglobin alpha adult chain 1
1453223_s_at	-8,39272	Dppa2	16 A1	developmental pluripotency associated 2
1433939_at	-8,487	Laf4	1 18.7 cM	lymphoid nuclear protein related to AF4
1417356_at	-8,49805	Peg3	7 6.5 cM	paternally expressed 3
1442379_at	-8,49945			Mus musculus transcribed sequences
1456208_at	-8,53697	A530057A03	2 F1	hypothetical protein A530057A03
1424470_a_at	-8,54878	9330170P05Rik	15 F1	RIKEN cDNA 9330170P05 gene
1433489_s_at	-8,54963	Fgfr2	7 63.0 cM	fibroblast growth factor receptor 2
1419542_at	-8,55919	Dazl	17 25.6 cM	deleted in azoospermia-like
1450736_a_at	-8,62295	Hbb-bh1	7 49.96 cM	hemoglobin Z beta-like embryonic chain
1433526_at	-8,67396	Klhl8	5 55.0 cM	kelch-like 8 (Drosophila)
1453133_at	-8,84055	1700034J06Rik	3 B	RIKEN cDNA 1700034J06 gene
1437269_at	-8,86921	9030019H09	15 E2	hypothetical protein 9030019H09
1451021_a_at	-8,97557	Klf5	14 E2.1	Kruppel-like factor 5
1453228_at	-8,97831	Stx11	10 A1	syntaxin 11
1430780_a_at	-9,01573	Pmm1	15 E1	phosphomannomutase 1
1429597_at	-9,02756	Dppa4	16 A1	developmental pluripotency associated 4
1456777_at	-9,09244	6030407P20Rik	6 B1	RIKEN cDNA 6030407P20 gene
1453395_at	-9,21205			Mus musculus adult male pituitary gland cDNA RIKEN full-length enriched library clone:5330403D14 product:unknown EST full insert sequence
1441187_at	-9,39836			Mus musculus transcribed sequences
1449298_a_at	-9,42293	Pde1a	2 C3	phosphodiesterase 1A calmodulin-dependent
1456559_at	-9,46953			Mus musculus 12 days embryo female mullerian duct includes surrounding region cDNA RIKEN full-length enriched library clone:6820401H22 product:unknown EST full insert sequence
1442101_at	-9,49468	A930017N06Rik	5 G2	RIKEN cDNA A930017N06 gene
1430368_s_at	-9,50192	1700019D03Rik	1 C1.1	RIKEN cDNA 1700019D03 gene
1420448_at	-9,52625	4930539I12Rik	X A3.1	RIKEN cDNA 4930539I12 gene
1436221_at	-9,54201	LOC381325	1 H2.3	LISCH7-like
1456307_s_at	-9,62968	Adcy7	8 40.0 cM	adenylate cyclase 7

1449502_at	-9,76642	Dazl	17 25.6 cM	deleted in azoospermia-like
1440000_at	-9,76954	E330013P04Rik	19 D3	RIKEN cDNA E330013P04 gene
1436978_at	-9,81971	Wnt9a	11 B1.3	wingless-type MMTV integration site 9A
1420410_at	-9,8967	Nr5a2	1 E4	nuclear receptor subfamily 5 group A member 2
1445952_at	-9,98796			Mus musculus transcribed sequences
1417184_s_at	-10,3271	Hbb-y	7 49.95 cM	hemoglobin Y beta-like embryonic chain
1441462_at	-10,5158	Dock4	12 A3	dedicator of cytokinesis 4
1431114_at	-10,5702	Dock4	12 A3	dedicator of cytokinesis 4
1444390_at	-10,6813			Mus musculus transcribed sequences
1418362_at	-10,706	Zfp42	8 24.0 cM	zinc finger protein 42
1456033_at	-10,9031	Tbx4	11 49.0 cM	T-box 4
1442549_at	-10,915	Mbnl3	X A4	muscleblind-like 3 (Drosophila)
1454768_at	-10,9225		12 A1.2	Mus musculus 10 days neonate cortex cDNA RIKEN full-length enriched library clone:A830052E03 product:unknown EST full insert sequence
1454904_at	-10,9787			
1417714_x_at	-11,0174	Hba-a1	11 16.0 cM	hemoglobin alpha adult chain 1
1448688_at	-11,2975	Podxl	6 10.0 cM	podocalyxin-like
1459279_at	-11,3899			Mus musculus 16 days embryo head cDNA RIKEN full-length enriched library clone:C130019H05 product:unknown EST full insert sequence
1455595_at	-11,4374		5 E1	Mus musculus similar to hypothetical protein C730031G17 (LOC231396) mRNA
1444292_at	-11,6267	Nalp12	7 A1	NACHT LRR and PYD containing protein 12
1416552_at	-11,6427	Dppa5	9 E1	developmental pluripotency associated 5
1416967_at	-11,8127	Sox2	3 15.0 cM	SRY-box containing gene 2
1454969_at	-11,9268	E130115E03Rik	2 C1.1	RIKEN cDNA E130115E03 gene
1433147_at	-12,1737	Cald1	6 11.5 cM	caldesmon 1
1424152_at	-12,2183	Sall4	2 H3	sal-like 4 (Drosophila)
1449204_at	-12,5704	Gjb5	4 57.5 cM	gap junction membrane channel protein beta 5
1421883_at	-12,6283	Elavl2	4 42.6 cM	ELAV (embryonic lethal abnormal vision Drosophila)-like 2 (Hu antigen B)
1424270_at	-12,7246	Dcamkl1	3 C	double cortin and calcium/calmodulin-dependent protein kinase-like 1
1456003_a_at	-12,7438	Slc1a4	11 10.92 cM	solute carrier family 1 (glutamate/neutral amino acid transporter) member 4
1449064_at	-13,3767	Tdh	14 C3	L-threonine dehydrogenase
1436392_s_at	-13,5462	Tcfap2c	2 H3-H4	transcription factor AP-2 gamma
1460084_at	-13,5721			
1440452_at	-13,6851	Drp2	X E3	dystrophin related protein 2
1452366_at	-13,8287	4732435N03Rik	8 B3.2	RIKEN cDNA 4732435N03 gene
1420433_at	-14,1126	Taf7l	X E3	TAF7-like RNA polymerase II TATA box binding protein (TBP)-associated factor
1450621_a_at	-14,1405	Hbb-y	7 49.95 cM	hemoglobin Y beta-like embryonic chain
1448962_at	-14,2616	Myh11	16 5.0 cM	myosin heavy chain 11 smooth muscle
1436837_at	-14,2952	4933405K18Rik	1 H2.3	RIKEN cDNA 4933405K18 gene
1426650_at	-14,3632	Myh4	11 35.0 cM	myosin heavy polypeptide 4 skeletal muscle
1429905_at	-14,9314			Mus musculus 13 days embryo head cDNA RIKEN full-length enriched library clone:3110009O07 product:unknown EST full insert sequence
1419719_at	-15,3978	Gabrb1	5 40.0 cM	gamma-aminobutyric acid (GABA-A) receptor subunit beta 1
1450065_at	-15,8802	Adcy7	8 40.0 cM	adenylate cyclase 7
1460627_at	-15,9672	D130067I03Rik	1 E4	RIKEN cDNA D130067I03 gene
1429366_at	-16,2227	1700007J06Rik	3 A3	RIKEN cDNA 1700007J06 gene
1448716_at	-16,3884	Hba-x	11 16.0 cM	hemoglobin X alpha-like embryonic chain in Hba complex

1419418_a_at	-16,7602	Morc	16 33.7 cM	microrchidia
1435314_at	-17,2302	Tph2	10 D2	tryptophan hydroxylase 2
1456552_at	-17,2403			Mus musculus transcribed sequences
1436319_at	-17,3772	Sulf1	1 A3	sulfatase 1
1420549_at	-17,7152	Gbp1	3 67.4 cM	guanylate nucleotide binding protein 1
1437810_a_at	-18,9247	Hbb-bh1	7 49.96 cM	hemoglobin Z beta-like embryonic chain
1419018_at	-19,3419	Psx1	X A3.1	placenta specific homeobox 1
1449540_at	-19,5452	Psx2	X A3.1	placenta specific homeobox 2
1417504_at	-19,6565	Calb1	4 10.5 cM	calbindin-28K
1427238_at	-20,3156	Fbxo15	18 E4	F-box only protein 15
1437990_x_at	-20,3661	Hbb-bh1	7 49.96 cM	hemoglobin Z beta-like embryonic chain
1438645_x_at	-20,4792			
1456673_at	-20,7124			Mus musculus transcribed sequences
1429701_at	-21,0692	2410003J06Rik	X A7.3	RIKEN cDNA 2410003J06 gene
1454138_a_at	-21,5891	Stk31	6 B2.3	serine threonine kinase 31
1424271_at	-21,8467	Dcamkl1	3 C	double cortin and calcium/calmodulin-dependent protein kinase-like 1
1421749_at	-21,9261	Lin28	4 D3	lin-28 homolog (C. elegans)
1446308_at	-22,4911	1700106J16Rik	11 C	RIKEN cDNA 1700106J16 gene
1434280_at	-22,92			
1447839_x_at	-23,6085			
1421882_a_at	-23,8732	Elavl2	4 42.6 cM	ELAV (embryonic lethal abnormal vision Drosophila)-like 2 (Hu antigen B)
1434458_at	-24,1609	Fst	13 D2.2	follistatin
1451289_at	-26,9889	Dcamkl1	3 C	double cortin and calcium/calmodulin-dependent protein kinase-like 1
1418517_at	-28,9836	Irx3	8 42.1 cM	Iroquois related homeobox 3 (Drosophila)
1427263_at	-34,88			Mus musculus 9 days embryo whole body cDNA RIKEN full-length enriched library clone:D030072M03 product:inactive X specific transcripts full insert sequence
1420970_at	-37,5552	Adcy7	8 40.0 cM	adenylate cyclase 7
1432031_at	-42,0019			Mus musculus adult male testis cDNA RIKEN full-length enriched library clone:4930563E18 product:unclassifiable full insert sequence
1436659_at	-54,06	Dcamkl1	3 C	double cortin and calcium/calmodulin-dependent protein kinase-like 1
1436936_s_at	-67,1224			Mus musculus 0 day neonate thymus cDNA RIKEN full-length enriched library clone:A430022B11 product:inactive X specific transcripts full insert sequence
1420771_at	-108,638	Sprr2d	3 45.2 cM	small proline-rich protein 2D
1427262_at	-144,417			Mus musculus 0 day neonate thymus cDNA RIKEN full-length enriched library clone:A430022B11 product:inactive X specific transcripts full insert sequence
1421365_at	-147,74	Fst	13 D2.2	follistatin

Solutions:

10x PBS
80g NaCl
2g KCl
14.4g Na2HPO4
2.4g KH2PO4
800ml DDW
Dissolve, pH to 7.4, add DDW to 1L, DEPC treat and autoclave.

PBT
PBS with 0.1% Tween-20

20x SSC
175.3g NaCl
88.2g Sodium Citrate
800ml DDW
Dissolve, pH to 7.0, add DDW to 1L, DEPC treat and autoclave.

Antibody Buffer
10% Heat Inactivated Goat Serum
1% Boehringer Block
0.1% Tween-20
Dissolve in PBS at 70°C, vortexing frequently, and then filter (0.45um).

Hybridization Buffer (50mL):
25mL Formamide (deionized with beads)
12.5mL SSC 20X pH5.0
2.5mL (5ml) SDS 20% (10%)
50uL tRNA 80mg/mL (in DEPC-H_2O)
50uL Heparin 50mg/mL (in DEPC-H_2O)
9.9mL (7.4ml) DEPC-H_2O
store at –20°C

H_2O-DEPC:
0.1% DEPC in H_2O, 37°C O.N. and autoclave.

PBS-DEPC:
0.1% DEPC in PBS, 37°C O.N. and autoclave.

PBT-DEPC:
0.1% Tween 20 and 0.1% DEPC in PBS, 37°C O.N. and autoclave.

Solution1 (500mL):
125mL SSC 20X pH5.0
250mL Formamide (deionized)
100mL (75ml) H_2O
25mL (50ml) SDS 20% (10%)
store at –20°C

Solution2 (500mL):
50mL SSC 20X pH5.0
250mL Formamide (deionized)
200mL H_2O
500uL Tween 20
store at –20°C

TBS 10X (500mL):
40g NaCl
1g KCl
125mL Tris 1M pH 7.5
complete to 500mL with H_2O
autoclave

TBST:
0.1% Tween 20. Filter.

TBST-Levamisole:
24mg Levamisole Hydrochloride/ 50mL TBST

Heat-Inactivated Serum (Goat or bovine):
at 56°C for 30min

MABT 5X (200mL):
11.6g Maleic Acid
8.7g NaCl
pH to 7.5 with solid NaOH
0.5% Tween
complete to 200mL with H_2O

10% BR:
10g of "Roche" Blocking reagent
100 ml of MABT 1X
autoclave and store in 1.5ml aliquots at -20 °C

Blocking Solution (2mL):
200uL Heat-Inactivated Serum
200uL BR 10%
1.6mL TBST-Levamisole

Ab Solution (1mL):
10uL Heat-Inactivated Serum
890uL TBST-Levamisole
100uL BR 10%
0.5uL (0.75U/ul stock) anti-digoxigenin-AP Fab fragment
(or 5ul of fluo-conjugated Dig antibody)

NTMT (50mL):
Make fresh
1mL NaCl 5M
5mL Tris 1M pH 9.5
2.5mL $MgCl_2$ 1M
50uL Tween 20
41.5mL H_2O

NTMT-Levamisole:
24mg Levamisole Hydrochloride/ 50mL NTMT

CMFET (100mL)
0.8g NaCL
0.02g KCl
0.115g Na_2HPO_4
0.02g KH_2PO_4
0.02g EDTA
100uL Tween 20
complete to 100mL with H_2O

ACCORD ET PERMISSION DES COAUTEURS D'UN ARTICLE

IDENTIFICATION DE L'ÉTUDIANT

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DÉCLARATION DES COAUTEURS

Déclaration		
À titre de coauteurs de l'article identifié ci-dessus, nous autorisons le microfilmage du mémoire et nous sommes d'accord qu' Aron T. Cory *inclut cet article dans son mémoire de maîtrise qui a pour titre* Purified Pre-Sertoli Cells Express Genes Involved in Cell Proliferation and Cell Signalling During a Critical Window in Early Testis Differentiation		
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