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**Use of a Conservation-of-Linkage Strategy
to Identify a Candidate for the Rat *Lymphopenia* Gene**

by

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B.S. Mathematics and Biology, Stanford University

Submitted to the Department of Biology in Partial Fulfillment
of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
in Biology

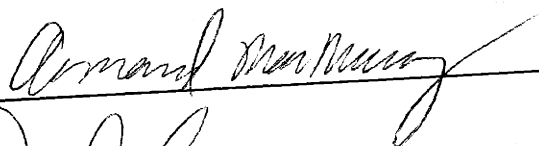
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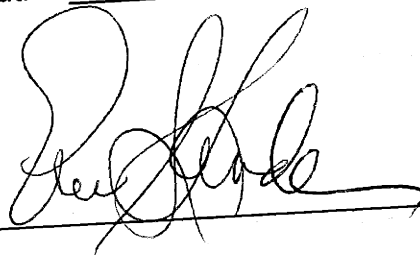
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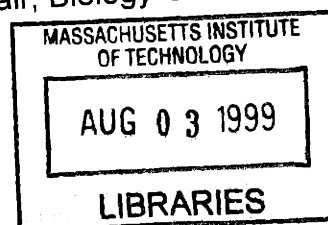


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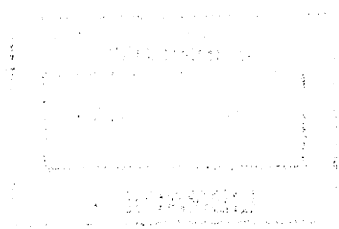


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ABSTRACT

The rat *Lymphopenia* gene, *Lyp*, is absolutely required for the development of Insulin-Dependent Diabetes Mellitus (IDDM) in the BioBreeding (BB) rat. In order to overcome the lack of positional cloning resources available in the rat to clone the *Lyp* gene, we developed and successfully implemented a conservation-of-linkage (COL) cloning strategy. Using the COL between the rat *Lyp* region and its murine counterpart, we developed large-scale YAC and fine-scale P1/BAC physical maps of the mouse *Lyp* region, and integrated these maps with a fine-scale genetic map of the rat *Lyp* region. Using cDNA fragments obtained through direct cDNA selection, we established a transcript map of the mouse *Lyp* region. One of these transcripts, PA, maps in the region fully-linked with *Lyp* and its transcript level is severely reduced in *Lyp/Lyp* homozygous animals. It is expressed chiefly in immune system organs, most strongly in the thymus, where *Lyp* is thought to act; within the thymus, PA is restricted to punctate expression in the thymic medulla -- again, the inferred site of *Lyp* action in late T-cell thymic maturation. Although no functional mutation has yet been identified in the *Lyp* haplotype allele of PA, it remains the leading candidate gene for *Lyp*. The successful cloning of the rat *Lyp* region validates the COL cloning strategy and suggests that this strategy may be widely applicable among mammalian species.

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PREFACE

This thesis is organized into seven main chapters: Chapter I describes the BB rat model of IDDM and the *Lymphopenia* (*Lyp*) mutation. Chapters II-VI describe the conservation-of-linkage cloning strategy and the various stages of its implementation, while Chapter VII discusses the lessons learned from this project and the more general applicability of the conservation-of-linkage cloning strategy. Some of the continuing work-in-progress on the *Lyp* project amplifies or extends the work described in the earlier chapters and so is included as a Supplement. Finally, the various details of oligonucleotide sequences, unabridged genotyping results, lists of cDNA and genomic clones isolated, and information about the sequences comprising the *Lyp* region transcription units are listed in the Appendices.

CHAPTER I

Introduction

Overview

Biomedical research depends on the use of animal models to study both normal function and disease processes. The usefulness of a given animal model to researchers increases as more research work is done with it, producing both more knowledge about the model itself and more infrastructure to facilitate future work. Because of these increasing returns as more research is done using a given animal model, researchers tend to standardize on a limited set of animal model systems that have characteristics well-suited to the work at hand, and to focus infrastructure and tool development on those systems.

In mammalian genetics, humans and mice have long been the major research organisms, and it is these two species that have, by far, the best-developed support infrastructures, with plentiful genetic maps, physical maps, sequence information, and so forth. The rat, which is in fact, the most-studied animal in biomedical research[1] (being used extensively in fields such as neuroscience and hypertension research where its larger-than-mouse organs are essential) has, by contrast, lagged in the development of the genomics infrastructure needed to efficiently identify important genes isolated in those species, and so the researcher working with rat models faces a special challenge in successfully performing genomic research.

In this thesis, I will describe my work in positionally cloning the rat *Lymphopenia* (*Lyp*) mutation in terms of the techniques required for performing genomic analysis in rats.

The BB rat model of IDDM

In Insulin-Dependent Diabetes Mellitus (IDDM, or Type I diabetes), an autoimmune response destroys the body's insulin-producing cells, the β cells within the pancreatic islets of Langerhans. Without the blood sugar-level regulation mediated by insulin, death follows rapidly. Insulin supplementation can prolong life to a substantial fraction of the normal lifespan in humans, converting an acute disease that was a significant cause of death in young people in the early 20th century into a major chronic disease, especially in the developed world. During that lengthened lifespan, however, the morbidity caused by imperfect sugar-level regulation leads to many complications (see [2] for a review): in developed countries, IDDM is now the leading cause of new cases of blindness [3], the leading cause of non-traumatic lower-extremity amputation (due to neuropathy) [4], the leading cause of end-stage renal disease[5], and a significant risk factor for atherosclerotic disease[6,7].

The BioBreeding (or BB) rat model of IDDM is one of the two major animal models of spontaneous IDDM (non-drug-induced, and thus potentially more relevant to human IDDM) in research use today. The other major animal model, the NOD mouse [8], is broadly similar, but also has some substantial phenotypic differences from the BB rat. In particular, the incidence of diabetes varies greatly between the sexes in the NOD mouse[9] and the BB rat shows a distinctive lymphopenia phenotype.

The naturally-occurring BB rat model of IDDM was first identified in the mid 1970's[10,11]. During the 1980's, breeding work developed the strains in use today [12,13,14,15,16,17,18,19], verified the genetic and environmental nature of the susceptibility to IDDM [14,19,20,21] and characterized the diabetic phenotype and its close similarities to naturally-occurring human IDDM [11,22,23,24].

Genetic Analysis of IDDM

Clearly, the identification of genes and particular alleles of those genes responsible for the genetic susceptibility to IDDM could be of great importance in understanding why and how IDDM develops, how it might be treated, and, more generally, the causes of other autoimmune diseases. Although humans pose special challenges for genetic analysis, a particular MHC Class II allele has already been implicated in susceptibility to IDDM [25].

In the NOD mouse model, the MHC has also been identified as a major influence on the development of IDDM[26] (indeed, more than one MHC gene may be involved in IDDM development [27,28]), along with 15 other unlinked genes [29,30,31,32], although the evidence for some of these loci remains equivocal. Fine mapping of these other genes is complicated by the large number of *Idd* genes segregating in the crosses, and their low penetrance; as yet, none of these other genes has been identified. The use of congenic strains, in which chromosomal regions from IDDM-resistant strains have been introgressed on a NOD background, may help in this regard. The difficulties for positional cloning are evident, though, in Denny *et al.*'s work indicating that *Idd3* may be IL-2 [33]: even the NOD-congenic line carrying only a 0.35 cM section of B6 chromosome (an IDDM-resistant line) still had a 20.3% frequency of IDDM in 7-month-old females.

Genetic Analysis of IDDM in the BB Rat

Genetic analysis of complex traits such as IDDM works best when the animal lines used are genetically uniform and the phenotypic variance due to environmental factors is minimized. Ideally, then, the lines used should be fully inbred and the phenotype examined should be fully penetrant and free of environmental phenocopies. Unfortunately, much early work with BB rats has been limited by a lack of complete inbreeding in many BB rat lines [34, Chappel, 1983 #3]

In addition, variable influence of environmental pathogens on the development of IDDM in the BB model has weakened studies of the causal connection between genotype and phenotype and thus made genetic dissection of IDDM more difficult. Pathogens have been observed both to prevent and to promote the development of IDDM: in the first case, Like observed an increase in the incidence of IDDM in BB-Dp rats after the elimination of environmental viruses by cesarean derivation[35]. In the second case, Thomas observed an outbreak of spontaneous IDDM in BB-Dr rats that coincided with evidence of viral infection[36].

Lernmark therefore developed IDDM-susceptible (Dp) and IDDM-resistant (Dr) BB rat lines that are fully inbred and raised them in Specific Pathogen Free (SPF) environments in order to prevent pathogen-caused and pathogen-prevented IDDM [18,19]. Using this technique, the rate of such environmentally-caused phenocopies (as measured by IDDM incidence in the Dr strain and lack of IDDM in the Dp strain) has been reduced to zero, greatly simplifying genetic analysis in crosses between these strains. A few instances where SPF environments became contaminated have resulted in diabetic Dr animals and non-diabetic Dp animals, verifying the potential confounding influence of environmental pathogens on the IDDM phenotype [37,38].

Jacob *et al.*[39] used these strains, along with standard inbred wild-type rat strains, to genetically dissect IDDM into at least three genetic components with specific alleles required for the development of IDDM in crosses between the BB diabetes-prone (Dp) strain and three inbred wild-type strains. Of these, the identification of the MHC, with its known role in IDDM and other autoimmune diseases (see above and [40]) was a confirmation of expectations; the Fischer allele of an additional gene, as yet unmapped, provided resistance to IDDM; finally, the homozygous presence of the mutant version of the lymphopenia (*Lyp*) gene, *iddm 1*, mapped near *Npy* on rat chromosome 4, was absolutely required for the development of IDDM in the

three crosses. This proven requirement for *Lyp* in the development of IDDM in the BB rat suggests that understanding the action of the *Lyp* gene could provide important insights into the development of IDDM itself.

Lymphopenia

The lymphopenia phenotype is principally a profound deficit in peripheral T cell levels[41,42,43,44,45]. The earliest stage of T-cell maturation at which differences have been noted in lymphopenic animals is in late thymic maturation. Although the lymphopenic thymus is grossly normal[44,46], a deficit of more mature thymocytes has been noted in the medulla, with the CD5^{low}/CD5^{high} CD8⁺ ratio increased[47] and the cellular organization and cytokine ratios somewhat altered in *in situ* hybridizations [48,49,50]. Studies that looked closely at the short-term fate of recent thymic emigrant T-cells found that even most of the reduced number of T-cells that do emigrate to the periphery die within 7 days[51] [52], again suggesting a defect in late thymic maturation. Peripheral T-cell numbers are drastically reduced, with almost all CD8⁺ and most CD4⁺ cells missing, while B-cell numbers remain normal.[42,43,44] This reduction in T-cell numbers is accompanied by immune response deficits including an inability to reject skin grafts [53] and a weak response in mixed lymphocyte reactions (MLR)[41,44], indicating that those T-cells that do remain have significantly altered functionality. Finally, Bellgrau *et al.* [54] report finding very few canonical Th2 cells in the periphery, while the remaining T_H cells express a phenotype that is neither Th1 nor Th2.

Since a T_H-cell (CD4⁺) -mediated autoimmune response is implicated in the destruction of the pancreatic β cells (reviewed in [55]; [56] provides evidence for Th1 primacy in IDDM), the increase in the peripheral CD4⁺/CD8⁺ T cell ratio and the altered T cell phenotypes produced

by *Lyp* in the BB rat are attractive candidate mechanisms for the susceptibility to IDDM conferred by *Lyp*.

The *Lyp* defect acts in one or more cell types of the hematopoietic lineage, as best illustrated in elegant experiments by Greiner *et al.* [57] in which SCID mice were reconstituted with fetal liver cells (a source of hematopoietic stem cells) from either wild-type or lymphopenic (*Lyp*) rats. The resulting reconstituted wild-type animals showed normal T-cell levels, while those reconstituted with *Lyp* fetal liver showed <20% of normal T-cell levels. Since the majority of cells in the thymus, including pre-T-cells and antigen-presenting cells such as macrophages and dendritic cells, are derived from the hematopoietic lineage [58], this result is fully consistent with the thymic abnormalities mentioned above and with a proposed principal site of action of the *Lyp* gene in late thymic (that is, medullar) maturation.

This is further supported by the phenotypes of a number of mouse lines with knockouts in genes important in thymic T-cell maturation (reviewed in [59]), most importantly that of CD45 exon 6 [60]. This knockout mouse has a phenotype very similar to that of *Lyp*, including the peripheral T-cell deficit with CD4⁺/CD8⁺ ratio increased, a normal level of B cells, and defects in LCM virus challenge and MLR response. In addition, the CD45 isoform containing exon 6, CD45RC, is normally expressed in the thymic medulla and on peripheral T-cells [61]. However, the thymocyte populations in CD45 exon 6 knockout mice are more skewed, with single-positive (SP) cells decreased and double-negative (DN) cells increased compared to those in *Lyp* animals. The CD45 gene maps to a different rat chromosome than does *Lyp* and thus cannot be the *Lyp* gene ([62], and see Chapter II for *Lyp* mapping in rat and mouse).

***Lyp* – A Model Target for Rat Positional Cloning**

The *Lyp* gene in the BB rat model of the disease IDDM is an excellent example of the situation described in the Overview. The identification of *Lyp* is of great interest, as it is required for the development of IDDM in the BB rat model[39] and has a clear, well-defined, but interestingly complex phenotype. *Lyp* is easy to map genetically: it is fully penetrant, fully recessive [14], and easily assayed by analyzing a blood sample[63]. Finally, it maps to within less than 1 cM from a known marker[39]. These characteristics make *Lyp* both a scientifically desirable and a technically feasible gene to attempt to positionally clone in the rat.

Although dense genetic maps and large-insert genomic libraries of the mouse have been available for some time[64,65,66], it is only very recently that equivalent resources are becoming available in the rat [67,68,69]. This lack of resources has prevented the positional cloning of rat genes. Therefore, I implemented a strategy based on conservation-of-linkage (described in Chapter II) and transferred positional information from the rat to the mouse and back again in order to employ the superior physical mapping resources of the mouse in identifying and narrowing a set of candidate genes for *Lyp*. As described in the following chapters, I identified and physically mapped the mouse chromosomal region orthologous to the rat *Lyp* region, identified transcription units within that region, identified the rat orthologs of mouse transcription units, and used those to identify rat genetic markers, further limit the rat *Lyp* region, and create a physical map of it. I discuss the transcription units identified, in particular the gene PA which is associated with late thymic T-cell maturation, transgenic experiments to attempt to rescue the *Lyp* phenotype, and the lessons learned during this project about positional cloning and conservation-of-linkage strategies.

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CHAPTER II

A Fine Genetic Map of the *Lyp* region in Rat and Mouse: Support for a Conservation-of-Linkage Positional Cloning Strategy

Introduction

The rat *Lymphopenia* gene, *Lyp*, is absolutely required for the development of Insulin-Dependent Diabetes Mellitus (IDDM) in the BioBreeding (BB) rat model of IDDM.[1,2,3] This gene causes a severe peripheral T-cell deficit,[4,5,6,7,8,9] and is recessive and fully penetrant[2,10]. Furthermore, it maps less than 1 cM from the rat *Npy* gene.[3] Thus, it is an excellent candidate for positional cloning.

More than 100 genes have been positionally cloned in mouse and human (some recent examples: [11,12]), but none yet in the rat. In large part, this has been due to the lack of the infrastructure necessary for positional cloning in the rat. High-resolution mouse and human genetic maps of 0.1-1.5 cM resolution [13,14,15] allow genes to be mapped to small genetic intervals, which can then be spanned physically using YAC libraries[16,17,18] or even complete physical maps[19,20] for easy coverage, and using high-redundancy smaller-insert genomic libraries (or even gene maps[21]) for fine-mapping, gene identification, sequencing, and genomic rescue [22,23,24,25,26]. Rat genetic map resolution is improving [27,28], but it is only very recently that good rat physical mapping resources have begun to become available [25,26,29]. Overall, rat resource development has lagged far behind that developed for the human and mouse.

A different strategy was needed to make rat positional cloning easier, given the current state of the infrastructure in the rat. In this chapter, I will describe such a strategy, the

“Conservation of Linkage” strategy, which has the advantage of leveraging for use in the rat the pre-existing infrastructure developed in mouse and human. I will examine its applicability in general for mammalian positional cloning projects, and provide evidence supporting its use in the particular case of the BB rat *Lyp* gene.

Conservation of Linkage (COL) Strategy

Description of the COL Strategy

The positional cloning strategy proposed here depends on the conservation over evolutionary time of local gene order. That is, for related species, a given small segment of a chromosome from one species will likely have an orthologous chromosomal segment in the other species with the same gene order. Such pairs of segments are said to exhibit “conservation of linkage”[30] (COL implies *both* that each segment in the pair contains orthologs of all the genes in the other segment *and* that the order of these genes is the same in both segments; eliminating the latter constraint leads to the weaker notion of “conservation of synteny” (see below)). Of course, given the current lack of extensive comparative genomic sequence data between mammalian species, the genomic segments are operationally defined by comparing individual gene sequences from within the segments.

As evolutionary divergence increases, the average size of the segments of conserved gene order will tend to get smaller with the increase in the number of chromosomal rearrangements between the two species. In addition, DNA sequence changes in the genes will accumulate over time, even though gene order may be maintained.

Given the existence of conservation of linkage between two related species, one with extensive positional cloning resources (a “map-rich” species, e.g. the mouse), and one much

poorer in such resources (a “map-poor” species, e.g. the rat), we can see a possible strategy for positional cloning in the latter: transfer the genetic mapping information from the rat to the mouse, and make use of the positional cloning resources in the mouse to identify the gene of interest.

For the COL positional cloning strategy to succeed, two questions must be answered in the affirmative: (1) is linkage in fact conserved between rat and mouse throughout the region of interest (in this case, the region recombinationally inseparable from *Lyp* (“the *Lyp* region”))? and (2) can genetic markers (the determinants of the boundaries of the *Lyp* region) be successfully transferred between rat and mouse to transfer that positional information? In this chapter, I address the first question.

I address this question in three parts: First, do we have reason to believe that, in general, there exist sizeable regions of conserved linkage between pairs of mammalian species? Second, given such conserved linkage, are typical genetic maps appropriate to allow a COL strategy? Third, in the Results and Discussion section, we specifically examine the rat and mouse *Lyp* regions in light of these considerations.

How Large are the Regions of Conserved Linkage Among the Mammals?

There is evidence to suggest that the average size of regions of conserved linkage between a pair of mammalian species is often sufficiently large to be useful for positional cloning purposes. Nadeau and Taylor[31] originally proposed a model in which the chromosomal rearrangements that determine the boundaries of these regions are randomly distributed within the genome. This allowed straightforward mathematical modeling of the process and resulted in an estimate of 8.1 +/- 1.6 cM as the estimate of the mean length of the autosomal regions conserved between mouse and man. As Nadeau and Sankoff[32] point out,

later estimates of 10.1 +/- 2.2 cM in 1989[33] and 8.8 cM in 1993[34], using much denser genetic maps, remain consistent with this original estimate and support the original model, as does an estimate using an independent method[35].

A weaker concept than “conservation of linkage” is “conservation of synteny,” in which the “nearness” of genes, but not necessarily gene order, is preserved across species. Because of the limited resolution of quick genomic physical mapping methods prior to the development of radiation hybrid panels, this "nearness" is typically defined as co-location of genes on a given chromosomal arm. Both nearness and gene order are disrupted by interchromosomal rearrangements, while conservation of linkage is also disrupted by intrachromosomal rearrangements. Because synteny information is available for a wider range of species than are good genetic maps with mapped reference genes, Ehrlich *et al.*[30], studied synteny disruption rather than linkage disruption in order to be able to simultaneously study interchromosomal rearrangements among a wide range of mammalian species. Interestingly, among the nine species studied (cow, chimp, human, baboon, Chinese hamster, mouse, rat, mink, and cat), cat and hamster showed the slowest rates of synteny disruption, while human, mouse and rat showed the highest lineage-specific rates of synteny disruption: an average of ~0.05 synteny disruptions for cat or hamster versus an average of ~0.90 synteny disruptions for human, mouse or rat, per million years.

Although this study did not measure linkage disruption, it did estimate ~78 synteny disruptions (breakpoints) between mouse and rat, and ~129 between mouse and human. If we make the reasonable assumption that the ratio of linkage disruptions to synteny disruptions between mouse and human (as estimated by different methods to be ~180/~140 = ~1.3 [30]) applies also between mouse and rat, we expect there to be ~78 x ~1.3 = ~101 linkage disruptions

between mouse and rat. Thus, the average length of conserved linkages between mouse and rat should be ~16 cM. Since the maximum pairwise number of syntenic disruptions in the study was between mouse and human, all the other pairs of species in the study should have average lengths of conserved linkages greater than the 8.8 cM between mouse and human. Thus, significant linkage conservation exists between the pairs of the species examined in this study, and in particular, between mouse and rat. Even pairs of mammalian species other than those studied by Ehrlich *et al.* that diverged at the same time or more recently than rodents and primates (~75 million years ago[36]) might reasonably be expected to have segments of conserved linkage on the order of 10 cM or greater in length. As discussed below, this distance compares favorably with a typical 5cM median gap between markers in a first- or second-generation genetic map, and, in particular, is substantially greater than the 1 cM interval containing the rat *Lyp* gene.

Are Typical Genetic Maps Appropriate for a COL Strategy?

Of course, the practical exploitation of linkage conservation depends on the quality of the genetic maps and crosses in each of the two species considered. The wider the limits of the interval fully linked to the gene (the “gene interval”), the less likely that both of those flanking markers will be contained within the same region of conserved linkage in the other species. There are two effects that tend to widen the size of the gene interval: First, the genetic map or the mapping cross for the gene of interest in the map-poor species places a lower limit on the size of the gene interval in that species. Second, transferring the genetic mapping data to the map-rich species typically involves identifying genetic markers from that species that immediately flank the orthologs of the genetic markers from the first species, thus resulting in a larger gene interval in the second species.

For most map-poor species, it is the spacing of the genetic markers, rather than the number of crossovers, that places a lower limit on the size of the gene interval. Although map-rich species such as the mouse and human have genetic maps with marker spacing on the order of 0.1-1.5 cM[13,14,15,37,38], the marker spacing of a typical first- or second-generation simple-sequence-repeat (SSR) marker genetic map in a map-poor species such as the sheep or rat is on the order of 5 cM.[27,39] In the sheep map in particular, 44.5% of the intervals between adjacent markers are ≥ 5 cM in length. Since it is usually quite easy to generate a cross with expected 1 cM resolution (approximately 100 informative meioses, or 50 F2 intercross animals for a recessive Mendelian trait), in these cases the lower limit on the size of the gene interval is usually imposed by the resolution of the genetic map itself, rather than the density of breakpoints generated by the cross. The COL strategy involves mapping a gene of interest in a map-poor species and then transferring that positional information to a map-rich species. Since the map-rich species typically has a high-resolution genetic map, the additional positional uncertainty added by transferring the positional information to the map-rich species does not usually significantly increase the size of the gene interval, as determined by the closest markers flanking the interval in the second species. Even in a case such as that of the mouse genetic map[13], where the total of 92 meioses limits the bin resolution to an average of 1.36 cM (even though average marker spacing is ~ 0.2 cM), the set of genetic markers in the few bins spanning a given region of interest can be easily typed on a mouse cross with sufficient crossovers to resolve the genetic markers into individual positions.

Thus, using a typical map-poor species gene interval of ~ 5 cM (using a typical map resolution of ~ 5 cM and assuming minimal expansion in the transfer of markers to the map-rich species), and the smallest average size for the regions of conserved linkage above of ~ 9 cM

(mouse-human), we would expect to have linkage conservation over the gene interval in about 44% of the cases – significant enough to be worth examining. For an expected mouse-rat conserved region size of ~16 cM and the same ~5 cM gene interval, we would expect to have COL over the gene interval more than two-thirds of the time (the probability of rat-human COL falls between these two values). In the particular case of the *Lyp* region, with *Lyp* contained within an approximately 1 cM interval on rat chromosome 4.[3,40] [41] and the expected mouse-rat conserved region size of ~16 cM, the prerequisites for a COL positional cloning strategy are quite likely to be fulfilled. The Results and Discussion sections below show that, indeed, genetic marker order is preserved (COL holds) between the rat and mouse *Lyp* regions. Thus, the mouse *Lyp* region should contain the orthologs of the genes in the rat *Lyp* region, including the *Lyp* gene itself.

Materials and Methods

Animals

The rat DpxDr F₂ intercross[3] and DpxFischer F₂ intercross[3], with 279 and 42 animals, respectively, were used previously to genetically dissect IDDM in the BB rat. An additional 247 DpxFischer intercross animals became available after that work and were also used for this project. Subsequently, the Lernmark lab (R.H. Williams lab, U. Washington, Seattle) has passaged the BB-Dp *Lyp* region haplotype through DpxDr cross-intercross breeding [42], generating more than 300 additional animals segregating the *Lyp* mutation.

The mouse B6 x *Mus spretus* backcross[34] consists of 205 (C57BL/6J x *M. spretus*) x C57BL/6J backcross animals. It has been used extensively for RFLP-based mouse mapping --

my thanks to Neal Copeland and Nancy Jenkins for kindly mapping the mouse Npy polymorphism I sent them.

The B6-*ob* x *Mus m. castaneus* F₂ intercross[43] was also used for fine-scale mouse genetic mapping. All animals in the B6-*ob* x *Mus m. castaneus* F₂ intercross (C57BL/6J-*ob* x CAST/Ei)F₂ were selected to be homozygous *ob/ob*; since this gene is approximately 10 cM proximal of the mouse *Lyp* region, there is an excess of B6-*ob*/ B6-*ob* homozygous typings and a deficit of *castaneus/castaneus* homozygous typings for markers in the mouse *Lyp* region. 211 animals from this intercross were successfully typed for the two markers MPC132 and D6Nds4 generously flanking the mouse *Lyp* region on left and right. The 40 animals recombinant between those two markers were then typed with the complete set of genetic markers in the mouse *Lyp* region. The complete typing data for these 40 animals are in Appendix A.

PCR Analysis

PCR primers were selected using the Primer 0.5 [44] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. STS and SSR marker assay primer information is listed in Appendix A. Standard PCR reactions were performed in 100 µl reactions under oil in 1x Perkin-Elmer PCR buffer, with each dNTP at 200 mM concentration and each primer at 200 nM concentration. Standard cycling conditions on the MJ Research 96-well PCR machine were: 94°C 3'; 25x 94°C 45s, 60°C 1', 72°C 3'; 72°C 7', with the annealing temperature adjusted as necessary to match the lower of the predicted melting temperatures for the two primers. For more sensitive amplifications, 35 cycles of amplification were performed.

SSR genotyping was as described previously[3], with the exception of alternate annealing temperatures (standard temp. 60°C), as described in the text. Briefly, radio-labeled PCR

products were visualized on a 6% denaturing acrylamide gel (Sequagel, National Diagnostics). For each reaction, one primer was end-labeled with ^{32}P γ ATP (spec. act. 6000 Ci/mmol, Dupont/NEN) using T4 polynucleotide kinase (NEB) according to standard protocols[45]. Genomic DNA (25 ng/reaction) was PCR-amplified in a 10 μl reaction with 100 nM each unlabelled primer and 20 nM labeled primer, under oil, using *Taq* DNA polymerase in the following program: 94°C 3'; 25x 94°C 45s, 55°C 1', 72°C 2'; 72°C 7'. Completed PCR reactions were mixed 1:1 with formamide containing added xylene cyanol and bromophenol blue dyes, denatured 5' 100°C, snap-cooled on ice, and electrophoresed on 0.4 mm 6% acrylamide gels. Completed gels were wrapped in Saran Wrap and exposed to X-ray film 4-24 hours at -80°C.

Map Construction

Except where noted, genetic maps were constructed using Mapmaker[46], MS-DOS version, with the default settings, three-point analysis on, error detection off.

YAC Analysis

YACs were isolated from the MIT mouse YAC library[16] by PCR. Either non-radioactive or radioactive PCR reactions using the primers from the assay of interest (see PCR Analysis above) were used to amplify pooled YAC DNA from the library. The presence of one or more bands of the appropriate size on an agarose (for non-radioactive) or acrylamide (for radioactive) gel identified one or more library sub-pools which contained the desired YAC clone(s). Further PCR on plate, row and column DNA pools from these subpools identified the well address(es) of the positive clone(s). Liquid yeast was DNA prepared as described[47]; essentially, the yeast were spheroplasted using zymolyase and then further processed by standard methods.

YAC end sequences were isolated using inverse PCR as previously described [48] and these end PCR products sequenced directly using standard fluorescent sequencing methods. In brief, in the inverse PCR technique, the YAC DNA is separately digested with frequent-cutting restriction enzymes (for the "left end" of these YACs, *Hae*III or *Taq*I were used, while *Hae*III or *Alu*I were used for the "right end"). The fragments are then circularized by self-ligation and amplified with PCR primers chosen from the end of the cloning site to point away from each other. In the circularized cloning site/insert junction fragments, these primers will amplify a product around the circle, forming a PCR product that contains mostly insert end sequence. The largest of these products were identified on a 2% SeaPlaqueGTG/0.5x TBE agarose gel, gel purified, and sequenced as described above.

Results

Previous mapping work in mouse and rat [3,34,49] had suggested that a region (the "mouse *Lyp* region") on proximal mouse chromosome 6 had conserved linkage with the *Lyp* region on rat chromosome 4: that is, the order of the mouse orthologs of the genes immediately proximal to the rat *Lyp* region (including the T-cell receptor β chain genes) was the same as the order of the rat genes adjacent to *Lyp*; in addition, the nearest gene flanking *Lyp* distally, *Igk*, 10-15 cM distal to the *Lyp* region, had its mouse ortholog mapping distal to the T-cell receptor β chain genes on mouse chromosome 6.

As shown in Figure 1(a) and 1(b) (along with the new mappings described in this section), the gene order on rat chromosome 4 was *Cpa* - *Tcrb* - R236 - *Lyp* - *Npy* - *Igk*, while the gene order on mouse chromosome 6 was the same: *Cpa* - *Tcrb* - *Igk* (R236 and *Npy* had not been mapped in the mouse, and *Lyp* does not exist in the mouse). The COL cloning strategy (which

requires that the same genes be present in the same order in the two species) might thus be suitable in this case.

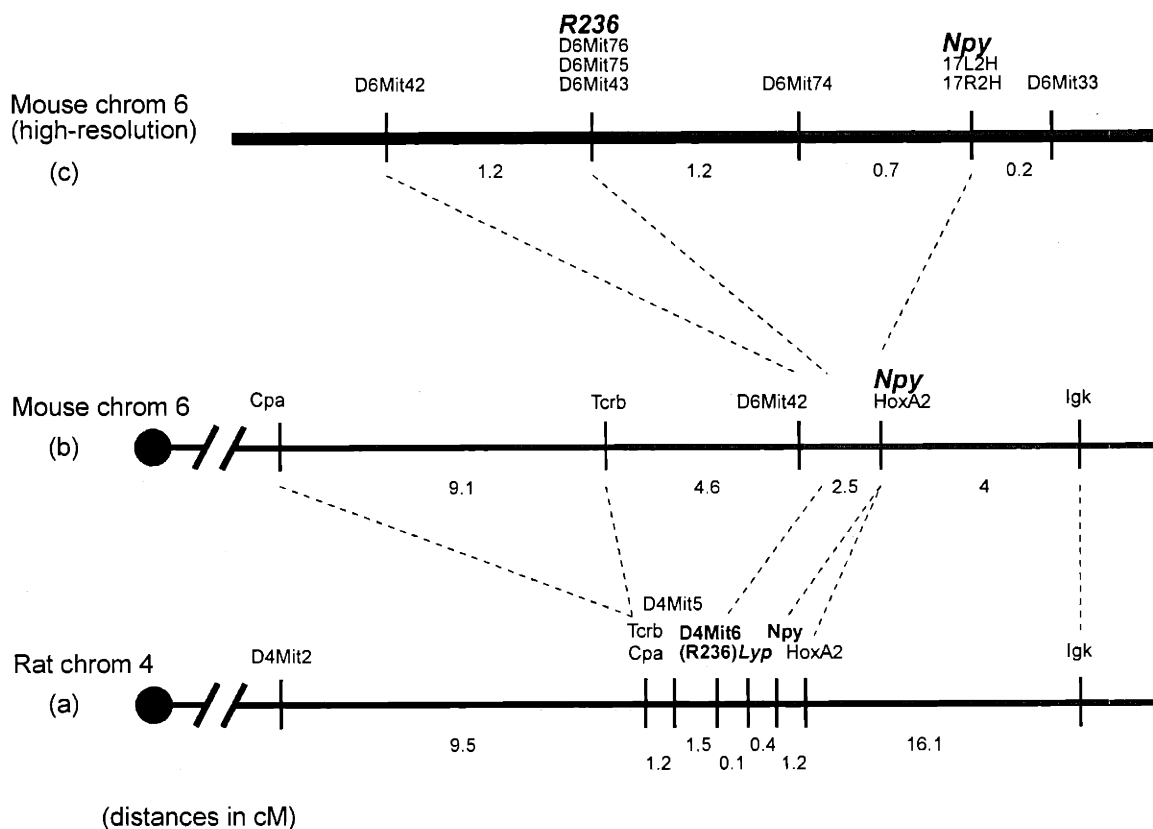


Figure 1: Comparison of rat and mouse genetic maps in the *Lyp* region: (a) Rat map. Framework (excluding *Lyp*) was generated by pooling data from 279 DpxDr F₂ intercross animals and 42 DpxFischer F₂ intercross animals, using Mapmaker with default settings (framework order supported by 500:1 odds over any alternative). Additional DpxFischer F₂ intercross and DpxDr cross-intercross animals were used to place *Lyp* within R236-*Npy* interval (see text). (b) Integrated mouse map. *Npy* and *Hoxa2* (Hox-1.11) were added to the standard map to complete the comparative mapping of all genes known in the mouse and rat *Lyp* regions. (c) Fine-scale mapping of mouse *Lyp* region. Orthologs of closest markers flanking rat *Lyp* region mapped on high-resolution mouse B6-*ob* x cast F₂ intercross along with mouse SSR genetic markers. R236 is the same PCR assay as in the rat, but with 50°C annealing temperature. Y17L2H and Y17R2H are genetic markers at opposite ends of a mouse YAC that contains the *Npy* gene; therefore, *Npy* maps in the same genetic position as Y17L2H/Y17R2H.

Genetic Marker Order in the Rat *Lyp* Region

In order to verify conservation of linkage in more detail in the *Lyp* region, and to narrow the ~15 cM interval containing *Lyp*, I attempted to identify additional rat genetic markers in the

Npy-Igk interval. Since direct genetic marker identification approaches using cloned genomic rat *Lyp* region DNA were unavailable, I instead used two approaches based on conservation of synteny between rat and mouse. In the first, I chose 8 anonymous SSR markers from the mouse genetic map[13] between *Tcrb* and *Igk* and attempted to amplify them from rat DNA under conditions of reduced stringency (55°C and 50°C annealing temperatures). This was unsuccessful: every one of these anonymous assays failed to amplify in the rat even under the conditions of reduced stringency.

Since sequences within and adjacent to genes, and in particular coding sequences, are much more highly conserved evolutionarily than random DNA outside genes, I next searched for additional gene-associated SSRs (either pre-existing or newly generated from database sequences) from genes in the mouse *Lyp* region. The mouse *Hoxa2* gene (old terminology Hox-1.11) genomic sequence[50] contains a CA-repeat downstream of the poly-A site. PCR primers were chosen from this sequence, and the resulting PCR assay, *hox1.11*, was successfully amplified at reduced stringency (55°C annealing temperature) in the rat. This success is most likely due to the selection acting on the sequences between the Hox complex coding regions, as they are known to play important roles in regulating Hox complex gene expression.

Subsequently, the 279 animals in the DPxDR F₂ intercross and the 42 animals in the DpxFischer F₂ intercross were typed for *hox1.11*, resulting in the rat genetic map shown in Fig. 1a. In addition, the critical recombinant animals placing *Lyp* distal to *Npy* were retyped. Re-genotyping and the new flanking marker *hox1.11* showed that one had been mis-genotyped, while the other two had been mis-phenotyped for *Lyp* (their diabetes phenotypes were, however, concordant with their genotypes). Of the remaining informative animals, one was recombinant between R236 and *Npy*, placing *Lyp* distal to R236, while six were recombinant between *Npy*

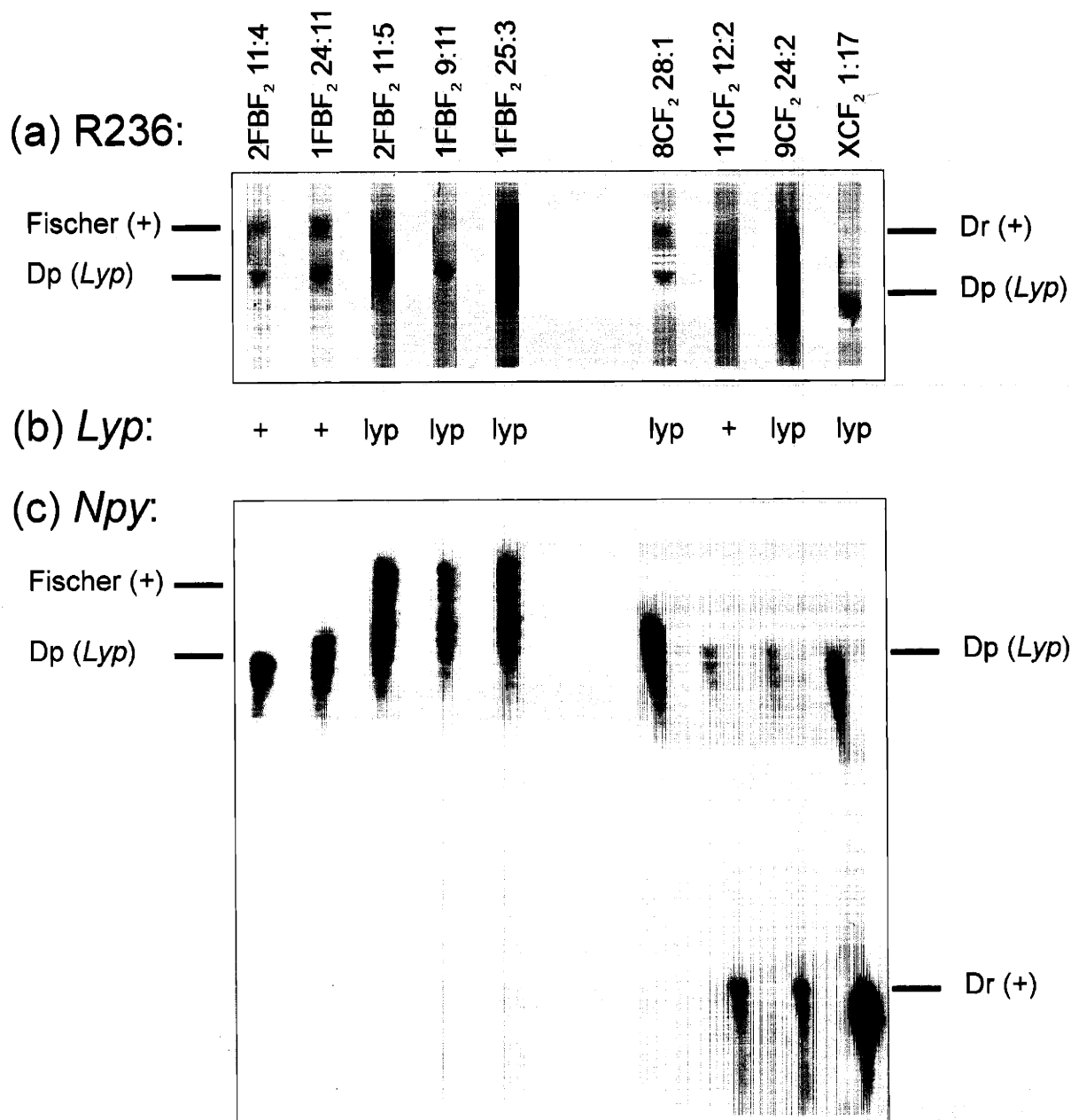


Figure 2: SSR genotypings of selected recombinant rats show that R236 and *Npy* flank the *Lyp* gene. (a) R236 locus genotypings of nine rats recombinant in the *Lyp* region. The rats are identified above each autoradiograph lane; “FBF” animals (leftmost five) are from a BB-Dp (*Lyp*) x Fischer (+) cross, while “CF” animals (rightmost four) are from a BB-Dp (*Lyp*) x BB-Dr (+) cross. The gel positions of the *Lyp* haplotype (BB-Dp) and + haplotype (Fischer or BB-Dr) SSR alleles are indicated along the sides of the autoradiograph lanes. As shown by the autoradiograph lanes, the genotypes at R236 for these nine animals are *+/Lyp*, *+/Lyp*, *Lyp/Lyp*, *Lyp/Lyp*, *Lyp/Lyp*, *+/Lyp*, *Lyp/Lyp*, *Lyp/Lyp*, *Lyp/Lyp*. (b) Lymphopenia phenotype of the same nine animals. (c) *Npy*-2 locus (*Npy* gene) genotypings of the same nine animals. As shown by the autoradiograph lanes, the genotypes at *Npy*-2 for these nine animals are *Lyp/Lyp*, *Lyp/Lyp*, *+/Lyp*, *+/Lyp*, *+/Lyp*, *Lyp/Lyp*, *+/Lyp*, *+/Lyp*, *+/Lyp*.

As shown above, animals 1-5, 8, and 9 have recombination breakpoints between *Lyp* and *Npy*, while animals 6 and 7 have breakpoints between R236 and *Lyp*. The *Lyp* gene thus maps between R236 and *Npy*. (see Chapter IV, Figure 6 for a schematic diagram of the genotypes of these recombinant animals in the R236-*Npy* interval)

and hox1.11, placing *Lyp* proximal to hox1.11. Further genotyping of an additional 247 DpxFischer F₂ intercross animals, and of a similar number of animals in the ongoing DpxDr cross-intercross (generating *Lyp*-Dr congenic animals) has placed *Lyp* in the 0.5 cM R236-*Npy* interval(see erratum for [3]): there are 17 additional recombinants between R236 and *Npy*, with 2 of these placing *Lyp* distal to R236 (for a total of 3: 1 previous and 2 additional) and 15 of these placing *Lyp* proximal to *Npy* (see Fig. 2 for critical region recombinant genotypings).

In summary, the genetic order in the rat *Lyp* region is (cen 4) - *Cpa/Tcrb* - D4Mit5 - D4Mit6(R236) - *Lyp* - *Npy* - *HoxA2* - *Igk* - (tel 4).

Genetic Marker Order in the Mouse Lyp Region

Conservation of linkage in the mouse was verified in two steps. First, gene order was determined on the 205 animal B6 x spretus backcross: hox1.11 (*Hoxa2*) and the mouse genetic marker D6Mit42 were mapped as standard SSR markers. Since the mouse *Npy* gene had no associated SSR, it was mapped as an RFLP to the same genetic location as hox1.11[51]. The resulting map is shown in Fig. 1b.

Second, in order to resolve the order of *Npy* and Hox1.11, and to map R236 directly on the mouse, a set of mouse SSRs, including D6Mit42, were typed on a B6-*ob* x *cast* F₂ intercross with 211 progeny. R236 was also typed on this panel: although R236 is not a gene-based assay, at reduced stringency 35% of the animals typed were scorable, placing R236 clearly to the right (distal) of D6Mit42 (see Table 1). In order to map *Npy* on this cross, a mouse YAC clone containing *Npy* was isolated by screening the YAC library with a PCR assay derived from the consensus rat and human *Npy* mRNA gene sequences (*Npy*-c; assay and primer information listed in Appendix A). SSRs were identified at each of its ends and converted into the assays

Y17L2H and Y17R2H. These markers were polymorphic in the cross and mapped to the same bin; thus, *Npy* also maps to that bin. The resulting map is shown in Fig. 1c.

In summary, the genetic order in the mouse *Lyp* region is (cen 6) - *Cpa* - *Tcrb* - D4Mit5 - D6Mit42 - R236/D6Mit43/MPC1305/MPC1683 - MPC1322 - (17L2H/*Npy*/17R2H)/*HoxA2* - *Igk* - (tel 6).

DNA Sample	D6Mit42	R236	MPC1305 D6Mit76	MPC1683 D6Mit75	D6Mit43-x	MPC1322 D6Mit74	17 L2H	17 R2H	D6Mit33
183	1	1	1	1	1	3	3	3	3
194	1	-	1	1	1	3	3	3	3
208	1	-	1	1	1	1	1	1	3
215	1	3	3	3	3	-	3	3	3
218	1	3	3	3	3	3	3	3	3
239	1	-	1	1	1	1	3	3	3
267	1	-	3	3	3	3	3	3	3
305	1	-	1	1	1	3	3	3	3
306	1	-	3	3	3	3	3	3	3
119	1	-	1	1	1	1	3	3	3
121	1	-	1	1	1	1	3	3	3
144	1	-	3	3	3	3	3	3	3
210	3	3	3	3	3	2	2	2	2
339	1	-	1	1	1	3	3	3	3

Table 1: Table of genotypings of 14 mouse high-resolution cross key recombinants with D6Mit42, R236, Y17L2H (*Npy*) and other markers, showing the order D6Mit42 - R236 - 17L2R(*Npy*) in the mouse (the two recombinants placing R236 to the right of D6Mit42 are shaded). Uninterpretable typings are denoted by hyphens; otherwise, a "1" represents a homozygous B6-*ob*/B6-*ob* genotype at the locus, a "3" represents a heterozygous B6-*ob*/*cast* genotype at the locus, and a "2" represents a homozygous *cast*/*cast* genotype at the locus. The excess of "1" genotypes and deficit of "2" genotypes is due to the fact that the animals in the cross were selected to be homozygous B6-*ob*/B6-*ob* at the *ob* gene approximately 10 cM proximal (left) of this region.

Discussion

As expected, conservation of linkage holds between rat and mouse for the *Lyp* region and the genes flanking it. The 20.2 cM *Cpa-Igk* interval in the mouse contains the same genes (or markers) *Tcrb*, R236, *Npy* and *Hoxa2* (Hox-1.11) within it as does the 20.5 cM *Cpa-Igk* interval in the rat. The ~20 cM known size of the conserved region is consistent with the predicted

average size of ~16 cM for intervals of rat/mouse linkage conservation. Although linkage is conserved, fine-scale genetic distance is not (*Cpa*, *Tcrb* are fully linked in the rat, 9.1 cM apart in the mouse; *Hoxa2-Igk* is 4 cM in the mouse, 16.1 cM in the rat); nor should we expect it to be conserved, given the variations in the genetic distances between pairs of genes even in different mouse crosses[34]. Interestingly, linkage between rodents and human over this interval is *not* conserved: the human orthologs of genes from *Pon1* near the centromere of mouse chromosome 6 through *Tcrb* map from 7q21-22 to 7q33-34, while the orthologs of genes from *Aqp1* (distal to *Hox* on mouse chromosome 6) through *Npy* map from 7p14 to 7p15-21.[52,53] The failure of anonymous (presumably non-gene) mouse STS PCR assays to work in the rat suggests that non-genic sequence divergence between mouse and rat is sufficient to prevent the easy transfer of positional information based on sequence similarity between species (see Chapter IV for more on this issue). This also confirms the impracticality of constructing a dense rat genetic map by the direct use of mouse STS PCR assay genetic markers.

The results indicate that a COL strategy should be a viable approach to positionally cloning the rat *Lyp* gene. The initial experience with transferring markers between species suggests that gene-based markers will be the easiest to transfer, presumably due to greater evolutionary sequence conservation than non-gene-based SSRs. The various sizes of the *Lyp* region (~0.5 cM in the rat, ~2 cM in the mouse; these are consistent with[41]) indicate a physical interval of 0.5 - 4 megabases, based on average physical distances per cM in rat and mouse. This physical distance is suitable for a YAC walk, and the ordering of mouse genetic markers spanning the mouse *Lyp* region in Fig. 1c above should contribute to the rapid assembly of such a contig, as the STS assays will provide a set of known anchor loci of defined order spanning the mouse *Lyp* region.

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CHAPTER III

Large-Scale and Fine-Scale Physical Maps of the Mouse *Lyp* Region

Introduction

Having identified genetic markers flanking the *Lyp* region in the mouse, according to the conservation of linkage strategy described in Chapter II, we expect that region to contain the mouse ortholog of the rat *Lyp* gene. Since no likely candidate genes for *Lyp* (i.e. genes affecting late thymic T-cell maturation) are known within this region, all genes within the region must initially be considered as equally possible candidates. It should be noted that numerous genes affecting T-cell function and development in the thymus have been "knocked-out", but few of these knockouts have phenotypes similar to that of *Lyp*; those that are superficially similar in the sense of producing some sort of reduction in the peripheral T-cell count (e.g., the CD45 exon 6 knockout mouse [1,2]) have, upon closer inspection, subtler phenotypic differences from *Lyp*, and map to chromosomes other than mouse chromosome 6.

Two main approaches are available to prune the list of candidates to be examined: First, new genetic markers developed from the rat orthologs of loci within this interval will more closely define (and thus, narrow) the critical genetic interval containing *Lyp* (see Chapter IV). Second, characterization of genes within the interval will indicate whether they are more or less likely to be *Lyp* (see Chapter V). In addition, transgenic rescue can limit the critical region to a single P1 or BAC genomic clone (see Chapter VI). For all of these approaches, a fine-scale physical map that includes transcript information is necessary in order to establish distances, order, and the boundaries of the critical region, and to provide appropriately-sized high-fidelity

genomic clones for transgenic rescue experiments. In order to serve these purposes, such a map should have a resolution on the order of the size of a typical gene -- ~30kb.

Developing such a map in the mouse is a three-step process: first, create a large-scale physical map of the *Lyp* region in order to assemble a set of DNA clones which contain the *Lyp* gene; second, use this large-scale physical map to isolate a dense set of gene fragments for use as sequence-tagged-sites (STSs) and third, use these STSs to isolate and order smaller genomic P1 and BAC clones for the actual fine-scale physical map of the region. This map provides both the high resolution necessary to order the candidates for *Lyp* (<100kb) and the high-fidelity representation of the underlying chromosomal interval that YACs, with their potential instability, chimerism, and internal deletions[3],[4], often cannot provide. The genomic clones available for construction of the physical maps include YACs (typical insert size range 500kb-1500kb), BACs (typical insert size range 100kb-250kb) and P1s (typical insert size ~85kb). In this chapter I describe the construction of both large- and fine-scale physical maps of the mouse *Lyp* region.

Materials and Methods

PCR Analysis

Unless pre-existing or otherwise noted, PCR primers were selected using the Primer 0.5 [5] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. STS and SSR marker assay primer information is listed in Appendix A, while exon and cDNA fragment sequence information is listed in Appendix B. PCR amplification was performed according to the conditions specified for each protocol (e.g. YAC library screening), or, if not specified, according to standard conditions as recommended by Perkin-Elmer, as described in Chapter II.

Southern Blot Analysis

Genomic DNA was prepared from mouse livers using standard methods. In brief, fresh or flash-frozen tissue was placed in PBS and homogenized. A proteinase K/ SDS-based lysis solution was added and incubated O/N at 65°C [6]. The resulting solution was centrifuged, and the supernatant phenol/chloroform-extracted twice and chloroform extracted once. Finally, the DNA solution was dialyzed 48 hours against multiple changes of TE at 4°C.

For Southern blots, 20 µg of C57BL/6JEi mouse genomic DNA per lane was digested to completion using *EcoRI* (NEB) according to the manufacturer's instructions (digestion was 12 hours at 37°C in 100 µl volume of 1x *EcoRI* buffer (NEB) using 140 units of enzyme). The digested DNA was ethanol precipitated by adding 1/10 volume of 3M NaOAc and 2.5 volumes 100% ethanol, pelleted 20' 12 krpm in an Eppendorf benchtop centrifuge, washed with 500 µl 70% ethanol, and resuspended in 20 µl TE. The resuspended DNA was electrophoresed on a 1% Seakem ME agarose gel (FMC)/0.5x TBE at a field strength of 2.5V/cm and then transferred to a Genescreen Plus nylon membrane and hybridized as described below under YAC Analysis. The membranes were washed under a final stringency of 0.1x SSC / 0.1% SDS at 60°C 20', then exposed to film for 1-2 days at -80°C.

Plasmid DNA Preparation

As described in [7], plasmid DNA preparation was by the alkaline lysis miniprep protocol. Briefly, bacterial cells were lysed in a NaOH/SDS solution, then neutralized with KAc. After centrifugation to pellet the precipitated proteins, genomic DNA, and other debris, the supernatant was isopropanol precipitated, washed in 100% and 70% ethanol, dried briefly, and resuspended in TE.

Sequencing

Unless otherwise noted, the sequencing described below was performed on PCR products amplified using M13-f and -r primers (see Appendix B) from 1/10 dilutions of 96-well-plate minipreps (alkaline lysis minipreps scaled down so that each prep is performed in a single well of a standard V-bottom 96-well plate; solutions are added or removed using multi-channel pipettors or by inverting the plate over absorbent material [8]), using 30 cycles of PCR with 50°C annealing temperature, and purified with Qia-Quickspin PCR spin columns (Qiagen) according to instructions (resuspending in 50 µl TE), or Ultrafree-MC filters -- 30,000 MW, PLTK membrane columns (Millipore) -- by diluting sample in 300 µl water, spinning, and resuspending in 25 µl TE. Cycle-sequencing reactions using M13-f or M13-r dye primers were performed exactly as recommended by ABI (5 µl and 10 µl reactions with 1 µl and 2 µl template PCR product DNA, respectively, cycle sequencing 15x 96°C 15s, 55°C 5s, 70°C 1'; 15x 96°C 15s, 55°C 1' on a PE9600 thermocycler) and run on an ABI-373A fluorescent sequencer. The resulting sequences were processed to remove vector or other contaminant sequences (e.g. hemoglobin exon sequences) using the computer programs EXSTRIP or FREEINST [9] and analyzed for overlaps using the programs COMPSEQ and COMP2SEQ[10]. Database searches to identify repeat sequences or homology with known genes were performed using Blast in both the email server (NCBI) and web (NCBI) versions[11].

YAC Analysis

YACs were isolated from the MIT mouse YAC library[12] using standard PCR screening methods and the YAC DNA prepared as described[13]. YAC end sequences were isolated using inverse PCR as previously described [14] and these few-hundred base-pair long end PCR

products sequenced directly using standard fluorescent sequencing methods as described above. See Chapter II for more details.

YACs were sized by preparing pulsed-field gel (PFG) quality YAC DNA[15] (briefly, the yeast are grown in AHC media, pelleted and rinsed, then spheroplasted using zymolyase; the spheroplasts are then mixed with an equal volume of molten low-melt 1% SeaPlaqueGTG agarose and formed into blocks. Once solidified, the blocks were digested in a Proteinase K/EDTA/beta-mercaptoethanol solution O/N 50°C and transferred to 0.5M EDTA for long-term storage. Immediately prior to use, blocks were equilibrated with desired buffer (e.g. 0.5x TBE)) and separating the intact chromosomes in the ~10kb - ~1200kb size range on a CHEF gel [16] using 1% SeaKem GTG agarose (FMC) gel in 0.5x TBE buffer with a ramped switch time of 40s-110s over 20 hours and a field strength of 6V/cm. The yeast DNA was then transferred and bound to a positively charged nylon membrane (Genescreen Plus, DuPont NEN) by an initial short-wave UV exposure in the presence of ethidium bromide and 40-hour capillary transfer with 0.4 N NaOH[17], and short-wave UV fixing to the membrane (Stratalinker auto setting, Stratagene). The YAC chromosomes were then identified by hybridization with ³²P-labelled[18] mouse C₀t-1 repetitive DNA (Gibco BRL) using essentially Church's protocol [19] and a final wash of 0.5x SSC / 0.1% SDS at 50°C. The membranes were exposed to film overnight at -80°C. Flanking yeast marker lanes (BioRad) and the faintly visible bands of the normal yeast chromosomes present in the YAC lanes were used to estimate the YAC chromosome sizes.

YAC walking was performed as follows: YAC ends were isolated and sequenced. Non-repetitive sequences were then used to create STSs, using the naming convention YYE2X, where YY is the YAC number, E is "L" for the YAC vector telomere arm designated as "left", and E is "R" for the YAC vector telomere arm designated as "right" [12], and 2X is "2" followed by a

single letter designating the enzyme used in the end-isolation inverse PCR. For example, the STS 17R2H is from the “right” end of YAC 17 using the *Hae*III restriction enzyme. These STSs were then tested for (a) their ability to successfully amplify mouse genomic DNA and (b) their concordance with mouse chromosome 6 markers in a hybrid cell line panel[20]. STSs that passed these criteria were then screened against the set of known YACs from the walk; those that did not amplify known YACs were inferred to extend outside the current contig limits and were thus used to rescreen the mouse YAC library. This cycle was then repeated using the newly identified YACs until the walk was closed by the coalescence of the YAC contigs into a single contig.

YAC subcloning into cosmids was performed as follows: (1) YAC block DNA partial digestion: one block (250 μ l = ~1.25 μ g DNA) of PFG-quality YAC DNA was successively equilibrated in 25 mM EDTA, 2 mM EDTA, and 1x New England Biolabs (NEB) restriction enzyme (RE) buffer #2. The block was placed in a 1.5 ml tube with 150 μ l 1x NEB RE buffer #2; the tube was incubated 10' 65°C to melt the agarose and cooled 10' to 37°C; 2 μ l beta-agarase (1 U/ μ l, NEB) were mixed in gently and thoroughly and incubated 20' 37°C to fully digest the agarose. 4 μ l of enzyme dilution (0.05 U/ μ l *Mbo*I (NEB), 0.5 mg/ml RNase A (USB) in NEB RE buffer #2) was then mixed in, the tube incubated 10' 37°C and the partial digestion stopped by the addition of 8 μ l 0.5 M EDTA. The DNA was then phenol/chloroform extracted, EtOH-precipitated, and resuspended in 50 μ l TE. (2) Size selection of partially-digested DNA: the partially-digested DNA was loaded and run on a 0.6% SeaPlaque GTG (FMC)/ 0.5x TBE prep gel and 2 V/cm 6 hours in the cold. Based on ethidium-stained flanking marker lanes, the exclusion band (>23 kb in size) was cut out, and this gel slice melted 10' 65°C and extracted with phenol at RT. Further phenol/chloroform extractions and EtOH precipitation was as above,

with the addition of 0.5 μ l 5 mg/ml glycogen (BMB) used as a carrier during EtOH precipitation. The size-selected DNA was resuspended in 25 μ l TE. (3) Cosmid vector preparation: 50 μ g SuperCos1 vector (Stratagene) was digested with 450 U *Xba*I (NEB) and 3 U CIP (NEB) in 1x NEB RE buffer #2 + BSA in 300 μ l total volume 70' 37°C. The reaction was stopped and the CIP inactivated by adding 10 μ l 0.5 M EDTA and incubating 10' 68°C; the vector was then EtOH precipitated and resuspended in 50 μ l TE. After verifying complete *Xba*I digestion and lack of significant self-ligation in a ligation assay, the cloning site was cut 60' 37°C using 2 U *Bam*HI (NEB) per μ g vector in NEB *Bam*HI RE buffer+BSA. After phenol/chloroform extraction, EtOH precipitation, and resuspension in 50 μ l TE, complete *Bam*HI digestion and ligatability of the *Bam*HI ends were verified using agarose gel electrophoresis and a self-ligation assay. (4) Ligation and packaging: ~1.25 μ g of size-selected DNA from (2) was ligated with 1.5 μ g prepared SuperCos1 vector from (3) overnight at 16°C using 40 NEB units of T4 DNA ligase (NEB) in the supplied ligation buffer. After EtOH precipitation and resuspension, the ligation was packaged using Gigapack II XL (Stratagene) according to the manufacturer's instructions, and stored in SM buffer over chloroform. (5) Transduction and identification of cosmids containing mouse DNA: XL-1 Blue MR cells (Stratagene) were prepared and transduced with the packaged cosmids according to manufacturer's instructions. In order to isolate cosmids containing mouse DNA rather than yeast genomic DNA (expected to be about 4% of the cosmids, assuming a YAC size of 700 kb), replica filters were made using standard procedures (DuPont Colony/Plaque Screen nylon filters, lifts as described[21], except that filters were pre-shrunk by autoclaving 1' 100°C on dry cycle, and processed after lifting by again autoclaving 1' 100°C on dry cycle to lyse and fix the clone DNA) and hybridized with either a mixture of mouse L1, B1 and B2 repeats, or with mouse C₀t-1 DNA (as described under YAC

sizing, above) to identify those cosmids containing mouse repeats, and thus, mouse genomic DNA.

P1 and BAC Analysis

Mouse bacteriophage P1 clones were isolated from two libraries: most from the P1 mouse RIII (2-3x coverage) and some additional clones from P1 mouse ES (3x coverage) libraries[22],[23] (Genome Systems). Mouse BAC clones were isolated from a library prepared by Bruce Birren (7x coverage)[24]. Each library was screened by a PCR-based or hybrid PCR- and hybridization- based protocol, as recommended by the library maker. P1 and BAC DNA was prepared according to standard protocols as recommended by Genome Systems. P1 end sequences were obtained using the protocol for cloning YAC ends[14], with modifications previously described[13]. STS content maps were assembled by using standard PCR techniques to determine the STS content of panels of miniprep DNA from the isolated P1s and BACs.

Exon Trapping

Exon trapping was performed on pools of cosmids using the pSPL3 plasmid essentially as previously described[25] using vector and reagents provided by Gibco BRL. RNA isolation was performed using Trizol (Gibco BRL) and cDNA synthesis and trapped-exon PCR were performed according to the manufacturer's recommendations. Putative exon PCR products were cloned in the pAmp10 plasmid vector using the uracil DNA glycosylase (UDG) cloning method (Gibco BRL).

cDNA Selection

cDNA selection was performed using a protocol modified from that of M. Lovett[26],[27] as follows: (1) Genomic template preparation: the genomic template DNA was

either a pool of mouse cosmids, or a CHEF-gel-purified mouse YAC band. This genomic DNA was separately digested to completion with the 3 blunt-cutting enzymes *AluI*, *HaeIII*, *RsaI* (NEB), and the inactivated digests pooled and ligated with a biotin-containing linker: Bio-Blunt-1 (5' biotin-gcggtagacccgggagatctgaattc 3') / Blunt-2 (5' gaattcagatc 3'). If necessary, this ligated genomic template was amplified by approximately 10 cycles of dual-temperature PCR (94°C/72°C) using the Bio-Blunt-1 primer, after an initial 5' extension at 65°C to extend the Blunt-2 ends, and purified using Qia-QuickSpin PCR (Qiagen; this product was used for all the other primer-removal and PCR-product-purifications in the protocol unless otherwise noted). (2) cDNA preparation: adult C57BL/6JEi mouse testis and thymus poly-A⁺-selected RNA was used as the template for cDNA synthesis. For each tissue, 1 µg of ds cDNA was synthesized using the SuperScript Choice system (Gibco BRL) with the supplied random primers producing an average cDNA fragment size of approx. 250bp. These fragments were then linkered with cDNA-1 (5' ctgagcggaattcgtgagacc 3') / cDNA-2 (5' P-ggtctcacgaattccgctcagtt 3'; note phosphate group on 5' end) and amplified 15 cycles with 64°C annealing temperature using the cDNA-1 primer. In addition, ribosomal cDNA for later use as a blocker was prepared and amplified from the poly-A⁺ selection flow-through RNA as above, then digested with *EcoRI* to remove the linkers. (3) Hybridization: 1 µg amplified cDNA + 2 µg mouse C0t-1 DNA (Gibco BRL) + 0.1 µg amplified ribosomal cDNA (to block selection by contaminating yeast ribosomal RNA genes) + 2 µg glycogen (BMB) were mixed, precipitated, resuspended, denatured, and prehybridized in 8 µl of Lovett's hybridization buffer at 65°C for 4-8 hours in order to anneal repeat and very-high-abundance cDNAs (the C₀t value of this prehybridization is 2x-4x greater than in Lovett's protocol. 0.1 µg amplified genomic template DNA + 2 µg glycogen (BMB) were mixed, precipitated, resuspended in 10 µl of hybridization buffer, denatured and snap-cooled on ice.

The prehybridized cDNA mixture was then mixed with the genomic template DNA and hybridized under oil 65°C 2.5-3 days. (4) Washing: 1 mg Dynabeads M280 streptavidin (Dyna) were blocked and washed in Bead Binding Buffer (BBB: 10 mM Tris pH7.5, 1 mM EDTA, 1 M NaCl) + 500 ng/ml mouse C0t-1 DNA + 1x BSA (NEB). The hybridization reaction was stopped by adding 50 µl BBB, then mixed with Dynabeads in 100 µl BBB. This mixture was incubated 15' RT to bind, then concentrated, and the beads washed 2x 15' RT in 1x SSC/0.1% SDS, and 3x 15' 65°C in 0.1x SSC/0.1% SDS. Selected cDNAs were eluted by adding 50 µl 50mM NaOH, mixing, inc. 10' RT. After concentration, supernatant containing eluted cDNAs was neutralized with 50 µl 1M Tris pH7 and purified in 50 µl TE. Eluted cDNA was amplified 30 cycles with 64°C annealing using the cDNA-1 primer, and rehybridized. After again washing as above it was amplified 30 cycles with 60°C annealing using the cDNA-U-1 primer (5' cuacuacuacua ctgagcgggaattcgtgagac 3'). (5) The purified double-selected cDNAs were then cloned into the pAMP10 vector (Gibco BRL) using the UDG cloning method with the dU-containing 5' ends created by the PCR with cDNA-U-1. Random clones were picked, minipreped, and sequenced as described above.

Results

Large-Scale Physical Map of the Lyp Region in the Mouse

The genetic markers in the mouse R236-*Npy* interval (described in Chapter II) were used to screen the MIT mouse YAC library. Since the rat R236 PCR assay works very poorly in the mouse, the D6Mit42 PCR assay (which flanks R236 on the far side from *Npy*) was used in its place as a proxy for the left (centromere-proximal) end of the interval. Due to the limited number of initial STSs available from the *Lyp* region, the YACs identified by this first library

screening did not form a single contig, so a YAC walking strategy was used to create a single contig that spanned the D6Mit42-*Npy* interval, as described in Materials and Methods. Genetic marker order information from Chapter II placed D6Mit43 between D6Mit42 and *Npy*, so the initial contig that contained both D6Mit42 and D6Mit43 needed to be extended only on the D6Mit43 side. The completed contig contains 24 YACs and spans approximately 2.5-3 megabases, based on YAC sizes and estimates of overlap (see Table 1, Figure 1). The *Lyp* region is at most ~2150 kb, using D6Mit42 and *Npy* to define the interval, and adding up the sizes for the overlapping YACs 31, 36, 43 and 27, without taking into account their degree of overlap.

YAC Number	Library Address	Size (kb)	"Left" Arm STS	"Right" Arm STS
35	22:2B6	650	repeat	n.d.
32	4:3C4	200	32L2T	repeat
41	4:6G7	120	repeat	41R2H
31	10:4G10	525	repeat	31R2H (partial L1)
70	25:6B1	825	n.d.	n.d.
68	10:2E1	>1250	n.d.	n.d.
56	8:7A8	600	repeat	56R2H
34	7:6H4	690	repeat	34R2A
57	6:6A12	1050	57L2H	repeat
36	5:5B9	600	36L2H	36R2A
61	23:8C11	90	n.d.	n.d.
60	21:5B6	700	n.d.	n.d.
44	7:7C12	600	44L2T	44R2H
43	5:2D4	500	repeat	43R2H
45	12:6H9	750	repeat	45R2H
27	5:8G9	540	27L2T	27R2H
15	9:2D3	775	repeat	15R2H
12	24:4A9	725	repeat	12R2H
17	7:7D9	975	17L2H (CT SSR)	17R2H (CA SSR)
53	24:8D12	625	n.d.	n.d.
50	24:5E2	400	n.d.	n.d.
46	7:6H5	650	n.d.	n.d.
47	12:3D9	700	n.d.	n.d.
63	17:5G2	n.d.	n.d.	n.d.

Table 1: List of mouse *Lyp* region YACs identified with library addresses, sizes, and "left" and "right" end STSs. These STSs were isolated from YAC ends using inverse PCR, as described in Materials and Methods. "repeat" indicates that the YAC end sequence was repetitive; named ends were not repetitive and were verified to map on mouse chromosome 6 using an RH panel; "n.d." ends not analyzed by inverse PCR. Note: YAC 17 contains *Npy*; since each YAC end also contains a polymorphic SSR, *Npy* must map between the genetic markers defined by those polymorphic SSRs.

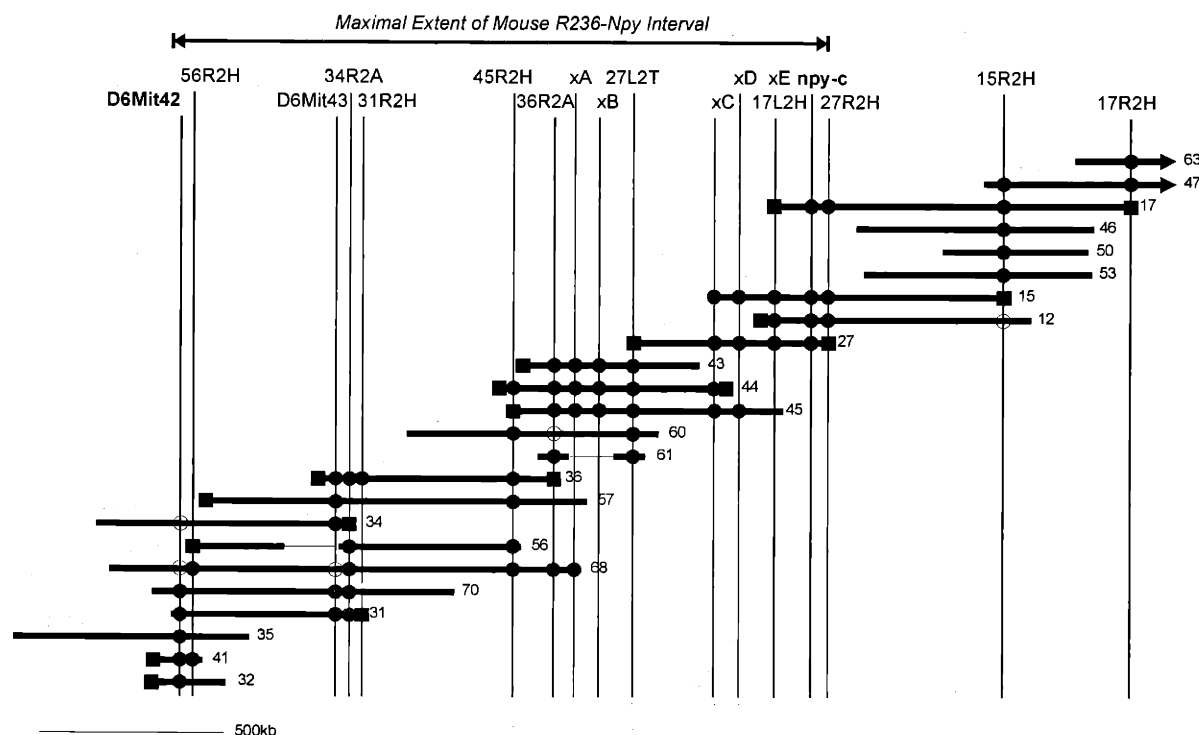


Figure 1: Diagram of STS content map of mouse YAC walk spanning mouse *Lyp* region. YACs are represented horizontally, approximately to scale; orientation of individual YACs is not determined, except when YAC end STS results are shown. STSs are represented as vertical lines. YACs containing a given STSs as shown by PCR are indicated by filled circles at the intersections of the STSs and YACs; those combinations not tested lack circles. YACs not containing a given STS are indicated by open circles. Verified non-chimeric YAC ends are indicated by filled squares; the status of the remaining ends is unknown: YAC end inverse PCR fragments were too short for analysis or contained only repeat sequences (e.g. L1, B1, B2), or were not analyzed (see Table 1). STSs are either MIT mouse genetic map markers (D6MitXX), assays derived from YAC ends (nomenclature in Materials and Methods) or transcript-based PCR assays (exon-trapping- and cDNA-selection-derived STSs (xA-E) and Npy-c, described in Chapter II). Transcript bins xA-E indicate the STS map positions of trapped exons and cDNAs selected from YAC 45 (bin mapping of individual clones indicated in clone listing in Appendix B); the mapping of later cDNA-selection-derived STSs is shown in Figure 2. The line at the top of the figure indicates the maximal extent of the region which is expected to contain the mouse ortholog of the *Lyp* gene.

STS Identification -- Exon Trapping

In order to identify STSs from the *Lyp* region, I first employed exon trapping on pools of mouse cosmid subcloned from a YAC in the center of the *Lyp* region, YAC 45. Table 2 summarizes the results of the exon trapping experiments performed (the non-vector and non-repetitive sequences identified are listed in Appendix B). Using 7 pools of 6 cosmids per experiment (3 for pool A), and grouping by sequence overlap, a total of 26 unique sequence groups were identified from 125 exons sequenced, and 512 exons initially screened. Probes from

12 out of the 15 unique sequence groups tested gave only one or two bands on B6 mouse Southern blots (not shown), verifying their non-repeat nature (further hybridization and other cross-species work on these clones is described in Chapter IV) As expected, STSs derived from the exon sequences were successfully amplified from YAC 45 (these mapped to bins xA, xB, xD and xE in Figure 1). The average size of the recovered exons was ~180 bp, although they ranged in size from 38 bp to 350 bp. The distribution of the recovered exons among the different unique sequence groups is shown in Table 2(b).

Pool	Cosmids/Pool	Putative Exons Examined	Non-Vector Exons (by size)	Exons Sequenced	Unique Sequence Groups (non-vector, non-repeat)
A	3	32	18	16	3 (19%)
E1	6	96	25	23	8 (35%)
F	6	192	37	35	6 (17%)
G	6	48	10	9	5 (55%)
H	6	48	28	25	3 (12%)
I	6	48	7	7	3 (43%)
J	6	48	11	10	4 (40%)
Totals	39	512	136	125	26* (21%)

Table 2(a): Summary of results of exon trapping on pools of mouse cosmids from YAC 45. Results by pools: random subclones from the exon trapping experiment (putative exons) were sized by PCR; those with inserts larger than the expected vector insert (non-vector exons by size) were then sequenced. Vector- and repeat- containing sequences were eliminated and the remaining sequences were then arranged into groups within pools based on sequence overlaps. The percentage of unique (non-overlapping) sequence groups among the exons sequenced is given in parentheses. * - Since some of sequence groups in different pools overlapped, the number of different sequence groups for the entire set of exon-trapping experiments is less than the sum of the poolwise-unique sequence groups.

Group Size	Number of Groups
1	11
2	5
3	3
4	3
5	2
12	1
15	1

Table 2(b): Summary of results of exon trapping on pools of mouse cosmids from YAC 45. Unique sequence groups from the set of all exons examined summarized according to the number of sequences per group.

STS Identification -- cDNA Selection

The second approach used to identify STSs from the *Lyp* region YACs was direct cDNA selection with genomic templates derived from the YAC walk. These experiments used pools of mouse cosmids subcloned from YAC 45 (the cosmids used in 45A and 45B together are the six cosmids used for pool I in the exon trapping described above) or the gel-purified mouse YACs themselves as the genomic templates (see Table 3 and Materials and Methods). For all the selections, random-primed primary cDNA from mouse testis and thymus was used as the cDNA source. Initial selection experiments on the cosmid pools and YAC 45 (45A, 45B, and 45_2 -- see Table 3) indicated that selection against a whole YAC rather than against cosmid subclones produced a greater diversity of selected cDNAs; therefore, later selection experiments were performed solely with mouse YACs as the genomic templates (ac_45, ac_44, ac_43, 34_36_56, and 31_35 -- see Table 3).

Since YAC 44 was the only one among the three overlapping YACs 43, 44, and 45 with both ends verified as non-chimeric, the bulk of the cDNAs selected from the middle of the *Lyp* region were selected by using it as the genomic template. STSs derived from selected cDNA sequences were successfully amplified from the YAC used as the template in their selection; some of these STSs and some of the exon-derived STSs were mapped on the YAC contig by STS content mapping and defined bins xA through xE of the contig (Figure 1). As described in Chapter IV, a rat cosmid containing the ortholog of one of these STSs, ajm207, was found to contain a simple-sequence repeat (SSR)-based genetic marker (207rpt1) that mapped to the right of *Lyp* in the rat, shortening the critical interval on the right. Ajm207 was mapped to region xB of the mouse YAC walk, excluding regions to the right from the mouse *Lyp* region; thus, further cDNA selection was limited to the genomic clones containing this bin and those to the left, bounded by

Experiment	cDNA Source	Selection Template	Selected cDNAs Sequenced	Non-Repeat Sequences	Unique Sequence Groups (non-repeat)	Singleton Groups	2-Sequence Groups	3-Sequence Groups	4-Sequence Groups
45A	B6 thymus + testis	3 mouse cosmids (YAC 45)	59	30	29 (49%)	28	1		
45B	"	3 mouse cosmids (YAC 45)	48	20	19 (40%)	18	1		
45_2	"	YAC 45	58	47	41 (71%)	37	3	0	1
ac45	"	YAC 45	60	37	34 (57%)	31	3		
ac44	"	YAC 44	130	121	104 (80%)	91	9	4	
ac43	"	YAC 43	38	31	30 (79%)	29	1		
34_36_56	"	YACs 34+36+56	146	94	86 (59%)	78	8		
31_35	"	YACs 31 + 35	142	99	87 (61%)	75	12		

Table 3: Summary of results of cDNA selection in the mouse *Lyp* region. Results by cDNA selection experiment: random subclones from the cDNA selection experiment were sequenced. Repeat-containing sequences were eliminated and the remaining sequences were then arranged into groups by experiment based on sequence overlaps. The percentage of unique (non-overlapping) non-repeat sequence groups among the cDNAs sequenced for a given experiment is given in parentheses.

	exons	45A	45B	45_2	ac45	ac44	ac43	34_36_56	31_35
exons	26	3	-	3	-	2	1	-	-
45A		29	-	1	-	-	2	-	-
45B			19	-	-	1	-	-	-
45_2				41	2	6	3	-	-
ac45					34	3	-	-	-
ac44						104	4	-	-
ac43							30	-	-
34_36_56								86	1
31_35									87

Table 4: Sequence overlaps between exons and/or cDNAs from the different experiments. The unique sequence groups shown in Table 3, plus the pooled set of all unique exon sequence groups from Table 2, were compared against each other as pairs of experiments. The number of sequence groups present in both experiments in the pair (that is, where sequence from a group in the first experiment overlapped with that from a group in the second experiment of the pair) is listed at the intersection of the row and column representing the two experiments. The diagonal contains the total number of unique sequence groups for each experiment, and a "-" indicates that no sequence groups from that pair of experiments were present in both experiments.

Experiment	Non-Repeat Sequences	Total Number of Sequence Overlaps	Average Sequence Length (bp)	Total Amount of Non-Overlapping Sequence (kb)	θ	n	c	Predicted Total Length of Transcribed Sequences in Subregion (kb)
45_2+ac45+ac44+ac43	236	124	240	~52	0.104	0.525	0.59	~88
34_36_56	94	16	234	~20	0.107	0.170	0.19	~105
31_35	99	24	234	~22	0.107	0.242	0.27	~81

Table 5(a): Estimation of the degree of coverage by selected cDNAs of three mouse *Lyp* subregions, using formula $c = n / (1 - \theta)$ restated from [37]. c is the estimated degree of coverage, while n is the average number of overlaps per cDNA fragment and θ is the minimum detectable fraction of overlap. The threshold for detection of overlap was 25 bp, so $\theta = \frac{25}{(\text{average fragment length})}$; $n = \frac{\sum (\text{overlaps per fragment})}{(\text{number of fragments})}$. The "Predicted Total Length ..." column was calculated by dividing the "Total Amount of Sequence" column by the estimated degree of coverage c .

Experiment	Predicted Number of Genes in Subregion	Predicted Degree of Gene Coverage by Selected cDNAs	Predicted Number of Genes Not Represented Among Selected cDNAs
45_2+ac45+ac44+ac43	29	8.1x	0
34_36_56	35	2.7x	2
31_35	27	3.7x	1

Table 5(b): Estimation of the coverage by selected cDNAs of the genes in the three mouse *Lyp* subregions. The second column was calculated by dividing the "Predicted Total Length ..." from part (a) by the assumed average transcript size of 3 kb. The third column was calculated using the number of cDNA sequences from each subregion in part (a) and the previously calculated number of genes. The last column represents a Poisson-distribution estimate of the fraction of genes with no hits (based on the degree of coverage in column three) multiplied by the estimated number of genes in column two.

D6Mit42 on the far left. In these cDNA selection experiments targeting the leftmost part of the mouse *Lyp* region, two or three overlapping YACs were pooled as genomic templates in order to minimize the chance of a subregion being excluded due to a rearrangement or deletion within a single YAC.

Overall, the average size of the selected cDNAs was ~242 bp, with a range from 133 bp to 438 bp (a listing of the selected cDNAs is in Appendix B). As with the exons, the selected cDNAs were compared against each other, as well as against the exons previously trapped, to identify groups of overlapping sequences (Table 3, Table 4).

Fine-Scale Physical Map of the Lyp Region in the Mouse

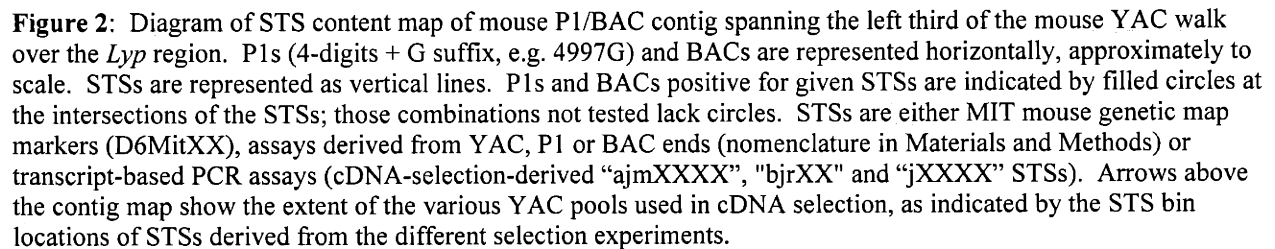
As described in chapter IV, the *Lyp* region had been limited to an interval corresponding to the region spanned by two sets of mouse YACs: 31/35 and 34/36/56. STSs were developed from the sequences of cDNAs selected from these two sets of YACs (and a few mouse genetic markers and YAC ends), and were used to screen two mouse P1 libraries (chiefly P1 mouse RIII, with P1 mouse ES for those STSs that did not produce clones from P1 mouse RIII -- see Materials and Methods). Typically, the library was screened with three or four mouse STSs at a time, the resulting P1 clones were then screened with the remaining mouse STSs, and the process was repeated. The P1 library screening produced 6 contigs containing, respectively from left/centromere-proximal to right/distal, 5, 3, 14, 2, 4, and 4 P1 clones, with, respectively, 7, 3, 22, 1, 2, and 4 STS bins per contig (see Figure 2, P1 genomic clones mostly in upper half of contig).

In order to connect the contigs, 53 BAC clones were isolated from the mouse BAC library (Materials and Methods) and 3 new STS bins were identified by using BAC clone end sequences (see Figure 2, BAC genomic clones mostly in lower half of contig). Most STS bins in

the central part of the contig contained more than one cDNA-based STS assay -- the complete ordered list of STSs in the bins from this region is listed in Table 6. The completed contig spans approximately one megabase.

STS Bin	STSs Mapped to Bin
D6Mit42	D6Mit42, 4825Gl
3935	ajm3935, ajm5402, ajm5436, ajm5708, ajm5729, ajm5932, ajm3916b
5422	ajm5422, 4825Gr
5414	ajm5414, ajm5709, ajm5928, ajm4303
5704	ajm5704, ajm3921, ajm4306c
5201	ajm5201, ajm5415, ajm5421, ajm5705, ajm5733, jln0236b
3905	ajm3905, ajm4133, ajm5433, ajm5723, ajm5820
5406b	ajm5406b
279 N21 SP6	279 N21 SP6
25 G7 SP6	25 G7 SP6
4318	ajm4318
4121	ajm4121, ajm5223, ajm5805, ajm5823, jln0129, 4866Gr
6110	ajm6110
5227	ajm5227
3801	ajm3801, ajm3806, ajm3811, ajm3910, ajm4123, ajm4125, ajm4313, ajm5222
3907	ajm3907
5207	ajm5207, ajm5221, ajm5224, ajm5813
3808	ajm3808
5808	ajm5808, jln0214, ajm4124, ajm3908
D6Mit75	D6Mit75
3810	ajm3810, ajm5206
5806	ajm5806, jln0210
jln0202	jln0202
5220	ajm5220
D6Mit314	D6Mit314
3804	ajm3804
3809	ajm3809
5814	ajm5814, ajm4126
D6Mit74	D6Mit74
3805	ajm3805
6005	ajm6005
5811	ajm5811, 6350Gr

Table 6: Ordered list of mouse STS bins spanning the central *Lyp* region from D6Mit42 through 5811 (left to right in Figure 2), including all mouse STSs mapped to each bin. "ajmXXXX" and "jlnXXXX" indicate mouse cDNAs obtained by cDNA selection. "D6MitXX" STSs are MIT mouse map genetic markers, while "XXXXGl/r" are mouse P1 genomic clone end assays ("l" for left end, "r" for right end) from the eponymous P1 clones.



completion of a mammalian genome sequence is still some years away. As work progresses toward that goal, however, a useful mapping infrastructure is being constructed. Although in the case of the mouse *Lyp* region none was available, in organisms such as mouse and man, large-scale physical maps and, in some cases, fine-scale physical maps and sequence contigs are becoming available (see, for example, the human chromosome 7p conservation of linkage region described in Chapter IV). Thus, positional cloning work is already beginning to concentrate on the identification of genes within a well-mapped region, and on the identification of the desired gene among that set of genes.

STSs as Gene Candidates

Since STSs are already present in a physical map, it would be useful if they could serve as gene candidates as well. However, the ESTs used to construct a physical do not generally constitute a comprehensive representation of all genes in the region. Thus, for a complete set of candidate genes in a region, a targeted gene identification approach must still be used, at least until genomic sequence becomes available.

As described in this chapter, I chose to identify a dense set of gene-based STSs in order to be able to use the same STSs both as markers to order the physical maps and as candidates for the *Lyp* gene. In addition, as described in Chapter IV, gene-based STSs are very important in transferring positional information between species using the conservation-of-linkage strategy. I employed two methods to identify gene-based STSs: exon trapping and cDNA direct selection.

Exon Trapping

Gene identification approaches may be evaluated on the basis of their completeness, their specificity and their bias. In positional cloning, *completeness* (i.e. identifying *every* gene in the target set) is important, because typical positional cloning projects end up with perhaps 20

candidate genes in the region of interest; overlooking a gene can thus lead to a 5% chance of missing the target gene of the positional cloning project. *Specificity* (i.e. identifying genes that *are* in the target set) is important from an efficiency standpoint: if only one out of four genes identified is in the target set, extra effort goes into winnowing away the other three. *Bias* (i.e. over- or under- representation of some genes) should obviously to be minimized. The ideal approach will have high specificity and completeness, so that the targets are all identified with few additional non-target sequences, and low bias, so that the targets are each present in similar proportions.

Exon trapping is a technique for identifying genes using genomic DNA, by identifying putative internal exons as the fragments remaining after a genomic DNA fragment has been transcribed and has undergone splicing in an artificial splicing vector. [31],[32] In principle, this technique has a great advantage: it requires only genomic DNA, avoiding the inherently biased populations of transcripts in mRNA. (This may, however, be a disadvantage as well, inasmuch as the bias in mRNA populations may in some cases improve specificity: thymic mRNA is likely to be more specific for T-cell genes than the results of exon trapping). In addition, exon trapping has two major disadvantages: (1) In excluding those genes that have no internal exons, it sacrifices completeness (in particular, the PA gene discussed in Chapter V appears to have no internal exons and is thus missed with exon trapping) (2) it usually does not scale well with the size of the region to be examined -- a large genomic fragment often yields significantly fewer distinct sequences than if it were cut in half and each half exon trapped separately. However, a number of groups have used the technique with some success[33],[34],[25],[35].

As shown in Table 2a, using the pSPL3 exon trapping system did provide a number of useful putative exons. Although the number of clones sequenced varied from pool to pool, the

yield of unique, non-repetitive clones was approximately one per cosmid in the source pool of genomic DNA. Since we expect approximately 1 gene per 30kb of genomic DNA (100,000 genes per 3×10^6 kb genomic DNA), this suggests a yield of approximately 1 exon per 1.5 genes. However, the low complexity of the selection products from each experiment is evident: increasing the number of clones sequenced did not substantially increase the number of unique sequence groups in that experiment (see especially pools F and H). The concentration of 27 (21%) of the sequences in just 2 unique sequence groups (see Table 2(b)) also indicates a substantial bias in favor of some genes.

In addition, the SPL3 exon trapping protocol is quite time-consuming, as one must first subclone a YAC into cosmids, then subclone a pool of cosmids into the trapping vector, then transform cos cells, prepare RNA, perform RT-PCR, and screen (rather than select) for non-vector products. To some degree, a different exon trapping vector, as referenced above, may improve on the complexity of sequences recovered and simplify the actual trapping process, but the exclusion of genes with fewer than two introns and the cumbersome nature of the trapping protocol prompted another choice for this work: cDNA direct selection.

cDNA Direct Selection

cDNA direct selection[26],[27] uses a cloned genomic region as a template for directly selecting matching cDNAs from a large pool of cDNAs. As shown in Table 3, the complexity of the cDNA fragments recovered is excellent, even for selections against large genomic targets such as 750kb YAC clones, and is much less labor-intensive than exon trapping. However, since the technique selects from a pool of expressed sequences, lack of completeness and bias are potential problems. There are three major potential problems that are relevant in this case.

In the first, if the gene of interest is not expressed in the tissue used to create the cDNA pool, the gene cannot be recovered. In the case of *Lyp*, however, there is good evidence that the gene is expressed at least in the thymus (see Chapter I). To the extent that the starting pool of cDNAs can be limited in complexity, while still containing the gene of interest, the specificity of the gene identification process is improved. Thus, having a specific tissue in which *Lyp* is expected to be expressed probably improves the specificity of cDNA selection over exon trapping.

The second potential problem in cDNA selection is the potential bias introduced by the wide variety of gene expression levels in the starting pool of cDNA. In this protocol, such bias is minimized by limiting the amount of genomic template DNA relative to the amount of cDNA so that even moderate-abundance cDNAs saturate the genomic template available and are not recovered in excessive numbers. [36] The data in Table 3 support this, as any highly over-represented gene would lead to a unique sequence group containing a much higher-than-expected number of cDNA fragments, as with the 2 exon-trapping sequence groups containing 12 and 15 sequences (Table 2b). The one unique sequence group composed of four cDNA fragments in the 45_2 experiment was the only possible example of this situation in the *Lyp* region selection experiments, suggesting that overabundance bias was not a major problem. On the other hand, bias caused by low representation of a particular cDNA in the starting pool is only indirectly compensated for in the cDNA selection technique by limiting the recovery of high abundance cDNAs, and remains a disadvantage in cDNA selection versus exon trapping. In this work, I judged the possibility of missing a gene due to the lack of internal exons or due to the relatively low complexity of the trapped exon fragment pools more significant than the possibility of being

unable to recover it using cDNA selection, and so emphasized cDNA selection as the source procedure for the bulk of the STSs generated.

The third potential problem (which affects exon trapping equally), is the fidelity of the genomic DNA used to specify the region of interest: any deletions will result in missed genes. YACs, especially, are often prone to infidelity, so the cDNA selection performed on the *Lyp* region used multiple YACs as the genomic templates for each region: YACs 43, 44 and 45 for the middle; YACs 34, 36 and 56 to the left of those; YACs 31 and 35 on the far left.

In the YAC-based selections, the yield of unique sequence groups was 60-80% of the sequences sequenced, much better than the 12-55% (21% overall) for exon trapping. In addition, since the starting cDNA pool was the same for all the selections, the incremental effort involved in doing an additional selection was only the effort in YAC clone preparation and the actual selection -- a 5x to 25x improvement over exon trapping.

Estimate of Transcript Coverage by Selected cDNAs

An important characteristic of the set of gene-based STSs is the degree of coverage it provides of the genes within the region. A preliminary estimate of the fraction of the transcribed portion of the genomic region represented in the selected cDNAs can be calculated by considering the sequence overlaps among selected cDNAs. If we consider the transcribed portion of the region as a single interval, and the selected cDNAs as random fragments from that interval, we can make use of the formulas developed in [37] to predict the degree of coverage of the interval. The formula $n = c(1 - \theta)$, where n is the expected number of overlaps per fragment, c is the degree of coverage of the interval, and θ is the minimum detectable fraction of overlap, can be restated to solve for c as follows: $c = n / (1 - \theta)$. Thus, we can estimate the degree of coverage of the interval by counting the average number of overlaps per selected cDNA

fragment. Table 5 shows the results of this calculation for the three groups of cDNA selection fragments from experiments 45_2+ac45+ac44+ac43 (pooled), 34_36_56, and 31_35. Based on the predicted degree of coverage and the amount of cDNA sequence that degree of coverage represents (the total amount of sequence recovered in each experiment), the final column in the table lists the predicted total size of the transcribed portion of each subregion.

The size of the genomic region spanned by the YACs used to select each of the three sets of cDNAs should be approximately 750-1000kb, based on the ~500-750kb size of the individual YACs. Naively, we would expect up to 10% of the region to be transcribed if we assume the genome contains 100,000 genes with an average transcript size of 3,000 bp in a 3,000 Mbp genome. In reality, only a subset of these genes will be transcribed in the tissue source examined. On the other hand, any unspliced mRNA incorporated into cDNA would be expected to increase this size. The 81kb, 88kb, and 105kb predicted transcribed region sizes (Table 5a) seem perhaps a bit high and may suggest an above-average gene density in this region.

Using these estimates and an estimated average transcript size of 3kb, we can make a conservative (that is, high) estimate of the number of genes expressed in each subregion in thymus and/or testis. As shown in Table 5(b), moving from right to left in the region, we estimate 29 genes in the YAC 43+44+45 region, 35 genes in the YAC 34+36+56 region, and 27 genes in the YAC 31+35 region. Using this estimate and assuming that the cDNA fragments are randomly distributed among the genes, the count of cDNA fragments per gene should follow a Poisson distribution. As shown in Table 5(b), the number of expressed genes we expect to have missed (that is, containing none of the selected cDNA fragments) is at most 2 per subregion, even given the probable overestimate of the count of expressed genes in each subregion.

Additional tests of the degree of gene coverage support these estimates, and are described in Chapter V.

Conclusion

cDNA selection is a robust, efficient method of generating gene-based STSs. Further analysis in Chapter V will provide additional evidence that coverage of genes in the region of interest is quite complete. Construction of large-scale and fine-scale physical maps of the mouse *Lyp* region, along with a diverse set of fragments of candidate genes from that region, completes the initial phase of the conservation-of-linkage positional cloning strategy -- using the extensive positional cloning resources in the mouse to isolate the DNA of the mouse *Lyp* region. The next phases are described in Chapter IV -- the transfer of positional and information between mouse and rat, and Chapter V -- characterization of the genes in the *Lyp* region.

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CHAPTER IV

Transferring Positional Information Across Species -- Use of Mouse Maps and COL to Create and Integrate a High-Resolution Genetic Map of the Rat *Lyp* Region

Introduction

The successful execution of a conservation-of-linkage (COL) cloning strategy requires that one be able to transfer positional and gene information between related species in order to identify in the second species cognates to the markers and genes identified in the first species. This allows one to continually verify that COL is maintained as finer and finer levels of resolution are achieved, and thus to integrate positional information (e.g. recombination breakpoints defining a gene interval in the rat) with STS and gene information (e.g. random genomic fragments or transcription units identified within a physical interval in the mouse). Ideally, we would like to extend the genetic mapping information described in Chapter II and use that information to define a region containing a set of candidate genes for *Lyp* (see Chapter V). The process of narrowing the critical region for a gene through genetic mapping is typically an iterative process: as genetic markers define the interval containing the gene of interest, that interval is more finely physically mapped and new genetic markers are generated from those physical resources. Eventually, once the genetic interval has been narrowed to the point where it is expected to contain sufficiently few genes to allow analysis of the individual genes, the effort focuses on identifying and analyzing the transcription units (the gene candidates) within that interval.

Genetic markers and physical maps within the region of interest come both as pre-existing ones from the mapping infrastructure already developed for the organism, and as those that must be newly created. In the case of the *Lyp* project, Chapter II illustrates the advantages of a pre-existing infrastructure: almost all the genetic markers and mapping information described there (for both mouse and rat) had been previously developed. Even the new genetic mapping information described there (e.g. *Npy* mapped in the mouse and *HoxA2* mapped in the rat) was generated in part through the use of sequence information from related species (i.e. the mouse *Npy* RFLP was identified using a rat *Npy* cDNA probe, and the rat *HoxA2* SSR PCR assay was developed from mouse *HoxA2* genomic sequence). Chapter III illustrates the second situation, where physical maps were newly created for the mouse *Lyp* region.

In the COL strategy adopted for this project, creating a fine-scale rat genetic map of the *Lyp* region was the first key step in the project to require the use of COL. Given the lack of rat physical mapping resources at the beginning of this project (no rat YAC library, limited coverage rat P1 library), directly generating genetic markers from a set of rat clones comprising a physical map of the rat *Lyp* region was impractical. Thus, taking advantage of COL with the mouse by generating rat *Lyp* region genetic markers from the mouse *Lyp* region physical map was key to the progress of the project. In this chapter, I describe my use of the mouse *Lyp* region physical map to generate a set of genetic markers targeted to the rat *Lyp* region. This rat *Lyp* region genetic map was integrated with the mouse *Lyp* region physical map through the ortholog-ortholog relationship of the mouse and rat cDNAs used to create it, and was then used to rapidly isolate a contig of rat YACs spanning the region as the rat YAC library recently became available. Finally, additional rat genetic markers were generated from the rat YACs in order to define the rat *Lyp* interval more precisely.

Strategy

As summarized above, creating a rat physical map directly from the R236 and *Npy* markers to span the 1 cM *Lyp* interval in the rat was impractical due to the lack of high-coverage rat YAC, BAC, or P1 clone libraries. Thus, a high-resolution rat genetic map could not be constructed directly from an underlying physical map. Similarly, as described in Chapter II, creating rat SSR genetic markers directly from anonymous mouse SSR genetic markers was unsuccessful. This was presumably due to the sequence divergence between mouse and rat, especially outside genes, and to the greater sensitivity to sequence divergence of PCR as opposed to hybridization techniques.

Therefore, I reasoned that the following strategy would be most effective: make use of sequence conservation to isolate the rat orthologs of sequences from the mouse physical map of the *Lyp* region. Then, use these rat sequences to identify rat genetic markers nearby; these rat genetic markers should map to the rat *Lyp* region and produce a high-resolution genetic map of that region. Specifically, I proposed to use hybridization techniques (with their greater tolerance for sequence divergence) in the first step of identifying rat orthologs, while using standard higher-throughput PCR techniques in the later steps of identifying rat genomic clones and genetic markers, once the orthologous rat sequences had been obtained.

Given a fragment of rat DNA, the techniques for identifying a rat genetic marker nearby are established, if at times cumbersome: isolate a genomic clone containing that rat sequence (e.g. a P1 clone) and identify genetic-marker-containing subclones of that genomic clone by screening for SSRs.

Thus, the challenge was in isolating rat DNA sequences that were the orthologs of mouse sequences from the mouse *Lyp* region. As the techniques for efficiently identifying the rat

ortholog of a fragment of mouse DNA had not been well-studied, I investigated four different approaches before settling on cross-species cDNA selection.

Finally, as a high-coverage genomic rat YAC library became available[1], the *Lyp* region rat YAC contig created from it was used to increase the number of genetic markers and to further narrow the rat *Lyp* interval.

Materials and Methods

PCR Analysis

Unless pre-existing or otherwise noted, PCR primers were selected using the Primer 0.5 [2] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. STS and SSR marker assay primer information is listed in Appendix A, while exon and cDNA fragment information is listed in Appendix B. PCR amplification was performed according to the conditions specified for each protocol (e.g. YAC library screening), or, if not specified, according to standard conditions as recommended by Perkin-Elmer (see Chapter II). SSR genotyping was as described previously[3] and in Chapter II, with the occasional exception of alternate annealing temperatures (standard temp. 60°C), as listed for each primer pair.

Animals

The rat BB-DpxBB-Dr F₂ intercross[3], DpxFischer F₂ intercross[3], and DpxDr cross-intercross[4] have been described previously and in Chapter II.

Southern Blot Analysis

Genomic DNA was prepared from mouse or rat livers using standard methods[5] as described in Chapter III. Unless otherwise noted, genomic Southern blots consisted of a marker lane, C57BL/6JEi mouse genomic DNA, Fischer F344 rat genomic DNA and Lewis rat genomic DNA. 20 µg of genomic DNA per lane was digested to completion using *EcoRI* (NEB) according to the manufacturer's instructions (see Chapter III). The digested DNA was electrophoresed on a 1% Seakem ME agarose gel (FMC)/0.5x TBE and transferred to a Genescreen Plus nylon membrane and hybridized as described below under Rat YAC Library. The membranes were washed under a final stringency of 0.1x SSC / 0.1% SDS at 60°C 20' (for cross-species work, such as mouse probes on a rat Southern blot, hybridization was carried out at 55°C to 60°C and final wash stringency was 0.5x SSC / 0.1% SDS at 55°C 20'), then exposed to film for 1-2 days at -80°C.

Sequencing

Unless otherwise noted, sequencing was performed on PCR products amplified using M13-f and -r primers (see Appendix A) from 1/10 dilutions of 96-well-plate minipreps[6], using 30 cycles of PCR with 50°C annealing temperature, and purified with Qia-Quickspin PCR (Qiagen) according to instructions, or Ultrafree-MC filters, 30,000 MW, PLTK membrane columns (Millipore) by diluting in 300 µl water, spinning, and resuspending in 25 µl TE. Cycle-sequencing reactions using M13-f or M13-r dye primers were performed according to recommended conditions (ABI) and run on an ABI-373A fluorescent sequencer. (Further details appear in the Materials and Methods for Chapter III) The resulting sequences were processed to remove vector or other contaminant sequences (e.g. hemoglobin exon sequences) using the computer programs EXSTRIP or FREEINST[7] and analyzed for overlaps using the programs

COMPSEQ and COMP2SEQ[8]. Database searches to identify repeat sequences or homology with known genes were performed using Blast in both the email server (NCBI) and web (NCBI) versions[9].

RH Mapping

A rat radiation hybrid (RH) mapping panel of hybrid cell lines is now available (T55 panel created in Peter Goodfellow's lab; available as RH07 from Research Genetics), but no final genome-wide framework has yet been established for it. Thus, in order to determine whether or not a given rat STS was in or near the *Lyp* region on chromosome 4, the panel was typed with a number of markers including R236, Npy-c, 5579CA1b, 5631CA3b/3c, ajm5513, and 207rpt1 in order to establish a reference set of typings for the *Lyp* region. As expected, these markers shared most typings in common, with the most differences between R236 and Npy-c. Typing was performed by PCR amplifying 2 µl of hybrid cell line DNA (50 ng) in a 10 µl reaction under standard conditions 35 cycles using the predicted primer melting temperature as the annealing temperature. 10 µl of each resulting PCR reaction was electrophoresed on a standard 2.5% Seakem ME agarose/0.5x TBE gel, stained with ethidium bromide, and photographed over shortwave UV transillumination.

New markers were tested for proximity to the *Lyp* region by comparing their typings with the reference set of typings -- a high degree of concordance indicated a physical map location in or near the rat *Lyp* region.

Rat Genomic Cosmid and P1 Libraries

A Wistar Kyoto male genomic rat cosmid library constructed in the SuperCos-1 vector (Stratagene) was purchased from Stratagene (#961502; 7x predicted genomic coverage). To

simplify screening, ~1.1x genomic coverage (total) was plated out on 23 LB-kanamycin plates, and the resulting colony material from each plate was collected by rinsing with LB and scraping. These 23 plate pools were then processed as follows: 2 aliquots from each pool each representing 5% of the collected material were mixed with equal volumes freezing medium (40% glycerol, 1x LB) and stored at -80°C. The remaining cosmid bacteria from each pool were processed as standard cosmid minipreps using the alkaline lysis method ([10], see Chapter III) with great care not to shear the cosmid DNA, to isolate the cosmid DNA from each pool. 1/5th of each cosmid miniprep was then digested to completion with 600U *EcoRI* (NEB) 3 hours at 37°C. These digested pooled cosmid DNAs were then used to make 4 duplicate Southern blots from standard 0.8% agarose gels (see Southern Blot Analysis above) with the following lanes: 10 µg mouse C57Bl6/J-*EcoRI* digested genomic DNA, 10 µg rat Fischer F344-*EcoRI* digested genomic DNA, 23 lanes of pooled cosmid-*EcoRI* digested miniprep DNA (1/4 of each digest per lane). Thus, this plating of the rat genomic cosmid library could be screened by hybridization as follows: (1) hybridize one of the Southern blots with the desired probe to identify which of the 23 pools contain cosmids positive for the probe and (2) plate out a sample of the frozen pooled cosmid stock for those positive pools for screening by standard filter lift methods (replica filters were made using standard procedures using DuPont Colony/Plaque Screen nylon filters, lifts as described[11], except that filters were pre-shrunk by autoclaving 1' 100°C on dry cycle, and processed after lifting by again autoclaving 1' 100°C on dry cycle to lyse and fix the clone DNA, then hybridized as described above). The plating could also be screened by PCR by replacing the hybridization in step (1) with PCR of the 23 individual pooled cosmid minipreps.

Rat bacteriophage P1 clones were isolated from a Sprague-Dawley P1 rat spleen library[12] (< 2.5x coverage) (available from Genome Systems). The library was screened by a

PCR-based or hybrid PCR- and hybridization- based protocol by the library maker (briefly, P1 clones were pooled as described in the previous paragraph, but in much larger scale. Initial screening was by either PCR of a panel of clone pools or by hybridization to DNA of the clone pools spotted on a nylon filter. The final round of screening was by hybridization to a set of filters lifted from the plating of a single pool of P1 clones, again as described above for the cosmid library). P1 DNA was prepared according to standard protocols as recommended by Genome Systems (again, using the alkaline lysis miniprep protocol, but using unsaturated cultures and great care to minimize any shearing; see Chapter III).

Rat YAC Library

YACs were isolated from the Whitehead Institute rat YAC library[1] using standard PCR screening methods and the YAC DNA prepared as described[13]. See Chapter II for more details.

YACs were sized by preparing pulsed-field gel (PFG) quality YAC block DNA[14](see Chapter III) and separating the intact chromosomes in the ~10kb - ~1200kb size range on a CHEF gel [15] using 1% SeaKem GTG agarose (FMC) gel in 0.5x TBE buffer with a ramped switch time of 40s-110s over 20 hours and a field strength of 6V/cm. The yeast DNA was then transferred and bound to a positively charged nylon membrane (Genescreen Plus, DuPont NEN) by an initial short-wave UV exposure in the presence of ethidium bromide and 40-hour capillary transfer with 0.4 N NaOH[16], and short-wave UV fixing to the membrane (Stratalinker auto setting, Stratagene). The YAC chromosomes were then identified by hybridization with ³²P-labelled[17] mouse C₀t-1 repetitive DNA (Gibco BRL) using essentially Church's protocol [18] and a final wash of 0.5x SSC / 0.1% SDS at 50°C. The membranes were exposed to film overnight at -80°C. Flanking yeast marker lanes (BioRad) and the faintly visible bands of the

normal yeast chromosomes present in the YAC lanes were used to estimate the YAC chromosome sizes.

YAC chromosomes were isolated by performing preparative CHEF electrophoresis using the same DNA block preparation and CHEF electrophoresis protocol as described above. For the yeast marker lane, and for each YAC-containing lane, the lane margins were cut away and soaked in ethidium-bromide solution so that the yeast and YAC chromosome bands would be visible under UV light. For each YAC lane, the position of the YAC band within that lane was determined by this UV inspection; the corresponding section of the unstained center strip of each lane was then excised and stored at 4°C. Due to co-migration of the YAC with similarly-sized endogenous yeast chromosomes, the excised gel slices often contained one or two yeast chromosomes in addition to the YAC.

Random rat subclones of the gel-isolated YAC bands were generated by cloning blunt-cutting 4-base-recognition restriction enzyme fragments of the YAC as follows: (1) YAC DNA digestion: three 100 µl aliquots of agarose gel slice containing the YAC band were equilibrated with the supplied digestion buffers for the three restriction enzymes *AluI*, *HaeIII*, and *RsaI* (NEB), diluted 2x with 1x restriction buffer, then melted and cooled to 37°C, and digested 4 hours with 10 U of the appropriate restriction enzyme. Remaining agarose was macerated and removed by centrifugation through Spin-X 0.22 micron pore size spin filter membranes (Costar) and the digested DNA then purified using the QiaQuickSpin PCR column purification system (Qiagen) and the three purified digests for each YAC band pooled together into a single mixture. (2) Linker ligation: 1/6 of each DNA digest mixture (~50 ng) was ligated with 10 pmol of annealed cDNA-b linkers (cDNA-1b/cDNA-2b; predicted 40x molar excess of linkers over YAC DNA fragments) 3 hours RT and QiaQuickSpin PCR purified. (3) dU-tail incorporation: the

linked fragments were PCR amplified 5-10 cycles with cDNA-U-2 primer at 60°C annealing temperature in order to provide the correct dU-containing fragment ends for (4) cloning into pAMP10 using the UDG cloning system (Life Technologies).

Assembly of the rat YAC contig was performed as follows: rat STS assays previously isolated from the *Lyp* region were used to screen the rat YAC library and identify an initial set of overlaps between the YAC clones. Selected YACs were then subcloned into small fragments (~500bp; as described above) which were sequenced. Using BLAST[9] to compare the resulting sequences with the complete database sequence of the *S. cerevisiae* genome allowed contaminating yeast genomic fragments (typically 50-80% of the total) to be discarded. The remaining clones' sequences were then used to develop additional STSs which were PCR amplified against the set of isolated rat YACs and/or used to screen the rat YAC library.

SSR Isolation From Genomic Clones

SSR isolation for the construction of genetic markers was performed as follows: cosmid, P1 clone, or YAC DNA was subcloned into random small fragments as described under "Rat YAC Library" above. The resulting small-insert plasmid library was screened for (CA)_n and (GA)_n-containing clones by hybridizing replica lift filters (Colony/Plaque Screen membranes, pre-shrunk by autoclaving 1' 100°C, lifts processed by autoclaving 1' 100°C; DuPont/NEN) with (CA)₁₀ and (GA)₁₀ ³²P end-labeled oligos as described above, and washing with 2x SSC/0.1% SDS 50°C 30'. Positive clones were picked and sequenced; flanking oligos were ordered for clones containing SSRs in order to create SSR genetic markers.

Cross-Species cDNA Selection

"Cross-Species" cDNA Selection was performed using a protocol modified from that for the cDNA selection described in Chapter III. In brief, cross-species cDNA selection uses a set of template cDNAs from one species (rather than genomic DNA fragments as in normal cDNA selection) to select cDNAs with a high degree of sequence similarity from a pool of cDNAs from a different species. The degree of sequence similarity between the selected cDNAs and the cognate template cDNAs can be varied by varying the wash conditions (see Results).

Cross-species cDNA selection was performed as follows: (1) Template cDNA preparation: all template cDNAs used were mouse cDNAs obtained through cDNA selection as described in Chapter III; these cDNAs thus already contained the cDNA-1 linker sequences at their ends. In order to biotinylate them for use as selection templates, each cDNA was separately PCR amplified 10-15 cycles (94°C /64°C /72°C) with the bio-cDNA-1 primer (5' biotin-ctgagcgggaattcgtgagacc 3'; 64.4°C predicted melting temperature) and purified using QiaQuickSpin PCR (Qiagen; this product was used for all the other primer-removal and PCR-product-purifications in the protocol unless otherwise noted). (2) cDNA preparation: adult Sprague-Dawley outbred adult rat brain, spleen and testis double-poly-A⁺-selected RNA (Clontech) was used as the template for cDNA synthesis. For each tissue, 1 µg of ds cDNA was synthesized using the SuperScript Choice system (Gibco BRL) with the supplied random primers (500ng per 1 µg RNA), producing an average cDNA fragment size of approx. 500bp. These fragments were then linkerized with cDNA-1b (5' gtcacgcaagcttctcacagg 3') / cDNA-2b (5' P-cctgtgagaagcttgctgactt 3'; note phosphate group on 5' end and sequence differences from cDNA-1/cDNA-2 oligos in order to prevent linker-based cross-hybridization) and amplified 20 cycles with 63°C annealing temperature using the cDNA-1b primer. (3) Hybridization: 1 µg

amplified cDNA + 2 μ g mouse C₀t-1 DNA (Gibco BRL) + 2 μ g glycogen (BMB) were mixed, precipitated, resuspended, denatured, and prehybridized in 8 μ l of Lovett's hybridization buffer at 55°C for 4-8 hours in order to anneal repeat and very-high-abundance cDNAs (the C₀t value of this prehybridization is 2x-4x greater than in Lovett's protocol. 2.5 ng of each of the biotinylated mouse cDNAs prepared in step (1) was pooled and 2 μ g glycogen (BMB) was added; the mixture was precipitated, resuspended in 10 μ l of hybridization buffer, denatured and snap-cooled on ice. The prehybridized rat cDNA mixture was then mixed with the mouse template cDNA and hybridized under oil 55°C 2.5-3 days. (4) Washing: 1 mg Dynabeads M280 streptavidin (Dynal) were blocked and washed in Bead Binding Buffer (BBB: 10 mM Tris pH7.5, 1 mM EDTA, 1 M NaCl) + 500 ng/ml mouse C₀t-1 DNA + 1x BSA (NEB). The hybridization reaction was stopped by adding 50 μ l BBB, then mixed with Dynabeads in 100 μ l BBB. This mixture was incubated 15' RT to bind, then concentrated, and the beads washed 2x 15' RT in 1x SSC/0.1% SDS, and 3x 15' in 0.1x SSC/0.1% SDS at one of the three wash temperatures 65°C, 55°C, or 50°C, depending on the stringency desired. Selected cDNAs were eluted by adding 50 μ l 50mM NaOH, mixing, inc. 10' RT. After concentration, supernatant containing eluted cDNAs was neutralized with 50 μ l 1M Tris pH7 and purified in 50 μ l TE. Eluted cDNA was amplified 30 cycles with 63°C annealing using the cDNA-1b primer, and rehybridized. After again washing as above it was amplified 30 cycles with 60°C annealing using the cDNA-U-2 primer (5' cuacuacuacua gtcacgcaagcttctcacag 3'). (5) The purified double-selected cDNAs were then cloned into the pAMP10 vector (Gibco BRL) using the UDG cloning method with the dU-containing 5' ends created by the PCR with cDNA-U-2. Random clones were picked, minipreped, and sequenced as described above.

Results

Cross-Species Ortholog Identification and Genetic Marker Isolation

In this work I sought to identify the rat orthologs of sequences from the mouse *Lyp* region, as the COL strategy predicts that those orthologs will populate the rat *Lyp* region. These orthologs could then be used to isolate rat genomic DNA from the region. Although the definitive determination that two homologous sequences are orthologs requires the determination of their phylogeny, I used a high degree of sequence similarity as a working proxy for the classification of orthologs. Further discussion of this topic can be found in the Discussion section below.

Hybridization of Rat Genomic DNA With Mouse Cosmid Fragments

As a test in order to determine whether one might be able to easily identify mouse genomic fragments that would specifically hybridize to their presumed rat orthologs, I attempted to isolate such fragments from mouse cosmids from the walk region by identifying cosmid fragments that lacked repetitive sequences. I chose 3 mouse cosmids subcloned from mouse YAC 45 and 4 mouse cosmids subcloned from mouse YAC 60 (see Chapter III for the subcloning). These cosmids were digested to completion with *Eco*RI and the fragments separated by electrophoresis and immobilized on nylon membranes by Southern blotting. The blots were then hybridized with body-labeled total genomic rat DNA (Fischer F344) in order to identify those cosmid fragments containing repetitive sequences. Although most fragments were positive for repeats, 8 fragments among the 7 cosmids seemed repeat-free (average size, approximately 2kb; data not shown). These fragments were then isolated from larger-scale digests of the cosmids by cutting out and purifying the bands from a preparative agarose gel.

When radioactively labeled and used as probes against rat genomic Southern blots, however, no clear bands were visible above the lane background (data not shown). I thus proceeded with the targeted isolation of DNA contained within the genes of the mouse *Lyp* region in order to obtain DNA fragments more likely to contain significant-sized regions of sequences very similar to their rat orthologs.

PCR of Rat Genomic DNA With Mouse Exon Primers

As mentioned in Chapter II, anonymous mouse SSR assay primers did not successfully amplify specific rat DNA fragments from rat genomic DNA. In the hope that perhaps mouse exon sequences would be sufficiently similar in sequence to their rat orthologs for the technique to work, I tested the early products of the mouse exon trapping experiments described in Chapter III. Primer pairs were selected for each of the mouse exons ajm2401(ex01), ajm2403(ex03), and ajm2410(ex10), with predicted melting temperatures of 65°C. These primer pairs were then used to amplify both mouse B6 genomic DNA at an annealing temperature of 65°C and rat Fischer F344 and Lewis genomic DNAs at annealing temperatures of 65°C, 60°C, 57°C and 54°C. Although a single band of the expected size was observed in the amplifications from B6 DNA, and background smear and the incidence of other bands did increase with declining annealing temperature (declining stringency), no band within +/-10bp of the expected band size was observed in any of the amplifications from rat genomic DNA (data not shown).

Hybridization of Rat Genomic DNA With Mouse Exon Fragments

As many of the mouse exons isolated produced only one or two bands when used as probes on mouse Southern blots, I reasoned that sequence conservation between the mouse exons and their rat orthologs might be sufficient for mouse exon probes to produce individual bands on

rat Southern blots as well. Such probes could then be used to screen the rat genomic cosmid library which I had prepared for pool screening by Southern blot probing (see Materials and Methods), and the resulting rat genomic clones used further to identify rat genetic markers.

I selected the ten mouse exon clones ex03 (ajm2403), ajm207, ajm224, ajm315, ajm401, ajm504, ajm703, ajm706, ajm707, and ajm713 as probes and hybridized these to the rat cosmid library screening Southern blots under reduced stringency conditions (see Materials and Methods). The probes ajm207, ajm224, ajm707, and ajm713 each showed one or more positive cosmid pools (see Figure 1 for ajm224 results); the frozen stocks of cosmid clones corresponding to those positive pools were then plated and rat cosmids positive for each of the three probes were isolated through secondary and tertiary rounds of cosmid colony hybridization screening. A single rat cosmid was isolated using each of the ajm207 and ajm224 probes, while two cosmids were isolated using the ajm707 probe and 3 cosmids (1 weakly hybridizing) were isolated using the ajm713 probe. *Eco*RI and *Bgl*II digests of the two ajm707 cosmids were identical, as were similar digests of the two strongly positive ajm713 cosmids, while the weakly hybridizing ajm713 cosmid had no non-vector bands in common with the other two ajm713 cosmids (suggesting it represented a different locus than the other two cosmids, one less similar to the ajm713 probe). Thus, the single ajm207 and ajm224 cosmids, the first ajm707 cosmid, and the first ajm713 cosmid were selected for further analysis.

In order to identify genetic markers within the cosmids, the cosmids were subcloned into small (~500 bp) fragments and these subclones screened for the presence of CA and GA repeats (Materials and Methods). The twelve strongest positive subclones from each cosmid were then sequenced; a CA repeat was identified from the ajm207 cosmid (sequences ajm1905, 06, 14, 17, 18), an imperfect CA/GA repeat was identified from the ajm707 cosmid (sequences ajm1907, 09,

11, 19, 20, 21, 22, 24), and no SSR was identified from either the ajm224 or the ajm713 cosmid. PCR assays were created from the two repeat sequences, but only the PCR assay 207rpt1 was polymorphic: it was polymorphic in the Dp x Fischer cross but not in the Dp x Dr cross. As shown in Figure 2, *Lyp* mapped to the left of the 207rpt1 marker. Thus, using a mouse exon to isolate a rat genetic marker was successful in the case of exon ajm207.

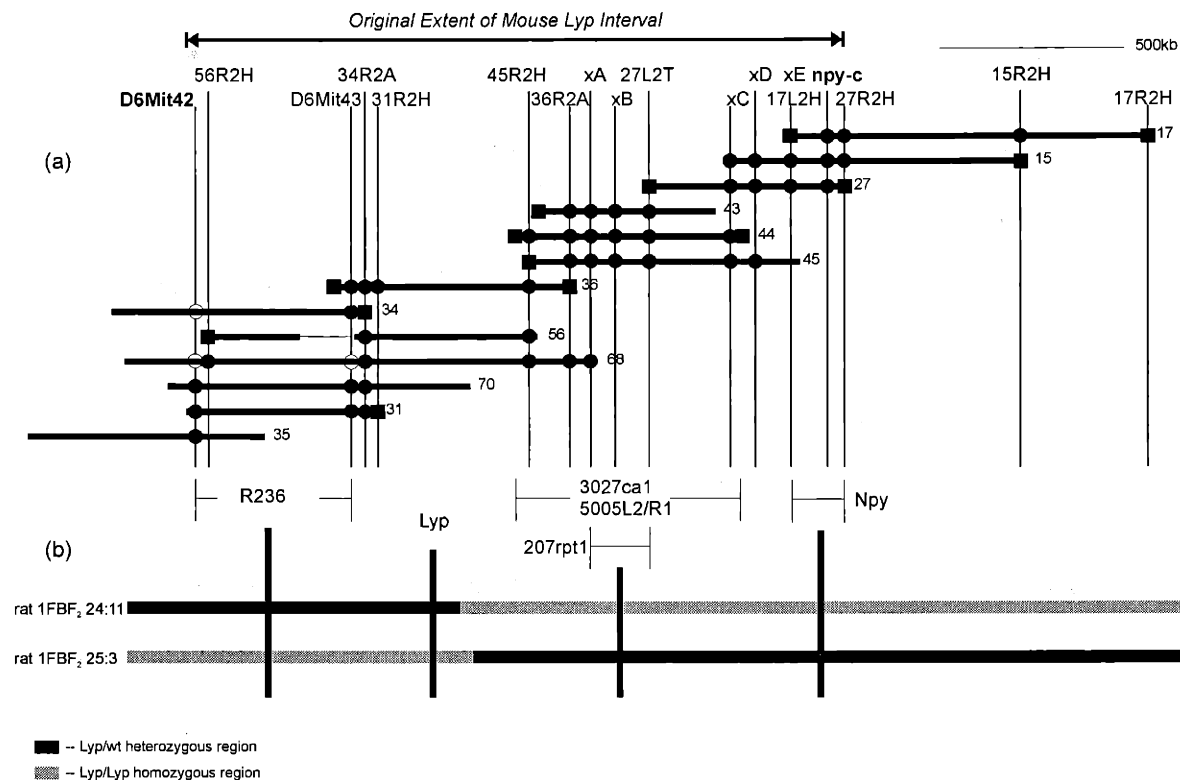


Figure 2: Integration of mouse *Lyp* region YAC map (a) with rat *Lyp* genetic mapping (b): initial rat genetic markers isolated by using mouse exons and rat cDNA orthologs of mouse cDNAs restrict the *Lyp* interval to the left part of the original R236-*Npy* interval. The approximate locations of the mouse exons and cDNAs used to identify the rat genetic markers are indicated on the mouse YAC map (a number of mouse YACs not material to the mapping have not been included in the mouse YAC map). The labels 1FBF₂ 24:11 and 1FBF₂ 25:3 identify the Dp x Fischer cross animal genotypes depicted in (b). The darkest region of each genotype represents a Dp/Fischer heterozygous region, while the lighter-colored region represents a Dp/Dp (*Lyp* haplotype) homozygous region. Vertical lines show the genotypes and inferred relative order of the rat genetic markers indicated and the *Lyp* gene. (Note: the genetic marker 207rpt1 has not been ordered relative to 3027ca1 and 5005L2/R1, but the original mouse exon 207 has been mapped to the region indicated as "xB", while the mouse cDNAs ajm2713 and bjr16 used to identify 3027ca1 and 5005L2/R1, respectively, have only been mapped to YAC 44)

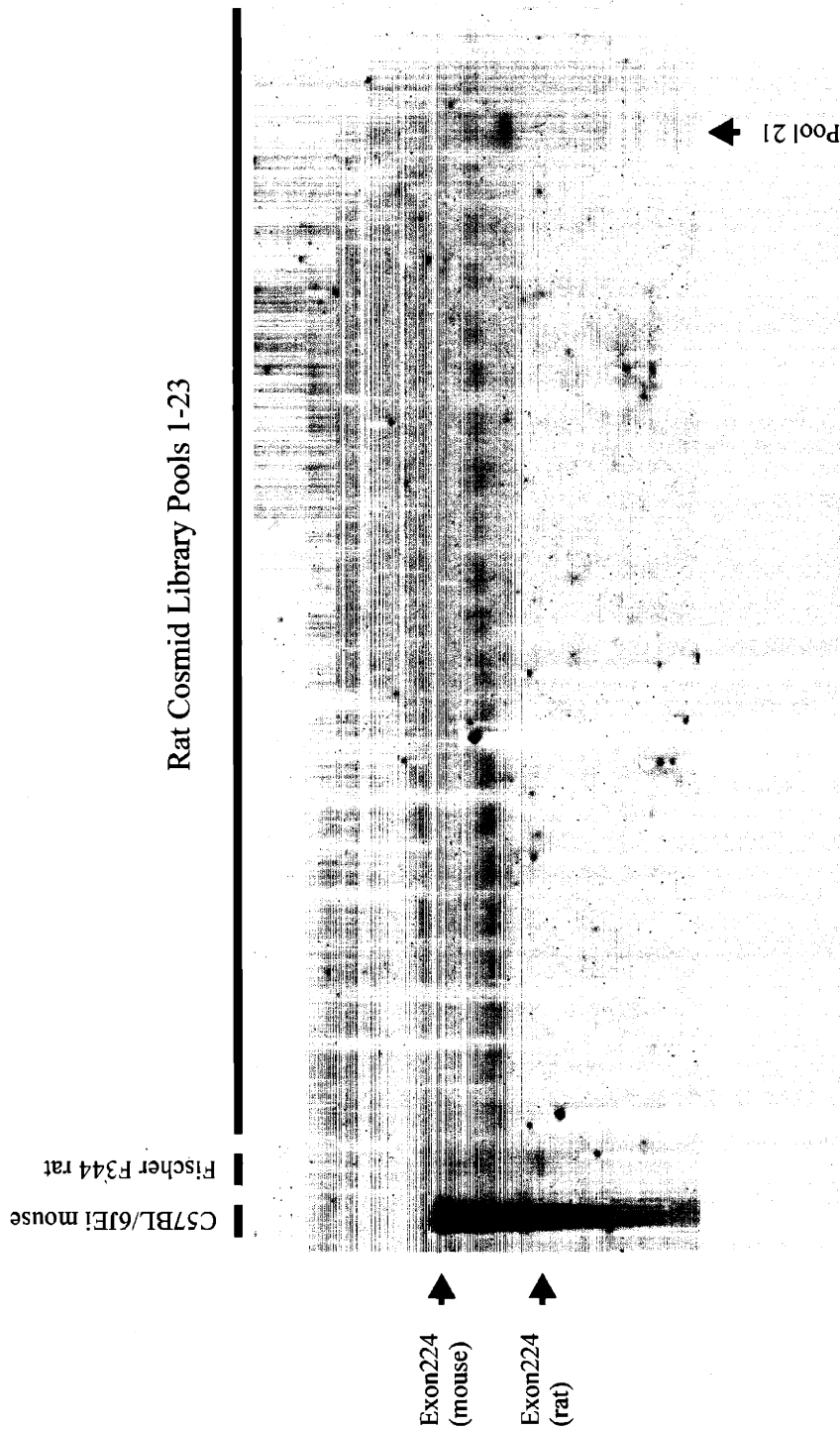


Figure 1: Sample rat cosmid library first-round Southern blot cross-species screening result. A ~1.1x genome equivalent plating of a rat genomic cosmid library prepared for Southern blot screening was hybridized with the exon224 mouse exon probe and washed under conditions of reduced stringency (see Materials and Methods for details). The positions of the mouse and rat fragments hybridizing with exon224 are indicated to the left of the image. A single cosmid pool (pool 21) contains a band of the same size as that in the rat control DNA lane; thus, cosmid pool 21 includes a rat cosmid that contains a rat homolog of mouse exon224. Lane contents are as follows: C57BL/6JfEi mouse control DNA, Fischer (F344) rat control DNA, and 23 lanes of pooled rat cosmid DNAs, each pool digested with *Eco*RI to completion. Under the reduced stringency hybridization conditions used (washed in 0.5x SSC / 0.1% SDS at 55°C 20'), lane background is evident in the control lanes, and common cosmid vector bands are visible in the cosmid pool lanes, along with certain repetitive DNA bands (cosmid vector bands and additional bands visible in pools 5 and 7 are also visible as intense bands in UV images of the ethidium-stained gel; not shown).

Using a Southern blot of pooled cosmid DNAs rather than initial colony hybridization provides a necessary degree of specificity, as the size of a true positive band in any of the 23 lanes of pooled cosmid DNA must match the size of the band in the Fischer rat genomic DNA lane; thus, false positives due to nonspecific hybridization such as that seen in this figure are greatly reduced.

As screening the rat genomic cosmid library with mouse rather than rat DNA fragments was difficult because of false positives and false negatives at the colony hybridization steps due to the reduced stringency necessary for cross-species hybridization, I developed a mouse→rat cross-species cDNA selection protocol to directly isolate rat orthologs from a set of mouse cDNA templates. As discussed in the next section, these rat cDNAs could then be used to easily isolate the rat genomic clones that contained them.

Mouse→Rat Cross-Species cDNA Selection

Cross-Species cDNA Selection Strategy

Although the genomic hybridization of mouse gene fragments described above did succeed in identifying rat orthologs, it has two major drawbacks: (1) it is difficult to evaluate the confidence in the identification of the putative ortholog by determining the degree of sequence conservation between the gene fragment probe and the resulting much larger clone (because of the large size of the clone), and (2) it is difficult to scale up the process to work with many gene fragments in parallel. Given the success of the same-species genomic cDNA selection procedure in identifying sequences that match exactly (see Chapter III), I reasoned that modifying that procedure by using a pool of mouse cDNA fragments as the selection template for rat cDNA fragments, and reducing the stringency of the hybridization and washing steps, would produce easily-sequenced rat cDNA fragments closely related in sequence to the mouse cDNA templates.

Details of the modified procedure are listed in the Materials & Methods section; briefly, the cDNA fragments to be used as the template (mouse) are PCR amplified with a biotinylated version of the linker primer originally used to amplify them, thus allowing them to be retained on streptavidin beads. New cDNA is prepared from mRNA from the second species (in this case,

rat) using linkers distinct in sequence from those used in the template cDNA, in order to prevent cross-hybridization based on the linker sequences. Prehybridization, hybridization, and washing of the cDNA is all performed at lower temperatures than for normal cDNA selection, so as to allow mismatches up to a certain degree.

Determining the Proper Level of Selection Stringency

In order to determine the proper level of stringency to produce rat orthologs of mouse cDNAs in the selection, an initial panel of 16 mouse cDNAs selected from YAC 44, pool "1_16", was prepared as templates for cross-species selection (see Table 1). As for all the mouse->rat cDNA selection experiments described here, the rat cDNA source was a 1:1:1 mixture of amplified primary rat spleen, brain and testis cDNA fragments. As the previous mouse->mouse cDNA selection experiments showed that a 65°C wash temperature produced almost exclusively fragments exactly matching the sequence of the template DNA (see chapter III), cross-species selection (with sequence mismatches expected) would require a less-stringent lower wash temperature. Based on the "...1% decrease in identity leads to 1-1.5 degree drop in melting temperature..." heuristic from [19] and the estimate of 90% sequence identity between mouse and rat genes (based on personal examination of database sequences, but verified in [20], which reports approx. 94% sequence identity in transcript coding sequences and 87% identity in transcript untranslated regions), wash temperatures of 50°C, 55°C, and 65°C were chosen for testing.

Tables 1 and 2 below list the template mouse cDNAs used for the selection (pool 1_16) and a summary of the selection results at the three different wash temperatures. Figure 3 parts (a)-(c) list the results in more detail, while parts (g)-(i) show sample alignments between the mouse template cDNAs and selected rat cDNAs for each of the three wash temperatures.

Additional Selection Results at 50°C Wash Temperature

As the alignment between ajm2722 and ajm3115 in the 50°C wash results above had too many mismatches to consider ajm3115 the rat ortholog of ajm2722, further reduced stringencies were not tested. 44% of the mouse cDNA templates in pool 1_16 produced plausible rat orthologs at the 50°C wash temperature, based on sequence similarity. Thus, the 50°C wash temperature was chosen for the remaining selection experiments as a good balance between sequence specificity and the number of orthologs produced. The results of these selections, using template pools 2_3, 3_2 and 4_24 are also summarized in Tables 1 and 2. Figure 3 parts (d)-(f) and parts (j)-(l) show more detailed results and sample alignments of the selected rat cDNAs with their mouse orthologs.

Mouse cDNA Template Pool	cDNA Fragments in Template Pool
1_16	ajm2705 ajm2707 ajm2708 ajm2709 ajm2711 ajm2712 ajm2713 ajm2715 ajm2716 ajm2717 ajm2718 ajm2720 ajm2721 ajm2722 ajm2723 ajm2724
2_3	ajm2705 bjr16 bjr24
3_2	ajm3302 ajm3313
4_24	ajm3803 ajm3805 ajm3806 ajm3809 ajm3810 ajm3811 ajm3913 ajm3915 ajm3918 ajm3924 ajm3926 ajm4129 ajm4132 ajm4133 ajm4134 ajm4136 ajm4303 ajm4308 ajm4313 ajm4315 ajm4321 ajm5202 ajm5222 ajm5225

Table 1: Clones used in cross-species cDNA selection experiments. This table lists the mouse cDNA fragments (derived by cDNA selection against mouse YAC clones) used as templates for mouse->rat cross-species cDNA selection experiments.

Mouse cDNA Template Pool	Wash Temperature	Number of Selected Rat cDNAs Sequenced	Number of <i>Selected</i> Rat cDNAs Similar to a Template cDNA	Number of <i>Template</i> cDNAs Similar to a Selected Rat cDNA
1_16	50°C	36	19 (53%) [+1 weak]	7 (44%) [+1 weak]
1_16	55°C	15	6 (40%)	2 (13%)
1_16	65°C	12	2 (17%)	2 (13%)
2_3	50°C	16	10 (63%)	1 (33%)
3_2	50°C	9	6 (67%)	1 (50%)
4_24	50°C	84	62 (74%)	9 (38%)

Table 2: Summary of results of cross-species cDNA selection experiments. The experiments with template pool 1_16 were used to determine that the 50°C wash temperature was best for maximizing the number of template cDNAs similar to a selected cDNA (diversity) as well as the number of selected cDNAs similar to a template cDNA (specificity).

Figure 3: Detailed results of cross-species cDNA selection experiments (below). (a)-(f): Similarity of selected rat cDNAs to mouse template cDNAs, by template cDNA. (g)-(l): Sample alignments of selected rat cDNAs with mouse template cDNAs; in each alignment, the upper sequence is that of the template mouse cDNA sequence, while the lower sequence is that of the selected rat cDNA:

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
ajm2716	1
ajm2722	1

Figure 3(a): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 1_16 / 65°C wash.

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
ajm2716	2
ajm2724	4

Figure 3(b): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 1_16 / 55°C wash.

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
ajm2708	4
ajm2713	4
ajm2715	2
ajm2716	3
ajm2720	1
ajm2723	4
ajm2724	1
ajm2722	1 (weak)

Figure 3(c): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 1_16 / 50°C wash.

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
bjr16	9

Figure 3(d): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 2_3 / 50°C wash.

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
ajm3302	6

Figure 3(e): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 3_2 / 50°C wash.

Mouse cDNA Template	Number of Sequenced Rat DNAs Similar to Template cDNA
ajm3810	1
ajm3805	2
ajm3809	7
ajm3913	14
ajm3926	4
ajm4129	2
ajm4133	24
ajm4303	1
ajm5225	7

Figure 3(f): Detailed results of cross-species cDNA selection experiments: Matches for Mouse cDNA Template Pool 4_24 / 50°C wash.

Figure 3(g): Sample Alignments for Mouse cDNA Template Pool 1_16 / 65°C wash (below):

```

AJM2716.PSQ vs. Rev Comp of AJM3011.PSQ
-----
ctcagcgagt cttttcagtt tcaacttcctc ttctaaataa acttccaggn
0   AJM2716.PSQ
50  Rev Comp of AJM3011.PSQ

-----tcc tacaaatcaa gaggggaaa attaaagtgt aaacccacgc
   ||| ||| ||||| ||||| ||||| ||||| |||||
gctctgctcc taccaatcaa gaggggaaa attaaagnn cnccccacgc
43  AJM2716.PSQ
100 Rev Comp of AJM3011.PSQ

agagttaatt atcttgcaaa agctcggttac tgcctaaaa ttagctttcc
   ||||| ||||| ||||| ||||| ||||| ||||| |||||
agagttaatt atcttgcaaa agctcggtta ccattcctta aaat-----
93  AJM2716.PSQ
144 Rev Comp of AJM3011.PSQ

ccacaagtcc tatttttagca caacgatctg aatgtactag agaaagatca
143 AJM2716.PSQ
144 Rev Comp of AJM3011.PSQ

atggttgcat atagtaagtt tcgagagtct tcttctctga agagcttgaa
193 AJM2716.PSQ
144 Rev Comp of AJM3011.PSQ

caaatgcttg ttgatctttc tagagactcc accaccttgt agggagtnga
243 AJM2716.PSQ
144 Rev Comp of AJM3011.PSQ

```

```

gccncctgga agnttattta gaagaggaag ntaaacttgg aaaagcttgg      293  AJM2716.PSQ
-----
144  Rev Comp of AJM3011.PSQ

ttgaagattc ctcttttctt tgggctcacc ggattcccgt cggaantatt      343  AJM2716.PSQ
-----
144  Rev Comp of AJM3011.PSQ

a    344  AJM2716.PSQ
-    144  Rev Comp of AJM3011.PSQ

||Alignment End||

AJM2722.PSQ vs. Rev Comp of AJM3007.PSQ
ccacactgag ggtattcaga aagtgaacgg cccgttttca ctgatccaca      50  AJM2722.PSQ
-----
                      ||
                      -gccggcaag      9  Rev Comp of AJM3007.PSQ

atactcttcc acacctctgt atcctctctt cctc--gtc tttcttccaa      98  AJM2722.PSQ
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
cttctcaaca ggcccntgaa aactctcatt cctctgggc tctcttccaa      59  Rev Comp of AJM3007.PSQ

tgtcctaact agacgccaaa gaaatccact actttattta ctctcatttg      148  AJM2722.PSQ
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
tgtcctaact agac-acaga gaaatctgcc actttatttg ttctcgtttg      108  Rev Comp of AJM3007.PSQ

tgt-cttttt cattccatat gtaaagatc aaagtcaca atcataagct      197  AJM2722.PSQ
| | | | | | | | | | | | | | | | | | | | | | | | | | | |
tgcccttttt cattccatag ggaaatgac gaagtttaca atcataa---      155  Rev Comp of AJM3007.PSQ

tattagtgtg attcaagtgt aattaaatga actagggtct ataaagaagt      247  AJM2722.PSQ
-----
155  Rev Comp of AJM3007.PSQ

cgtgtcttgg ggctggcgaa acgggtcaag tggtaagag cacgggactt      297  AJM2722.PSQ
-----
155  Rev Comp of AJM3007.PSQ

ctcttccgga aggtccctga gntcaaatcc tagcaatcac atggnnngct      347  AJM2722.PSQ
-----
155  Rev Comp of AJM3007.PSQ

caaaacccat ccgtaatgag ntcttgacat cctcttctng gggttttctt      397  AJM2722.PSQ
-----
155  Rev Comp of AJM3007.PSQ

naaggcaagg tactt      412  AJM2722.PSQ
| |
-----c ttatt      161  Rev Comp of AJM3007.PSQ

||Alignment End||

```

Figure 3(h): Sample Alignments for Mouse cDNA Template Pool 1_16 / 55°C wash (below):

```

AJM2716.PSQ vs. AJM3016.PSQ
-----
0  AJM2716.PSQ
ctcagcgagt cttttcagtt tcaacttctc ttctaaataa acttccaggc      50  AJM3016.PSQ

```

```

-----tcc tacaaatcaa gaggggaaaag attaaagttg aaaccacgc      43  AJM2716.PSQ
      ||| ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
ggngngntcc taccaatcaa gaggggaaaag attaaagttg aaaccacgc      100  AJM3016.PSQ

agagttaatt atcttgcaaa agctcggttac tg-tcctaaa attagctttc      92  AJM2716.PSQ
||||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||
agagttaatt atcttgcaaa agctcggttac cattcctaaa at-----      142  AJM3016.PSQ

cccacaagtc ctatttttagc acaacgatct gaatgtacta gagaaagatc      142  AJM2716.PSQ
-----
-----
142  AJM3016.PSQ

aatggttgca tatagtaagt ttcgagagtc ttcttctctg aagagcttga      192  AJM2716.PSQ
-----
142  AJM3016.PSQ

acaaatgctt gttgatcttt ctagagactc caccaccttg tagggagtng      242  AJM2716.PSQ
-----
142  AJM3016.PSQ

agccncctgg aagnttattt agaagaggaa gntaaacttg gaaaagcttg      292  AJM2716.PSQ
-----
142  AJM3016.PSQ

gttgaagatt cctcttttct ttgggctcac cggattcccg tcggaantat      342  AJM2716.PSQ
-----
142  AJM3016.PSQ

ta      344  AJM2716.PSQ
--      142  AJM3016.PSQ

||Alignment End||

AJM2724.PSQ vs. AJM3021.PSQ
gggatgtggg gggggaggac ctcatgtgat gtcagtcgat tacactccaa      50  AJM2724.PSQ
-----
0  AJM3021.PSQ

gtcagaattg atccccagga attagtgaga aacaaagcgg agtttacagt      100  AJM2724.PSQ
-----
0  AJM3021.PSQ

gagtcacagg gaattattgc tctgcctcat gatgtcaagt ccaatgcagc      150  AJM2724.PSQ
-----
0  AJM3021.PSQ

ttaagacagt ctaagtcttt aaaaagtaga agagaatgat agcttcattt      200  AJM2724.PSQ
-----
0  AJM3021.PSQ

tgtaactag agttacacac anggaaggga ngccttattt cctgaccatg      250  AJM2724.PSQ
      ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
-----aggag ataatgactc cctgccatt      25  AJM3021.PSQ

```

```

tgaaaacaca agaaggaag agtcatggg accaatacaa ggtaaaaccg 300 AJM2724.PSQ
||||||| | | | | | | | | | | | | | | | | | | | | |
tgaaaacaca --gaaggaag attcatggg- accaatncng gtaaaca--- 69 AJM3021.PSQ

gtgccaaactt cttcccaagc aagagggtta accactttaa gggaggagca 350 AJM2724.PSQ
||||||| | | | | | | | | | | | | | | | | | | | | |
gtgccaaactt tcccagcaga ga-----tta accactg--a gggaggagca 112 AJM3021.PSQ

----- 350 AJM2724.PSQ

gaggggtaag gtgtgtacac acacacacaa cacacacaca cacacacaca 162 AJM3021.PSQ

--agagaggg tntcacggaa ttcccg 375 AJM2724.PSQ
| | | | |
cctatatata atttattata ctcttg 189 AJM3021.PSQ

||Alignment End||

```

Figure 3(i): Sample Alignments for Mouse cDNA Template Pool 1_16 / 50°C wash (below):

AJM2708.PSQ vs. AJM3203.PSQ

```

----- -tcct ttngttata 15 AJM2708.PSQ
| | | | |
ggggacacaa ccatctgcaa aatcaatgtt cttttgcttg tnetgttcta 50 AJM3203.PSQ

aactgaaatc aagccttcct tcttcaagtt gtttgtgctt aggnatcaat 65 AJM2708.PSQ
| | | | | | | | | | | | | | | | | | | | |
gttagaaacc aagccttcct tctcaagtt gtttgtggtc aggtatcaat 100 AJM3203.PSQ

caactcatca ataagaaaag taacaagagt ctctgtgaac catcatcata 115 AJM2708.PSQ
| | | | | | | | | | | | | | | | | | | | |
tcattaatg- ---agaaaag taacaagagt ctctgtgagc cgtcatcgta 146 AJM3203.PSQ

aaagccagga ctgctgaata -atgaggaac gtatgggaaa tgatggctctg 164 AJM2708.PSQ
| | | | | | | | | | | | | | | | | | | | |
aaagccagaa ctggttgaat gatgaggaac gtgtgaggaa tgatggcttg 196 AJM3203.PSQ

agaatctgaa acagaatcct taagaaagcc agtttgggct tctattgtgt 214 AJM2708.PSQ
| | | | | | | | | | | | | | | | | | | | |
agaatctgaa acagaatcct taaggaganc agaatgcctg tgagaagttt 246 AJM3203.PSQ

ccag----- 218 AJM2708.PSQ
|
nggggcacct agaga 261 AJM3203.PSQ

||Alignment End||

```

AJM2713.PSQ vs. Rev Comp of AJM3025.PSQ

```

gcaaattatc ccaactggg cagtttataa acagcagaag tctatcatc 50 AJM2713.PSQ
----- 0 Rev Comp of AJM3025.PSQ

atcattttgg aggctgatag tccactgtca aggtgtttca ggtttggtgt 100 AJM2713.PSQ
| | | | | | | | | |
-----gntcca ggtttggtgt 16 Rev Comp of AJM3025.PSQ

```

```

gtgttgaagg ctttgtgcct cacaagatgg tctttcctct gtctcggggc 150 AJM2713.PSQ
||||| ||| ||||| ||| ||||| | ||||| ||||| |||||
ttgttgaagg ctgtgtgcct nac-agatgg tg-ttcctct gtctcagggc 64 Rev Comp of AJM3025.PSQ

agacatcaag gcaaggactt gcatactagg gaccctttaa aatc--aagg 198 AJM2713.PSQ
||||| ||| | ||||| | ||||| | | ||||| |||||
agacaagcag ggcaggac-t gcataccag gt-cctttaa aatcnnccct 112 Rev Comp of AJM3025.PSQ

taatcgtgct ctagactaat tactgctcag aggcttacct aatccctcac 248 AJM2713.PSQ
| | | ||||| ||||| ||||| ||| | | |
natcctgcgc gtagactaat aactgctcac aggatgacac ctaaacacca 162 Rev Comp of AJM3025.PSQ

tgtnggtgac taaggtntca tctgtgtga ccacgggggt ngatggggct 298 AJM2713.PSQ
|
ctgaga---- - - - - - - - - - - - - - - - - - - - - 168 Rev Comp of AJM3025.PSQ

```

```

cacggattcc ggtggattag taantagt-- --- 326 AJM2713.PSQ
||| ||| |
----- --tgattag gtgagacctc tga 189 Rev Comp of AJM3025.PSQ

```

||Alignment End||

AJM2715.PSQ vs. AJM3109.PSQ

```

cttaaaaggg ctcaggataa gtgcttcccg tggatttcgc tagatgctct 50 AJM2715.PSQ
| |||| | || |||
----- -gattttcaa gaggtgttct 19 AJM3109.PSQ

gttgcttcan atacagttac aaaggtatgc attaaagtat tatggatcaa 100 AJM2715.PSQ
||||| ||| ||| ||||| ||| | ||| ||| ||| |||
gttgcttcan ataaacttat aaaggtatgc atnanggtat tacggataaa 69 AJM3109.PSQ

acactagaaa gcagggtctg gcaggatggc tcatgccac aatcccaagc 150 AJM2715.PSQ
||| |||| | ||||| | ||| ||| || ||||| ||||| |||
acagcagaaa acggggctgg ggaggacggc tcgtgccat aatcca-gc 118 AJM3109.PSQ

acttagcaag aagaggcaga agagaaagtt cagcctggag ccacataaca 200 AJM2715.PSQ
||| ||| || ||||| |||||
actaagccag aagaggcag- - - - - - - - - - - - - - - 137 AJM3109.PSQ

ataagcaaat g 211 AJM2715.PSQ
----- - 137 AJM3109.PSQ

```

||Alignment End||

AJM2716.PSQ vs. AJM3036.PSQ

```

----- - 0 AJM2716.PSQ

tcagcgagtc ttttcagttt cacttctct tctaaataaa cttccggggn 50 AJM3036.PSQ

-----tcct acaaatcaag aggggaaaga ttaaagttga aaccacgca 44 AJM2716.PSQ
||| || ||||| ||||| ||||| ||||| ||||| |||||
ntctgtcctt accaatcaag aggggaaaga ttaaagttga aaccacgca 100 AJM3036.PSQ

gagttaatta tcttgcaaaa gctcgttact g-tcctaaaa ttagctttcc 93 AJM2716.PSQ
||||| ||||| ||||| ||||| ||||| |
gagttaatta tcttgcaaaa gctcgttacc attcctaaaa t----- 141 AJM3036.PSQ

```

ccacaagtcc	tat t t t t a g c a	caacgatctg	aatgtactag	agaaagatca	143	AJM2716.PSQ
-----	-----	-----	-----	-----	141	AJM3036.PSQ
atggttgcat	atagtaagtt	tcgagagtct	tcttctctga	agagcttgaa	193	AJM2716.PSQ
-----	-----	-----	-----	-----	141	AJM3036.PSQ
caaatgcttg	ttgatctttc	tagagactcc	accaccttgt	agggagtnga	243	AJM2716.PSQ
-----	-----	-----	-----	-----	141	AJM3036.PSQ
gccncttga	agnttat t t a	gaagaggaag	ntaaacttgg	aaaagcttgg	293	AJM2716.PSQ
-----	-----	-----	-----	-----	141	AJM3036.PSQ
ttgaagattc	ctcttttctt	tgggctcacc	ggattccctg	cggaantatt	343	AJM2716.PSQ
-----	-----	-----	-----	-----	141	AJM3036.PSQ

a 344 AJM2716.PSQ
- 141 AJM3036.PSQ

||Alignment End||

AJM2720.PSQ vs. Rev Comp of AJM3031.PSQ

ttgcactggc	tgtaagtngc	tccttgaatc	tgccctgta	ttctgttaat	50	AJM2720.PSQ	
tnaagagctc	tttttgtag	agc-----	-----	-----	23	Rev Comp of AJM3031.PSQ	
gtctccca	gcgcggtggg	ccctgggcag	tgtnactttt	atcacgtact	100	AJM2720.PSQ	
-----	-----	-----	-----	-----	23	Rev Comp of AJM3031.PSQ	
cccttctga	cacatgagat	agttctgggc	catcttggtc	atttctgccc	150	AJM2720.PSQ	
-----	-----	-----	-----	-----	23	Rev Comp of AJM3031.PSQ	
cactcccg	atcagccggg	accttgagaa	acagagcccc	ttttcattag	200	AJM2720.PSQ	
-----	-----	-----	-----	-----	23	Rev Comp of AJM3031.PSQ	
naacttgga	taagaaactg	tatttgagca	gtaaggtgct	cctagccacc	250	AJM2720.PSQ	
-----	-----	-----	-----	-----			
-----	tgga	taaggaactg	tatttgagca	gta-ggtgct	cctgcccact	67	Rev Comp of AJM3031.PSQ
aagttctntt	tacaacctaa	cggaaatatt	aacnggggta	aaactngcgt	300	AJM2720.PSQ	
gagttctcct	acacctattg	gaagtgttaa	catgttgaaa	ctgtgttcna	117	Rev Comp of AJM3031.PSQ	
tcccggtctc	acggatttcc	gtnggnagna	antnngnant	tntngnaang	350	AJM2720.PSQ	
ncccaagcaa	agagctagct	actcaaaaaa	ctgtcagaat	ccaggtcaac	167	Rev Comp of AJM3031.PSQ	
ttnggaggcc	cgnggaaatt	tcccggnccc	ggtaacctg		389	AJM2720.PSQ	
atggttaacac	ttccaa----	-----	-----		183	Rev Comp of AJM3031.PSQ	

||Alignment End||

AJM2723.PSQ vs. AJM3028.PSQ

```
gatagggtaa agagatttct atagaacgaa tttgggaggc cagtgagatc 50 AJM2723.PSQ
----- 0 AJM3028.PSQ

ggctcggcag gcacaggcac ttattgtgtg aacctggcac tgggtccagt 100 AJM2723.PSQ
----- 0 AJM3028.PSQ

ctccaggacc tatctaaaag atagaaggaa agagccaact cccagagtgt 150 AJM2723.PSQ
      ||| ||||| ||||| | |||||
-----aaag gtagaaggag agagcanctg cg-agagtgt 33 AJM3028.PSQ

accgtgtca caccagataa ccaactgcac ttgtgaagt ttccacaagt 200 AJM2723.PSQ
||| | | || | | | | | | |
accgacggga cagcgcaag ttctcacagg gggncg----- 69 AJM3028.PSQ

ngagtttttg aagggtgaat caagtaagtc tttggagtc tacaagtgc 250 AJM2723.PSQ
      ||| |||| |||| || |||| ||| ||| ||||| ||
-----ttg aagg-tgaat cagc--agtc ttt-gtcgtc tacaagtnac 108 AJM3028.PSQ

tgtcatttta agcaactcat taaatatng gttcattttt tttttcccat 300 AJM2723.PSQ
||||||| ||| |||| ||||| | ||||| | | |||
tgtcatttta agcacctcat taaatatt-g gttcatttat t-----ccat 152 AJM3028.PSQ

gtttgtaaga ggcaccaaag atgaaccgna aggtcttca ccgattccc 350 AJM2723.PSQ
||| | | | | | | |
gttatggata cacttgagat tgaaccagcn a----- 183 AJM3028.PSQ

ngtgggaant antaantaaa taagnccggc ccctgggatt tnccggnccc 400 AJM2723.PSQ
----- 183 AJM3028.PSQ

ggnanccttn aagggnnacc aagtttttcc cctatttt 438 AJM2723.PSQ
----- 183 AJM3028.PSQ
```

||Alignment End||

AJM2724.PSQ vs. AJM3105.PSQ

```
gggatgtggg gggggaggac ctcagtgatc gtcagtcgat tacactccaa 50 AJM2724.PSQ
----- 0 AJM3105.PSQ

gtcagaattg atccccagga attagtgaga aacaaagcgg agtttacagt 100 AJM2724.PSQ
----- 0 AJM3105.PSQ

gagtcacagg gaattattgc tctgcctcat gatgtcaagt ccaatgcagc 150 AJM2724.PSQ
----- 0 AJM3105.PSQ

ttaagacagt ctaagtcttt aaaaagtaga agagaatgat agcttcattt 200 AJM2724.PSQ
      | ||| | |||| |||| | ||||| |||||
-----ttt ttaaagactt aaaatgtaga aatgaatgat agcttcattt 43 AJM3105.PSQ
```


NOTE: Sequence quality is poor for AJM2722.PSQ after base 330 and for Rev Comp of AJM3115.PSQ after base 180; thus, the actual degree of sequence similarity between the two sequences may be significantly higher in that region than is apparent.

Figure 3(j): Sample Alignments for Mouse cDNA Template Pool 2_3 / 50°C wash (below):

```

BJR16.PSQ vs. AJM4011.PSQ
ngcagattcc atttggggaa atnagtcaat gctatgagca gttggagant 50 BJR16.PSQ
  |||
tgca----- 4 AJM4011.PSQ

tctcgatgaa tagactagac atcctcgctc aggaaagtta ctgtgtgctg 100 BJR16.PSQ
----- 7 AJM4011.PSQ

aaagagacaa gatggcatac aactacagta cagntagant atgntaaatn 150 BJR16.PSQ
||||| ||||| ||||| ||| ||| ||| ||| |||
aaagagaca- gatggcatac aactacagta cagatagaat gtgataaatg 56 AJM4011.PSQ

ccataataaa attacaaatn cctttgggcg cagngagana aatggaatgc 200 BJR16.PSQ
||||| ||||| ||| ||| ||| ||| ||| |||
ccataataaa ngtacaaatg cctgtgggag cggagagaga t---gaatgc 103 AJM4011.PSQ

cagntcctcc ataaaaacngt ttccgggctc acga----- 234 BJR16.PSQ
||| ||||| ||||| ||| ||| ||| |||
cagttcctcc ataaagcgtt gcctaatttc cccaatgga atctgcagtg 153 AJM4011.PSQ

----- 234 BJR16.PSQ
aacttgaaag atggatggaa tgtaacaga agaggaaaga agggcctctc 203 AJM4011.PSQ

----- 234 BJR16.PSQ
ctccaaggtg acagattccc tgaaggtcaa aggggaaaac atccaccatc 253 AJM4011.PSQ

----- 234 BJR16.PSQ
tgaagaggac aaattatagc aagatgagct tcttggtcac tgggccctgt 303 AJM4011.PSQ

----- 234 BJR16.PSQ
gaggagnta 312 AJM4011.PSQ
||Alignment End||

```

Figure 3(k): Sample Alignments for Mouse cDNA Template Pool 3_2 / 50°C wash (below):

```

AJM3302.PSQ vs. AJM4222.PSQ
----- 0 AJM3302.PSQ
agcggagaga gatgaatgcc agtncctcca taaagcgttg cctaatttcc 50 AJM4222.PSQ

----- 9 AJM3302.PSQ
ccaaatggaa tctgcagtga aacttgaaag atggatggaa tgtaacaga 100 AJM4222.PSQ

```



```

tcaaaggaaa aatgtagata tgaggcggtc tcacgaat      235  AJM3810.PSQ
-----
270  Rev Comp of RMO0124.PSQ

||Alignment End||

AJM3805.PSQ vs. Rev Comp of AJM5513.PSQ
ggagatcttg aggaaaattt acacttgctt cttcagcctt tgctccgcgt      50  AJM3805.PSQ
-----
0  Rev Comp of AJM5513.PSQ

ccttgatac gggatgggag aagtagagac caccattggt ctgcaccagg      100  AJM3805.PSQ
          |   |||   |   |||||
-----cc gntcanttnt gcgcaccagg      22  Rev Comp of AJM5513.PSQ

gtttctacaa gcccacatcag ctccctgcacc tgcgtttccc tctcagcc-c      149  AJM3805.PSQ
| ||||| || | ||||| || ||||| || ||||| || ||||| || |||||
gattctanaa acnccatcag ntcctgcacc tgnatttcnc cctcagccnc      72  Rev Comp of AJM5513.PSQ

tctctgcttt gttattgatg gccaggcagc gattgccaca ctcccttgatg      199  AJM3805.PSQ
||| ||||| | ||||| ||||| ||| | || |||| |||| |||||
cctccgcttt gntattgatn gccagatagc ggctgtcaca ctccntgatg      122  Rev Comp of AJM5513.PSQ

atgcttttna ggtttgtatc tgggtctcac gaatncggg      238  AJM3805.PSQ
|
a-----
123  Rev Comp of AJM5513.PSQ

||Alignment End||

AJM3809.PSQ vs. AJM5515.PSQ
-----
0  AJM3809.PSQ

gtagatnngg ggtggnggca gctctccac accaacacca tcagagccaa      50  AJM5515.PSQ

-----ttagc aaggggcagg actagctctc ctgctgcaag      35  AJM3809.PSQ
          |  ||  ||| |||| | ||||| || |||||
tntcccatn ctgcctaggc gaggtgcagn actagctctc ctnttgcaag      100  AJM5515.PSQ

aaggggtg-g ggccagctca tcacagtctt aggacatcaa cccagcctca      84  AJM3809.PSQ
| || | ||||| || ||||| || || |||| | || |||
caagngtag ggccagctca ccacagtact gggncaccaa cacagnctta      150  AJM5515.PSQ

-ggtggcagc ccagaacaga cacatctgta tggcctttgg tggtagcatg      133  AJM3809.PSQ
||||||| ||||| ||| ||||| || ||||| || ||||| || |||||
nggtggcagc ccagagcagn cacatctgtn tggcctnngg tggtagcatg      200  AJM5515.PSQ

aacctggaac atcaacacag accatggctg ccataggacc acagaccag      183  AJM3809.PSQ
| |||||
atcctggg-- -----
208  AJM5515.PSQ

||Alignment End||

AJM3913.PSQ vs. AJM5518.PSQ
ctggctgggg cctgtggtt catggtcctt tgcttgctt tgccttcctc      50  AJM3913.PSQ
          ||||| ||||| ||||| ||||| || |||||
----- ---tgtggtt catggtcctt tgcttgctt tnccttcctc      37  AJM5518.PSQ

```

agtctcaagg	cagacaggct	gtgtcagag	tagagatggc	acctctggag	100	AJM3913.PSQ
agtctcaagg	caggcaagct	gtgtcagag	tagagatggc	acctctggag	87	AJM5518.PSQ
ggtaccagag	ctaggtggat	acatggaccc	aggggcagaa	ggagcaagaa	150	AJM3913.PSQ
ggcaccagag	ctaggnagac	gcatagaccc	aggg-canaa	ggagcaagaa	136	AJM5518.PSQ
gtaaaagatg	catatccatc	actgcagtgg	gatgctactt	gctaccgcc	200	AJM3913.PSQ
gtaacag---	-atatccatc	actg-tagtg	ggtgctattt	gctaccggcc	181	AJM5518.PSQ
atgatcctga	ggttctgtan	ggcgcggtga	gcagcatcac	ctcgggctgc	250	AJM3913.PSQ
atgatcctga	ggtactgtag	tgcgcgncgn	gcagcatcac	ctcgggntgn	231	AJM5518.PSQ
ctggtt----	-----	-----	-----	256	AJM3913.PSQ	
cttctggggt	agtgccctg	tnaagaagct	ttgcqta	268	AJM5518.PSQ	

AJM3926.PSQ vs. AJM5611.PSQ

gtagggtctg	gaaggctctg	ggctggcgtc	cggtgggccc	tctccttttc	50	AJM3926.PSQ
-----	-----	-----	-----	-----	0	AJM5611.PSQ
tggctctact	ccctgctccg	cagattctga	tggggttttn	aagttgtctg	100	AJM3926.PSQ
-----	-----	-----	-----	-----	0	AJM5611.PSQ
ttttgacagc	tgtcttctcg	aagtcagcca	gcttcttctc	cgctgaccct	150	AJM3926.PSQ
-----	-----	-----	-----	-----	0	AJM5611.PSQ
gcacgcccct	ccaggctctg	cagtcgggca	gctgtgtatt	ccatctttct	200	AJM3926.PSQ
-----ccct	ccaggctctg	cagtcgggca	gctgtggatt	ccatctttct	44	AJM5611.PSQ
ctccacggct	tgaatagnan	ccaccact-g	tccaaagtga	gatttctgtg	249	AJM3926.PSQ
ctccacagct	tgaatagcag	ccaccactng	tccaaagtga	gatttctgtg	94	AJM5611.PSQ
gtctgaa---	-----	-----	-----	-----	256	AJM3926.PSQ
gtctggaagt	actggctttt	ctctgtttgc	tgtgaaggtg	ggtgtggctg	144	AJM5611.PSQ
-----	-----	-----	-----	-----	256	AJM3926.PSQ
gagctaacgg	tgtggagcgc	cttttctctc	tggtgccgag	caggcgctcg	194	AJM5611.PSQ
-----	-----	-----	-----	-----	256	AJM3926.PSQ
ctccgncatg	gtcacagtng	gcccacaaca	gcgactgqcg	ggqagagtgc	244	AJM5611.PSQ

```

----- 256 AJM3926.PSQ
ttcgggactg tgcttgcttc accagtcgcc cnggacctg ctggaagccg 294 AJM5611.PSQ

----- 256 AJM3926.PSQ
gcanngtgtg agaaagttna nggttngcta ggtagtagnn nnngggncce 344 AJM5611.PSQ

----- 256 AJM3926.PSQ
ngggntnntn ttngngccc ttagctgnc angaggnacn cngctttgtc 394 AJM5611.PSQ

----- 256 AJM3926.PSQ
ccttggttgt gggggtcgt ttgaagtttg 424 AJM5611.PSQ
||Alignment End||

AJM4129.PSQ vs. Rev Comp of AJM5602.PSQ
----- 0 AJM4129.PSQ
nncncnacc cggatacttt cntagttgac actcagcaac tgtacctgtg 50 Rev Comp of AJM5602.PSQ

----- 0 AJM4129.PSQ
nggaccattc ctctttacct tagccccaga gcccaatatc ccacacagac 100 Rev Comp of AJM5602.PSQ

----- 0 AJM4129.PSQ
nactgaccac nccaactata aagactacgc tgctctgact gtggtcatcc 150 Rev Comp of AJM5602.PSQ

----- 0 AJM4129.PSQ
tgctgattct gcacagnccg actcctnaca aatgactcac tctccctcag 200 Rev Comp of AJM5602.PSQ

-----cg gaatgccac ctctaggggt tttctgtggc tgcctcgggg 42 AJM4129.PSQ
| ||||| ||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
gagaagncca gaatgctcac ctctaggggt tttctgtggc tgcctcgggg 250 Rev Comp of AJM5602.PSQ

agcatgtttc tnacaatgca tgcttggtta ctcggtcgca tccaccactg 92 AJM4129.PSQ
|||| |||
agcacgtt-- ----- 258 Rev Comp of AJM5602.PSQ

cacactgagc tccctggtta accaccaggt ctgagaagaa gaccctttac 142 AJM4129.PSQ
----- 258 Rev Comp of AJM5602.PSQ

ctccagttaa ttaggatcgg ccantantnc ggtcagcatg tgcattacag 192 AJM4129.PSQ
----- 258 Rev Comp of AJM5602.PSQ

cgatgctaaa ccaaccttga ccagtaagtt aatggtgaac accttggtatt 242 AJM4129.PSQ
----- 258 Rev Comp of AJM5602.PSQ

```

ctncagctcc c 253 AJM4129.PSQ

----- - 258 Rev Comp of AJM5602.PSQ

||Alignment End||

AJM4133.PSQ vs. Rev Comp of AJM5505.PSQ

```
----- --ccgtgt gcgttcggta gtggtcgatg agcttggaac 36 AJM4133.PSQ
      | ||| ||||| | ||||| ||||| |||||
aagggccncc acacctgtgt gcgttcggna gtggtcgatg agcttggaac 50 Rev Comp of AJM5505.PSQ

gctctgtgaa gcgcttctcg cactcgggtgc aggggaagg cctctnacca 86 AJM4133.PSQ
||||| ||||| ||||| ||||| ||||| ||||| |||||
gctctgtgaa gcgcttctcg cactcgggtgc aggggaagg cctctcacia 100 Rev Comp of AJM5505.PSQ

gtgtgtagca tgcggtggcg gatgaggtgt gcaggcgctg tgaagcagng 136 AJM4133.PSQ
||||| ||||| ||||| ||||| ||||| ||||| |||||
gtgtgtagca tgcggtgacg gatgaggtgt gcaggcgctg tgaagcagcg 150 Rev Comp of AJM5505.PSQ

nccacactcc ccacacctca gagcgtgct gtcccgtgca ctgetcccac 186 AJM4133.PSQ
||||| ||||| ||||| ||||| ||||| ||||| |||||
gccacactcc ccacacctna gagcgtgct gtcccngca ctgettccaa 200 Rev Comp of AJM5505.PSQ

ctccgtgct gctctctct cgcgcgntgc ggtctcacga at 228 AJM4133.PSQ
|
cctgcgctg- ----- 209 Rev Comp of AJM5505.PSQ
```

||Alignment End||

AJM4303.PSQ vs. RMO0115.PSQ

```
ggtttgcaa caggctatga agcagacggc actaaattcc actccaaaca 50 AJM4303.PSQ
----- 0 RMO0115.PSQ

aggactcaca gctctcccac tgagagaaaa cctccaggt tctagcagag 100 AJM4303.PSQ
----- 0 RMO0115.PSQ

gcacacacta cttactgggc ccagagtcca gtccaagctc cccttcatt 150 AJM4303.PSQ
      |||| |||||
-----agctc tccttcatt 15 RMO0115.PSQ

gagtcacgtg ggccccatgg gtacgggtgc tcttctgct tgatccagga 200 AJM4303.PSQ
||||| ||||| ||||| ||||| ||||| ||||| |||||
gagtcacgtg ggccccatgg gtacgggtgc tcttctgct tgatccagga 65 RMO0115.PSQ

caaatgtc- ----- 209 AJM4303.PSQ
|||||
caaatgtca tgtgctnaga tgagagattc tgaattagg tctntgggaa 115 RMO0115.PSQ

----- 209 AJM4303.PSQ

tatctctctc tgccaaatcc tgctgatccc atacacactg ctgctcctcc 165 RMO0115.PSQ

----- 209 AJM4303.PSQ

tgcttaatcc gggacaaaag gtnctcggca gagatggggg agtcaagtga 215 RMO0115.PSQ
```

```

----- 209 AJM4303.PSQ
tggattctgt ggggatggct ctttcttcca ggntccgctt tgagaagttt 265 RM00115.PSQ

----- 209 AJM4303.PSQ
cgtgactaga gagttagtcg ccccggaatt ccgaccgtac ntcaagggtta 315 RM00115.PSQ

----- 209 AJM4303.PSQ
ccantttccn atagggagtc gattagagtt ngggata 352 RM00115.PSQ

||Alignment End||

AJM5225.PSQ vs. Rev Comp of AJM5507.PSQ
agaaagacca gcctccctcc tgtaagaccc tgttcgtctg tgccctgtct 50 AJM5225.PSQ
||
agcg----- 4 Rev Comp of AJM5507.PSQ

ctggcgcac tc caagagtc aacagtaatn gctgttctgc ggaagaccgt 100 AJM5225.PSQ
|| ||||| ||| ||| ||||| ||||| |||
-----tc tccaagagtc aacganaata cctgttctgc cgaagaccct 46 Rev Comp of AJM5507.PSQ

gagagaacag agcccaggga ctgcagcagc ctcagcgcag gaagagcaga 150 AJM5225.PSQ
||||| ||| ||||| ||| ||||| ||| ||||| ||| ||||| |||
gagagagcag agcccagggc ctgcagcaac ctcagtgcag gaagagnaga 96 Rev Comp of AJM5507.PSQ

agagaagccc caccaccca gaagagaaga tgggtgcagag cgcacacgnc 200 AJM5225.PSQ
||| ||| | ||||| ||| ||| ||| ||||| ||| ||| |||
aganaagaca caccaccca gnaganagga tgggtgccag ctgcctcacc 146 Rev Comp of AJM5507.PSQ

agcctgggccc tgtcaccaat gctgaag--- ----- 227 AJM5225.PSQ
| | || |
ttttccttct tttccttcag cactgctgac agggccaggc tggcatgtgc 196 Rev Comp of AJM5507.PSQ

----- 227 AJM5225.PSQ
gctct 201 Rev Comp of AJM5507.PSQ

||Alignment End||

```

Rat Cosmid/P1 Clone and Genetic Marker Isolation using Selected Rat cDNAs

With the availability of rat cDNA orthologs of mouse cDNAs from the *Lyp* region isolated as described above, genetic marker isolation was greatly facilitated. Initial rat cDNAs were used to screen the rat genomic cosmid library, again by hybridization, but with much clearer results, as the hybridizations could be performed at high stringency to greatly reduce the number of false positives. In addition, the availability of sequence information from these rat cDNAs made PCR screening of the genomic libraries possible as well.

Initial Genetic Marker Isolation using cDNAs Confirms *Lyp* in Left of Interval

For the initial rat cDNA work I chose two rat cDNAs: ajm3027 (a rat ortholog of ajm2713 from the first cross-species selection) and ajm4011 (a rat ortholog of bjr16 from the second cross-species selection). These cDNAs were used to screen the rat cosmid library, and each was used successfully to identify a rat cosmid hybridizing to it. These rat cosmids were then used to isolate SSR genetic markers (Materials and Methods), resulting in 3027ca1 (from the cosmid positive for ajm3027) and 5005L2/R1 (from the cosmid positive for ajm4011). When mapped on rats recombinant in the *Lyp* region, both genetic markers mapped to the right of *Lyp*, confirming the location of *Lyp* in the left portion of the original interval (see Figure 2 for key recombinant typings and the regions on the mouse YAC physical map corresponding to ajm3027 and ajm4011).

Genetic Markers Isolated using cDNAs in Left Third of Interval are Closer to *Lyp*

Based on the successful genetic marker isolations described above and the location of *Lyp* in the left part of the original interval, I directed further effort toward the left third of the original *Lyp* interval. The final cross-species cDNA selection involved template pool 4_24, which contained 24 mouse cDNAs from the left third of the mouse YAC walk, in the region of the dense mouse P1 and BAC physical map. Rat cDNAs obtained from this selection (see Figure 3 (f) and (l)) were used to screen the rat P1 genomic library (see Materials and Methods). Although the library had limited coverage and a rat P1 contig spanning the entire region could not be constructed, many islands of coverage were obtained (see Figure 4). These P1 clones were screened for SSR genetic markers, and two such markers (polymorphic in both mapping crosses) were identified: 5579CA1b and 5631CA3b/3c. When mapped on the panel of recombinant rat DNAs, 5579CA1b mapped immediately to the left of *Lyp*, representing the new

Rat Lyp Region YAC Contig Construction and Genetic Marker Isolation

The availability of a new high-coverage genomic rat YAC library[1] provided the opportunity to quickly isolate a rat YAC contig spanning the (now smaller) *Lyp* interval and to use it to saturate the region with additional rat genetic markers. The closest genetic markers flanking *Lyp* (5579CA1b and 207rpt1), two rat cDNA PCR assays within the region fully linked to *Lyp* (ajm5507 and ajm5513), and R236 were used to screen the rat YAC library and assemble a contig (see Figure 6, Materials and Methods). This contig contained the *Lyp* region (5579CA1b - 207rpt1) on two overlapping YACs.

In order to saturate the region with new rat genetic markers in order to map *Lyp* more finely, a number of YACs, including 320f9, 28e5, and 101f4 were cut into small (~500bp) fragments and subcloned (see Materials and Methods, Rat YAC Library). These libraries were then screened for simple-sequence repeat-containing clones (see Materials and Methods, SSR Isolation from Genomic Clones), which were sequenced. After eliminating those clones containing yeast sequences or non-SSR repeat sequences by BLAST database searching, PCR assays were constructed from the clone sequences that contained SSRs (these assays are named XXYY, where XX is the source YAC clone, and YY is the subclone ID; e.g. 28e5e09 was created from the sequence of subclone e09 from YAC 28e5). Those assays that were polymorphic between either Dp(*Lyp*) and Fischer(F344; *wt*) or between Dp(*Lyp*) and Dr(*wt*) were then mapped using the panel of rats with recombination breakpoints in the neighborhood of *Lyp*. The most important of these genotypes are shown in Figure 6 (key SSR typings are shown in Figure 7). Although no new genetic markers mapping between 5579CA1b and *Lyp* were identified, a number of new markers mapping between 5631CA3b/3c (fully linked with *Lyp*) and 207rpt1 were identified (four shown in Figure 6). Of these, 28e5e09 was the closest to *Lyp* and

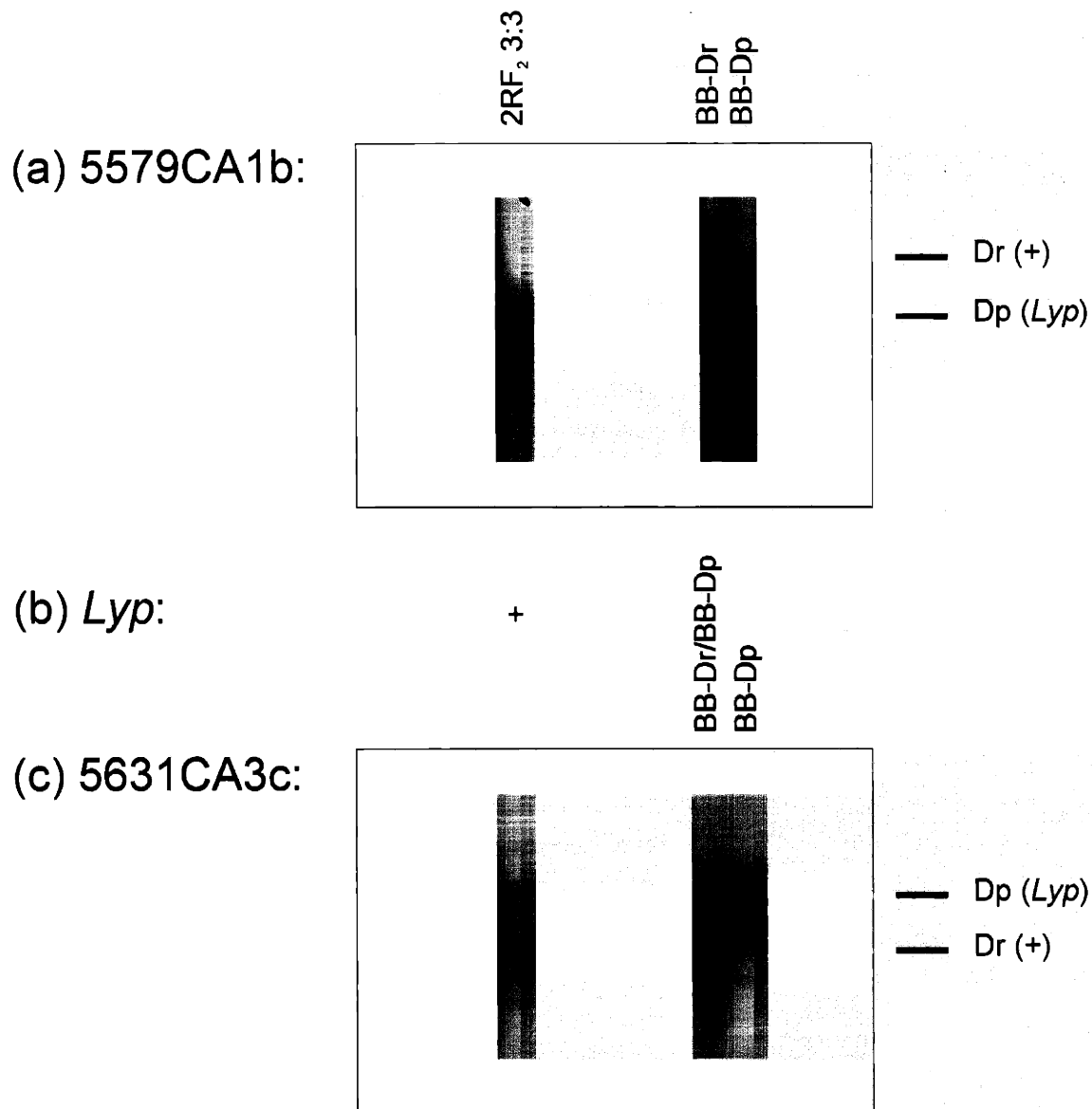


Figure 5: SSR genotypings of rat 2RF₂ 3:3 show that *Lyp* is to the right of the 5579CA1b genetic marker, and fully linked with 5631CA3c. 2RF₂ 3:3 is a descendant of 11CF₂ 12:2 that has the same recombinant genotype in the 5579CA1b / 5631CA3c region. The gel positions of the *Lyp* haplotype (BB-Dp) and + haplotype (BB-Dr) SSR alleles are indicated along the sides of the autoradiograph lanes. (a) 5579CA1b locus genotypings of 2RF₂ 3:3 and control rats. The 2RF₂ 3:3 genotype at 5579CA1b is clearly *Lyp/Lyp*, but the phenotype of that animal (b) is normal (+), showing that *Lyp* maps to the right of 5579CA1b. As shown in (c), 5631CA3c is fully linked to *Lyp*, as 2RF₂ 3:3 has the +/*Lyp* genotype at that marker expected from a normal (+) phenotype.

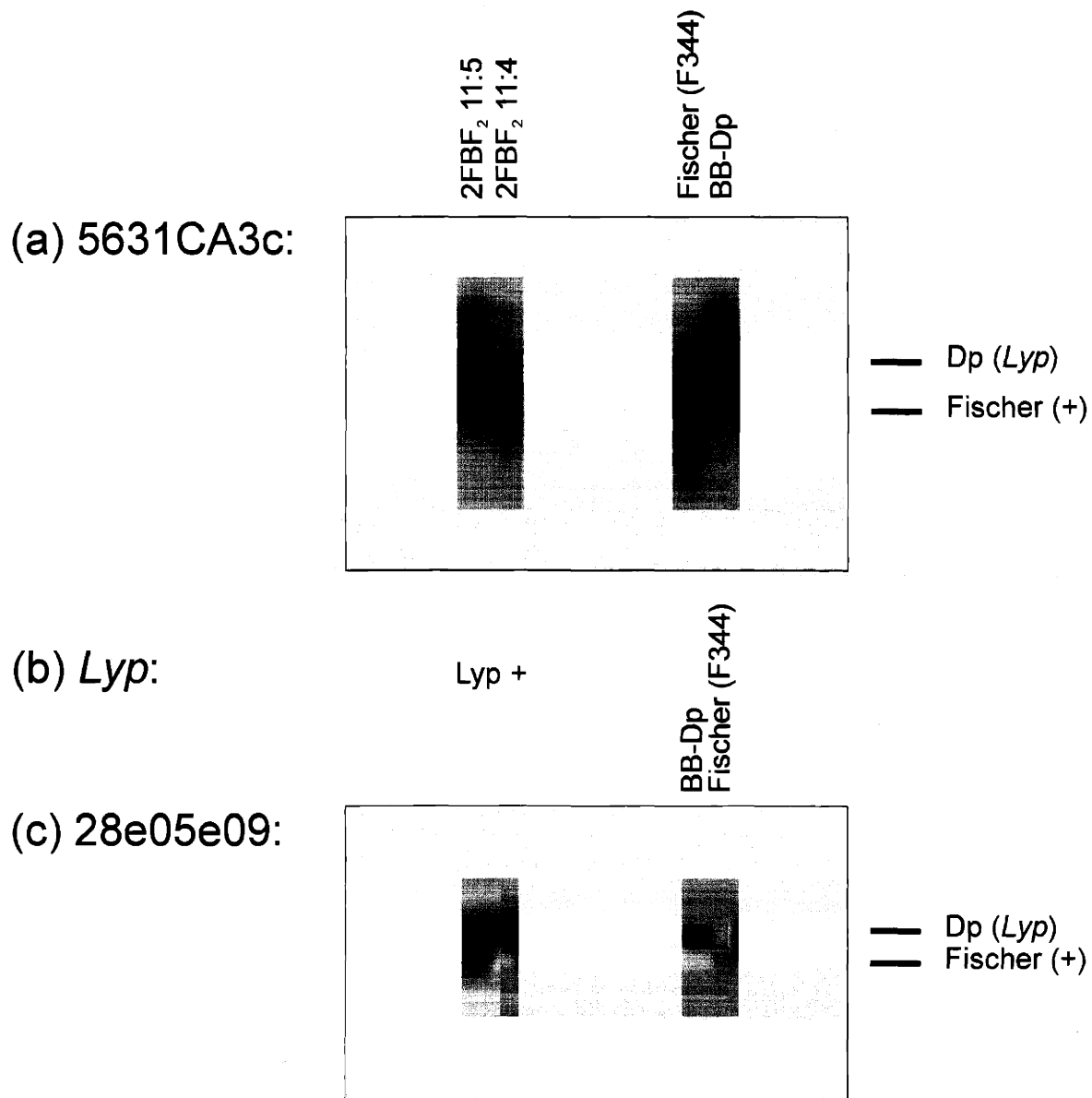


Figure 7: SSR genotypings of rats 2FBF₂ 11:5 and 2FBF₂ 11:4 show that *Lyp* is to the left of the 28e05e09 genetic marker, and fully linked with 5631CA3c. The gel positions of the *Lyp* haplotype (BB-Dp) and + haplotype (Fischer) SSR alleles are indicated along the sides of the autoradiograph lanes. (c) 28e05e09 locus genotypings of 2FBF₂ 11:5 and 2FBF₂ 11:4 and control rats. The 2FBF₂ 11:5 genotype at 28e05e09 is clearly +/*Lyp*, but the phenotype of that animal (b) is *Lyp*, showing that *Lyp* maps to the left of 28e05e09. Conversely, the 2FBF₂ 11:4 genotype at 28e05e09 is clearly *Lyp*/*Lyp*, but the phenotype of that animal (b) is +; this also shows that *Lyp* maps to the left of 28e05e09. As shown in (a), 5631CA3c is fully linked to *Lyp*, as both 2FBF₂ 11:5 and 2FBF₂ 11:4 have the genotypes (*Lyp*/*Lyp*, +/*Lyp*) at that marker expected from their respective phenotypes (*Lyp*, +).

defines the current right-hand boundary of the *Lyp* interval. Since both 5579CA1b and 28e5e09 are contained on rat YAC 28e5, this YAC contains the entire current rat *Lyp* interval.

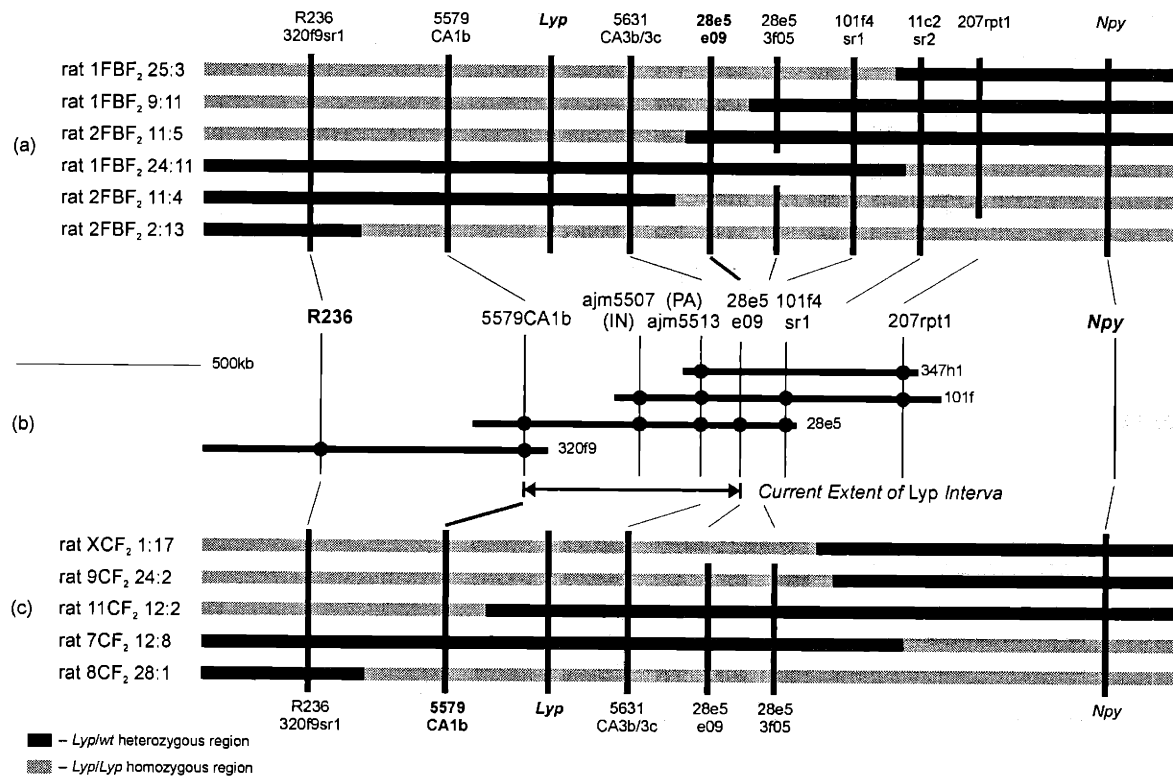


Figure 6: Rat YAC physical and high-resolution genetic maps of *Lyp* region of chromosome 4: (b) Center of figure shows rat YAC framework spanning *Lyp*, indicating PCR assays (except for 28e5-e09 and 101f4-sr1) used to identify the YACs and assemble the framework. (a) Upper third of figure shows the genotypes of key *Lyp* region recombinant animals from the rat Dp x Fischer (F344) cross, while (c) lower third of figure shows the genotypes of key *Lyp* region recombinant animals from the rat Dp x Dr cross (not all *Lyp* region genetic markers are polymorphic between Dp and Dr). The darkest region of each horizontal line represents a Dp/wt heterozygous region, while the lighter-colored region represents a Dp/Dp (*Lyp* haplotype) homozygous region. Breaks in the vertical lines indicate genotypes not done. Diagonal lines connect the genetic markers with the rat YAC framework, indicating the physical location of the genetic markers (5631CA3b/3c is an SSR obtained from a rat P1 that also contains 5513(PA)). As indicated in the figure, animals 2FBF₂ 11:5 and 2FBF₂ 11:4 (a) define the current rightmost extent of the *Lyp*-containing interval in the rat, while animal 11CF₂ 12:2 (c) defines the current leftmost extent of the *Lyp* interval.

Discussion

Sequence Conservation and the Identification of Orthologs

A pair of similar sequences are homologous if they share their similarity due to common ancestry. In the case of similar mouse and rat sequences, high levels of sequence similarity (>80% identical) can be presumed to indicate homology. Two homologous sequences may be related as orthologs, in which case they have diverged only after speciation events, or they may be related as paralogs, in which case they have diverged after at least one duplication of an ancestral sequence. Since paralogs may remain very similar in sequence, orthology cannot be conclusively determined by sequence similarity alone. Rather, phylogeny must be examined in order to minimize the incidence of paralogs misclassified as orthologs (see [21] for a discussion of this issue). Unfortunately, determining phylogeny is substantially more complex than a simple pairwise sequence comparison, and key related sequences from other species required to determine the phylogeny are often unavailable.

Thus, my working definition of orthology as a high degree of sequence similarity was likely to misidentify some paralogs as orthologs. However, as I was studying a large group of rat cDNAs isolated by cross-species cDNA selection, I expected the bulk of "orthologs" to truly be orthologs. These true orthologs, unlike most paralogs, should map to the rat *Lyp* region under COL; thus, the verification of COL described below also supports the general utility of my identification of orthologous sequences. As the verification of COL is based on the mapping of a number of sequences, any individual false orthologs should not disrupt the process.

As substantial number of human, mouse and rat ESTs have been sequenced, empirical data is now available on the degree of sequence conservation to be expected between mouse and rat genes.[20] In the 470 mouse-rat ortholog pairs studied, median coding region sequence

identity was 94%(range 75%-99%), while 5' UTR and 3' UTR median sequence identities were 87% and 88%, respectively. Thus, typical cDNA sequence identities between mouse and rat clones would be expected to be 85% or higher. Given the inaccuracies introduced by extensive PCR amplification during the cDNA selection process and rough, high-throughput single-pass sequencing, experimental sequence identities of 80%-90% would not be unreasonable between mouse and rat orthologs. The sample alignments of rat cDNAs isolated by selection against mouse cDNA templates were consistent with these values (see, for example, Figure 3 (j)). Thus, I expect that most of the rat cDNAs isolated through cross-species cDNA selection were the orthologs of the mouse template cDNAs.

Ortholog Isolation

Of the different methods of isolating orthologous rat DNA from mouse sequences whose use is described in this chapter, cross-species cDNA selection was clearly the best. The other methods were more labor-intensive, could process only a few samples in parallel, and were not particularly successful. In particular, PCR amplification of rat genomic DNA with mouse primers, although simple and important for mapping HoxA in the rat (see Chapter II), was unsuccessful as a general method.

Hybridization of mouse sequences against a rat genomic Southern blot (or rat genomic cosmid library screening Southern blot) did show limited success, but is hampered by the fact that repetitive sequences in the mouse probe are also likely to be conserved in the rat (e.g. mouse-human sequence conservation of L1 repeat sequences is on the order of 70%[22]), and to produce background hybridization, lessening the signal/noise ratio. Smaller mouse probe fragments limit the chance of including repetitive sequences in the probe, but also decrease the chance of including in the probe a region of sequence conserved in the rat.

Cross-species cDNA selection, on the other hand, produced a wide range of rat cDNAs in parallel (for the latter three selections, the rat cDNAs isolated corresponded to 33-50% of the template mouse cDNAs; Table 2) with a high degree of sequence similarity to their mouse template cDNAs (see Figure 3, (g)-(l)), and with a high degree of specificity (again, for the latter three selections, 63-74% of rat cDNAs isolated matched one of the mouse template cDNAs). One key advantage of this technique is that, since the mouse cDNAs used as templates were fully sequenced, repetitive or otherwise undesirable sequences could be removed from the template pool by inspection. The other key advantage is that this technique is a selection (for sequence similarity with the template sequences) and thus much more efficient than a screening technique such as assaying random mouse sequences (or even exons) as probes on rat Southern blots.

Of course, as EST sequence data is gathered from more and more species, isolating orthologous sequences across species will become a matter of database searching and PCR primer design; this scenario is already nearing feasibility for mouse and human cDNA sequences from many tissues. However, until this becomes practical for many more species, cross-species cDNA selection will provide an efficient method to isolate large numbers of orthologous sequences in parallel.

Integration of Mouse Physical Map with Rat Genetic Map -- COL Verification

The five rat *Lyp* region genetic markers newly identified from mouse exon and cDNA sequences in this chapter had the same relative order in mouse and rat (Figure 2, Figure 4), thus extending the confirmation of COL between rat and mouse *Lyp* regions. As the placement of the mouse *Lyp* gene and its neighbors in the mouse *Lyp* region depends on COL, it is important to verify continued COL between rat and mouse as new markers become available.

Interestingly, recent human chromosome 7 genomic sequencing results indicate that COL between mouse and human seems to break down precisely in the middle of the mouse *Lyp* region, as described more fully in the Supplement.

A Full-Coverage Physical Map and High-Resolution Genetic Map in the Rat

As shown in Figure 2 and Figure 4, the rat cosmid and rat P1 libraries were quite insufficient to span the rat *Lyp* region. The P1 clones did provide enough genomic DNA to identify new rat genetic markers, but even with the dozen or so new rat STSs generated in the left part of the *Lyp* region, were insufficiently long to create a contig with that limited set of STSs. The newly available rat YAC library[1], with an mean insert size of 820 kb, easily produced a contig spanning the region (Figure 6). Although the genomic fidelity of YACs is less than optimal, this YAC contig was sufficient to develop a dense set of genetic markers (as shown in Figure 6) that narrowed the *Lyp* interval substantially, to a portion of a single YAC. Further development of a high-resolution, high-fidelity rat physical map of the *Lyp* region is described in the Supplement.

Conclusion

The development of cross-species cDNA selection described in this chapter made practical and efficient the transfer of positional information between mouse and rat required for a COL cloning strategy, and the transferred markers served to further confirm the COL between mouse and rat *Lyp* regions at higher resolution. Cross-species cDNA selection is sufficiently versatile (by adjusting stringency) to be applied to other COL-based projects where ortholog identification is required.

Cross-species cDNA selection produced enough rat STSs from the *Lyp* region so that a rat YAC contig spanning the region could be rapidly assembled once an appropriate rat YAC library was available. This rat YAC contig has already produced a large number of new rat genetic markers which have narrowed the rat *Lyp* region to a portion of a single YAC clone.

The integration of rat genetic mapping information with the mouse high-resolution physical map was sufficient to permit the inclusion and exclusion of mouse transcription units from consideration as *Lyp* gene candidates based on their position. Thus, transcription units can now be intelligently evaluated as *Lyp* candidates (Chapter V) while the *Lyp* region is narrowed even further and the reagents prepared for future comparative genomic sequencing of mouse and rat *Lyp* regions (Supplement).

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CHAPTER V

Transcript Analysis and Identification of PA, a *Lyp* Candidate Gene Associated with Late Thymic T-Cell Maturation

Introduction

Once rat positional information had narrowed the *Lyp* critical interval substantially, we set out to characterize the transcription units within the critical interval in order to determine which might be likely candidates for the *Lyp* gene. Continuing the COL strategy, we elected to study transcription units in the mouse *Lyp* region, making use of the physical mapping and cDNA resources previously developed (Chapter III) and concentrating on the mouse region corresponding to the rat region fully linked to *Lyp* (Chapter IV). As described in the latter part of this chapter, among the transcripts in that region was PA, a gene fully linked with *Lyp* whose transcript level is reduced in *Lyp/Lyp* animals and which is associated with the final stages of T-cell maturation in the thymus.

Strategy

As described in Chapter III, the cDNAs comprising the bulk of the mouse *Lyp* region STSs were selected from a mixture of testis and thymus cDNA, and thus the *Lyp* gene, acting in the thymus, would be likely to be represented among them (see Chapter III for some calculations on gene coverage by the cDNA fragments). We therefore began with the set of STS-mapped cDNAs mapping in the mouse *Lyp* region and performed three stages of analysis.

Consolidate Transcription Units

First, we attempted to group the cDNA fragments into transcription units. Various techniques were used for this, including determining sequence overlap, isolating and sequencing larger cDNA clones from a cDNA library and comparing BLAST sequence similarity search results for clones in the same or neighboring STS locations.

Evaluate Transcription Units

Transcription units were then evaluated as candidates for *Lyp*. Since *Lyp* is thought to act in the thymus, we assayed for expression in the thymus, analyzed clone sequence for clues to function, and attempted to identify functional polymorphisms (between *Lyp* and wild-type) in the candidates.

Study Good Candidates

Finally, with the identification of the PA gene as a good candidate for *Lyp*, we studied it in more detail, determining a substantially complete cDNA sequence, the entire sequence of its coding region (and resequencing it in BB-Dp (*Lyp*) and BB-Dr (wild-type) cDNA), and studying its expression pattern in more detail.

Materials and Methods

PCR Analysis

Unless pre-existing or otherwise noted, PCR primers were selected using the Primer 0.5 [1] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. STS and SSR marker assay primer information is listed in Appendix A, while exon and cDNA fragment information is listed in Appendix B. PCR

amplification was performed according to the conditions specified for each protocol (e.g. YAC library screening), or, if not specified, according to standard conditions as recommended by Perkin-Elmer (see Chapter II).

High-fidelity or long-range PCR was performed using Expand Long Template PCR System (BMB), which includes a mixture of *Taq* and *Pwo* DNA polymerases to provide proof-reading activity during amplification, and TaqStart (Clontech) an antibody that blocks the polymerase active site until the initial denaturing phase, thus providing true "hot-start" PCR. PCR conditions were modified slightly from the standard when using this polymerase mixture: supplied PCR "Buffer 1" was used (1.75 mM final MgCl₂ concentration), individual nucleotide concentrations were 350 µM, primer concentrations were 300 nM each, and the 2.6 U polymerase mixture was used for each 50 µl reaction. PCR was performed in a Perkin-Elmer 9600 thermal cycler as follows: 94°C 2'; 35x 94°C 15s, 64°C 30s, 68°C 5'; 68°C 7'. Note: for primers with predicted melting temperatures other than 60°C, an annealing temperature 4°C above the predicted melting temperature was used. This elevated annealing temperature greatly reduced the amplification of non-specific products.

Sequencing

Unless otherwise noted, sequencing was performed on PCR products amplified using M13-f and -r primers (see Appendix A) from 1/10 dilutions of 96-well-plate minipreps[2], using 30 cycles of PCR with 50°C annealing temperature, and purified with Qia-Quickspin PCR (Qiagen) according to instructions, or Ultrafree-MC filters, 30,000 MW, PLTK membrane columns (Millipore) by diluting in 300 µl water, spinning, and resuspending in 25 µl TE. Direct sequencing of PCR products was also performed on PCR products amplified using the Expand system described above after purifying the PCR products with Qia-Quickspin PCR columns.

Cycle-sequencing reactions using M13-f or M13-r dye primers were performed according to recommended conditions (ABI) and run on an ABI-373A fluorescent sequencer. Dye terminator sequencing was performed similarly, using the ABI standard dye terminator sequencing kit. The annealing temperature used for dye terminator sequencing was the predicted melting temperature of the primer used. (Further details appear in the Materials and Methods for Chapter III)

The resulting sequences were processed to remove vector or other contaminant sequences (e.g. hemoglobin exon sequences) using the computer programs EXSTRIP or FREEINST[3] and analyzed for overlaps using the programs COMPSEQ and COMP2SEQ[4]. Database searches to identify repeat sequences or homology with known genes were performed using Blast in both the email server (NCBI) and web (NCBI) versions[5].

RNA Analysis

45-50-day-old BB-Dp (*Lyp/Lyp*), BB-Dr (*wt/wt*), or Fischer F344 rat thymus total RNA was isolated using Trizol (Life Technologies). Briefly, fresh tissue was homogenized in a 10x volume of Trizol, briefly incubated, and any debris removed by centrifugation. The cleared lysate was then chloroform extracted; finally, total RNA was recovered from the aqueous layer of the extraction by isopropanol precipitation, washed with 70% ethanol, and resuspended in water.

Poly-A⁺ RNA was prepared using Oligo(dT) cellulose columns (Life Technologies) by: 0.1M NaOH-washing the column, equilibrating it with 4 ml binding buffer (10 mM Tris-HCl pH7.5, 1 mM EDTA, 0.3M NaCl, 0.1% SDS), denaturing (5' 70°C) and quenching the RNA sample (in 3 ml binding buffer), and applying it to the column. The flow-through was collected and re-applied to the column before the column was washed with 4 ml binding buffer to elute

Poly-A- RNA. Bound poly-A+ RNA was eluted with 1.5 ml elution buffer (10 mM Tris-HCl pH7.5, 1 mM EDTA, 0.1% SDS). If a second round of selection was desired, the column was re-equilibrated with binding buffer, the eluted RNA was denatured, quenched, warmed to room temp. for 20', and 90 μ l 5M NaCl added. This RNA solution was then immediately applied to the column, and the column was processed as before. After elution in 1.5 ml of elution buffer, the poly-A+ RNA was precipitated by adding 90 μ l 5M NaCl and 3 ml ethanol, washed with 70% ethanol, briefly dried, and resuspended in water.

Northern blots were prepared by electrophoresing either 1 μ g poly-A+ RNA or 5 μ g total RNA per lane (denatured, in formamide loading buffer) on a 1% SeaKem ME agarose formaldehyde gel, as well as a marker lane of 3 μ l RNA ladder marker (Life Technologies). After electrophoresis, RNA was transferred to a GeneScreen Plus nylon membrane through capillary transfer using either 20x SSC or 5 mM NaOH as the transfer buffer. After transfer, the membrane was UV-crosslinked (Stratalinker; auto setting). Hybridizations were as described in Chapter III.

cDNA preparation was performed using the Marathon cDNA amplification kit (Clontech) on doubly-selected poly-A+ RNA according to the manufacturer's instructions. Briefly, a modified lock-docking oligo(dT) primer containing two degenerate nucleotide positions at the 3' end was used to prime first-strand synthesis with an RNase H- MMLV reverse transcriptase. Second-strand synthesis was performed using a Gubler & Hoffmann procedure, the cDNA ends were blunted using T4 DNA polymerase, and the Marathon cDNA adapter was ligated to the cDNA.

cDNA Libraries

The mouse cDNA library used was Stratagene catalog # 938305, mouse female B6 thymus library in Lambda ZAP Express/EcoRI. The library is oligo-dT and random-primed, insert size >0.4 kb, av. insert size 1 kb, 2×10^6 primary recombinants, estimated titer 3×10^6 per μ l. The rat cDNA library used was Stratagene catalog # 936502, rat male Sprague Dawley (6 week old) thymus library in Lambda ZAP II. The library is oligo-dT-primed, insert size >0.5 kb, av. insert size 1 kb, 2×10^6 primary recombinants, estimated titer 3×10^7 per μ l.

Library screening was performed as follows: each library was plated onto 10 large petri dishes using XL-1Blue MRF' plating cells (grown in LB-Mg) to a density of 5×10^5 to 1×10^6 plaques/dish. These were then lifted onto pre-autoclaved Colony/PlaqueScreen filters (DuPont/NEN) and processed by autoclaving 1' 100°C dry cycle. Hybridization and washing were performed as described in Chapter III, with a final wash of 0.1xSSC/0.1% SDS at 60°C. For secondary purification, positive regions of the plates were plugged (1 cm diameter), diluted in SM buffer, and replated at a density of 300-1000 plaques/plate. Lifts were made as described above, hybridized, washed and exposed. Positive plaques (or in some cases, two adjacent plaques) were plugged (1 mm diameter) and stored in SM at 4°C over a drop of chloroform. In order to purify an insert fragment without tertiary screening, PCR primers from the probe sequence used to isolate the clone were used along with plasmid vector primers flanking the cloning site (pBM13for and pBM13rev, or t3bsext and t7bsext) to amplify the four combinations pBM13for/probe -f primer, pBM13for/probe -r primer, pBM13rev/probe -f primer, pBM13rev/probe -r primer, using the BMB proofreading polymerase mixture described above and an annealing temperature 4°C above the predicted primer melting temperature in order to insure specificity. This produced products in two of the four reactions (e.g. pBM13for/-r and

pBM13rev/-f), which together spanned the insert of the cDNA clone, overlapping only in the region bounded by the probe's -f and -r primers; this also allowed the cDNA orientation with respect to the vector cloning site to be determined. These DNA fragments were then sequenced from the vector end using M13 dye primer reactions and from the probe-specific primer end using the probe-specific primer and dye-terminator reactions; some fragments were also used as hybridization probes. The requirement for successful amplification of the cDNA clone with the probe-specific primers served to screen out false positive cDNA clones containing sequences only related to the probe sequence, but not matching it exactly.

Results

Consolidation of Transcription Units

We began our transcription unit analysis with the more than 70 mouse *Lyp* region cDNA fragments isolated and fine-mapped in Chapter III. These were initially grouped by sequence overlap, requiring at least a 25 bp long perfect-match overlap. All such overlaps proved to be between cDNAs in the same physical location ("bin") or in adjacent bins, confirming the robustness of this technique. Additionally, clones with similarity to known repeats (e.g. IAP-like sequences adjacent to NM) were excluded at this point.

Clones were then pooled as groups of 4-6 probes and used to screen a mouse thymus cDNA library. The screening technique allowed the unambiguous identification for each cDNA clone recovered of which probe had recognized it (see Materials and Methods). Recovered cDNA clones were then sequenced and the sequence iteratively used as before to group clones by overlap. BLAST database sequence similarity searching was also performed, and used along with physical adjacency to group non-overlapping contigs together. For example, two contigs,

Transcription Unit Name	Candidate Status	Mouse cDNAs Isolated	Rat cDNAs Isolated	Expressed in Thymus	<i>Lyp/Lyp</i> vs wild-type polymorphism	BLAST Matches
AZ	excluded	No	No	-	-	No
WA	excluded	Yes	Yes	Yes	No	Protein disulfide isomerase
OR	excluded	Yes	Yes	Yes	No	zf
AK	excluded	No	No	-	-	No
CA	excluded	No	No	-	-	No
NV	excluded	No	No	-	-	zf
CO	excluded	Yes	Yes	-	-	related ESTs
NM	excluded	No	Yes	-	-	No
NE	excluded	No	No	-	-	zf
MT		No	Yes	Yes	No	No
KS		No	No	-	-	ESTs
TX		Yes	Yes	Yes (matches thymus cDNAs)	-	No
OK		No	No	-	-	No
ND		No	No	-	-	No
SD		No	No	-	-	No
IL		Yes	No	Yes	No	No
WY		No	No	-	-	No
IA		Yes	No	Yes (matches T-cell cDNAs)	No	ESTs
IN		Yes	Yes	Yes (matches thymus cDNAs)	No	ESTs
MI		No	No	-	-	No
MO		Yes	No	Yes (matches T-cell cDNAs)	No	spondin-like
MS		No	No	-	-	No
WI		Yes	No	Yes	No	zf
KY		Yes	No	-	-	ESTs
OH		No	Yes	-	-	No
OH		No	Yes	-	-	No
TN		Yes	No	Yes	No	zf
TN		Yes	Yes	Yes	No	No
NC		Yes	Yes	Yes	<u>Yes</u>	ESTs
PA	strong candidate	Yes	Yes	Yes	<u>Yes</u>	ESTs
MD		No	No	-	-	No
NJ		No	No	-	-	No

Table 1: Summary of transcription unit data: Transcription units are presented in map order (as shown in Figure 1). At least the leftmost transcription units have been excluded as candidates for *Lyp*, as *Lyp* is to the right of a breakpoint to the right of the rat genetic marker 5579CA1b (see Figure 1). Mouse and Rat "cDNAs Isolated" columns indicate whether mouse or rat cDNA clones overlapping the initial STS were isolated. The next columns indicate whether the transcript is expressed in the thymus (by Northern blotting or otherwise, as indicated) and whether there is a visible size or abundance polymorphism between wild-type and *Lyp/Lyp* transcripts. The final column indicates whether any database matches to the transcript were found by using BLAST (against both NR and dbEST databases). Hyphens indicate the lack of a visible band on the Northern blot; no expression or polymorphism evaluation was thus possible.

Evaluation of Transcription Units

As shown in Table 1, these transcription units were then evaluated as candidates for *Lyp* on the basis of expression in the thymus (the inferred site of action of *Lyp*) by Northern blot or presence in a thymus or T-cell cDNA library, difference in transcript size or expression level between *Lyp/Lyp* and wild-type thymus on a Northern blot, and any functional inferences made on the basis of database sequence similarity. Of the 13 transcription units with significant BLAST matches, one was WA, the previously known gene, 5 matched only exactly-matching ESTs, without providing any functional information, and 6 matched sequences that provided functional insight (i.e. zf domains or spondin domains; the remaining 1 clone had only a non-identical match with an EST of unknown function).

After the grouping into 30 transcription units, a new recombinant animal placing *Lyp* to the right of the rat 5579CA1b genetic marker eliminated 9 of the 30 transcription units from consideration: those transcription units were all well to the left of the mouse map location corresponding to 5579CA1b (see Figure 1); these are so noted in Table 1.

After completing the expression analysis, 19 transcription units (chiefly the singletons mentioned above) gave no visible band on the Northern blots; of the remaining transcription units, one, PA, showed a significant (approximately 4x) reduction in its 1.3 kb transcript level in *Lyp/Lyp* vs. wild-type thymus RNA (see Figure 2). We therefore selected this clone for more extensive analysis.

Characterization of PA, a Transcript Whose Level is Reduced in Lyp/Lyp Thymus

By screening the mouse and rat thymus cDNA libraries as described in Materials and Methods, and by making use of database EST sequences, we were able to assemble an 1191 bp-long sequence of the rat PA transcript (excluding the poly-A tail). The transcript contains a

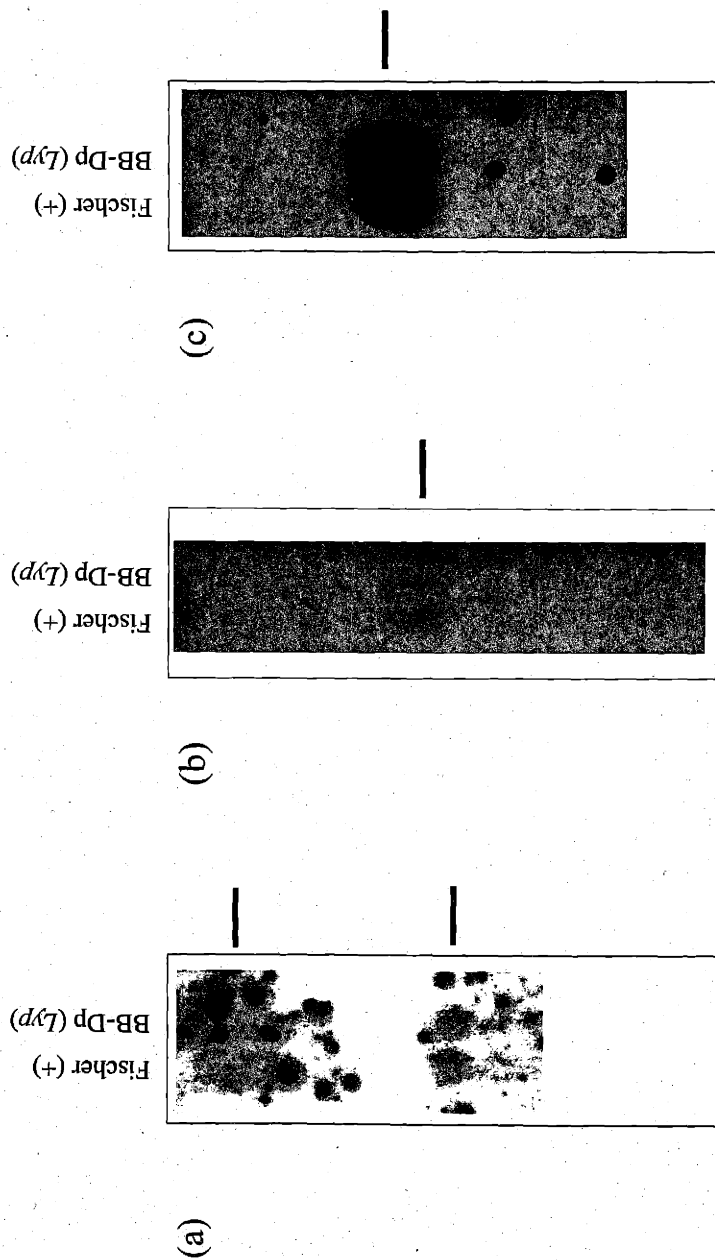


Figure 2: Rat thymus Northern blots showing expression differences between *Lyp* and wild-type for the PA transcript. In each case, wild-type (Fischer F344) thymus RNA is on the left, and *Lyp* (BB-Dp) thymus RNA is on the right. (a) ajm5507 (IN transcription unit) probing showing equal-intensity bands in wild-type and *Lyp* thymus RNA. (b) ajm5513 (PA transcription unit) probing showing an approximately 3x reduction in transcript level in *Lyp* versus wild-type thymus RNA. Northern blots contained ~2 μ g poly-A+ per lane, equalized to show the same β -actin probe signal (c). Markers along sides of autoradiograms indicate location of bands in lanes.

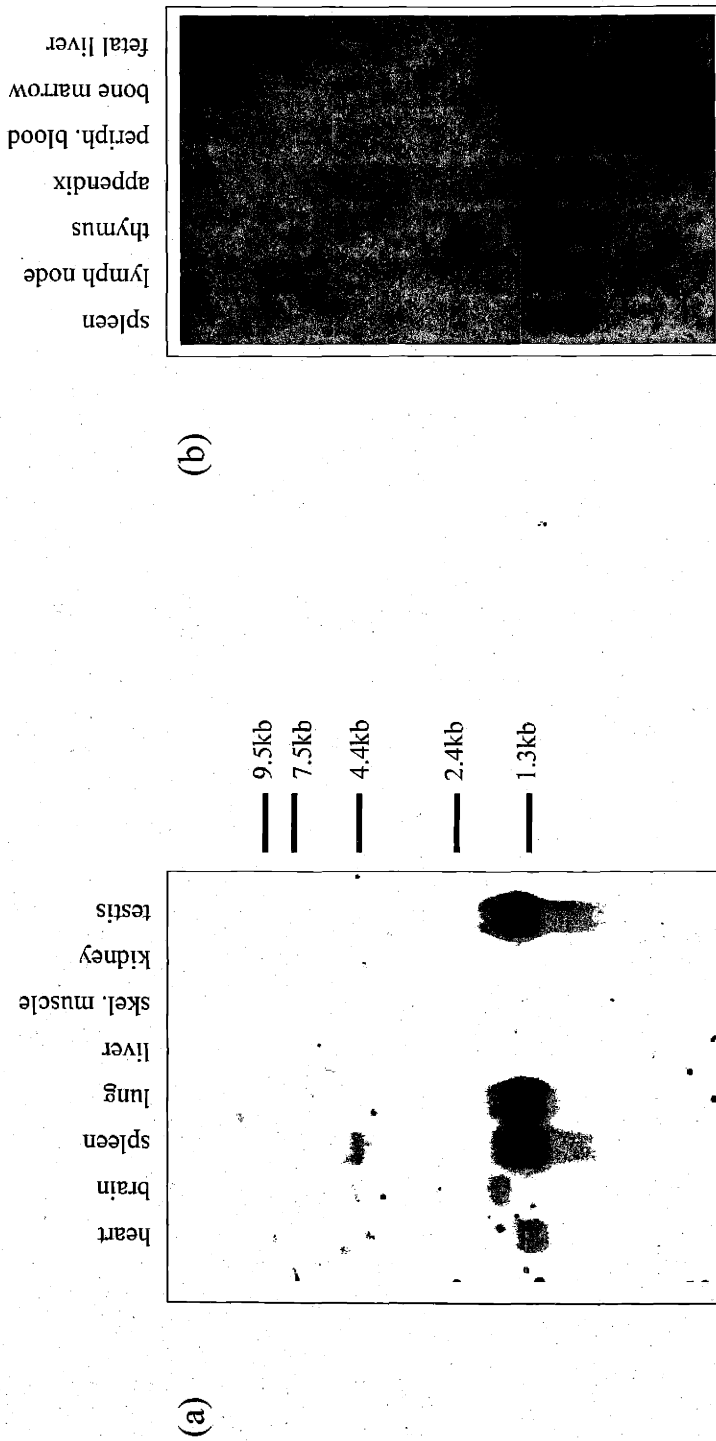


Figure 3: Northern blots showing the rat and human tissue distribution of the PA transcript. As expected for *Lyp*, PA expression is strongest in thymus, strong in other immune-related organs such as the spleen, and weak elsewhere. Poly-A+ Northern blots were probed with the rat PA cDNA clone and washed at 0.1x SSC/55°C (rat) or 0.1x SSC/50°C (human). (a) Rat multiple-tissue Northern shows a major transcript consistent in size with the ~1.3kb of known PA sequence (see Chapter V, Supplement), with a possible larger-sized transcript in the brain. (b) Human immune-system Northern, though showing high background due to lower-stringency cross-species probing, shows highest levels of expression in thymus, bone marrow, and fetal liver tissues, with a similar-sized transcript. Northern blots were from Clontech, containing ~2 µg poly-A+ per lane, equalized to show the same β-actin probe signal.

single long open reading frame predicted to encode a protein 293 amino acids in length (see Figure 4), a polyadenylation signal and a poly-A tail. This sequence may be somewhat incomplete at the 5' end, as the orthologous mouse sequence extends approximately 60 bp further upstream of the 5' end of the rat cDNA clone; however, the transcript size of ~1.3 kb on Northern blots suggests that any additional sequence at the 5' end is quite limited in length (see also Supplement). The two contigs of mouse sequence partially spanning the rat sequence are each about 90% identical with the rat sequence, and the portions within the long open reading frame are predicted to code for protein segments with >95% identity with the rat protein.

We then examined the expression pattern of PA in order to determine whether it was consistent with that expected of *Lyp*. As shown in Figure 3, using the rat cDNA as a probe, PA expression is strongest in the thymus, strong in immune-related organs (esp. spleen), and weaker elsewhere. In addition, the two closest database matches to PA (Figure 6)[6,7], are to GTP-binding-domain-containing proteins involved in plant host defense and in mammalian testosterone-mediated changes in immune response. These results strongly suggest that PA is involved in immune system function. *In situ* hybridization experiments using PA as a probe on rat thymus sections (personal communication, [8]) show that PA is expressed in punctate form (on only one or a few cells in a cluster) in the medulla of the thymus, and not expressed in the thymic cortex. As the final stages of T-cell maturation occur in the thymic medulla, this is again consistent with what we expect of *Lyp*.

Finally, we resequenced the PA transcript in *Lyp/Lyp* and wild-type rat thymuses by amplifying cDNA from each genotype with the 5513-5 and 5513-3 primers derived from the ends of the sequence in Figure 4 (see also Supplement). This resequencing spanned the entire coding region and most of the rest of the transcript except for the terminal 20 bp on each end. As

shown in Figure 5, a single polymorphism was identified; however, as it falls in the third base of a codon, there is no predicted amino acid change between the two sequences -- each encodes valine at that position.

Figure 4: cDNA Sequence of Rat and Mouse PA Transcripts (below)

Rat		
MouseC1	1	AGTCCTGGTGCAGTCAGATTCTTTGAAGAAGGTGTGAGAACTCAAAGGCC
Rat	1	GCACCTGGATTCCAGAGCTTCCACATCAGACCATGGCTGAA
MouseC1	51	CTGCAGGAG.....G.T.....G
Rat	1	METAlaGlu
MouseC1	1	. . .
Rat	42	CTGGATGATGACTCTCTGAGGATTGTTCTGGTAGGGAAAACAGGAAGTGGA
MouseC1	101	.CCAG...CA.....
Rat	4	LeuAspAspSerLeuArgIleValLeuValGlyLysThrGlySerGly
MouseC1	4	ProSer . Asn
Rat	93	AAGAGCGCCACAGCAAACACCATCCTGGGGCAAAGATATTTACTTCTAGA
MouseC1	152G....GT.....
Rat	21	LysSerAlaThrAlaAsnThrIleLeuGlyGlnLysIlePheThrSerArg
MouseC1	21 Arg . Val . .
Rat	144	ATCGCACCCACGCTGTCACCCAGACCTGTCAGAAGGCATCCCGCAGGTGG
MouseC1	203T.....A.....GT.AC.....
Rat	38	IleAlaProHisAlaValThrGlnThrCysGlnLysAlaSerArgArgTrp
MouseC1	38 Asn . . SerAsp
Rat	195	AAGGAAAGAGACCTCCTGGTTGTTGACACCCAGGACTCTTCGACACCAAG
MouseC1	254T.....G.....
Rat	55	LysGluArgAspLeuLeuValValAspThrProGlyLeuPheAspThrLys
MouseC1	55
Rat	246	GTGAAGTGGAAACCACCAGCATTGAAATCAGCCGGTGTGTCCTTCAGTCCT
MouseC1	305	..A.....T..C.....C.....
Rat	72	ValAsnTrpLysProProAlaLeuLysSerAlaGlyValSerPheSerPro
MouseC1	72 Ser . .
Rat	297	GCCCCGGGCCTCATGCCATCATTCTTGTCTGCAACTGGAATCGCTTCACG
MouseC1	356T.....G.....-..C.....
Rat	89	AlaProGlyLeuMetProSerPheLeuPheCysAsnTrpAsnArgPheThr
MouseC1	89	. Leu Trp . . . ?
Rat	348	ATAGAAGAGCAAGAGACCGTTACTAGGATCAAGGCTATCTTTGGGAAGGCA
MouseC1	406	G.G.....A..T....TC.....G...A.
Rat	106	IleGluGluGlnGluThrValThrArgIleLysAlaIlePheGlyLysAla
MouseC1	106	Val Ile GluGlu
Rat	399	GTCATGAAGTACCTGATCATCTTGTTCACCCGCAAAGACGAGTTGGAGGAC
MouseC1	457A....TG.....T..TC.....
Rat	123	ValMetLysTyrLeuIleIleLeuPheThrArgLysAspGluLeuGluAsp
MouseC1	123 Met . Val Asp . . .
Rat	450	CAGAACCTAAACGATTTCATTGAAGACTCAGATACTAACCTTAAAGCATC
MouseC1	508G.T...G...C.....CT.....
Rat	140	GlnAsnLeuAsnAspPheIleGluAspSerAspThrAsnLeuLysSerIle
MouseC1	140	. Ser . Ser Ala . . .
Rat	501	ATCAAGGAGTGTGACAGCCGCTATCTGGCCATCAATAACAAAGCGGAGGGG
MouseC2	1	AA..
Rat	157	IleLysGluCysAspSerArgTyrLeuAlaIleAsnAsnLysAlaGluGly
MouseC2	1	Arg

Figure 6: Closest BLAST database matches to the rat PA transcript sequence. (below)

(a) gi|1550784|emb|Y08026|MMAIP38
M.musculus mRNA for immunity associated protein 38
Length = 1632 Score = 94.8 bits (232), Expect(2) = 1e-34
Identities = 43/131 (32%), Positives = 84/131 (63%),
Gaps = 5/131 (3%) Frame = +1

Query: 103 RFTIEEQETVTRIKAIFGKAVMKYLIILFTRKDELEDQNLNDFIEDSDTN-LKSIKECD 161
RFT+++ + + +K +FGK VM +++FTR+++L +L D++ +D L+ ++ EC
Sbjct: 637 RFTMQDSQALAAVKRLFGKQVMARTVVVFTRQEDLAGDSLQDYVHCTDNRALRDLVAECG 816

Query: 162 SRYLAINNKAEGAEGEMQVQELMGFVSLVRSNGGLYFSDPIYKYAEQRL----KKQVGI 217
R A+NN+A G+E E Q ++L+G V LVR +GG ++S+ +Y+ + + QV
Sbjct: 817 GRVLCALNNRATGSEREAQAEQLLGMVACLVRHGGGAHYSNEVYELVQDTRCADPQDQVAK 996

Query: 218 LREIYTDVLEKEIRIV 233
+ EI + +++ R++
Sbjct: 997 VAEIVAERMQRRL 1044

Score = 75.3 bits (182), Expect(2) = 1e-34
Identities = 39/83 (46%), Positives = 55/83 (65%), Gaps = 1/83 (1%)
Frame = +2

Query: 9 LRIVLVGKTGSGKSATANTILGQKIFTSRIAPHAVTQTCQKASRRWKERDLLVVDTPGLF 68
LR++LVG+TG+GKSAT N+ILGQK F SR+ VT++C ASR W + VVDTP +F
Sbjct: 353 LRLILVGRGTGTGKSATGNSILGQKFLSRLGAVPVTRSCTLASRMWAGWQVEVVDTPDIF 532

Query: 69 DTKV-NWKPPALKSAGVSFSPAPG 91
+++ P +++A APG
Sbjct: 533 SSEIPRTDPGCVETARCFVLSAPG 604

(b) gi|1127803|gb|U40856|ATU40856
Arabidopsis thaliana AIG1 mRNA, complete cds
Length = 1381 Score = 103 bits (254), Expect = 7e-21
Identities = 86/274 (31%), Positives = 142/274 (51%),
Gaps = 14/274 (5%) Frame = +1

Query: 11 IVLVGKTGSGKSATANTILGQKIFTSRIAPHAVTQTCQKASRRWKERDLL-VVDTPGLFD 69
IVLVG+TG+GKSAT N+I+ K+F S+ VT C E +L V+DTPGLFD
Sbjct: 178 IVLVGRTGNGKSATGNSIVRSKVFKSKTKSSGVMTMECHAVKAVTPEGPIILNVIDTPGLFD 357

Query: 70 TKVNWKPALKSAGVSFSPAPGLMPSFLFCN-WNRFTIEEQETVTRIKAIFGKAVMKYLI 128
V+ + + GL L + R + EE+ ++ ++ +FG ++ YLI
Sbjct: 358 LSVSAEFIGKEIVKCLTLADGGLHAVLLVLSVRTRISQEEEMVLSTLQVLFSGSKIVDYLI 537

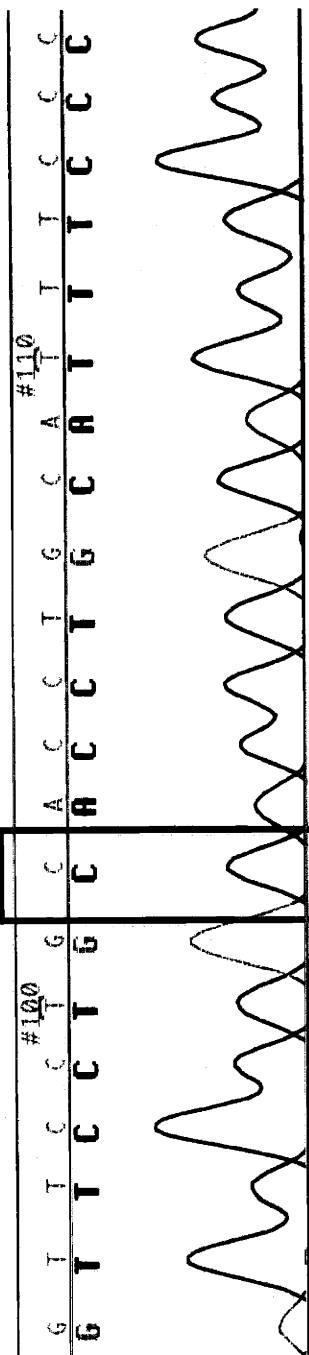
Query: 129 ILFTRKDELED--QNLNDFIEDSDTN-LKSIKECDSRYLAINNKAEGAEGE-MQVQELM 184
++FT D LED L D++ D+ + LK ++ C R + +NK + E + QV EL+
Sbjct: 538 VVFTGGDVLEDDGMTLEDYLGDNMPDFLKRVLILCGQRMILFDNKTDDKKTQVHELL 717

Query: 185 GFVESLVRSNGGLYFSDPIY---KYAEQRLKKQVGILR-----EIYTDVLEKEIRIVEEE 236
++ + + N + ++D +Y K +R KK+ L E L KE++I+ E
Sbjct: 718 KLIDLVRKQNNNIPYTD EYHMIKEENERHKKEQEELSKGHSEEQLAALMKELQIMNER 897

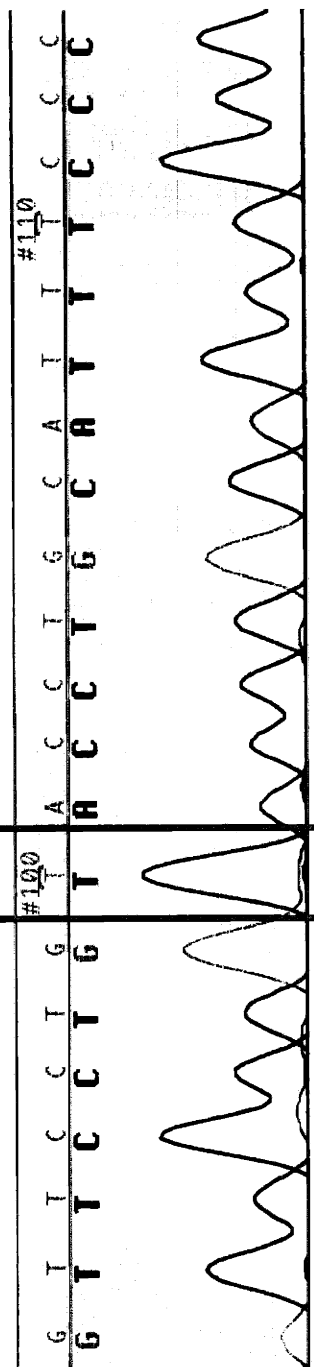
Query: 237 CGLGKLSTQEGEEKIQAIREKYNLKGNLREAAEKNIPTWIVEEVKKV 284
+ E EK I + K+ RE A++ + +E +K+
Sbjct: 898 ---NLKAMAEMMEKNMKIAMEAQEKLFQREKAQEMSYQQMEMQEK 1032

Figure 6: Closest BLAST database matches to the rat PA transcript (a,b). Interestingly, both AIG1 and IAP38 are associated with immune or defense responses (with bacterial infection of plants in the first case and with testosterone-mediated variations in immunity in the second), although very little else is known about each's function. The strongest matches, near the N-terminal end of the predicted protein, represent a GTP-binding domain, as shown by other, more distantly related matching sequences (not shown). The overall amino acid identity of only ~30% with PA suggests, however, that even detailed knowledge of the function of these genes might not be of much help in interpreting the function of PA.

PA Base 572



(a) wild-type PA sequence



(b) *Lyp* PA sequence

Figure 5: Sequence polymorphism between wild-type (a) and *Lyp* (b) alleles of the PA transcript. ABI 373A sequencer traces show the thymus cDNA single-base polymorphism identified between BB-Dr (wild-type) and BB-Dp (*Lyp*) alleles of PA at position 572. These traces show the sequence of the reverse complement of the sequence listing in Figure 4; the wild-type sequence at base 572 in this figure is a C, while the *Lyp* allele sequence contains a T. As shown in Figure 4, the polymorphic base is the third of its codon, and so the polymorphism is silent -- each codon encodes a valine.

Discussion

Transcription Unit Coverage

After this grouping, one previously known gene had been identified (WA -- protein disulfide isomerase, recognized by two cDNA fragments) and 13 transcription units remained as singletons, without either a significant database match or a matching cDNA clone from the thymus cDNA library. However, as the cDNA library screening process was designed to maximize throughput and not to maximize coverage for each individual probe used (since probes were pooled for initial screening), the number of singletons remaining is not rigorous enough to be used for analysis. Instead, we can calculate what fraction of the previously known WA gene was covered by cDNA sequence fragments: $433 \text{ bp in two fragments} / 2365 \text{ bp gene length} = 18.3\%$ coverage. Similarly, we can examine the sum of contigs from some of the other transcription units. For example, the 2.1 kb of sequence generated for IL includes about 400 bp of two cDNA selection fragments. In this case, coverage is approximately 19%. Both these numbers are in reasonable agreement with the predicted coverage of cDNA fragments in the YAC31+35 and YAC34+36+56 regions as calculated in Chapter III: 27% and 19%, respectively. Thus, as discussed in Chapter III, we may have missed one or two genes in each region, but should have recovered the vast majority of thymus-expressed genes in the region.

Transcription Unit Analysis

As shown in Table 1, the more practical problem in transcription unit analysis is that the transcription units identified only by single cDNA fragments often cannot be successfully analyzed on Northern blots. Presumably, this is because of a combination of low transcript abundance and small probe size. A more thorough cDNA library screening might improve the

second problem, but in my view, the more efficient approach is to put additional resources into precisely defining the minimum critical *Lyp* interval, sequencing that genomic interval, and identifying all polymorphisms at the genomic level. This approach prevents missing any gene that can be identified in the genomic sequence, and, more importantly, identifies all the possible locations of the *Lyp* mutation by identifying all polymorphisms between the two strains in that region.

PA as a Candidate for Lyp

PA remains a good candidate gene for *Lyp*. It is in the region fully linked with *Lyp* (the neighborhood of the rat 5631CA3b genetic marker -- see Figure 1). The significantly lower expression of PA in *Lyp/Lyp* thymus remains unexplained: All that is lacking is either a clear mutation in the *Lyp* allele of PA, or genomic rescue showing that the PA region can rescue the *Lyp* phenotype. Further work is currently underway to (a) identify the entire PA transcript and resequence that in both strains and (b) obtain the genomic sequence of the *Lyp* region in both mouse and rat (see Supplement).

Conclusion

The COL cloning strategy is successful. By working in the mouse region orthologous to the rat *Lyp* region, we were able to create physical and genetic maps of the mouse *Lyp* region, isolate cDNA fragments from that region, group them into transcription units, and transfer positional information from the rat in order to further reduce the size of the region to be considered. Even at the gene level, COL was conserved between rat and mouse in the central *Lyp* region (see Supplement).

The mouse transcription units were successfully screened for a gene that met the criteria expected of *Lyp*: expression in the thymic medulla and perhaps other immune tissues, and a difference in transcript size or expression level in *Lyp/Lyp* vs. wild-type thymus. Another method to test whether PA is *Lyp* is described in Chapter VI.

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CHAPTER VI

Transgenic Rescue to Narrow the *Lyp* Region – Identifying a Gene of Interest in the Rat

Introduction

Based on the closest rat genetic markers flanking the *Lyp* critical region and the positions of their orthologs in the fine-scale physical map of the mouse *Lyp* region, the physical size of this region is less than 750 kb (see Chapter IV). In practice, narrowing this region even further requires either the exclusion of part of the region by the identification of new recombinant animal breakpoints within it, or the restriction to an individual genomic clone based on its ability to complement the *Lyp* defect in a transgenic animal. Although Åke Lernmark's lab continues to identify new recombinants in the critical region in the continuing BB-Dp(*Lyp*) x BB-Dr(*wt*) cross-intercross, the need to produce several hundred offspring for every new recombinant animal identified severely limits the number of such recombinants expected to be identified in practice.

As described in Chapter I, *Lyp* is a recessive mutation, where heterozygotes are overtly normal and non-lymphopenic. Since the single wild-type copy of the *Lyp* gene present in heterozygotes is sufficient for normal function, a *Lyp/Lyp* homozygote with an additional wild-type *Lyp* transgene would be expected to show a normal phenotype. Thus, one could in principle narrow the *Lyp* critical region to a single genomic clone by creating a series of rat lines transgenic for genomic clones spanning the *Lyp* region, and identifying that line or lines that showed a wild-type phenotype even in animals with a *Lyp/Lyp* genotype outside the transgene. Recent work by Bruce Hamilton, among others, has shown the power of this approach.[1]

Therefore, in collaboration with Dr. Victoria Herrera's laboratory (Whitaker Cardiovascular Institute, Boston University School of Medicine), we chose to attempt to complement the *Lyp* defect by the creation of rats transgenic for a genomic clone containing the *Lyp* gene.

Strategy

Due to the lack of availability until just recently of rat P1/BAC libraries with many-fold genomic coverage, the rat *Lyp* region is fully spanned only by YACs (see Chapter IV), which are subject to chimerism and internal deletions and rearrangements. However, the physical map of the mouse *Lyp* region is complete, and is spanned by high-fidelity, relatively small-insert P1 and BAC clones (see Chapter III).

In addition, polymorphism between the introduced genomic DNA clones and the host genomic DNA is necessary in order to identify and track successful transgenic animals. While the P1 and BAC vector sequences are not present in the recipient embryo's genomic DNA and can serve to identify the transgene, transgenic animals may also contain only an internal segment of the injected DNA clone. Polymorphism between this segment, or its chimeric boundary regions, and the endogenous orthologs then provides the only way to identify the presence of the transgene. For smaller transgenes, polymorphism at the chimeric boundaries is often sufficient for detection of the transgene, but with larger genomic P1 and BAC clones, the greater the polymorphism rate between the introduced genomic DNA and the recipient embryo, the greater the chances of identifying a suitable polymorphism. In the case where the introduced transgene is from a different species, polymorphisms are easily identified, as indicated by the sequence differences between mouse cDNA fragments and their rat orthologs described in Chapter IV.

Thus, any mouse genomic DNA introduced as a transgene in rats should be easily identifiable, even with less sensitive methods of detecting polymorphism, such as Southern blotting.

Based on the conservation of linkage between rat and mouse *Lyp* regions demonstrated in Chapter IV, we expect the mouse ortholog of the *Lyp* gene to be present in the mouse *Lyp* region. In addition, as shown by previous cross-species transgenic complementation experiments[2,3,4] and by the fact that wild-type and *Lyp* rat hematopoietic stem cells successfully restore, respectively, wild-type and *Lyp* immune systems to SCID mice (see Chapter I), we would expect the wild-type mouse *Lyp* gene to correct the *Lyp* defect in a *Lyp/Lyp* rat recipient.

Thus, we chose to use mouse P1, BAC, and cosmid clones in attempting to create rats transgenic for wild-type genomic clones within the *Lyp* region.

Materials and Methods

Animals

BB-Dp, BB-Dr, and BB-DpxBB-Dr F₁ rats [5] were obtained from Åke Lernmark's lab and bred in the Whitaker Institute facilities. Lewis and Fischer (F344) rats were obtained from Charles River Laboratories (Wilmington, MA) and bred in the Whitaker Institute facilities. Rat genomic DNA was prepared from tail sections[6].

BAC and Cosmid DNA Preparation

The selected mouse genomic clone DNA was prepared according to standard methods for large-insert vectors such as P1s and BACs, as recommended by Genome Systems (Chapter III). The genomic clones were digested to completion with *NotI* restriction enzyme (NEB), linearizing the clone by cutting in the vector (except for cosmid clone 9000G, where *NotI* excised the insert from the SuperCos-1 vector), and extracted to remove impurities. The purified

DNA was then resuspended in 10mM Tris pH 7.5 and extensively drop-dialyzed on 25 mm diameter VS filters (0.025 μ m pore size; VSWP 025 00; Millipore, Bedford, MA) against 10mM Tris pH 7.5, precipitated, and resuspended in Brinster's medium at 3 ng/ μ l[6].

Transgenic Construction

BB-Dp, BB-Dr, or BB-DpxBB-Dr F₁ males were mated with BB-Dp, BB-Dr, BB-DpxBB-Dr F₁, Lewis and Fischer females to provide fertilized eggs. These eggs were then microinjected with BAC or cosmid DNA as described previously[3,6,7], and implanted in recipient females. Potentially transgenic offspring were then subjected to PCR and Southern blot analysis to determine their transgenic status. Procedures followed were in accordance with institutional animal care guidelines.

PCR Analysis

Potentially transgenic rats were analyzed by PCR for the presence of transgenic BAC vector sequences in their genomic DNA. Unless pre-existing or otherwise noted, PCR primers were selected using the Primer 0.5 [8] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. The BAC vector primer pair used is listed in Appendix A. PCR amplification was performed according to standard conditions (using 60°C annealing temperature) for amplification from genomic DNA as recommended by Perkin-Elmer (50 μ l reaction volume, 100 ng genomic DNA template; 35 cycles 50s 94°C, 90s 60°C, 150s 72°C).

Southern Blot Analysis

In addition to PCR testing for the presence of genomic clone vector sequences, potentially transgenic rats were tested for the presence of the injected mouse genomic DNA by

Southern blot analysis. Each putative transgenic rat genomic DNA (20 ug), or control genomic DNA (either 20 ug C57BL/6JEi mouse DNA, or 20 ug Fischer F344, BB-Dp, or BB-Dr rat DNA, or 1 ng P1/BAC/cosmid clone DNA), was digested to completion using *TaqI* or *EcoRI* (NEB) according to the manufacturer's instructions. The digested DNA was electrophoresed on a 1% Seakem ME agarose gel (FMC) and then transferred and bound to a positively charged nylon membrane (Genescreen Plus, DuPont NEN) by an initial short-wave UV exposure in the presence of ethidium bromide and 40-hour capillary transfer with 0.4 N NaOH[9], and short-wave UV fixing to the membrane (Stratalinker auto setting, Stratagene). The Southern blots were then hybridized with ³²P-labelled[10] mouse cDNA fragments contained within the P1 or BAC clone used to microinject the rat embryos, using essentially Church's protocol [11] and a final wash of 0.5x SSC / 0.1% SDS at 50°C. The membranes were exposed to X-ray film overnight to five days at -80°C.

Results

Suitability of Rat Strains for Microinjection

Due to the limited supply of BB-Dp and BB-Dr animals, we obtained additional rats from other strains to provide sufficient embryos for the microinjection experiments. In order to facilitate potential future experiments to analyze diabetes incidence in any successful transgenic animals, we chose to use the Lewis and Fischer F344 strains, whose genetic interactions with *Lyp* and IDDM in the BB model of IDDM had been determined to be limited to one and two loci, respectively, besides *Lyp*. [12] For successful microinjection, fertilized embryos must be injected within a few-hour timespan during their limited growth *in vitro* before their reimplantation into foster females. Thus, synchronization of the fertilized embryos, which must all be collected

simultaneously from a given donor, is important to ensure that all embryos are at the appropriate stage of development for microinjection during the limited *in vitro* culture period.

Lewis rats were the initial non-BB-Dp, non-BB-Dr strain used, and embryos from these females were not synchronized -- typically, only approximately one quarter of the embryos collected were suitable for microinjection. Interestingly, this lack of synchronization was independent of the male genotype, occurring in embryos from both Lewis x Lewis and BB-Dp x Lewis matings. No such lack of synchronization was seen in embryos from BB-Dp, BB-Dr, BB-DpxBB-Dr F₁, and Fischer F344 females, and so the bulk of the microinjected embryos were collected from these non-Lewis females.

Mouse Genomic Clone	Number of Rat Pups Born	Mouse Probes Used in Analysis
B301C20	25	ajm3801, ajm3907, ajm3808, ajm5808, ajm3810
B230A7	6	ajm3935, ajm5422, ajm5414, ajm5704, ajm5201
B315E6	20	ajm3810, ajm5806, jln0202, ajm3804, ajm3809, ajm5814, ajm3805, ajm6005
B205C12	11	ajm3805, ajm6005, ajm5811, ajm5728
9000G	15	ajm3805, ajm6005

Table 1: List of mouse *Lyp* region genomic clones used in microinjection experiments. The first four clones are mouse BACs; for mapping info, see Chapter III. 9000G is a mouse cosmid isolated from an AKR SuperCos-1 library (Stratagene) using the ajm6005 PCR assay. For each clone, the number of potentially transgenic rat pups born is indicated, as well as the mouse cDNA probes used on Southern blots of the pups' DNA to test for presence of the mouse genomic clone.

Analysis of Microinjected Rats

Table 1 shows the genomic clones used for the microinjection experiments, along with the number of rat pups born from microinjected embryos for each clone. The pups were analyzed for the presence of transgenes by two methods, as described in Materials & Methods: (1) PCR analysis of genomic DNA for the presence of BAC vector sequences, which are unique to the injected clones and (2) Southern blot analysis of genomic DNA for the presence of

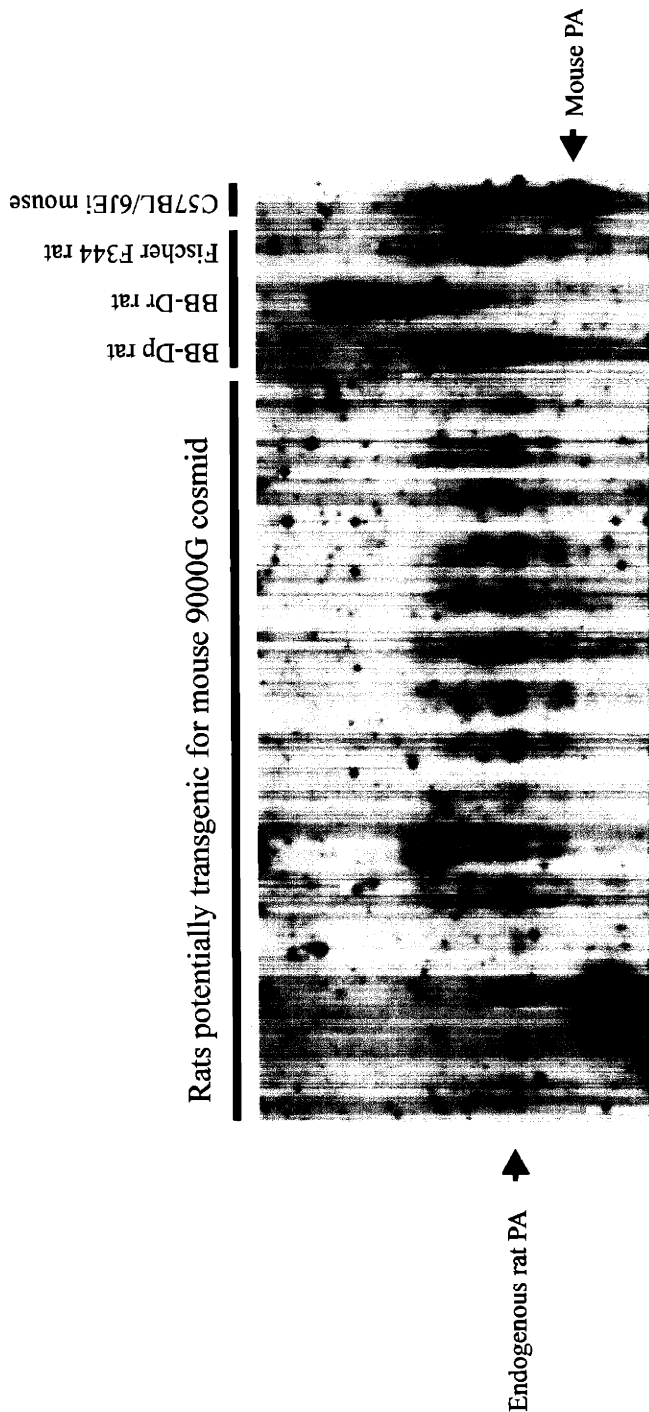


Figure 1: Determining transgenic status of rat pups born after microinjection of rat embryos with mouse *Lyp* region genomic DNA clones. In this example, DNA from rats potentially transgenic for the mouse 9000G cosmid clone (which contains the mouse ortholog of the PA gene) is tested by hybridizing a Southern blot of *TaqI*-digested DNA (15 rats born from mouse cosmid 9000G transgenesis experiment along with control DNAs) with the mouse *ajm3805* cDNA probe (mouse PA gene -- see Chapter V). No mouse-PA-specific band is present in the 15 experimental rat DNAs, indicating that none is transgenic for the mouse 9000G cosmid clone (strong signal partially obscuring the lower part of lane 3 is a non-specific "spot" signal outside the lane).

Lanes 1-15: putatively transgenic rats 1-15; lanes 16-18: control rat DNA from BB-Dp, BB-Dr (incompletely digested) and Fischer F344 strains; lane 19: control C57BL/6JEt mouse genomic DNA. Strong band in mouse control lane indicates size and intensity expected of any band representing a successfully integrated transgene of cosmid 9000G. Weaker larger band in rat lanes is the cross-hybridizing fragment containing the endogenous rat PA gene; this serves as an internal control for the hybridization experiment.

mouse-specific restriction fragments from the injected clones. Both methods returned negative results for all 77 pups tested (see Figure 1 for sample Southern blot result; PCR results not shown).

Discussion

Approaches to Identifying a Gene of Interest in the Rat

One can prove which gene in a segment of DNA is responsible for a certain phenotype in three ways: (1) the knockout approach – create a novel allele of the mutation by converting a wild-type endogenous gene to the mutant genotype (by e.g. homologous recombination) and verify the resulting mutant phenotype, (2) the complementation approach – correct the defect by adding an extra wild-type copy of a specific gene (e.g. transgenesis) and verify the resulting wild-type phenotype and (3) comparison approach – one or more of the sequence differences, within the segment, between the genomic sequences of the parental wild-type strain and the mutant strain must cause the mutant phenotype.

The lack of embryonic stem cells (ES cells) in the rat effectively prevents the direct use of approach (1) in the rat for at least the near future; in any case, given the time and expense needed to create a knockout for a single gene, this approach must wait until the gene of interest is narrowed to one or only a few good candidates.

Approach (3) requires DNA from parental pre-mutation and mutant post-mutation lines for comparison. Alternatively, in the case where pre-mutation DNA is not available, but a number of independently-arising mutant alleles are available, one would expect to consistently find significant mutations (e.g. nonsense and missense coding region mutations) only in the gene of interest. Of course, this variant lacks the logical certainty of comparing pre- and post-

mutation DNA. In the case of *Lyp*, the mutation was pre-existing in a non-inbred line when identified, so no immediate parental (pre-mutation event) DNA is available for comparison. In addition, no other alleles of *Lyp*, in rat or other mammals, are available for sequence comparison. Thus, approach (3) will not allow definitive identification of the gene, but may well yield candidates that contain significant sequence differences between the allele found in the BB-Dp (*Lyp*) strain and the alleles found in other, wild-type strains.

Therefore, we attempted approach (2) with a number of genomic clones from the mouse *Lyp* region. Unfortunately, creating rats transgenic for large-insert clones is not yet an established technique; I am aware of only one published large-insert clone transgenic rat (in this case, a cosmid) [13]. As the results show, rat strain variations can also create significant obstacles in addition to the expected difficulties of embryo microinjection.

Proposed Future Strategy

These experiences, combined with the decreasing cost of genomic sequencing, suggest the following strategy for identifying a gene of interest in the rat: (1) Narrow the critical region as much as possible with genetic recombinants, (2) sequence the critical region in wild-type and mutant rats and, based on the mutation candidates identified by sequencing, (3) verify the correct candidate either through transgenic complementation in the rat or through a knockout of the orthologous gene in the mouse. This approach has the advantages that the initial sequencing step covers the entire critical region without bias and is rapidly decreasing in cost. If DNA from a parental (pre-mutation) line or from multiple alleles is available, this initial analysis becomes even more effective, as discussed above. Given the current state of transgenesis in the mouse and rat, verification of a given candidate gene by making a knockout of the orthologous mouse

gene is more likely to be successful than transgenic rescue in the rat, but this will likely change as transgenesis techniques improve in the rat.

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CHAPTER VII

Conclusion

Summary of Results

Characterization of the Lyp Region

In this thesis I report the characterization of the genomic regions containing the *Lyp* gene in rat and mouse[1,2]. These *Lyp* regions were mapped and cloned through the identification and mapping of new genetic markers in these regions, and through the construction of large-scale YAC-based physical maps spanning those markers. These maps were then used to develop STSs and to construct fine-scale P1- and BAC-based physical maps of the *Lyp*-containing subregions of the large-scale maps. As part of this, positional information was transferred from rat to mouse and back to rat by the identification of DNA sequence orthologs between the two species, thus allowing integration at a fine scale of the rat and mouse *Lyp* region physical maps. The fine-scale rat physical map and its full integration with the mouse map, as well as current comparative sequencing results between the mouse *Lyp* region and human genomic regions orthologous to it are described in the Supplement.

Identification of Candidate Genes for Lyp

The characterization of transcripts from the mouse *Lyp* region, enriched for those expressed in the thymus (the expected site of *Lyp* gene expression), produced a set of candidates for the *Lyp* gene[3]. Based on current sequence and expression pattern information, one of these genes, PA, stands out as a candidate for *Lyp*[3]. It is the only candidate whose transcript level is observed to differ between wild-type and *Lyp* animals (being substantially decreased in the *Lyp*

animals), and its expression pattern matches that expected of the *Lyp* gene. It is expressed in immune-system-related tissues, most strongly in the thymus; in the thymus, it is expressed in the medulla, the site of the latter stages of T-cell maturation. This expression pattern is consistent with expression in T-cells or dendritic cells (major antigen-presenting cells involved in T-cell maturation); a defect in either of these cell types could explain the *Lyp* phenotype, where specifically T-cell and not B-cell maturation is defective, drastically reducing the number of T cells emigrating from the thymic medulla, and leaving successful emigrants in an abnormal reactive state.

Although a functional polymorphism has not yet been demonstrated between the wild-type and *Lyp* rat alleles of the PA gene (with ~90% of the transcript, including the entire coding region, comparatively sequenced), further sequence analysis is proceeding, as described in the Supplement.

Targeted Isolation of Gene Orthologs

In order to transfer positional information between mouse and rat, I tested a number of strategies for identifying the genes orthologous to a given set of genes in a closely related species[2]. In this thesis, the main cross-species ortholog-identification goal was to be able to compare the relative order of the genetic markers defining the *Lyp* interval in the rat with the rat orthologs of mouse cDNA fragments from the mouse *Lyp* region in order to determine which of those genes fell in the *Lyp* interval and thus were candidates for the *Lyp* gene.

The most successful ortholog-identification strategy developed was a modification of cDNA selection I denoted "cross-species cDNA selection." In this technique, a pool of specific cDNA clones (in this case, from the mouse) are used as the template to select from a large population of cDNAs from a related species (in this case, from the rat). The technique showed

good specificity, and produced a good diversity and quantity of rat cDNA orthologs when performed on a number of mouse cDNA pools from the mouse *Lyp* region. With adjustment to its stringency, it should be useful for the targeted isolation of related sequences between a wide range of species pairs.

Experimental Proof-of-Validity of the COL Strategy

In this thesis, I show that, for successively finer levels of resolution, genetic and physical order in the *Lyp* region is preserved between rat and mouse[1,2,3,4,5]. This allowed me to use the mouse *Lyp* region as a more-easily manipulated proxy for the rat *Lyp* region, and then to clone the rat *Lyp* region by using reagents developed from the mouse *Lyp* region. In sum, then, the work described here is a successful use of the conservation-of-linkage strategy to leverage mouse genetic and physical mapping resources for similar work in the rat.

Interestingly, this work has also shown that the human would not have been a suitable proxy for studying the rat *Lyp* region, as conservation of linkage between the rat and human breaks down precisely in the *Lyp* region (see Supplement for details).

As further genetic and physical mapping resources have been developed for the rat[6,7,8](see Supplement for use in this project), it has now become quite practical to embark on a positional cloning project in the rat directly with rat resources. However, other mammalian species (e.g. many livestock species) remain relatively map-poor and could benefit from a COL approach for positional cloning projects in the future.

Future Study of the Lyp Gene

Further research with the goal of definitively identifying the *Lyp* gene is ongoing. Such research includes both a continuation of the research described above, as well as some new

approaches. Recent work in the same vein as that described in this thesis includes further narrowing of the genetic interval that must contain *Lyp* through the accumulation of additional recombinant animals, and refinement to a fine scale of the rat *Lyp* region physical map and its further integration with the mouse *Lyp* region physical map. This work is described in the Supplement. It provides even stronger support for the assertion that the mouse ortholog of the rat *Lyp* gene is present in the mouse *Lyp* interval, and the fine-scale rat physical map should soon provide the rat genetic markers necessary to closely define the positions of the closest recombinational breakpoints bounding the *Lyp* interval.

By contrast, there are three techniques that have the potential to prove the identification of a given gene as the *Lyp* gene. These are transgenic rescue, "knockout" construction, and large-scale comparative genomic sequencing.

Transgenic Rescue

As described in this thesis, rescue of the *Lyp* phenotype by complementation with a segment of wild-type genomic DNA (by creating a transgenic rat) would provide proof that the *Lyp* gene was contained on that segment of DNA[9]. Unfortunately, rat transgenic construction still suffers from a lower success rate than mouse transgenic construction and from tenfold higher costs. As rat transgenic technology improves and as the *Lyp* gene is restricted to fewer candidates, this approach may become easier for final confirmation of the identity of the *Lyp* gene. In order to achieve this, and again in collaboration with Dr. Victoria Herrera's laboratory (Whitaker Cardiovascular Institute, Boston University School of Medicine), we are currently preparing a proposal to optimize the creation of large-insert genomic clone transgenic rats, using the mouse BAC clones spanning the mouse *Lyp* region.

Mouse Gene Knockout Construction

The converse of transgenic complementation, gene knockout construction, also has the potential for identifying the *Lyp* gene with a high degree of confidence. Unfortunately, rat stem cells do not yet exist, and so any knockout experiments would be performed in mice. As the work in this thesis shows, though, all indications are that the mouse ortholog of the *Lyp* gene is present in the mouse *Lyp* region. Because of the close relatedness of mouse and rat, we would also expect a knockout of the mouse ortholog of *Lyp* to exhibit a phenotype very similar to that of *Lyp* itself (the fact that the transfers of hematopoietic precursors from wild-type and *Lyp* rats to irradiated mouse recipients result in, respectively, wild-type and *Lyp* blood phenotypes in those recipients[10] also supports this contention). Thus, one could identify the *Lyp* gene by serially knocking out the mouse orthologs of the rat genes in the critical interval for *Lyp*. This approach is more technically complicated than the transgenic rescue approach above, but may in the end be easier and cheaper due to the high success rates and relatively low costs of mouse knockouts, especially if the number of candidate genes can be narrowed to 2-4.

In any case, even if the PA gene is excluded as a candidate for the *Lyp* gene, PA remains an attractive candidate for a mouse knockout. Most current knowledge of thymic T-cell maturation involves the early and middle steps in that process; since PA is associated with the late stages of T-cell maturation by its specificity of thymic medullar expression, the phenotype of a homozygous PA knockout mouse would likely provide insights into the understudied late stages of that maturation process.

Genomic Sequencing

Finally, sequencing the entire genomic region included in the recombinant-defined *Lyp* critical region is now becoming practical. Such sequence for the mouse or rat alone would allow

the identification of possible additional candidate genes in the region that were missed by the cDNA selection work[1,2]. In the rat, this sequencing would also allow the identification of all possible simple-sequence repeat genetic markers by inspection, facilitating the narrowing of the *Lyp* critical region to the maximum extent possible. Sequencing both mouse and rat would provide additional insights by identifying the regions of sequence conserved between the two species since their divergence from their common ancestor (as a bonus, it would also verify COL in the *Lyp* region down to the sequence level).

In my view, genomic sequencing's greatest promise lies in resequencing the critical region in multiple rat strains, including the wild-type and *Lyp* strains used in these experiments. Such resequencing of the *Lyp* critical region would have two possible results. In the first case, there might be only a single polymorphism between a given wild-type strain and the *Lyp* strain -- in this case, the *Lyp* gene must contain that polymorphism and the *Lyp* gene would thus be conclusively identified.

However, since the parental, pre-*Lyp* strain is unavailable, it is more likely that resequencing will identify a number of polymorphisms between *Lyp* and wild-type strains. In this case, one of the polymorphisms might be especially suggestive (e.g. a nonsense mutation in an open reading frame in the *Lyp* haplotype), and not present in any of the wild-type strains, narrowing attention to one or a few candidate genes. Even if none of the polymorphisms is immediately compelling, the *Lyp* gene or its immediate neighborhood must contain one of those polymorphisms that is not present in the wild-type strains, and so the number of candidate genes could be reduced even in this case.

Thus, genomic sequencing is currently the most practical of these three approaches for work on *Lyp*. Genomic sequencing of the mouse *Lyp* region has already begun (see Supplement)

and rat genomic sequencing will begin shortly as well, using the PAC clones from the fine-scale physical map described in the Supplement.

Lessons for Positionally Cloning Genes

My experiences on this project have provided practical insights into positional cloning in experimental laboratory mammals. Some of the most time-consuming steps in my work on *Lyp* have been or will shortly be made faster and easier through technological advances, while others remain difficult or time-consuming.

Careful Choice of Animal Model is Important

Much of the ease or difficulty of a positional cloning project stems from the initial choice of the animal model to be used. Techniques will continue to improve the cloning process, but the initial production of animals (for phenotypes and genomes) and the final identification of a gene (again, most often using live animals as in transgenic complementation) remain less susceptible to the development of infrastructure and technological advances than do the intermediate steps of assembling and characterizing the set of candidate genes in an interval (in principal, with the development of whole-genome physical and transcript maps and sequences, these steps might be completely absorbed into the infrastructure).

For the initial genetic mapping, generation of meioses is often a limiting factor -- being able to produce large numbers of offspring in a short time is extremely helpful. In addition, the mode of inheritance and penetrance of the phenotype, as well as the ease of its assay, determine how difficult it is to assemble the necessary number of phenotypes and informative genomes.

As mentioned above, the middle stages of the project -- identifying candidate genes in the region defined by genetic mapping -- are becoming vastly easier due to the development of

genomic resources in human and mouse, especially, and to a lesser degree in the rat and some other species. Thus, the choice of model will substantially affect the ease of completing these middle stages, but, as shown in this thesis, even resource-poor species can sometimes leverage the resources available in mouse and human.

For the last stages of the project -- identifying the target among a set of candidate genes -- the resources available for a given animal model are of the greatest importance. Identifying the gene of interest among a set of candidates will more and more be the rate limiting step in positional cloning. The initial choice of strain can have a great impact on this process (e.g. the availability of a parental, pre-mutation strain, or multiple independent affected strains, greatly enhances the chance of success for a comparative resequencing strategy), as can the choice of species. Thus, if the model of interest is a mouse model, reliable and relatively inexpensive transgenic rescue services are available; these are much less reliable in the rat and non-existent in other species. Alternatively, the existence of a tissue culture model or assay for the gene of interest can greatly ease gene identification in a wide variety of mammalian species.

Of course, often only a single animal model is available for the gene or disease of interest and no choice is possible. In these cases, the considerations above can still serve as guidelines to the anticipated relative ease or difficulty of a positional cloning project.

Other Lessons for Positional Cloning

Maximize Meioses to Minimize Physical Interval

Ruling out candidate genes at the end of the process is always almost more difficult than eliminating them through minimization of the size of the genetic interval containing the gene of

interest. Careful planning of the breeding and assay schedules will maximize the number of informative meioses available.

In this project, the production of recombinants after the initial set used in the genetic dissection of diabetes in the BB rat[11] was limited for a time due to breeding difficulties in the rat colony associated with moving the colony and nearby construction (the necessity for maintaining these somewhat immunocompromised animals in Specific Pathogen Free conditions also complicated the situation). More recently, additional recombinant production has been good and has produced the recombinant animal limiting *Lyp* to the region to the right of the 5579CA1b marker, rather than right of R236. Any new project, however, would benefit from a specifically-designed protocol to maximize the number of litters produced, with assays to be completed by weaning age so that the steady-state number of animals would not grow excessively.

Make Use of Most Representative and Most Stable Genomic Libraries Available

An early investment in the reliability of physical mapping reagents will simplify analysis and prevent later backtracking to verify early results.

At least two good genomic libraries are necessary: one with very large insert size in order to rapidly span the Mbp-sized regions represented by a typical initial critical genetic interval in mammalian positional cloning, and a second, high-coverage library with high-fidelity smaller clones in order to assemble a fine-scale physical map.

The MIT mouse YAC library had good coverage and was sufficiently stable to provide a framework map of the mouse *Lyp* region, but the fine-scale high-fidelity physical map necessary to order STSs and identify transcription units required the high-coverage medium-insert-size mouse genomic P1 and BAC libraries. As the work in Chapter IV showed, the COL strategy used allowed the project to proceed even without a large-insert rat genomic YAC library and

with only a low-coverage rat genomic P1 library (although clearly, the development of rat *Lyp* region genetic markers was greatly facilitated by the recent availability of a rat genomic YAC library).

Make Use of RH Panels to Reverify Position and to Rapidly Fine-Map Ttranscript Fragments

As above, the early use of RH panels or other means of identifying physical position will lead to a robust contig and the earliest possible classification of whether or not individual candidate genes are in the genetic interval of interest.

The key requirement is to be able to rapidly determine whether a given fragment of DNA maps (even quite roughly) to the region of interest. During most of this project, limited-resolution chromosomal hybrid panels served this purpose sufficiently well, but the newly-available RH mapping panels for human, mouse, and rat have additional advantages. For example, very high-resolution RH mapping panels can provide rough ordering even within a region of interest. Most importantly, though, combined with the rapid expansion of multi-species EST sequence databases, the RH mapping panels allow one to quickly map putative orthologs and verify or disprove COL (see the break in COL between mouse and human *Lyp* regions described in the Supplement).

Sequence Genomic Region as Early as Feasible

As discussed earlier, genomic sequencing has the potential to rapidly identify all possible candidate genes. Comparative resequencing of haplotypes can efficiently narrow the choices among those candidates. Such sequencing requires a high-coverage medium-insert-size genomic clone library to provide the sequencing templates; although the BACPAC Resource Center

(Roswell Park Cancer Institute, Buffalo, NY) continues to add to its large selection of such libraries, a library may not be available for the species of interest.

The capability to rapidly sequence regions ~500kbp in size is only now beginning to become available, and so was not attempted for the *Lyp* project until very recently. As noted in the Supplement, sequencing of the mouse genomic *Lyp* region is now in progress, with the rat to follow.

Collect as Many Alleles as Possible

Having multiple independent affected alleles available for resequencing and other candidate characterization greatly increases the credibility of gene identification if these multiple alleles have defects in the same gene. Conversely, candidates with an identifiable defect in only a single allele are less likely to be the gene of interest.

In the case of *Lyp*, many wild-type strains are available, but all BB-derived strains are thought to share the same *Lyp* mutation. No other *Lyp* allele is known: unfortunately, in the absence of the IDDM produced by *Lyp* in combination with the BB genetic background, the *Lyp* phenotype is almost unnoticeable without explicit blood analysis.

Make Candidate Screening Methods Very Efficient to do in Parallel

Due to the typical number of candidate genes (often dozens), candidate screening is often a rate-limiting step -- make it as efficient as possible.

In this work (Chapter V), duplicate "strip" Northern blots as well as sequence analysis were used to facilitate screening; however, the optimal strategies remain transgenic rescue in order to narrow the set of candidates to one or a few, or genomic resequencing of wild-type and *Lyp* haplotypes in order to narrow the candidates to only those with sequence polymorphisms.

Start Transgenic Rescue Experiments as Early as Possible

Transgenic rescue experiments take months to perform, and even more time in the majority of cases where additional breeding of transgenic animals is required to construct the appropriate genotype for assay of phenotypic rescue.

As mentioned above, transgenic rescue remains a technique of choice; as large-clone transgenesis in the rat remains problematic; it has not yet been used in a large scale for the *Lyp* region. The small-scale experiments described in Chapter VI were unsuccessful; as described in the Supplement, further experiments to perfect the process in the rat are planned.

Evaluation of the Lyp Project

Based on the lessons above, I would in the future place more early emphasis on increasing the number of recombinant animals available for genetic mapping. In addition, transcript fragment screening and characterization (such as cDNA fragment isolation, Northern blotting and cDNA library screening[2,3]) could be performed in a more parallel, higher throughput fashion in order to rapidly maximize the amount of information about each candidate gene.

As large-scale genomic sequencing is now becoming available, comparative resequencing of rat *Lyp* region haplotypes is the best prospect for significantly narrowing the set of *Lyp* gene candidates to the point where a single rat transgenic rescue or a few mouse gene knockouts can conclusively identify the *Lyp* gene.

Implications of the Success of a COL-Based Cloning Strategy on Choice of Model Systems

The recent practicality of genome sequencing and other large-scale investments in genomic infrastructure have prompted some to call for what is essentially a two-level approach for future infrastructure investments in mammals -- to restrict infrastructure funding to a limited set of species such as the mouse and human in order to conserve limited funding resources. These would then become the standard model systems.

This raises the question of the nature of the value of multiple model systems in biology. Purely physical characteristics of a given species that are advantageous for research can, in principle, be compensated for through the development of new technology. For example, the larger physical size of blood vessels in the rat versus the mouse is a major factor in its popularity with cardiovascular researchers; this size advantage could in principal be overcome through the miniaturization of equipment.

The true value of alternative mammalian model systems such as the rat lies elsewhere: in their genetic diversity and in the past history of their use as model systems, not in the details of technical manipulation. The body of knowledge built up during the history of use of a model system is much more difficult to transfer to a new system than are the actual techniques of manipulating that system. Impossible to transfer is the pre-existing genetic diversity of a species: both the divergence of one species from another (for example, the different roles of MHC Class I genes in different mammals) and the variation within a species. It is this variation that is the basis for much biological research -- as the *Lyp* mutation does not exist in the mouse, it would be difficult to identify it without making use of the rat! Thus, it is important to continue to make use of the available alternative mammalian model systems.

The COL-based cloning strategy validated in this thesis provides at least a partial solution for this problem. The ever-expanding genomic resources available for certain species (such as the mouse and human) can be leveraged for use in related species without immediately having to develop a complete infrastructure for all. As described earlier in this chapter, given an initial investment in a genetic map, a genomic clone library, and a method to at least roughly map DNA fragments, one can in principal use the COL strategy to identify the mouse or human ortholog of the gene of interest.

Therefore, the key infrastructure investments in alternative mammalian model systems should include genetic maps, high-coverage genomic clone libraries, and RH panels or analogous physical mapping reagents. Precisely these resources are now being developed for many mammalian model systems (see for example the BACPAC Resource Center, Buffalo, NY); thus, the COL cloning strategy may prove useful in a number of today's alternative mammalian model systems.

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SUPPLEMENT

Recent *Lyp* Project Progress

Introduction

This chapter describes recent significant results from work-in-progress. These results extend those described in the previous chapters and continue to verify conservation of linkage (COL) between the rat and mouse *Lyp* regions at ever finer levels of resolution.

The first result is from the continuing study of the PA gene (Chapter V): additional sequence information from the rat PA gene has shed light on its genomic structure and indicated a limited genomic region to examine for functional polymorphisms between the BB-Dr and BB-Dp alleles.

The other recent results stem from the rapid expansion of genomics infrastructure; a number of resources have recently become available that have greatly facilitated experimental work in the rat, and have redressed some of the key deficiencies in the experimental infrastructure. In particular, the availability of a high-coverage, medium-insert-size (~100-200 kb) rat PAC library has allowed the construction of a fine-scale physical map of the rat *Lyp* region and the further verification at very high resolution of the COL between mouse and rat in the central *Lyp* region. By contrast, the continuing expansion of genomic sequence information from human chromosome 7 and the easy radiation hybrid (RH) mapping of PCR assays from human ESTs orthologous to mouse and rat transcription units has shown that COL is broken between mouse and human in the *Lyp* region.

Materials and Methods

PCR Analysis

Unless pre-existing or otherwise noted, PCR primers were selected using the Primer 0.5 [1] program to choose primers with predicted melting temperatures within 1°C of 60°C and to avoid regions with repeat- or self-similarity. STS and SSR marker assay primer information is listed in Appendix A, while cloned fragment information is listed in Appendix B. PCR amplification was performed according to the conditions specified for each protocol (e.g. YAC library screening), or, if not specified, according to standard conditions as recommended by Perkin-Elmer. SSR genotyping was as described in Chapter II, with the occasional exception of alternate annealing temperatures (standard temp. 60°C), as listed for each primer pair.

Sequencing

Unless otherwise noted, sequencing was performed on PCR products amplified using M13-f and -r primers (see Appendix A) from 2 µl of 1/10 dilutions of 96-well-plate minipreps[2], using 30 cycles of PCR with 50°C annealing temperature in a 50 µl reaction, and purified with Qia-Quickspin PCR (Qiagen) according to instructions (eluting with 50 µl TE), or Ultrafree-MC filters, 30,000 MW, PLTK membrane columns (Millipore) by washing in 300 µl water, spinning, and resuspending in 25 µl TE. Cycle-sequencing reactions using M13-f or M13-r dye primers were performed according to recommended conditions (ABI) and run on an ABI-373A fluorescent sequencer. The resulting sequences were processed to remove vector or other contaminant sequences (e.g. hemoglobin exon sequences) using the computer programs EXSTRIP or FREEINST[3] and analyzed for overlaps using the programs COMPSEQ and

COMP2SEQ[4]. Database searches to identify repeat sequences or homology with known genes were performed using BLAST in the web (NCBI) versions[5].

RACE

5' RACE was performed using the Life Technologies kit (#18374-058). 45-50-day-old BB-Dp (*Lyp/Lyp*) and BB-Dr (*wt/wt*) rat thymus total RNA was isolated using Trizol (Life Technologies), DNase treated and poly-A selected using oligo-dT columns (Ambion). Specific reverse transcription for the PA gene was done using the "5513 RT R" primer (5' CAAGAATGATGGCATGAGGC 3') on 1 µg total RNA. The cDNA was GlassMilk (GM; Bio101) purified, RNase treated, GM purified again, then TdT (Promega) tailed with dCTP and used as a template for PCR. PCR was performed using the primers 5'CTTTCCACTTCCTGTTTTCCC3' (5513 RACE 2 Nested) and 5' GGGIIGGGIIGGGIIG 3' in a 100 µl reaction, as follows: 94°C 5'; 35x 94°C 35s, 52°C 30s, 72°C 1'; 72°C 7'. When run on a 1% agarose/0.5x TBE gel, the PCR products were approximately 200-300 bp in size. These products were directly cloned using pGEM4Z (T-tailed; Promega). Several hundred colonies were picked and gridded on DuPont/NEN Colony/Plaque Screen filters. These were hybridized with the rat PA cDNA (ajm5513-5/ajm5513-3 PCR product; see Figure 1(a)) to identify RACE products from the PA gene (65°C 0.1xSSC/0.1% SDS final wash 20'). Positives were picked and minipreped (see Sequencing, above), and PCR of the minipreps with M13-f/M13-r primers used to amplify the inserts. The largest inserts were sequenced on an ABI 373A sequencer using SP6 and T7 end dye primer sequencing; the assembled sequences from 20 such inserts produced the consensus 265 bp-long sequence shown in Figure 1(a).

Rat Genomic PAC Library

The RPCI-31 rat PAC genomic library (~10 coverage overall; segment 2, used in this work: 4.9x coverage, 147 kbp average insert size; available from BACPAC Resources, Roswell Park Cancer Institute, Buffalo, NY [6,7]) was screened to isolate overlapping rat genomic clones spanning the region of interest. A set of hybridization filters containing PAC colonies gridded in duplicate was hybridized with a pool of one or more radioactively-labeled probes using a variant of Church's solution at 65°C, as described in Chapter III. (The probes were screened by analysis of sequence prior to hybridization to eliminate any that contained repetitive sequences) The filters were washed 3x 45' at 65°C in 10 mM EDTA/400 mM NaHPO₄ (pH7.2)/1% SDS and exposed to X-ray film at -80°C O/N to 48 hours. Addresses of positive clones were determined from the orientation and position of positive colonies on the filters using the instructions provided by BACPAC Resources, and stab cultures of these ordered from BACPAC Resources.

STSs from rat YAC 28e5 (Chapter IV) used for screening the RPCI-31 library were isolated by preparing random rat subclones of the gel-isolated YAC band as described in Chapter IV. Subclones were picked at random and sequencing performed as described above. Of the 62 resulting good-quality sequences, 41 were eliminated as either yeast genomic sequences (presumably the result of the contamination of the gel-purified rat YAC chromosome with co-migrating yeast chromosomes or chromosome fragments) or as rat repetitive sequences. 18 STS PCR assays were created from the remaining 21 sequences; these 18 STSs were then amplified from the corresponding plasmid miniprep templates by PCR and purified with Qia-Quickspin PCR (Qiagen). The amplified STS DNA fragments were then individually radioactively PCR-labeled (Chapter III) and pooled for hybridization screening of the RPCI-31 rat PAC library. The STSs used were as follows (see Appendix A for primer sequences): (pool 1) ryac0309,

ryac0327, ryac0330, ryac0335, ryac2e01, ryac2e03, ryac2e05, ryac2f01, ryac2f09 and (pool 2) ryac2a01, ryac2a11, ryac2b01, ryac2b06, ryac2c02, ryac2c06, ryac2c07, ryac2e04, ryac2f02.

Rat PAC Clone Grid Hybridization

In order to facilitate the hybridization of multiple individual probes to the PACs isolated above, the PAC clones were inoculated on standard LB-Kan Petri dishes in duplicate grid patterns at standard 96-well plate spacing, with the duplicate patterns offset diagonally by half the well-well spacing. A set of DuPont/NEN Colony/Plaque Screen nylon filters were labeled and pre-shrunk by autoclaving 1' dry cycle at 100°C. Once the colonies were grown, they were lifted onto the filters and again processed by autoclaving under the above conditions. After rinsing the filters 5' RT w/ 0.1% SDS to remove bacterial debris, each filter was hybridized with a radioactively-labeled probe using a variant of Church's solution at 62°C, as described in Chapter III. The filters were washed 2x 10' with 0.5xSSC/0.1%SDS at RT, and finally 1x 15' with 0.5xSSC/0.1%SDS at 37°C, then exposed to X-ray film (DuPont/NEN Reflection, w/enhancing screen) at -80°C O/N. Addresses of positive clones were determined from the position of positive colonies on the filters.

Human Genomic Sequence Comparisons

Mouse cDNA sequences (see Appendix B) as well as mouse gene sequence contigs from the *Lyp* region (Chapter V, Appendix C) were compared against the "non-redundant" (NR) GenBank database using the BlastN sequence comparison program (ungapped web version; [8,9]). E-values less than 10^{-10} (or less than 10^{-5} if matching a human genomic clone already identified by another cDNA with E-value less than 10^{-10}) were the cutoffs for identifying the putative human orthologs of mouse cDNA and transcript sequences, with the exception that

obvious repeats (i.e. those matching multiple human sequence entries from different regions of the genome; this includes sequences such as non-orthologous zinc-finger domains) were discarded.

Radiation-Hybrid (RH) Mapping

The Stanford GeneBridge4 panel [10] was used for human RH mapping. In order to map the human orthologs of the PA and TX gene transcripts (Chapter V), PCR assays (hPApos and hTX2, respectively; see Appendix A) were created from the human EST sequences orthologous to the mouse sequences and mapped on the GeneBridge4 panel as recommended[11]. Briefly, 5 ul (25 ng) of each radiation hybrid DNA in a 10 ul reaction was amplified by 30 cycles of PCR at a 60°C annealing temperature. Amplified samples were run 45' on standard 1% SeqPlaque / 1x TBE agarose gels at 3V/cm, stained with ethidium bromide, and photographed on a shortwave UV transilluminator. Duplicate amplifications for each marker were scored and the data mapped on the Whitehead Institute/MIT Center for Genome Research map of the human genome[12,13], using the WI/MITCGR web-based human RH mapping server[14].

hPApos mapped 8.7 cR past AFMA082XC9 and 6.1 cR before FB16B4 on the framework map of human 7q (approximately 18 cR from the most distal mapped framework marker; lod score of 15 used for linkage to framework map, placement lod >3 vs. next most likely order). hTX2 mapped 21.8 cR past FB16B4 (the most distal mapped framework marker) on the framework map of human 7q (lod score of 19 used for linkage to framework map, placement lod >3 vs. next most likely order). Clearly, hTX2 is closely linked to hPApos near the distal end of 7q. However, as hTX2 was a weak assay, its scoring may contain a number of false negatives (in fact, its positives are an almost perfect subset of the positives for hPApos). Given a marker with a map location very near the telomeric end of the 7q map, false negatives might tend

to "push" the calculated map position for the marker past the end of the map in order to avoid introducing new breaks within a fairly dense set of existing markers in the underlying framework [15]; this is likely to be the case with hTX2 and so its local order with respect to hPApos is uncertain.

Results

PA Gene Characterization

Jon Schaefer, working in Åke Lernmark's lab, has recently sequenced an additional 265 bp at the 5' end of the rat PA gene transcript using RACE (Materials and Methods). The 5'-most 169 bp of this sequence extends 5' of the PA gene cDNA sequence described in Chapter V, while the 96 bp of sequence overlapping with the previous sequence matches it exactly (see Figure 1). Thus, 1359 bp of the cDNA has been sequenced, exclusive of the poly-A tail. In addition, preliminary genomic sequence from subclones of the rat 5631G P1 clone (which contains the PA gene; sequence from Jon Schaefer and Ben Snyder), when compared with the PA gene cDNA sequence, indicates the presence of an intron between bases 163 and 164 of the composite PA cDNA sequence, approximately 5 bp upstream of the previously known 5' extent of the PA cDNA sequence, and approximately 35 bp upstream of the start codon for the single long open reading frame (frame 3, bp 201). The cDNA and intron sequences are shown schematically in Figure 1(a), with the complete sequence of the first 200 bp of the cDNA and sequence from the ends of the intron shown in Figure 1(b). As shown in the figure, exon/intron junction sequences match the expected consensus splice site sequences, confirming the intron identification.

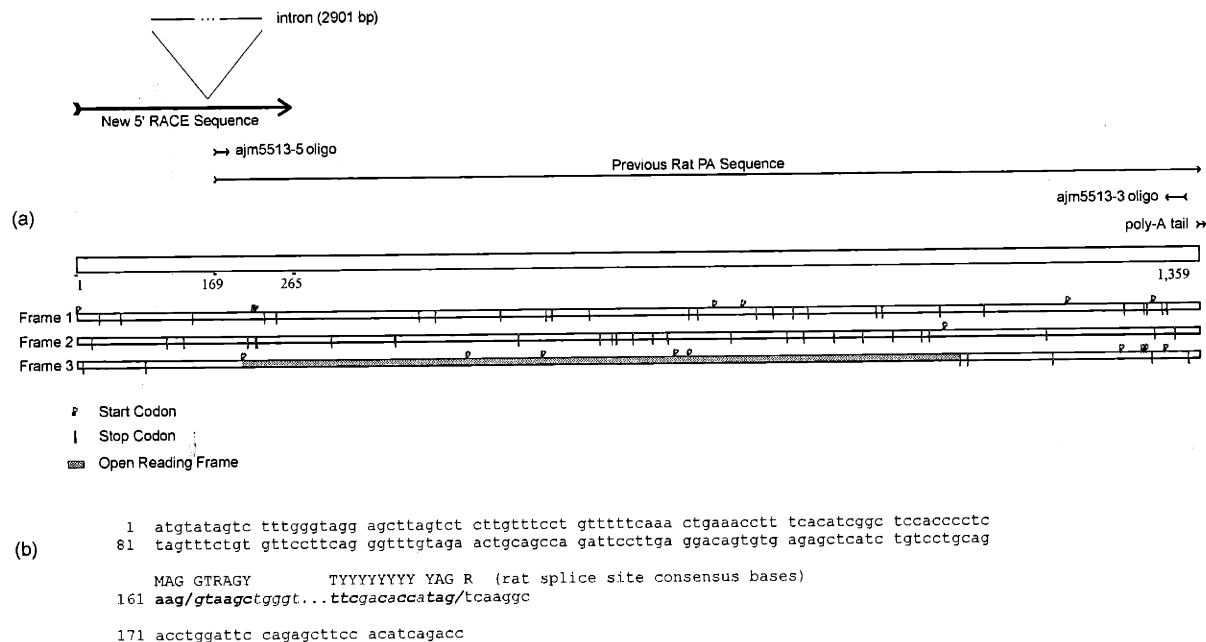


Figure 1: Currently known sequence of the rat PA gene: the most recent information about the structure of the rat PA gene is diagrammed here. (a) Recent RACE work by Jon Schaefer and Ben Snyder on wild-type transcripts of the rat PA gene has produced 265 bp of sequence at the 5' end of the PA cDNA, including 169 bp extending 5' of the 5' end of the previously isolated cDNA clones, resulting in a new sequenced length of 1359 bp for the PA gene, excluding poly-A tail. This sequence is shown as the bold arrow above the arrows representing the previously-determined PA gene sequence, two oligos marking the ends of that previously-determined sequence, and an arrow indicating the poly-A tail. The new composite cDNA sequence is shown below as the long open rectangle with base positions indicated. Below the schematic cDNA sequence, start and stop codons of the three forward reading frames are indicated. The single long open reading frame in frame 3 (beginning at base 201) is filled from start to stop to indicate its extent. Comparison of this new sequence with preliminary rat genomic sequence from subclones of the 5631G rat P1 genomic clone containing the PA gene (sequence from Jon Schaefer and Ben Snyder; detailed sequence not shown) revealed a divergence downstream of base 163 in the new cDNA sequence. As the downstream transcript sequence again matched the genomic sequence after a gap of 2901 bp, the presence of a 2.9 kb intron in the genomic sequence was inferred. This intron is shown schematically at the top of (a). (b) The full sequence of the new 169 bp RACE sequence is shown in part (b). For comparison with the consensus rat splice site sequences, sequence from the intron ends has been included; it is in italic, while the consensus splice site sequences [18] above are shown in uppercase. Those bases in the cDNA and intron matching the consensus are indicated in bold. The long open reading frame begins at base 201, immediately following the last base shown in part (b) (base 200; base numbering excludes sequence from the intron).

Fine-Scale Physical Map of Rat *Lyp* Region

Recently, a high-coverage rat PAC clone genomic library has become available (RPCI-31 [7]), providing the opportunity to assemble a fine-scale physical map of the rat *Lyp* region. Unlike the rat P1 library described in Chapter IV, which often had gaps in its coverage, 5x genomic coverage of this library was screened, with another ~5x coverage available if necessary

(10x total coverage). As the RPCI-31 library is screened by hybridization, the previously isolated SSR rat genetic markers (Chapter IV) could not be used directly to screen the library. Rather, the four rat cDNA STSs ajm5513, ajm5515, ajm5518, and rmo0124 (see Chapter IV, Appendix A) were pooled and used to screen the library to isolate clones from the central *Lyp* region. In addition, as rat YAC 28e5 (Chapter IV) contains the current *Lyp* interval, 18 random

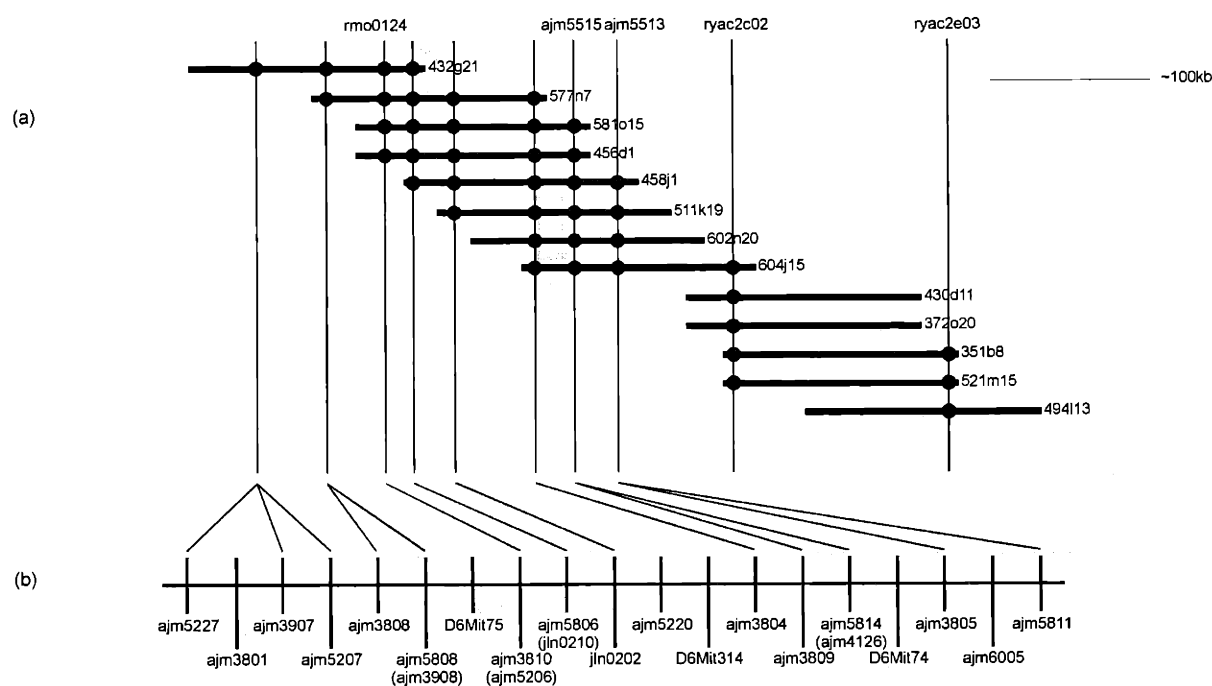


Figure 2: High-resolution rat PAC map of the central *Lyp* region (around ajm5513 -- PA) and its conservation of linkage (COL) with the central mouse *Lyp* region: (a) Rat PAC STS content map of central *Lyp* region on rat chromosome 4. Rat STS assays used to identify contig PACs are indicated above the vertical lines indicating STS content bins. Rat PACs are depicted as uniformly 150kb in length; although actual sizes vary, the average size of PACs from the RPCI-31 rat PAC library used is ~150 kb. (b) Central mouse *Lyp* region physical map STS bins arranged in order. Labels show the name of the STS defining each bin. In order to integrate this mouse map with the high-resolution rat PAC map, Ben Snyder hybridized mouse cDNA clones from these bins to the rat PAC clones gridded on a filter. A positive hybridization signal from a PAC clone indicated the presence of the putative ortholog of the mouse cDNA within that PAC, thus allowing the STS content mapping of the mouse cDNA clones on the rat PAC panel. These mappings are indicated by diagonal lines connecting mouse STS bins with rat PAC STS bins. In certain cases, an alternate mouse cDNA from a mouse STS bin was used in place of the cDNA naming the bin; this alternate probe is indicated in parentheses. Lack of a diagonal line for certain mouse STS bins indicates that the associated probe was not used for hybridization, usually due to its repetitive nature (e.g. D6Mit74).

As shown in the figure, all mouse cDNAs mapped on the rat PAC contig in the same order as in the mouse (to the resolution available), thus confirming COL between mouse and rat in the central *Lyp* region. In particular, the mouse orthologs of the rat cDNAs rmo0124, ajm5515, and ajm5513 (ajm3810 -- OH, ajm3809 -- NC, and ajm3805 -- PA, respectively) mapped to the same positions as did the rat cDNAs themselves.

non-repetitive rat STSs isolated from that YAC (see Materials and Methods) were separated into two pools, and each pool used to screen the RPCI-31 library in order to isolate additional rat PAC clones from the *Lyp* interval. The three screenings identified a total of 69 rat PAC clones. The central rat *Lyp* region is spanned by a contig of these rat PAC clones (Figure 2(a)); additional single-probe screening of the rat PAC library is now in progress in order to extend this contig to completely span the *Lyp* interval (the interval between rat genetic markers 5579CA1b and 28e5-e09).

Fine-Scale COL Between Mouse and Rat Lyp Regions

Previous confirmation of COL between the mouse and rat *Lyp* regions, as described in Chapter IV, was limited in resolution due to the lack of a fine-scale physical map for determining order at high resolution in the rat *Lyp* region. Therefore, I collaborated with Ben Snyder of Åke Lernmark's laboratory (U. Washington, Seattle) to map mouse cDNAs from the central *Lyp* region on the fine-scale rat PAC physical map. Since PCR amplification of rat genomic templates with mouse cDNA PCR primers had previously proved problematic (Chapter IV), we chose to hybridize individual mouse cDNA clones to a gridded filter array of the rat PAC clones isolated above in order to map the rat orthologs of the mouse cDNA clones.

Mouse cDNA clones were chosen from the mouse STS content map bins identified in Table 6 of Chapter III, and individually hybridized against the gridded rat PAC clones. Strong positive hybridization signals were taken to indicate the presence of the rat ortholog of the mouse cDNA probe (see Figure 3 for examples). These positives were used to order the mouse cDNA probes, with the results shown as the diagonal lines connecting in Figure 2(a) with 2(b). As shown in Figure 2, the STS order determined on the rat PAC panel was in complete agreement with the STS order previously determined in the mouse in Chapter III.

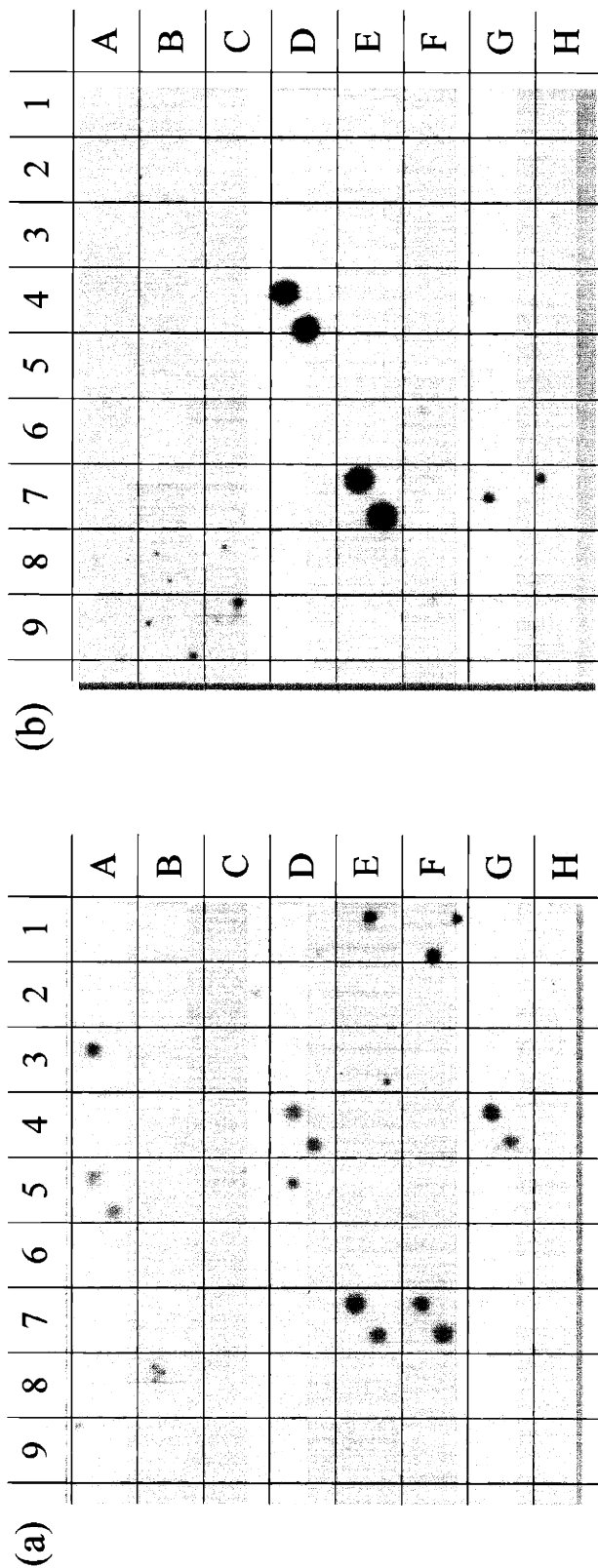


Figure 3: Sample autoradiograms of gridded rat PACs hybridized with mouse cDNA probes (source: Ben Snyder). Gridded panel of *Lyp* region rat PACs (69 total; arranged in duplicate as 8 rows (A-H) with 8 full columns plus a ninth column in rows A-E) were hybridized with mouse cDNA probes. Positioning grids have been superimposed on the autoradiograms in this figure to simplify scoring. In order to prevent single spurious spots from being scored as (false) positives, each PAC was spotted twice, with the second spot offset diagonally from the first. (a) Gridded panel hybridized with mouse jln0210 cDNA probe. Positives (in duplicate) are PACs 458 j1 (position A5), 432 g21 (D4), 577 n7 (E7), 581 o15 (F7), and 456 d1 (G4). (b) Gridded panel hybridized with mouse ajm3908 cDNA probe. Positives are PACs 432 g21 (D4) and 577 n7 (E7).

Breaks in COL Between Mouse and Human Lyp Regions

In addition to the rat work described above, recent advances have also facilitated the study of COL between mouse and human. As noted in the Discussion of Chapter II, COL between mouse and human breaks down precisely in the neighborhood of the *Lyp* gene, with the previously identified human orthologs of genes some distance to the left and right of the *Lyp* region mapping to chromosome arms 7q and 7p, respectively.

With the aid of newly available genomic sequence information from human chromosome 7 [16,17], and RH mapping of the human orthologs of mouse transcripts from the central *Lyp* region, I have now been able to investigate COL and its breakdown between mouse and human in the *Lyp* region at much finer resolution than before.

In particular, over 1 Mbp of genomic BAC/PAC clone sequence from the region of human 7p corresponding to parts of the region to the right of the PA gene in the mouse is now available, with a number of gaps currently linked only by the underlying human chromosome 7 physical map YAC clones. In addition, a ~220 kbp BAC/PAC clone sequence contig from human 7q, corresponding to part of the region to the left of the PA gene, has recently been deposited in Genbank. These fully-sequenced clones are represented to scale as horizontal arrows in Figure 4(a).

Given these human genomic sequences, I then used BLAST similarity searching to compare these sequences with the extensive mouse cDNA sequence information obtained from the mouse *Lyp* region (see Materials and Methods) in order to identify putative orthologs. Two groups of mouse cDNAs matched current human chromosome 7 genomic sequences: first, mouse cDNAs selected from mouse YACs 43, 44 and 45 (to the right of the mouse *Lyp* region) matched sequences in human clones A-J in Figure 4, and, second, a set of mouse transcript

sequences from the central *Lyp* region matched sequences in human clones Y and Z. Mouse cDNAs producing matches with Blast E-values less than 10^{-10} (or less than 10^{-5} if matching a human genomic clone already identified by another cDNA with E-value less than 10^{-10}) are indicated by vertical lines beneath the human BAC/PAC clones in Figure 4(a), showing the match locations within the human clones. Sample alignments for selected mouse/human matches are shown in Figure 5.

Additional map order information was provided by human RH panel mapping, as shown in Figure 4(c). Certain genes, such as the *HoxA* complex and *Evx* have long-established mappings on human chromosome 7, while the RH mapping information associated with the YAC contigs of the human chromosome 7 physical map[16] provided chromosomal localization for the human genomic clones A-J and Y-Z in Figure 4(a). Finally, BLAST database similarity searching had produced putative human ortholog ESTs for sequence from the TX and PA genes (Chapter V). PCR assays from these human ESTs were RH mapped to the end of chromosome 7q (Materials and Methods, Figure 4(c)).

Although the relative order of the mouse cDNAs selected from YACs 43, 44, 45 is unknown in the mouse, the mouse transcripts IL, IN, IA, and MO show COL with their human orthologs on a very fine scale: the transcription unit order IL - IN - IA - MO in mouse is conserved in their human orthologs in human clones Y and Z (Figure 4(a),(b); Chapter V).

On the other hand, the integrated mapping information shown in Figure 4 indicates the existence of at least two breaks in the COL between mouse and human in the greater *Lyp* region. From the previous mapping of genes flanking the *Lyp* region, it was known that those on the left side of the mouse interval had human orthologs that mapped to human 7q, while those on the right side of the mouse interval had human orthologs that mapped to human 7p. The more recent

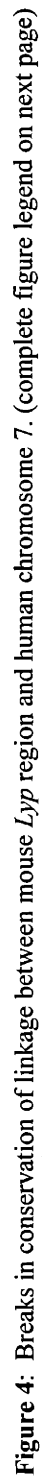


Figure 4: Breaks in conservation of linkage between mouse *Lyp* region and human chromosome 7. (a) Positions of human orthologs of mouse cDNAs selected from mouse YACs. Finished genomic sequence data is now available for segments of human chromosome 7 orthologous to portions of the mouse region spanned by mouse YACs 43, 44 and 45, and by YACs 34, 36 and 56. Horizontal arrows (labeled A-J, Y-Z) indicate the relative orientations and extent of finished clone sequences from a portion of human chromosome 7p14-15 and a portion of chromosome 7q31-35 as released by [17] while vertical lines with "sWSS" or "D7xxxx" labels above the arrows indicate the names and locations of STSs from the human STS content map spanning these regions (contig E -- 7p14-15 -- and contig R -- 7q31-35 -- of the human chromosome 7 physical map[16]; numbers in parentheses are the STS bin numbers within the contig, or "-" if not assigned a bin). Vertical lines with labels below the arrows indicate the names of mouse cDNA sequences (selected from mouse YACs 43, 44 and 45 or from YACs 34, 36, and 56) that are the probable orthologs of the human sequence at that position, as determined by BLAST sequence comparison. These mouse cDNA orthologs are labeled either by cDNA fragment name (e.g. ajm2030) or cDNA contig name (e.g. INc1 is Indiana contig 1); contig locations on the mouse physical map are also described in Chapter V. Human BAC/PAC sequence lengths and the positions of STSs within the sequences are to scale; gaps between arrows indicate gaps of unknown size in the sequence contig (these gaps are spanned by multiple human YACs in the underlying STS content map). The identities of the human genomic BAC and PAC clones are as follows: A – RG118E13; B – DJ1055C04; C – RG341D10; D – RG385F02; E – RG424N05; F – RG034F08; G – DJ0899B21; H – RG152H24; I – RG136N17; J – DJ0978I12; Y – DJ0811N16; Z – DJ0751H13. (b) Schematic mouse genetic/physical map of chromosome 6 *Lyp* region. Genes and transcription units shown here are linked by lines to human orthologs in part (a) (by genomic sequence comparison) and to portions of human chromosome 7 in part (c) (by RH panel mapping of human ortholog PCR assays, or by reported chromosomal locations of the human contigs in part (a)). (c) Ideogram of human chromosome 7.

Breaks in COL between mouse and human are as follows: (1) To right of mouse PA gene: TX/PA human orthologs both map to human 7q35/36 (c), as do mouse contigs between them (a); adjacent mouse cDNAs from YAC 43/44/45 region and *Npy* map to human 7p (a). (2) Based on physical and genetic mapping, *Npy* maps between the YAC 43/44/45 cDNAs and *HoxA* and *Evx* in the mouse (chapter II, III); as shown in (a) and (c), *Npy* maps to the left of both the YAC 43/44/45 cDNA orthologs and *HoxA* and *Evx* in the human. The relative order of the YAC 43/44/45 cDNAs is not known in the mouse; additional breaks in COL between mouse and human may exist in that region.

sequence comparison work confirms this and shows that the break is to the right of the PA gene.

This work also indicates that at least one more break in COL exists in the *Lyp* region: in the mouse, the YAC 43/44/45-derived cDNAs, *Npy*, and *HoxA/Evx* have the map order 43/44/45 - *Npy* - *HoxA/Evx*. In the human, however, the order is *Npy* - 43/44/45 orthologs - *HoxA/Evx*. Thus, the 43/44/45 region - *Npy* order is inverted between mouse and human with respect to *HoxA/Evx*.

Figure 5: Sample BLAST alignments of *Lyp* region mouse cDNA sequences with human BAC/PAC genomic clone sequences from chromosome 7. (below)

Figure 5(a): ajm2628 alignment against human BAC clone E (Figure 4; RG424N05). The multiple non-overlapping segment matches of BAC E with cDNA ajm2628 suggest the presence of introns in the genomic sequence. This is supported by the match of the segment 2/segment 3 and segment 3/segment 4 end sequences with consensus splice junction sequences[18]. Original BLAST output was edited as follows: "/" was inserted at predicted splice site junctions, sequences from BAC E and ajm2628 immediately flanking the matching segments were added in lowercase to clarify splice site consensus sequences, and bases matching the consensus splice junction sequence were underlined. The splice consensus matches indicate that the alignments below represent a

transcript portion in a 3' to 5' orientation, as 5' splice site consensus are at the left ends of segments 3 and 4, and 3' splice site consensus are at the right ends of segments 2 and 3. Added bases in lowercase are not counted in the BLAST match statistics and not counted in the base numbers flanking each stretch of sequence. Segment 1 may represent a spurious match, as its longest uninterrupted stretch of matching bases is a single stretch of 14 bases, and neither the left end of segment 2 nor the right end of segment 1 match splice site consensus sequences.

Figure 5(b): IAcontig1 (see Chapter V, Appendix C) alignment against human BAC clone Z (Figure 4; DJ0751H13). The multiple non-overlapping segment matches of BAC Z with cDNA contig IAcontig1 suggest the presence of introns in the genomic sequence, as listed in the annotations for BAC clone Z. The predicted exon/intron junctions appear at the ends of the matching segments below (in 5' to 3' order as shown), as expected, with the exceptions of the segment 3/4, 4/5, and 6/7 breaks. As the right end of segment 3 and the left end of segment 4 are immediately adjacent on both sequences (with a 1-bp offset in one), these presumably represent the same exon which has been split into two matching segments by a single-bp offset in one sequence relative to the other. The large gaps between the right end of segment 4 and the left end of segment 5 in both sequences suggest that the relatively poor sequence in IAcontig1 in this region prevents the detection of a proper alignment. The left end of segment 7 contains the expected 3' splice site consensus sequence, but the right end of segment 6 stops in another region of poor sequence quality in the IAcontig1 sequence, which presumably obscures the matching 5' splice site. Interestingly, an additional exon in both mouse and human not noted in the BAC Z annotation is indicated by matching segment 6. Original BLAST output was edited as follows: "/" was inserted at predicted splice site junctions, sequences from BAC Z immediately flanking the matching segments were added in lowercase to clarify splice site consensus sequences, and bases matching the consensus splice junction sequence were underlined. The splice consensus matches indicate that the alignments below represent a transcript portion in a 5' to 3' orientation, as 5' splice site consensus are at the right ends of segments, and 3' splice site consensus are at the

IAcontig1:	533	AG/CCTGGGTGTCTGGATGCGGTGGAAGTGGCTTGGGGCTGTNACANCCTC	582
BAC Z:	34827	tctgcAG/TCTGGGTGTCTGGATGCAGTGGAGGTGGCCAGGGCTGTGACAGCCCC	34876
Matching Segment 7:			
Score = 218 (60.7 bits), Expect = 2.5e-87, Sum P(6) = 2.5e-87			
Identities = 56/73 (76%), Positives = 56/73 (76%), Strand = Plus / Plus			
IAcontig1:	685	CAG/GTTCANCCAGATGAGTACCATGAGACCTGTCTCTTTGCCTACTGTGTTGGANCCACA	744
BAC Z:	35251	CAG/GTTTTCCCTGCTGAGTACCACGAGGCCTGTCTCTTTGCCTACTGCGCAGGGGCCATG	35310
IAcontig1:	745	GCAGGCAAATGGC	757
BAC Z:	35311	GCAGGCAAGTGGGC	35323

Discussion

PA Gene Characterization

As the transcript size of the PA gene is approximately 1.3 kb (see Chapter V), the 1359 bp composite sequence shown in Figure 1(a) should represent almost the complete PA transcript. All three reading frames include multiple stop codons upstream of the single long open reading frame, confirming that the complete coding sequence has been identified and is that described and analyzed in Chapter V. Although most of the transcript (flanked by the two oligos shown in Figure 1(a); this includes the entire coding region) has been resequenced in BB-Dp and BB-Dr affected and wild-type rats without identifying a predicted amino acid polymorphism, the newly identified sequence at the 5' end, and in particular the newly-identified intron, identify new portions of the PA gene where sequence differences between BB-Dp and BB-Dr may cause functional differences that produce the *Lyp* phenotype. We are currently in the process of resequencing in BB-Dp, BB-Dr, and other wild-type strains the rat genomic region containing the entire known PA gene with intron, from upstream of the 5'-most cDNA sequence to downstream of the transcript end. This resequencing will identify any polymorphisms in the immediate neighborhood of the rat PA gene; although the parental pre-lymphopenic chromosome is not available, comparison of the BB-Dp sequence with a number of wild-type

sequences will allow identification of those polymorphisms likely unique to the *Lyp* haplotype, which can then be studied further for any functional effect.

Fine-Scale Physical Map in the Rat

Subclones from the rat YAC clones described in Chapter IV allowed the rapid isolation of clones from the *Lyp* region from the new, high-coverage rat PAC library. These clones covered in full the central *Lyp* region which had only been partially spanned by P1 clones from the limited-coverage rat P1 library, confirming the importance of the availability of a high-coverage, medium-insert-size genomic clone library for the species of interest.

Initially, these PAC clones have been used to verify COL at a very fine scale (see below) while PAC coverage is extended to span the nearest recombinant breakpoints flanking *Lyp* in the rat (between the SSRs 5579CA1b and 28e5e09). Once that span is complete, a tiling path of rat PAC clones will be sequenced to complement the sequencing (currently in progress) of mouse BAC and P1 clones spanning the central *Lyp* region. This sequencing will (a) allow the identification by inspection of all SSRs in the region, facilitating the identification of the closest SSR genetic markers flanking the *Lyp* region, (b) allow the identification by sequence inspection and database comparisons of any additional genes in the *Lyp* interval that were not identified through cDNA selection but are of interest as candidates for *Lyp* and (c) allow the identification, by comparison of the orthologous rat and mouse (and perhaps human, as well) genomic sequences, of regions of conserved sequence within and, perhaps more importantly, outside of open reading frames. These conserved sequences are likely to be of functional importance (e.g. regulating the expression of adjacent genes) and can be quite difficult to identify by other means; it may be that the *Lyp* mutation lies in one of these sequences.

Fine-Scale COL Verification

As shown in Figure 2, COL between rat and mouse in the central *Lyp* region has now been verified to gene-level resolution. Each mouse cDNA probe tested in the region has identified a related sequence in the rat PACs spanning the region and those rat sequences are in the same order as the corresponding mouse cDNAs. This strongly supports the original premise of using the COL approach for the *Lyp* project and suggests that the approach may be equally fruitful in other projects where the pair of species has diverged within the last 20 million years, as for the rat and mouse.

Conservation of Linkage Between Mouse and Human – Where is the Human Lyp Region?

The situation between mouse and human in the *Lyp* region is, however, quite different. As shown in Figure 4, there are at least two breaks in COL between the mouse *Lyp* region and its human orthologs. Although COL seems to hold at the very fine scale of the IL-MO transcript interval (Figure 4(a), far right), the existence of rearrangements between mouse and human for the genes on human 7p over the 10-100 gene scale means that COL must be verified down to a fine level between mouse and human in this region for any practical use to be made of it. As sequence comparisons and human RH panel mapping show that all tested transcripts in the mouse TX-PA transcript interval (as well as the T-cell receptor beta gene) map to end of human 7q (Figure 4), the human ortholog of *Lyp* most likely is located there as well (certainly, if it is in fact PA).

More generally, the lack of complete COL between human and mouse in the *Lyp* region serves as a reminder that not every genomic region is suitable for a COL cloning approach between a given pair of species. It may be that the average size of blocks of conserved linkage

between mouse and human are just significantly smaller than those between mouse and rat, or it may just be that there happens to be a concentration of breaks in COL between mouse and human in the *Lyp* region. Fine-scale COL data from other regions of the genome should resolve this question; the efforts to sequence the human and mouse genomes will finally resolve this issue.

Conclusion

The recent work described in this chapter has confirmed the original premise of COL between rat and mouse in the *Lyp* region and lends confidence to the assertion that the mouse ortholog of the rat *Lyp* gene is among the set of transcripts identified in the mouse *Lyp* region.

Further study of the rat PA gene, in particular resequencing of the BB-Dp and various wild-type haplotypes, and ongoing transgenic rescue work (as described in Chapter VI), will show whether it is *Lyp* or a separate gene involved in late-stage T-cell maturation in the thymus. In the latter case, genomic rescue work and the sequencing of the entire mouse and rat *Lyp* intervals should lead to the final identification of the *Lyp* gene.

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APPENDIX A

Table A-1: The following table contains the sequences of primers used in this work. For some, the predicted melting temperature is also listed (calculated as described in the appropriate chapter).

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
101f4sr1-f	TCAAAAATTCCAGCCTTCTAATG	59.60
101f4sr1-r	GGTGTCTGACAGTTTCCTGA	60.10
11c2sr2-f	CCATCCCATAATCTGGTACAAGA	60.10
11c2sr2-r	TGTGTACATGAATGTGGATATAGGAA	59.60
12 R2H-f	ctccatgcccttgcatagtt	
12 R2H-r	ctccatgcccttgcatagtt	
15 R2H-f	cttggtgattcagactgcca	
15 R2H-r	gacaatgggaaggcactgtt	
17L2H-f	aaaggtctggaggtcaggg	
17L2H-r	ccccaaggctcttcacctt	
17R2H-f	ttattggcgacttgtgcttg	
17R2H-r	gaattccaggacagccagag	
1807-f	AGGTTGAGGAGGACTTTTCAAAC	60.00
1807-r	GTCATCAGCAGGATTATCTCCAC	60.00
1808-f	GATCGAGCACTATGGCGAA	59.90
1808-r	TTCGATCTCGTGGGCATTA	60.20
1809-f	CAAAGGTAAATGTGGCCTTGA	60.00
1809-r	TATGTTTACATGACCTGGAGCCT	59.90
1810-f	GCTCCAAAATTCAATTTAAACAAA	59.80
1810-r	TCCTCCAGGGGTCTTCATC	60.00
1811-f	GTGTGGATGGAAGAATGAAGAAG	60.00
1811-r	TTTACTTTTCCAATGGTCCCC	60.00
1812-f	ACACTGGCACAGCACTTCAC	59.90
1812-r	CTTCCTTAAGCAAGTTGAAGTGGT	60.20
1813-f	GTCTTGTAGCACCAACAACAAAC	60.00
1813-r	AGACCAACTGCTCAAACCTCTGAC	60.00
1816-f	AGCCTTTCCCTGGAAGGAT	60.00
1816-r	AAGTTCCTTCAAAGCCAGCTC	60.00
207con1a-f	TGGGCCATTAGACATTTTAAATT	60.00
207con1a-r	TCAGACCTCCTCATGCACAG	60.00
207con1b-f	CTGTCACTTCTGGGCCATTAG	59.70
207rpt1-f	tgggccattagacattttaattt	60.00
207rpt1-r	tcagacctcctcatgcacag	60.00
2313-f	CCCACATTCTTTGCCTG	60.00
2313-r	CCACAGGGTCACACAAGTTCT	60.10
2316-f	TCCCTTCAGCACTCCAGTG	60.00
2316-r	CCCTATAAAGTCAGAGGAAGAGTCC	60.00
2317-f	GCTCAATATCAGCTTGGCTCTT	60.00
2317-r	GAAATGACTTACACCCAGGAGC	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
2318-f	CCTACTATGCTGCTGACTCCTGT	60.00
2318-r	AATCCCTGTTACTTAGCTATGGCTT	60.00
2319-f	CTGTGTCCCTCAGCCTCAA	60.00
2319-r	GTAGACGGGGCATTAAATGTTGT	60.10
2320-f	GTCTCAATCAGTGAAGCCACC	59.70
2320-r	CCTATTCACCTGTGCTGGAAA	60.10
2321-f	AACCTCCTAAAACCTTTGCTCC	60.00
2321-r	TAGGACCTAGACCTGTAAAATCGTG	60.00
2322-f	TGTACTIONAGCCCCCAGTTCTACA	60.10
2322-r	TCTTCAAATGATTACAGCCCCT	60.00
27 L2T-f	ttcaggctcctcttcttacctgc	
27 L2T-r	cgaagagatctgttctctaaaagga	
27 R2H-f	tccagtctgtactccccacac	
27 R2H-r	tgagtgaacagacaatttatcagg	
2709-f	CCACCCCTGAGTTTGTGACT	60.00
2709-r	GTTTTGTCCAGTTGTGGGAGA	60.00
2710-f	CCTATCAGAAACCATGCTAGGG	60.00
2710-r	CTGAAGCCATTCCAAATTTAGACT	60.00
2711-f	AACTGCCACAGGAGAGAGACA	60.00
2711-r	GTGTCTACGGGCAGATGGTAA	60.00
2712-f	TTAAGGATGCAAGGGTTGTTCT	60.00
2712-r	CTATATCCAAACATGCTGCTTCC	60.00
2713-f	TTCAGGTTTGGTGTGTGTTGA	60.00
2713-r	ACGATTACCTTGATTTTAAAGGGTC	60.00
2714-f	GACTGCATGGTTCTTGAGCA	60.00
2714-r	CCATGGAGGTTGGCTAGAAA	60.10
2715-f	CCCGTGGATTTTCGCTAGAT	60.00
2715-r	CTTCTTGCTAAGTGCTTGGGAT	59.90
2716-f	TCAAGAGGGGAAAGATTAAAGTTG	60.00
2716-r	ATTCAGATCGTTGTGCTAAAATAGG	59.90
2717-f	AAAACAGAAGCCGGTAAACAAA	60.00
2717-r	AGTCAGCAATTTCCCATCTTGT	60.00
2718-f	CTGTCCTCCCGGTGATTCT	60.10
2718-r	TACAGCCAGCCCCAATGT	60.10
2719-f	CATAGTGGGACCAGAGAAGGTC	60.00
2719-r	TTATTGGTCCATACACCACATCA	60.00
2720-f	CTTGAATCTGTCCCTGTATTCTGTT	59.90
2720-r	AAATGAACAAGATGGCCAG	59.90
2721-f	CACCAGATGTTTTAGTCGATTTTTT	59.80
2721-r	TGGGTCAGAGGTGACCAAA	60.10
2722-f	CGTTTTCACTGATCCACAATACTC	59.90
2722-r	TGAGCTTTGATCATTTACATATGGA	59.90
2723-f	ACAGGCACTTATTGTGTGAACCT	60.00
2723-r	AAGTGCAGTTGGTTATCTGGTGT	60.00
2724-f	TCAAGTCCAATGCAGCTTAAGA	60.00
2724-r	TGTGTTTTCACATGGTCAGGA	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
28e053f05-f	CATGCGTGTTTGCAGCA	
28e053f05-r	TCATGTTAGGGGAGGATGTAAGA	
28e05e09-f	GAACACCTTTGACAGTGAAAACCTC	
28e05e09-r	CTGTAGAAACCTAACCAAGACAGGT	
3027con1a-f	AACAATTCACAGTTTATTGTTTTAGG	60.00
3027con1a-r	ATTGAGTCCCACAAGTTGTCCT	59.90
3027con1b-f	GGTTTCTATTTATCTATACCTCATACTCATGA	59.90
3027con1b-r	TCCATGTGTGCCATGCAT	60.50
3031con1a-f	AAAAGGTGCTCACCCAAGC	60.20
3031con1a-r	CACCAGGAGTAGTTCTGCCTCT	59.90
31 R2H-f	gccctctcaggagacagcta	
31 R2H-r	atcaaatagctgagaaacaccaaag	
3105con1a-f	ACTTCTGGGCCATTAGCATT	60.00
3105con1a-r	TCAGACCTCCTCATGCACAG	60.00
32 L2T-f	tcctctgacctcctcagcat	
32 L2T-r	atctcatacggagggtgcagg	
320f9sr1-f	GAAAAATTTCTAGTTGTTGATCACAGG	60.60
320f9sr1-r	TTCAACTTCAAAGAGACAGCCA	60.00
34 R2A-f	gggtactgtgggtcttgaatgt	
34 R2A-r	atttagtaattgtggcccatgtg	
36 L2H-f	tggtagatgccttgagtc	
36 L2H-r	tctggaaaaggacactgc	
36 R2A-f	cacttggatgtgcaggcta	
36 R2A-r	aaggcctgcttcaagtcag	
4011_2t3-2f	ccctccagatccttccttc	60.00
4011_2t3-2r	gcctcaaacctaaagcaggc	60.00
4011_2t3-f	gcaatctcctacaaccccttc	60.00
4011_2t3-r	gtaaacttaatgcagggtgcaaatg	60.00
4011_2t7-2f	tttttgacttttaatatccaagc	59.90
4011_2t7-2r	caagaacctagaggtcatcga	60.00
4011_2t7-f	agtgtctcagcagactacgc	60.00
4011_2t7-r	gcttggatattaaaagtgcacaaaa	59.90
4011ssr1a-f	TGGGAAAGGGAAACTGAGG	60.00
4011ssr1a-r	CTGCGTGCTGTGTGAAGAAT	60.10
41 R2H-f	catccagtcccagtcctgtct	
41 R2H-r	gccctgataaccacattgct	
43 R2H-f	catagcattgctgccag	
43 R2H-r	cactcctcaagacaaggctg	
4372GI-f	TTTCAATGGACCATCTGAACAG	60.00
4372GI-r	TGCCTTTTGGGGACTAAGTTT	60.00
4372Gr-f	GATTGCAGAGTTATCCAGGTTAGAA	60.00
4372Gr-r	TTAACAAAAACCCATCTCTACTTGC	60.00
44 L2T-f	aggaattgcttacatggtagagc	
44 L2T-r	ggcaggctgctgtttgtaa	
44 R2H-f	cagtgaaccagggtattgtga	
44 R2H-r	gagttcatacatgctgatggga	

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
45 R2H-f	GCTGCTAGTGGTGTCTCATTCTT	60.00
45 R2H-r	AGTCAGATCGCCAGCAAAGT	60.00
4825GI-f	GCCACAACACAACCACTCC	60.00
4825GI-r	GAAGTGGGGCAAGGGTCT	60.00
4825Gr-f	AATTAAGGTAAGTCTCTGAGAGGTATGC	60.00
4825Gr-r	ATGCCTTCCTGGTACTCCTACA	60.00
4829GI-f	TGGGACACGAAGCTAGATTTG	60.30
4829GI-r	TACCTCACCGGACAGACTTTG	60.20
4829Gr-f	TGCATTTGGACTTTTGTGTTTC	60.00
4829Gr-r	GGCAGAGGCAGTTGATTCTC	60.00
4866Gr-f	CCTGGAAGTTTTCTTCTGATGATT	60.00
4866Gr-r	TGGGGAAGGGGAAGAGAC	60.00
5578CA_d-f	CTTACCACTAAAAGTATGACCTGAA	60.00
5578CA_d-r	TGTGTGTGTGCACATGTGC	59.70
5578CA_d-f1	CTTACCACTAAAAGTATGACCTGAA	60.00
5578CA_d-f2	TTCAGATCCCACAAGTTGTCC	60.00
5578CA_d-r1	TTGTGCAAAATTGAAAAACAAA	59.90
5578GA1-f	TCTAAGAGAACAGAGTCTGAGAGTTC	60.10
5578GA1-r	AACCTGCTCTGTTTGTAGAAGAAAA	59.90
5578GA2-f	TCTCAAAGCGCTCATCCC	60.00
5578GA2-r	CATCTTCTGTGGGTATCTACA	52.70
5578GC-f	TCTTGGCTTCTCTCCCTATCTG	60.00
5578GC-r	ACTGGGTACTATGACACACACCTTT	60.10
5579CA1b-f	GCAAAGCACTTGCCTAGCAT	60.60
5579CA1b-r	ATACTAATATACAAATTCCAGTCTCGATA	56.60
5579CA2-f	gtaCAAAAAACATTTTAATTACACACA	55.90
5579CA2-r	CGGGGAGTTCCAGGACTAC	59.50
56 R2H-f	tatgttcacatgcagcttccc	
56 R2H-r	aaagaaagtaggcaatgtcaaag	
5621CA1-f	TGGAAGAAGAAAAAAGTTGTTAAAGA	60.10
5621CA1-f2	AAGATCTTGTTTCATTTACCAAGATTG	60.20
5621CA1-r	CAGTTCAGGCTCTATATCCCCTT	60.00
5631CA3b-f	CTGGAGAGGTAAGAACAGCTCTG	59.70
5631CA3b-r	CTCTTGGAAGAACATTCTCAAGC	59.50
5631CA3c-r	TCCTTCCATGAGGGGTTATTC	60.10
57 L2H-f	gcacataaatctctcccttcagt	
57 L2H-r	gtatttgattgtcattgtcatcca	
5807GI-f	GGTTGAACACAGGGCTTTGT	60.00
5807GI-r	TGCATTTACCACTGAGTCATC	60.00
5984/85ca_b-f	ACAGATGCTCCGCTGTGAC	60.00
5984/85ca_b-r	AAAGCTGAAAAGCATATTAATCTAAGC	59.10
5984/85ca_c-f	TCAGAATGTTGGCATCAACTATG	60.00
5984/85ca_c-r	CAGTGGCTCTGTGTAATTCTCG	59.90
5984/85ca_f-f	TCACCAGAACACAGCAGACTG	60.10
5984/85ca_f-r	ACAGTGTGTCAGAGCTTCAGTCA	60.10
5985Gr-f	TGTGAGTAGGAAGTTCTAAATGCG	59.80

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
5985Gr-r	CACGTGGACCACATTCCC	60.80
6239Gr-f	TGGTTGCTGGAATTTGAAGTC	60.10
6239Gr-r	GCCCTAGTCAGTGCCTAATTTTAA	60.00
6241GI-f	CACCTGTGCAGACTAGTCTTGG	60.00
6241GI-r	TCTTGAGAGGAGAGGTGTCCA	60.00
6241Gr-f	TTTAATTTTTTAAAAATGGTTTAACCCC	60.00
6241Gr-r	GGAACATTAAAGGATTGAATTGTG	60.00
6241ca_f-f	CGTGAGTAGTGAGGCTTCCTTT	59.90
6241ca_f-r	TCATCTGCTTGGAGATTGCTT	60.00
6241ca_h-f	CACTGAGGACAGCTTCCCA	60.00
6241ca_h-r	GATCTGAGGGTTGTTGTGATGA	60.00
6350GI-f	TTGCAGAACCGGACCATT	60.00
6350GI-r	CAACTCAATCGACAGCTGGA	60.00
6350Gr-f	GACTTGGGATTCCACTAAGTGC	60.00
6350Gr-r	AACAAGGCTTAGGTTTTCTCACC	60.10
6450GI-f	AATGTTTGTGGCTCCAGGAC	60.00
6450GI-r	TCCGTACACAGGGAGTTATGC	60.00
7134GC1-f	CAACACCTCTGGCCTCTACAG	59.90
7134GC1-r	GAGCAGCCTGCTGGTCTC	59.80
7425GD2-f	GCAGGCTTACAGAAAGGGG	59.80
7425GD2-r	ATCTGCAATGTGTCCAAAAGG	60.00
AP1	ccatcctaatacgactcactatagggc	63.30
AP1_60	ATCCTAATACGACTCACTATAGGGC	58.30
AP2_60	TCACTATAGGGCTCGAGCG	59.10
AP2low	CACTATAGGGCTCGAGCGGC	64.40
D6Mit314-f	CCAATCAGTCACTTATTTTCATCTCA	
D6Mit314-r	GGCTATTGTGTGACTTTGAGAGA	
D6Mit33-f	ACACATGTGCGCATACACACACA	
D6Mit33-r	TTTTATCTGGAAACCTATGGATT	
D6Mit42-f	cctcaccttgctctctttg	
D6Mit42-r	ttttgccagagtagggc	
D6Mit43-f	tccagtcttgacacacatca	
D6Mit43-r	cagggatgctctgtgactca	
D6Mit74-f	CATGTGCAGTGTAAGTAAGACCTC	
D6Mit74-r	TCTCCTCCATCCTTCTCCAT	
D6Mit75-f	ctgtgcatgatccaagtgt	
D6Mit75-r	gcagcaggtgggaacaaata	
D6Mit76-f	TCCTCCCAGGCATCTTTTAA	
D6Mit76-r	TTGTTCTCTGACCACTTCATGC	
M13 for	tgtaaacgacggccagt	
M13 rev	caggaaacagctatgacc	
Npy-c-f	tgctaggttaacaaacgaatgggctgtgt	
Npy-c-r	gtgatgagattgatgtagtgtcgagagcg	
R236-f	TCAATCCCTGAAACCCATGT	60.20
R236-r	GTATCCCATTAAATTCTCACATCG	58.80
R300-f	CTGGCCTCCAGACATGTGTA	

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
R300-r	TTTCATGGATGTAATTGTCACTG	
T3	see t3bs	
T7	see t7bs	
aa174917-f	TAACCTCCAACCCTCACACAC	59.90
aa174917-r	TCAGGCCTGTAATTAGTTTTGGA	60.00
ajm1502-f	ACATGGTTGCCTGGTTCTTC	60.00
ajm1502-r	GATGATGCTAAGGTCCTGTTCC	60.00
ajm1505-f	CCAGCCTTCTCTAGGCATCTT	60.00
ajm1505-r	ATATATCCATGATGCTTTCCAGGT	60.00
ajm1506-f	GCATCTCCTGGACTACTCAGCT	60.00
ajm1506-r	CAGGACATACACTTGCTTTATTGG	60.00
ajm1515-f	ACAGACAACAGTCCAGATAATGGA	59.90
ajm1515-r	GCTACAATGAGCAAGCCCTC	60.00
ajm1516-f	GATTCCCTCCAAGCATTGTA	60.00
ajm1516-r	CAGCAAACCTCCCATTCATTACA	60.00
ajm1517-f	CTAGGCTCTGTCTCAGTCACTCTG	59.90
ajm1517-r	CTGCAGTTTGTCTCTGCTTCC	60.00
ajm1518-f	AAATATTAATCTGTCCAAGTGGTGC	59.70
ajm1518-r	GCTCTTGCCCTCTTTTGAGAT	60.00
ajm1521-f	ATAGTACAAGCGGGTCATGTTCTAG	60.00
ajm1521-r	ACAAAGATTTCAACCCTCAAGTGA	60.00
ajm1701-f	GGCAGATCCCTCCCCTT	60.00
ajm1701-r	AGGATCTGGAGAGTCTGAGGC	60.00
ajm1705-f	CCATTTCTGGGTCTGAAGTAG	60.00
ajm1705-r	ATAACCCGTAAACCCAGAAGGT	60.00
ajm1708-f	ATTTGGTTGCTACTGGGAGAAA	60.00
ajm1708-r	TTCTCACTTCCCATTTCAGTAA	60.00
ajm1711-f	GCCCTTCTGTAGAACAGACTTCA	59.90
ajm1711-r	TCAGAGGGCTAAACGTGCT	60.00
ajm1802-f	AACAGAATACAGGGACAGATTCAAG	59.90
ajm1802-r	ACCTATTTCAAACCTTGTTGGGTGTT	60.10
ajm1803-f	ATTTTAGCTGAAGGAGTGTTCCAC	60.10
ajm1803-r	TTGAATCTTGGTGGGGACA	59.90
ajm1804f-1	ATCGCTGTCCGCTCAAGA	60.70
ajm1804r-1	GTGGAGATGGGAAAAAATAAATTCT	60.00
ajm1804r-1b	ACGCTGAGTCCGGAAACTT	59.90
ajm1805-f	CTTTGCGGTGTTGCTTGTT	59.90
ajm1805-r	TCTTGTCTACTTGGACCCCT	60.00
ajm1806-f	CGAAGCCACCATGTTTCC	60.00
ajm1806-r	AGCAAACGGGAAGGAATTG	60.10
ajm2028-f	CACCCAGATTGGTTATGCACT	59.90
ajm2028-r	AGGCATGATAATGAGGTTCTGTAAG	59.90
ajm2028f-1	TGTCTTACACCCAGATTGGTTATG	60.20
ajm2028r-1	AAGACACTCCTTGCCCAGG	60.20
ajm203-f	ggggacactttcccaactttgag	65.20
ajm203-r	tggtagcggggttagtagtaa	64.90

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm203b-f	CCAGCTCTTCGCCAGTGAGAG	64.90
ajm203b-r	GAGTTAGAAAGAAAGTCAGAGAAACCAG	60.60
ajm207-f	ccgggatgtcaatcactttttctc	65.00
ajm207-r	ggcacagatcctcctgcatctt	65.00
ajm207b-f	CACAGAATGTACCGGCAGAATGAG	65.10
ajm207b-r	TCTCCAAAGTCCAGCGTCGAC	65.00
ajm210-f	tcacgtgagtcgtttgataaggga	65.00
ajm210-r	gtggtatcgtgtaccgagcgaac	65.00
ajm210b-f	CTCGAAAACAACAATGCGTGAAAG	65.00
ajm210b-r	TCAACATGACAACACTACTGACAACCAAGA	65.00
ajm214-f	ttgaccgtgcctgtgtcaaact	65.10
ajm214-r	ggcccgatgtagcgagatttc	65.20
ajm221-f	agggtactggagcagtgccg	64.90
ajm221-r	ggagacgtcgaaccctactcc	65.50
ajm224-f	acggagtagacgtctaggtatcgacg	64.90
ajm224-r	acccgtcaagaacggaaacgt	65.10
ajm224b-f	TAGGTATCGACGAGGTCCGGAA	65.00
ajm224b-r	caatgagagacacgtccagaagta	65.00
ajm2312f-1	ACTCTCTCCTTGTCTGCTACGG	60.10
ajm2312r-1	CAGTTTGAGTTCTTTCTGGGAA	59.90
ajm2324-f	CGATAAAGAAAGAGGGCATCC	60.00
ajm2324-r	CATAAGCCCCCAAAGCTA	60.00
ajm2501-f	CTCTTCCTCATGCTCTTTGCTT	60.20
ajm2501-r	AGAGGTTTGGCATCGTGC	59.80
ajm2502-f	CCTTGGCATGGACCTGTC	60.00
ajm2502-r	CAGGCAGGGTGCTAACTCA	60.00
ajm2504-f	GAATTTACACGGTGTTTATTTTCCTAA	60.00
ajm2504-r	TAGGGACTTTCAAGTGTCTGCA	59.90
ajm2505-f	GCAAACACATGGGGGTATTC	60.10
ajm2505-r	GTGATCTGTGGGGACAGAGAA	60.10
ajm2506-f	TCTTGTGTTGCAAAGCCTTG	60.00
ajm2506-r	GGGCACTTTGGATCACAGA	59.60
ajm2508-f	GAGAGAGAGAGATGGGAGGACA	60.00
ajm2508-r	CCCAAGATATATCCACCTTGA	60.00
ajm2510-f	AAGACCACAGGGGTGAAGTG	60.00
ajm2510-r	AGAATTCCCAGGTCCATCG	59.90
ajm2511-f	AGGAAGGCACCGTGACTG	59.80
ajm2511-r	ACATACAGAGAGTGGACAGTCTTCC	60.10
ajm2513-f	CATACTAAGCAAAATCAGCCACC	60.00
ajm2513-r	CAACCTAGTCATGCTTTCCCA	60.10
ajm2514-f	GGATGTGTTTGTAACTGACTCGA	60.00
ajm2514-r	AGGCCCAAAAAGGTGGTATT	59.70
ajm2515-f	GAGATGGGTTACACGCAGTACA	60.10
ajm2515-r	GTCAAGATGCAAGCACAGACA	60.00
ajm2516-f	ATACACAAAGTCCTCACACTAAGTGC	60.10
ajm2516-r	GAAAAGTGCCTGTCTGAGCC	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm2519-f	CAGAATGGCTGCTCTGAATTC	60.00
ajm2519-r	TTCCTATCATGTAGGAAACATGGA	59.70
ajm2523-f	CTCCAACCTTCCCTAGACCCTAA	60.00
ajm2523-r	CTTTCCAACCTCTAGATCAGAAGCTG	60.10
ajm2524-f	TCCACACCAATAAATAAACCTGG	60.00
ajm2524-r	AGAACACAGGAGTTGAATGAGGA	60.20
ajm2619-f	agtcgagaattaccaggtgtgtaa	60.00
ajm2619-r	cttaattccaagcgaagatcattt	60.00
ajm2620-f	aactgtaaaccacaccttcctt	60.10
ajm2620-r	ggcctgattgttacacagga	60.00
ajm2621-f	cctaactactctgtacatcctccaca	60.00
ajm2621-r	tgacctggctccatgacat	60.10
ajm2622-f	cctgctcactccactcaaca	60.00
ajm2622-r	gaggcaggggatctgctag	59.90
ajm2623-f	ggctcattgcagttggct	59.90
ajm2623-r	ttggccctatatatcccaagg	60.00
ajm2624-f	tgacagctgatagctgggc	60.10
ajm2624-r	attaaaacttgccatgtccaattt	60.00
ajm2625-f	atattgcagtcctgtaacctagc	60.00
ajm2625-r	tgagacccttgagtagagagcc	60.00
ajm2626-f	cttctaagaggctggattccaa	59.90
ajm2626-r	cctgggtagagtgagcacag	59.90
ajm2627-f	gcaaacacatgggggtattc	60.10
ajm2627-r	caccaccagtgatcttggtg	60.00
ajm2628-f	tctcttgccagccttttaa	60.00
ajm2628-r	gtgtctcaaaagtgttcacctt	60.00
ajm2629-f	ttggcaaggacttcgagtg	60.00
ajm2629-r	agagcaagggtccaacattt	60.00
ajm2630-f	gggtgcatatagtaagtttcgagagtc	60.00
ajm2630-r	caagaggggaaagattaaagtga	60.00
ajm2701-f	GGATATACTATCTGATGTTGCCAGG	60.10
ajm2701-r	GATTTAGCCAGGGATAAATCCAC	60.10
ajm2702-f	GTGGCTGGCTGCTTTGTT	60.00
ajm2702-r	TCACTGTGAGGCCTTTTGC	60.00
ajm2703-f	GAGGCATCACATGGTAACAGAA	60.00
ajm2703-r	GAAAAATCCACAACCTGAGCAATC	60.00
ajm2705-f	AGAGCCTGCTCCCTAAGACC	60.00
ajm2705-r	AAAGGGCTATAATATATCGAGGGTG	60.00
ajm2706-f	AATTCCCCTTACAACACATTGTCT	60.00
ajm2706-r	CCTGCTTGTGCTCTCTCCTT	59.70
ajm2707-f	CTGGTTTAGTCCTCAAGCGC	60.00
ajm2707-r	TTCAGATTTTCCTTGCCCC	60.00
ajm2708-f	AAACTGAAATCAAGCCTTCCTTC	60.10
ajm2708-r	CATACGTTTCCTCATTATTCAGCAG	60.00
ajm3027-f	CATTTTGGAGGCTGAATAGTCAC	60.00
ajm3027-r	ATGATTTTAAAGGACCTGGGTGT	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm3031-f	TTGACCTGGATTCTGACAGTTTT	60.00
ajm3031-r	CTCCTGCCCCACTGAGTTCTC	60.00
ajm3036-f	CAGCGAGTCTTTTCAGTTTCACT	60.10
ajm3036-r	GTAACGAGCTTTTGCAAGATAATTAAC	60.00
ajm305-f	ctcacgtaaactcatggaccgacc	64.80
ajm305-r	ataatgaggacgacaacaacagtcagt	65.00
ajm305b-f	aaggggtggcagaggaagaac	65.00
ajm305b-r	cgtggtgggtggagaatcatc	64.90
ajm306-f	tcaattgagggtctccttcaacat	65.10
ajm306-r	gtacggccagaaagagcagtgat	65.00
ajm306b-r	tcttcgcagagattaagtgcgc	65.00
ajm307-f	ttcacgtgactaaacttcttactatga	59.00
ajm307-r	aatacgaacacgactataactttctcg	60.30
ajm3105-f	CTTAAATGTAGAAATGAATGATAGCTTCA	60.60
ajm3105-r	GTATTGGTCCCATGAATCTTCC	59.50
ajm3109-f	ATTTTCAAGAGGTGTTCTGTTGC	59.70
ajm3109-r	CTGGGATTATGGGCACGA	60.40
ajm3119-f	AGAGGATGTCAGATCCCATGAT	59.80
ajm3119-r	CTCTTAATCACTGACTGTTCTTCCTG	59.90
ajm315-f	ccactgatttatgacgtcaggacag	65.00
ajm315-r	gccatcttcttatacgaccacac	64.90
ajm315b-f	tgggatggcttaagccactgat	65.10
ajm315b-r	aacactctacctaataattctctggcgc	65.00
ajm3201-f	GTGGTGCGTTCCCGTAGT	59.60
ajm3201-r	CAAGGTGGAGCATGGAGC	60.40
ajm3202-f	ATCATTCAACCAAGTTCTGGCTT	60.00
ajm3202-r	AAAATCAATGTTCTTTTGCTTGTTT	59.90
ajm3206-f	CGGATACACTGAGATGAACCAA	60.00
ajm3206-r	ACCACTTCAAAGGTAGAAGGAG	60.00
ajm3301-f	cctgaagggtcaaaggggaa	60.00
ajm3301-r	ttccgtgagacctgtttcc	60.10
ajm3302-f	ggtgacagatttcctgaaggtc	60.00
ajm3302-r	attctaggactctgatccatcctt	59.90
ajm3303-f	tccctctggctcctctgtc	60.50
ajm3303-r	ctttagtagagcccagtagctaagg	60.40
ajm3304-f	ggggcatgtaaaaagcagag	59.70
ajm3304-r	atctgtcaagccttagcaattg	59.80
ajm3305-f	cagggtcagaccacattgtct	60.00
ajm3305-r	gtcagactgtaatgagttccaggtt	60.00
ajm3308-f	attctgggacggaaagtgc	60.10
ajm3308-r	atggtattgaaatgggaaggc	60.00
ajm3311-f	cacttttgcccaataattatagt	60.00
ajm3311-r	tgcttcctgaagttgcatg	60.00
ajm3312-f	gcttctaacctcaagccc	60.00
ajm3312-r	ggaaaccagtgagtgatgga	60.00
ajm3313-f	ctcctccttgacttcagagaa	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm3313-r	ctgttttcctagcaagtctcca	59.90
ajm3314-f	cagctgatctgagatgaaaacg	60.00
ajm3314-r	gccataaaacccgaagttga	59.90
ajm3315-f	cactcagagccatgtgctgt	60.10
ajm3315-r	ccccttttaaaaagcttggtg	60.00
ajm3316-f	gggtagagtcttgatgggga	60.30
ajm3316-r	gaagattggccatactagcc	59.90
ajm3317-f	tgtttagagacgaaatgaggtctg	59.80
ajm3317-r	tgaatagtctggcatctcct	59.90
ajm3320-f	cactccagtggctcagacaa	60.00
ajm3320-r	aaccctctcaagaggatggaa	60.10
ajm3322-f	gactacaagcaggcagacatctt	60.00
ajm3322-r	cttctgtccaaagcccaag	59.80
ajm3327-f	tggggttctggtacctcttg	60.00
ajm3327-r	ctgagatggctgcttgcac	60.10
ajm3329-f	aaaaacaattctttggcaaaca	59.90
ajm3329-r	cctgtgtccagggtgagg	60.10
ajm3331-f	aggctcccagaacaggct	59.90
ajm3331-r	ggactaaagactgtaaagtcacaa	59.90
ajm3335-f	cgctctcatatataaagtgggtg	60.00
ajm3335-r	caatgaccattgtttctgct	59.10
ajm3336-f	tctctgtctagatgtctggtactcag	59.60
ajm3336-r	tggcctctattgacatgc	59.90
ajm3701-f	tatgaaggcttctgaactagagactc	60.00
ajm3701-r	atatacagtcattggagggg	60.00
ajm3702-f	gactagatgccaccataaacactg	60.00
ajm3702-r	ctgtgtaataaccagccaagtgttc	60.00
ajm3703-f	cgtcaccacagaatgtaccg	60.00
ajm3703-r	tgtctgtgaagaagtcgtctaagt	60.00
ajm3704-f	aaaggacaccagcattacgg	60.00
ajm3704-r	gcagacacactgacagagaacc	60.00
ajm3705-f	aatgtgaagggtgtttcatagc	59.70
ajm3705-r	ctgatgactcgcttgcat	60.00
ajm3706-f	agagcatggattccaagg	60.00
ajm3706-r	ttctggctttaacatacaaacctc	60.00
ajm3707-f	gctctgccctctttgagatt	60.00
ajm3707-r	agaatcaccgggaggacag	60.10
ajm3708-f	gctctgccctctttgagatt	60.00
ajm3708-r	agaatcaccgggaggacag	60.10
ajm3709-f	gtgactgtccagatcgctga	60.00
ajm3709-r	gaggaccctgtccactacca	60.00
ajm3710-f	agacactcctgacatgcgc	60.00
ajm3710-r	tcagccttatcaagcatgagaa	60.00
ajm3711-f	tcatgtgcaagttacaggaacc	60.00
ajm3711-r	agttttaccttctactttccgtgaa	60.00
ajm3712-f	cacatggcatgaatgaagactt	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm3712-r	atccaaggtattgtgtggattg	60.00
ajm3713-f	ctgtgtctgtgttttagataaggtga	60.00
ajm3713-r	aagtcttcattcatgccatgtg	60.00
ajm3714-f	aagtcacagggcagcagg	60.00
ajm3714-r	tgtccaaactgtggacaaaca	60.00
ajm3715-f	gtgatatgctgaatgaggggtga	60.00
ajm3715-r	cgttatcattagatgcaggacc	59.90
ajm3716-f	ctcagtcocatggcatcc	60.00
ajm3716-r	gagatcccaggcctggtt	60.00
ajm3717-f	tgtgtctctagacaccagaagagg	60.00
ajm3717-r	cgagatgacctgggttcg	60.20
ajm3718-f	gagaggaatgttttagcaaccg	60.10
ajm3718-r	gttcagtgggcagggtatgt	59.90
ajm3719-f	cacctgaagttgggcttgtt	60.10
ajm3719-r	cccgtgtgtcacctctgta	59.60
ajm3721-f	ccctgcacagagcctgtc	60.60
ajm3721-r	cacagtgaagactgacagcttaa	60.00
ajm3722-f	tcagggtgaagctggggac	60.10
ajm3722-r	gcagagctccaagcttataaa	60.00
ajm3723-f	caggagcatgctggctct	59.90
ajm3723-r	caccagctgggtacagg	60.10
ajm3728-f	ctgaaaagacagcacagtaaactca	60.00
ajm3728-r	ttgtatgccctctgtagaagctc	59.90
ajm3729-f	caagggtaggtcagaagattgg	60.00
ajm3729-r	gactgccatgctgtgagaa	60.00
ajm3801-f	gctcatctgacaggacacttctt	59.90
ajm3801-r	gttaaggatcccatcgaga	60.00
ajm3802-f	ggattctaccactgtcgga	59.90
ajm3802-r	caggtcaggctaagtgaacc	60.00
ajm3803-f	gtctccgtgtccacagtt	60.00
ajm3803-r	aaagtgcattggcagcttg	60.00
ajm3804-f	agcaagagaactctgaatgcc	60.00
ajm3804-r	ggccactgtgatgagattgtt	60.00
ajm3805-f	atcttgaggaaaattacacttgctt	60.00
ajm3805-r	tggccatcaataacaaagca	60.10
ajm3805b-f	ATGGGAGAAGTAGAGACCACCA	60.00
ajm3805b-r	AGGTGCAGGAGCTGATGG	59.90
ajm3806-f	atggtcatcctgactgtctgg	60.00
ajm3806-r	cagctccatctgggcagt	59.90
ajm3806i1-f	tcaggatgactgtggtgacg	60.70
ajm3806i1-r	ggcagagaaggcagagagc	60.40
ajm3806i2	atatgctgcccctggaaag	60.00
ajm3806i3	gtttcgtctgccaattcaca	59.70
ajm3806i4-f	tgtgaattggcagacgaaac	59.70
ajm3806i4-r	cccacaggggtcagaacaag	60.50
ajm3807-f	tcgggggcacacatatatg	60.20

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm3807-r	accgtgtgcttgagaaacc	60.20
ajm3808-f	atccaggggacctgattatg	60.00
ajm3808-r	accccttgagtgaactgaaaca	60.10
ajm3809-f	aaggggcaggactagctctc	60.00
ajm3809-r	cctatggcagccatggtc	60.00
ajm3810-f	ttgagttaaagtcttgttctctgaca	59.90
ajm3810-r	tgagaccgcctcatatctacatt	60.00
ajm3811-f	gtttcacgggtgcaccagg	60.00
ajm3811-r	tggtcagcagtagcccagg	60.00
ajm3812-f	cctcatccaggggacct	59.80
ajm3812-r	ggctcctgatgtaggaccag	59.70
ajm3901-f	agattccagaccaggattgt	59.80
ajm3901-r	gcctctatagctggtctttgtga	59.90
ajm3902-f	tgcttgaatcgctcctgtg	60.00
ajm3902-r	caacctggtatctctgtcca	60.00
ajm3902b-f	cctggtccccctggactg	64.00
ajm3902b-r	gagttcctgctgtcaccttca	63.90
ajm3902c-f	tgggacagagataccagggtg	
ajm3902i1	ggagtgagggccagggtc	60.60
ajm3903-f	aggatggacatttgaccagatg	60.10
ajm3903-r	ggactcaggaggacatcaa	60.00
ajm3904-f	tagccctcaggagtggct	60.00
ajm3904-r	cctctgcagagctcactgc	60.00
ajm3905-f	tcaaaaagggtgtacgtgtacg	60.00
ajm3905-r	tctcaaacagaagctccaaa	60.00
ajm3906-f	acatacacaaaaggcaaacagaa	60.00
ajm3906-r	caggtagctcagagttggcc	60.00
ajm3907-f	caccacaaggcatcacagg	60.00
ajm3907-r	ttttgtgaggacctcagg	60.10
ajm3908-f	ggagatgtggtgagaccacc	60.40
ajm3908-r	ccattgtgaaccactcatgc	60.00
ajm3909-f	acactttcctaaccatggcg	60.00
ajm3909-r	aggtagctgctggtttt	60.10
ajm3910-f	ctagccactgctctctgcct	59.90
ajm3910-in-f	tactcctcctggggcat	
ajm3910-in-r	cctccagggcagcatatc	
ajm3910-r	cttccttctgagatactcactcgtc	60.00
ajm3913-f	cctgtgccttcctcagtctc	60.00
ajm3913-r	caggatcatggcgggtag	60.00
ajm3913b-f	gccctgtggtcatggctc	64.00
ajm3913b-r	agcaagtagcatcccactgcag	64.00
ajm3914-f	cagaaagcaggctatctgtg	60.00
ajm3914-r	gcagcagcaagaggaagc	60.00
ajm3915-f	cagctcagtggttaaaggagtc	60.30
ajm3915-r	tgtgtgcatcagtgcatacg	60.30
ajm3916-f	ctccaccaaataatgtctgca	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm3916-r	gggacaggccgttgactat	59.90
ajm3916b-f	CAACATCGTCAAAGTTATCTTTAGTCA	60.00
ajm3916b-r	GGAAGAAATTGTTGCCAAAGTC	60.00
ajm3917-f	atccccttcagccagattg	60.00
ajm3917-r	acaacctggtgtagacaagacaa	60.00
ajm3918-f	acaggctcagagccaacact	60.10
ajm3918-r	acatgccctgaagtgaac	60.00
ajm3919-f	gtctgtgtacatgcatgtgtg	60.00
ajm3919-r	ggtgaacactccctctgagc	59.80
ajm3920-f	tacagccatggcttaacatgt	59.50
ajm3920-r	aaggcaccagcactactgagg	60.30
ajm3921-f	tcttctccgctgaccctg	60.10
ajm3921-r	ccaaaaagcccgtacttca	60.10
ajm3921i1a-f	cggagtcgacgcctactc	59.50
ajm3921i1a-r	ctccaggctctgcagtcg	60.90
ajm3921i1a-r2	ctgctccgagaaacagatcc	60.00
ajm3921i1b-f	tcaagaatgggacatggatg	59.30
ajm3921i1b-r	ctggctagctgggcatc	59.50
ajm3921i1b-r2	cgaggacccagaagttc	59.30
ajm3921i3a	gcatctgtttctcgagcag	60.00
ajm3921i4a	cccaggactgaactcttagctt	59.10
ajm3921i5a-f	aagctaagagttcagtcctggg	59.10
ajm3921i5a-r	ccagacaagagtacattcacatatga	59.50
ajm3922-f	cattgtttgtgtgtttgagaca	60.00
ajm3922-r	gacccaaagctgtgggact	60.10
ajm3923-f	ggagctgctcctagatggtct	60.00
ajm3923-r	cataaccatgttcagatcacatg	60.00
ajm3924-f	gacaagcaagagatggcctc	60.00
ajm3924-r	tttctgcatcccagtgaaactt	60.00
ajm3927-f	ggggatgatatggtgagttataaact	59.80
ajm3927-r	gactcagcagctgtggctc	59.90
ajm3928-f	gcaggagagagctcaggct	60.00
ajm3928-r	tactccagtttccagggc	60.10
ajm3929-f	aatagagtgtatttgaccatttcacc	60.00
ajm3929-r	agtggatgccctacaccaag	60.00
ajm3930-f	tgaaaatgcttagccgtgc	60.00
ajm3930-r	agcgagttcagtgagctcaag	59.90
ajm3931-f	agggattaaaggtgtgcgc	60.10
ajm3931-r	ggtcttcagttgtgaacccaa	60.00
ajm3933-f	tgcaggattatgtcagtccttaaca	60.20
ajm3933-r	ctttggtagagccatgaacaca	60.20
ajm3934-f	gatttgaactcagaacagccg	59.90
ajm3934-r	ctttattccaagccccc	59.30
ajm3935-f	cttcaggggaagctctgtg	60.00
ajm3935-r	ccaactacaacatggtggagaa	59.90
ajm401-f	AAGGAAACCACCTGAGGGTGAATT	65.20

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm401-r	TCTCTTAATACCAATTTCCAATGCTTGTG	65.20
ajm4011-f	gcactgaaagagacagatggc	60.00
ajm4011-r	ctcttcagatggtggatgtttc	60.00
ajm4013-f	aatttcccaaatggaatctg	60.00
ajm4013-r	ttccccttgaccttcagg	60.00
ajm4018-f	aatttcccaaatggaatctg	60.00
ajm4018-r	ccaagaagctcatcttgctaaaa	60.00
ajm401b-f	TTGGAAAGCAGGTTACATGAGATT	65.10
ajm401b-r	AGGAGGTAGAATGTGGAGAAGACAGAGA	65.00
ajm4020-f	atggatcctgatgataacttagaacc	60.00
ajm4020-r	gaagagagagagggatagaggaaga	60.00
ajm4021-f	gtgaaaggaagtattcaggcaatt	59.90
ajm4021-r	gaatgatgaaaagcgtaaaggc	60.10
ajm4022-f	tgggacaggggtagagag	60.00
ajm4022-r	gctgtcataggtctctgacatcttt	60.20
ajm4023-f	tatttcgcccatagtgttctaagtc	59.90
ajm4023-r	ataacgtttaggtgcttctgtgttc	60.00
ajm4025-f	agatgtcaaatcaccttcaaata	59.90
ajm4025-r	catgtcagtaatgctgctgtca	59.90
ajm4026-f	tctctctagttcaggctctcttctaa	59.90
ajm4026-r	aatgttagtggccttctctcc	60.00
ajm4027-f	aagaagggttcttcatcacatga	60.00
ajm4027-r	cccacctttatccaattcaa	60.00
ajm4028-f	ggattttaggagctgtgtacatg	60.00
ajm4028-r	ttaacaatcccacacatctg	60.00
ajm4029-f	ccactactctgtctctctacacctg	59.90
ajm4029-r	agcatgtttgtctatagttctggg	60.00
ajm4030-f	atggctgctgaggagtact	60.00
ajm4030-r	atcttcaagtatgtgtaattgca	60.00
ajm4031-f	tgtccaccatgaacattgct	60.00
ajm4031-r	tccccgaattaaaagcag	60.00
ajm4033-f	gagaacagagtctggggcag	60.00
ajm4033-r	tctgcactcccatctgca	60.10
ajm4034-f	gtatctgggaggcagccat	60.00
ajm4034-r	atgaatgaattcctgaattggg	60.00
ajm4036-f	ggttctccaagtggcct	60.00
ajm4036-r	ctgtctaattctcatcccacttg	60.00
ajm409-f	TGAGAAGCAGAACGTGACTAACATGTC	65.00
ajm409-r	AGGCACAGAGTGAGCCCACAG	65.20
ajm409b-f	TCAGGTCAGCCTTGCTTGGTC	65.00
ajm409b-r	CCCACAGCGCATCCAGTTTAC	65.00
ajm4119-f	taccggaacatctcgtgtaagtt	59.90
ajm4119-r	ctcacacatgtccagaatgtga	59.60
ajm4121-f	acccatccatcctctcagc	60.00
ajm4121-in-f	ctgggacctgctgggtac	
ajm4121-in-r	atccccagatggctggac	

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm4121-r	caaagtgcacggcaacag	60.00
ajm4122-f	gccaactactgtgtactgcc	60.00
ajm4122-r	gtccaagtgtaccgcgg	60.10
ajm4123-f	tcacatagggaaccagaagg	59.90
ajm4123-r	tctccctaacgtctacataggtagaa	60.00
ajm4124-f	CTCCCCTCGCCTGGTAAC	60.60
ajm4124-r	TTCTCACCTCCCCATGTCTC	60.00
ajm4125-f	tcacatagggaaccagaagg	59.90
ajm4125-r	tctccctaacgtctacataggtagaa	60.00
ajm4126-f	ctgaagccacctcagtcctc	60.00
ajm4126-r	gaggattatttcttgaccattt	60.00
ajm4127-f	ccagtttctgcatctaaaacc	60.00
ajm4127-r	aaacccccatccttagg	60.00
ajm4128-f	gcatgatcctgggaggtg	60.00
ajm4128-r	tgtggcaagagtctcagtg	60.00
ajm4129-f	ctctaggggtttctgtggct	59.80
ajm4129-r	cgctgtaatgcacatctg	60.00
ajm4131-f	ttccacctgtctctcacatc	60.10
ajm4131-r	ctggttgtgaggaatacctaatt	60.10
ajm4132-f	ttgtttaggcagggctcatt	60.00
ajm4132-r	cttctgtgtcttaagggtgg	60.00
ajm4133-f	atgagcttgaacgctctgt	60.00
ajm4133-r	agcagcgctctgaggtgt	59.90
ajm4134-f	catgagaccaaccagtggc	60.10
ajm4134-r	tgatggagctctggcctc	60.00
ajm4135-f	aagagctacaaagataagtaagcgtg	59.90
ajm4135-r	ccagagagagtccagacatgc	60.00
ajm4136-f	actaccctcttggttaacaactcc	60.20
ajm4136-r	aggcgtgagaccaaacaac	60.20
ajm419-f	GTGTGGTCGTATAAAGGAAAGATGGC	64.90
ajm419-r	GTCCATGTTTATTCTCTCTGAAGGAGCTT	65.20
ajm419b-f	GCCGACAGATGCTAGTTGAGACAA	65.00
ajm419b-r	GGGAGCCAGTTCTCCCAGC	64.60
ajm4221-f	accaagaagctcatctgtataatt	60.10
ajm4221-r	ccataaagcgttgccctaattc	60.00
ajm4227-f	attgtctcttcagatggtgga	60.00
ajm4227-r	aatttcccaaataatgaatctg	60.00
ajm4301-f	aacaacaagcgaggaagtatgtatc	60.00
ajm4301-r	agtattagccatctccgaccaa	60.00
ajm4303-f	tgccaacaggctatgaagc	60.00
ajm4303-r	aatggaaggggagcttgg	60.00
ajm4306-f	cctaaagccctgctgagagtaa	60.00
ajm4306-r	aagtgatgcctggtgaatagagtc	60.00
ajm4306b-f	gaagcccctaaagccctgct	64.10
ajm4306b-r	ggaaagtctaatactgcacaacctcaa	63.90
ajm4306c-f	GTTCAAGTCCTGGGACCTTAGTG	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm4306c-r	TGTAATAGAGTCAAGCTCAGTCTTTCA	60.00
ajm4307-f	ctgctgatgaggttgggtt	60.10
ajm4307-r	tgacattttgcctggatcaag	60.00
ajm4308-f	gtgggttttggtgattggac	60.10
ajm4308-r	tctcctatagaaagctatcctgca	59.90
ajm4308b-f	gactcaggctgccaggcat	63.90
ajm4308b-r	aagctatcctgcaaagatcaaaataca	64.00
ajm4309-f	agattcacctagaagtcaaaacgg	60.10
ajm4309-r	aatccctgcacttctccctt	60.10
ajm4313-f	ttacctggaagcaacaggatg	60.10
ajm4313-r	gagagtaagaactgcagaagagtgg	60.10
ajm4315-f	ccaaacaagagctgactgacc	59.90
ajm4315-r	tgggctgctgtagctactt	60.00
ajm4315b-f	ccagccaagtcattgtcattcac	63.90
ajm4315b-r	gtacccatgggctgctgtag	63.90
ajm4317-f	ccagaatatcatcatggccc	60.10
ajm4317-r	gagagagatacccaagtggccc	60.10
ajm4318-f	tagaacaatggatgacgtcaagtt	59.90
ajm4318-r	attcagctgactagactcagaagattc	60.00
ajm4319-f	aacacttagaagcagatgaagagga	60.00
ajm4319-r	gctcttggctccctaagtc	59.90
ajm4320-f	gatgaacagggtgtggccac	60.00
ajm4320-r	gacccccctcttcagcat	60.00
ajm4321-f	gagccagatcccagaggag	59.90
ajm4321-r	agccatcaacctcaccca	60.00
ajm5005L2	ACTGTGAACTGGCTTTCTACAGC	60.00
ajm5005R1	GTGCCACTATATATACATATGCCCA	59.10
ajm5012-f	catgacgtatttttaatgttacagtgg	60.00
ajm5012-r	aggacaataggacaggactcca	60.00
ajm5015-f	atattgggacccggttaagg	59.90
ajm5015-r	cctgacaagcaaaggcgt	60.00
ajm5018-f	cagatgagctctgcaacggaa	60.00
ajm5018-r	atcttcacacgaaaaaccg	60.00
ajm5024-f	tctatcttcagaaatatcctccacg	60.00
ajm5024-r	agtactccgccacatatgcc	60.00
ajm5029-f	tagttggcttagaaagacgtattcatt	60.00
ajm5029-r	aggcttctagggaaatcca	60.00
ajm5030-f	ttagaaggatcaaactgaggatcc	60.00
ajm5030-r	gccggacacagctactcttc	60.00
ajm518-f	AACACAAGGCACATTAACATCAGGAA	64.80
ajm518-r	AGCAAGTGACTTCACAATAGGATTCTCC	65.00
ajm5201-f	agccaacctccatctccc	60.00
ajm5201-r	cctgaggcttaataacaaaggaa	60.00
ajm5202-f	gtggtcgagatgtgaccagtt	60.00
ajm5202-r	gttcaattgtagcaaaggagg	60.00
ajm5203-f	actacacaattgccatgagcc	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5203-r	ctttcctaagttaagtctgtcactc	60.00
ajm5206-f	acatgatcaacactactaagtatcccc	60.00
ajm5206-r	ctctttgaagatactgccagttagc	60.00
ajm5207-f	aaactgctcctgtgttcacgg	60.20
ajm5207-in-f	gctctatgggtgaatgctcc	
ajm5207-in-r	gggaacaaaggctccacat	
ajm5207-r	ggtaccaacccaccaca	60.10
ajm5209-f	ttcttgcaaaactctgactcca	60.00
ajm5209-r	gagtatcaacaccgaggaggtc	60.00
ajm5212-f	aacttccatctgtgcaagaa	60.00
ajm5212-r	ataaagtgactgctaagtatggactg	60.00
ajm5213-f	gatgccctataaccaccatgtg	60.10
ajm5213-r	tatgctttggctacacatccc	60.00
ajm5218-f	agaatccaaatacaactgagcca	60.00
ajm5218-r	ggaaactgggttgctataaattt	60.00
ajm5220-f	CTGCAGAGTTCCCAGTCCTC	60.00
ajm5220-r	TAGGAAGGGCAGTGCAGG	59.90
ajm5221-f	gagtcctggaagtgcagagg	60.00
ajm5221-r	ccctccctctgacccatt	59.80
ajm5222-f	tcctgggccagtgacctac	60.00
ajm5222-r	agacctctccatcttgatcaa	60.10
ajm5222i1	gctagctgtacctttgga	59.60
ajm5222i2	taccatgagacctgtctctttgc	60.70
ajm5222i3	gatttataacccatggcccc	60.20
ajm5222i4-f	ggggccatgggttataaatc	60.20
ajm5222i4-r	tagtggcctaggcggtcact	59.90
ajm5223-f	ctttccctgctctgaggactt	60.00
ajm5223-r	catggcactgatgaaaagtctatc	60.00
ajm5224-f	gaacagctgaggtaccgactg	59.90
ajm5224-r	tcagtagctaaggaatctttgagtga	60.00
ajm5225-f	cgcatctccaagagtcaaca	60.00
ajm5225-r	ctgcacatcttctcttctgg	60.00
ajm5227-f	tgggtctctgcctctatcctagc	60.00
ajm5227-r	gatgcccacagagtgatgg	60.10
ajm523-f	CCAGTGCACTCAGCAAATATTCC	64.30
ajm523-r	GTGACACTGGGAAAGGCCCA	65.60
ajm524-f	AAGAACACTTTGGGCGGGTG	64.50
ajm524-r	CTGTGTCTGGACCGTTCTACCAGA	64.90
ajm5401-f	cttggatccagcctactga	60.30
ajm5401-r	ccttccattccttcacctga	60.00
ajm5402-f	cttgctgtttgtttgggt	60.00
ajm5402-r	ctgtccataccctcgtgctt	60.10
ajm5403-f	ctgtccacagacgcatct	59.80
ajm5403-r	acaatgcctgctggcttc	59.90
ajm5404-f	actgcactacgatgtaggaagc	59.90
ajm5404-f2	GAAGCAGTCATACTTAAACATCGTC	59.70

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5404-f3	ACAGAAGAGCGTATGAAATGAATG	59.70
ajm5404-r	ccaaacatcgatggaccaa	60.30
ajm5404-r2	CAAATCTGTTCAGAGGGAGCA	60.40
ajm5406-f	tatggagaggaagattgaatcaca	60.00
ajm5406-r	gctgcagcagcccatatt	59.90
ajm5406b-r	CCACAGCTGTCTTCTCACAGTC	60.10
ajm5408-f	cttggctctcatcttctgtatgg	60.00
ajm5408-r	accccagacagcactgtga	60.30
ajm5411-f	aatcacaatctcccgcca	60.00
ajm5411-r	gatgtctcccttagggcca	60.00
ajm5413-f	actgctgggagtcttaaatgga	60.10
ajm5413-r	tgactgctacgttcggagg	60.00
ajm5414-f	tttctctcttcggggtga	59.90
ajm5414-r	aaggagaactacaaaaccctgatg	59.90
ajm5415-f	tccgcttgcatacagtctg	60.00
ajm5415-r	ggccgttcaccagtatgagt	60.00
ajm5416-f	cacggttcacagagaagtagctt	60.00
ajm5416-r	catatacagctgggacaacatctc	59.90
ajm5416b-f	CCCAGCCTCAAACACCAC	60.10
ajm5416b-r	AGTCATATACAGCTGGGACAACATC	60.70
ajm5417-f	CTTTGCTATCACTGTGAAGTGCTT	60.00
ajm5417-r	TCCCAGCAGTGTTGGGTT	60.10
ajm5418-f	tgaatgacctactttcagacagtcc	60.00
ajm5418-r	gatagagaacagttcaaaacttaccg	60.00
ajm5419-f	aggccatacctcattctcg	59.10
ajm5419-r	agctactaccctggggctcg	59.60
ajm5421-f	ctcattggaagaggactctgaga	60.00
ajm5421-r	ctgcacaaagggcctctaag	60.00
ajm5422-f	ggccaggcagtgTTTTg	60.20
ajm5422-r	tgtgcttttcttgaactatggtatc	60.00
ajm5424-f	actgagatggatgctcacc	60.10
ajm5424-r	agaaggagtggtgatgtg	60.00
ajm5425-f	gccactctacttcttggagaca	59.90
ajm5425-r	ctgctaggcctgggacttc	60.00
ajm5427-f	cagctggtacaacaagacgaag	60.00
ajm5427-r	atagctagaaggagatctctgca	60.00
ajm5429-f	gagcaggagacatctccacag	60.00
ajm5429-r	aggctctggagcaccagtt	60.00
ajm5429b-r	CTCCTGATGCAAATCAAGCA	59.90
ajm5431-f	tgagacaagttggaacattcatt	59.90
ajm5431-r	agtccatttgcatctcacag	60.00
ajm5432-f	agcaatggaaaccctaagacaa	60.00
ajm5432-r	acctgggataaacgctcactt	60.00
ajm5433-f	ctagtatgcataagcacacacag	59.90
ajm5433-r	ggttttgagatcttaccctctgaa	60.00
ajm5434-f	tggattctgccccctaaag	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5434-r	tctggatgtggagtcattgga	60.00
ajm5436-f	acagtcagactgggaccc	59.90
ajm5436-r	acacgagcttccggacag	60.00
ajm5506-f	aggtggaagcagtgctcg	60.10
ajm5506-r	atgagcttggaaacgctctgt	60.00
ajm5507-f	gaaggaaaagaaggaaaaggtga	60.10
ajm5507-r	tgccgaagaccctgagag	60.10
ajm5507b-f	CTTCCTGCACTGAGGTTGCT	60.60
ajm5507b-r	GCGTCTCCAAGAGTCAACG	59.60
ajm5513-3	AAAGATAGCAGTGTATTTTGGGC	59.90
ajm5513-5	GCACCTGGATTCCAGAGCT	60.40
ajm5513-f	GGAGTGTGACAGCCGCTATC	60.80
ajm5513-r	TGCGCACCAGGGATTCTA	61.40
ajm5513b	CGCTATCTGGCCATCAATAAC	
ajm5513c	TATGCAGAGCAAAGGCTGAA	
ajm5513d	AATGGAAAAAAATTTCAGACTCCTT	
ajm5513e	TAAGGAGTCTGAATTTTTTTTCCATT	
ajm5513f	GAGCCTGACACTGAGTCCTGA	
ajm5513f1	TCCACATCAGACCATGGCT	60.10
ajm5513f2	ACGCTGTCACCCAGACCT	59.20
ajm5513f3	GGCCTCATGCCATCATT	60.00
ajm5513f4	CATCAAGGAGTGTGACAGCC	59.30
ajm5513f5	CAATGGCGGGCTCTACTTC	60.70
ajm5513f6	GCCATTAGGGAAAAATACAACCT	59.70
ajm5513f7	TGAGCATTTCTTCCAGCTCA	59.70
ajm5513f8	AGTGTGAGGCTCCAAGGCT	60.00
ajm5513g	TGAACTGGGAAACCACCAG	
ajm5513h	CTGGTGGTTTCCCAGTTCAC	
ajm5513r1	CATGGTTACAAAGTCATTTTCAGTTC	59.00
ajm5513r2	GCATTGAATTCCTGGGTTTG	60.30
ajm5513r3	TCTCTGCTGCTTCACGAGG	60.40
ajm5513r4	CCCTCAAGATCCCCACTTG	60.40
ajm5513r5	CCCCATCAGTTCCTGCAC	60.00
ajm5513r6	TCGTCTTTGCGGGTGAAC	60.80
ajm5513r7	ACCACACCGGCTGATTC	59.50
ajm5513r8	AATATCTTTTGCCCCAGGATG	60.20
ajm5515-f	ccacaccaacacccatcagag	60.00
ajm5515-r	agtactgtggtgagctggcc	60.30
ajm5518-f	aggcaagctgtgtcagaggt	60.10
ajm5518-r	ctacagtacctcaggatcatggc	60.00
ajm5602-f	ttctcctgaggagagtgagtc	60.00
ajm5602-r	ttcctctttaccttagcccca	60.10
ajm5602b-f	GTGCAGAATCAGCAGGATGA	59.90
ajm5602b-r	TTGACACTCAGCAACTGTACCTG	60.40
ajm5610-f	gggcatacaaagaaggtgga	59.90
ajm5610-r	ctggggttatggctgtgc	60.10

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5611-f	cagcctgggattccatctt	60.00
ajm5611-r	cgctccacaccgtagct	60.00
ajm5614-f	ctgctgtcattgctgcaga	59.90
ajm5614-r	cttctcacaggaccaggagc	60.00
ajm5614b-f	CTGCCCCTGTTCCATCTG	60.20
ajm5614b-r	CCTCCCATGGAGATGCAG	60.20
ajm5618-f	aagtaggagacaaaccttactgaaa	60.00
ajm5618-r	agcttctcacaggtaggcg	60.10
ajm5618b-f	TTACTGGAAGACCCAAATAATGAC	59.70
ajm5618b-r	CACAAGTTTTTGAGGTGTTCCA	60.00
ajm5704-f	ccacccttatccccac	60.00
ajm5704-r	ttgtaatatagctgtggtcaaagaa	60.10
ajm5704b-f	AGAGAGGAGAGAGGAATTATGAAAATC	60.00
ajm5704b-r	TCCGTGAGACCGGTCATT	60.00
ajm5705-f	tggcaagcttgacttggtg	59.90
ajm5705-r	caatccaagcactcgct	59.90
ajm5708-f	atagtaacagtcataaaccagaatggg	60.00
ajm5708-r	tttgtagccagcttgagt	59.90
ajm5709-f	cctcatcattcatcaccgc	60.00
ajm5709-r	tcttctcacactcggagcatt	60.00
ajm5710-f	ggtcacattgaggcagggtg	60.10
ajm5710-r	ctccctaaggcccagctagt	59.90
ajm5711-f	gctacagacagcagttcctgg	60.10
ajm5711-r	gcatagtactgcctttaacttaggg	60.00
ajm5712-f	ttgaatgccagtgctagc	60.00
ajm5712-r	tgccataaaggcttctctctc	60.00
ajm5715-f	tgtcagctagtctaactgaaacgc	60.10
ajm5715-r	tgagcacatgtgctggcta	60.20
ajm5718-f	tagtagcatctgggaattgcatt	60.00
ajm5718-r	atcttggtcagcagactataacctt	60.00
ajm5720-f	atttcagaatcactcaggatctcc	60.00
ajm5720-r	gcatgtgtacacaccagtactgaa	60.00
ajm5722-f	gccgactggcatctaagatc	59.80
ajm5722-r	cctcagcttgttcaagtgg	59.90
ajm5723-f	ggtagtgacactcaggcc	60.10
ajm5723-r	aactttcaagccagctggaa	60.00
ajm5724-f	tcaagacatagccagaagactgtc	59.90
ajm5724-r	ggcatggtagatggctgg	60.00
ajm5728-f	agtttctcggacctgtatcca	60.00
ajm5728-r	actgcagcaatggactctacttc	60.00
ajm5729-f	gttcccatcatttaagacccaa	60.10
ajm5729-r	ggccagatactcagatacagaaaa	60.00
ajm5731-f	ggcccttggtagaagggtca	60.10
ajm5731-r	agcagctccattatccgct	60.00
ajm5732-f	ctgcattcaggtgtcgct	60.00
ajm5732-r	aactggatgcagcttccg	59.90

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5733-f	ctgcaattgaaggagacttcg	60.00
ajm5733-r	agcttgaccaagcaccagat	59.90
ajm5734-f	tgacccttcaccctccttc	60.00
ajm5734-r	cagagaatgtcaagctcaggg	60.00
ajm5805-f	cctctgcaggtagacaggaa	60.00
ajm5805-r	tctacagagacgtgatgcgg	60.00
ajm5806-f	tccttggaacagggtgt	60.10
ajm5806-r	agaagaagcatgatacctagggg	60.00
ajm5807-f	tgcgctgatggagatga	60.00
ajm5807-r	tttcgcatctcctcctgc	60.00
ajm5808-f	cagtaggggtggtgggtg	59.80
ajm5808-in-f	gcacatgaggaggctgt	
ajm5808-in-r	aaggccctggagcacaat	
ajm5808-r	catgagtgggtcacaatggc	60.00
ajm5809-f	ggctgatgatggcaaacc	60.00
ajm5809-r	gcgatattcgaccactggat	59.90
ajm5810-f	agatggccaagtgtaatggg	59.80
ajm5810-in-f	ggtagggcccccaagag	
ajm5810-in-r	cccactgagcctgaatcct	
ajm5810-r	ttccactgtcatggacatagc	60.00
ajm5811-f	ttgagcctggactcgaatg	59.90
ajm5811-r	aaggtagctgcacctcag	60.00
ajm5813-f	gagtggcaagagacacctctg	60.00
ajm5813-r	tgtccatcctgcactagctg	60.00
ajm5813i1-f	tgctggagcagctgagaag	60.60
ajm5813i1-r	gtctcttgccactctccagc	60.10
ajm5813i2	ttgcacatgcaatcaatgg	60.10
ajm5813i3	tgcatgaacaattatggcagt	59.10
ajm5813i4-f	actgccataattgttcatgca	59.10
ajm5813i4-r	gccttcatactgtttcaatcg	60.00
ajm5814-f	gcttcaaagaagagtgaggaa	60.00
ajm5814-r	aggcctgcaggtagagata	60.00
ajm5815-f	gtcaggcgagtgatcatcta	60.00
ajm5815-r	agctctgcatcctaagtcaaactaa	60.00
ajm5820-f	atgcacgtcgaggtagctag	60.00
ajm5820-r	ccttaaatacactgggagtttctga	59.90
ajm5821-f	gatcttaatcacgatgccatca	59.90
ajm5821-r	ctatggactatgcaatatccaaacc	60.00
ajm5823-f	gagctttgtcctgataaccaatg	60.00
ajm5823-r	ccgaaatgggtcatgctc	60.00
ajm5925-f	tcgattccagatgtggatctc	60.00
ajm5925-r	ggaagtctgcgtagcagt	60.00
ajm5926-f	ggagctgctcctagatggtct	60.00
ajm5926-r	tgtgtgcatcatgtgatcatg	60.00
ajm5928-f	aattggcataaaattccccgc	60.00
ajm5928-r	atgagtatgggagattctcgtga	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm5929-f	tggcaaaaaagagtggcag	60.00
ajm5929-r	gtccaaagtccatgctaacctc	60.00
ajm5932-f	ccagtgccccaaagtg	60.10
ajm5932-r	tggacaaagctcttgccac	60.00
ajm5934-f	acgattgaaccaagcatgttc	60.00
ajm5934-r	tccagatcaatctgaattgctaaa	60.10
ajm5935-f	accaaagtagagcgctctaattc	60.00
ajm5935-r	ttttctgggtaaatggatttca	60.00
ajm5936-f	cctccaaaaggagttcctcc	60.00
ajm5936-r	cctttctgaagaacatgtgacatc	60.00
ajm6003-f	ttccacctgccaccaagt	60.10
ajm6003-r	aggcaagatccccctcag	60.20
ajm6004-f	ttcttatcagcacacgggtc	60.10
ajm6004-r	tactaagtcctcaaaccagaccaacc	60.00
ajm6004b-f	ACGATCATGAATCATGGACTTG	59.80
ajm6004b-r	AATTGAGTCCTCCGAAGATAAGATT	59.90
ajm6005-f	aacggcttctctctgtttctg	60.10
ajm6005-r	gcactatacacagtgaggcagc	60.00
ajm6006-ex-f	tcatgatgctgctgtcgg	
ajm6006-f	ggcattgctgtgctgtg	60.00
ajm6006-in-r	tcctgagtaccatcgaccg	
ajm6006-r	accacaggctctaactattatgtgc	60.00
ajm6110-f	acttcacctgccaccaa	60.10
ajm6110-r	acaggcaagatccccctc	60.00
ajm6124-f	ataactaagcaccaccctgagga	60.00
ajm6124-r	ataaggccagtgcatcaaataga	60.00
ajm623-f	GGAAGTCGTGGAAGGATTGA	60.00
ajm623-r	CAGCTTGGCTCTTGGCATA	60.10
ajm701-f	TTCCATGTATCCCCAGGGTTACC	65.00
ajm701-r	ATCATGGAGAACTTTGTGTGCCAA	65.00
ajm703-f	TACCGAAAGTTGTTTTGAATCCCG	65.10
ajm703-r	AAGACTGCTTTCCTGTTAGAAGATTGGA	64.70
ajm706-f	GTGTGGTCGTATAAAGGAAAGATGGC	64.90
ajm706-r	ACGTCCATGTTTATTCTCTCTGAAGGAG	65.00
ajm707-f	TCTTCATTACTCGCCAGTCCCTG	64.80
ajm707-r	CTGTGTCTGGACCGTTCTACCAGA	64.90
ajm709-f	TAAGGGAAGCTGCTGAAGCCC	65.10
ajm709-r	CAACTAGTCAGTGACGGAGGTAGACTGA	65.20
ajm711-f	CATCCATTATCATCATGGCAGCA	65.00
ajm711-r	TCACTTATGGTGGTTTGAATGTGTTTG	65.00
ajm713-f	GTCCCTCTAAGCGACATGAAATCCT	64.90
ajm713-r	AAATATGCCAGGCAGGCCAGT	65.10
ajm716-f	AGCAGATGGTTTTACCGAAAGTTGTTT	65.10
ajm716-r	AGAATGTCTTCTCCTCAAGTATCGTTGG	65.00
ajm719-f	GCACTCAAGCAAATATTCCCTGG	65.20
ajm719-r	CACCATAGCACATGGCTCGC	65.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ajm7725if1	AGTCGGCATCGTTTATGGTC	60.00
ajm7725ir1	AAGACGATCAGATACCGTCGTAG	59.70
ajm7729if1	CCTCTCTAGAGGAGCACGGA	59.70
ajm7729ir1	TGCTGTTTCTCCTCCTTGCT	60.10
ajm7732mf1	CCAGAGAAGGAGATTAGAATAGTGGA	60.50
ajm7732mr1	GCTTGTATTTTTTCTCTCTTTCCTG	60.20
ajm7803mf1	AAGAGATAGCAAAGCCAGGGA	60.30
ajm7803mr1	tccctggcttgcctatctctt	60.30
am20719-f	ACTACCACAGAAAATAACTCAGTTTTGA	59.90
am20719-r	TGACAGATGCTGAAGCAGAATT	60.00
am20732-f	TCTTAAGCTCAAGAAAAGTTGATTCA	60.00
am20732-r	AATGCGCACAGAGTCATCAG	60.00
am20741-f	CTATCTTGAACACTTGGGAGGG	60.00
am20741-r	AACTACTGACCACTCATGTAAGGC	60.00
am20806-f	CGCTTGTGCATGTATGTGTATG	60.10
am20806-r	GTGAGAGCAATAGAGCTTAGACAATG	60.00
am20821-f	GATCTCAGTCCTAGCCTCAGTCA	60.00
am20821-r	ACTTAGCAATAATTGTTTCCTGGC	59.90
am20824-f	AAGCTCAAAGCAATGAGGTGA	60.00
am20824-r	TTGACTTTGTTTAAAACATTTTGCA	60.00
am20830-f	CCCATCACCTTCGAGG	60.00
am20830-r	AGAGCATGGGCTTCGTTCT	60.00
belbacl-f	CGGCATCAGAGCAGATTGTA	60.00
belbacl-r	ATCGCCCTTCCCAACAGT	60.50
belbacr-f	TTGTTATCCGCTCACAATTCC	60.00
belbacr-r	CACGACAGGTTTCCCGAC	60.10
bjr16-f	GTCAATGCTATGAGCAGTTGGA	60.30
bjr16-r	AGTTGTATGCCATCTTGTCTCTTTC	60.10
bjr17-f	TTAGTTCAGGGATATCGCTCTACC	60.00
bjr17-r	AAAGCCACCCAAAGAGTGTTT	60.00
bjr18-f	CTTTCTGAACCGCTTTATACACATT	60.00
bjr18-r	CATTCGTGAGACCGGGAC	60.00
bjr24-f	GCAATGCTGCTGGTCCTT	59.90
bjr24-r	CCTACAATGTGGCCACAGG	60.00
cDNA-1	ctgagcgggaattcgtgagacc	
cDNA-1b	gtcacgcaagcttcacagg	
cDNA-2	P-ggtctcacgaattccgctcagtt	
cDNA-2b	P-cctgtgagaagcttgcgtagctt	
enx3-1	TTCAAATTTGAAAATGTACCATACTG	59.70
enx3-2	cagtatggtacattttcaaatttgaa	59.70
enx3-3	ATGTTTTAAAATCAACTTTTTATTGCC	59.30
enx3-4	CACTGGTACAAAACACTTTTGACG	59.80
enx3-5	CAGCTGCAAAGTGTTTTGTACC	59.90
enx5-1	CGACACCCAGTGGGACAT	60.40
enx5-2	atgtccactgggtgtcg	60.40
enx5-3	ATGATTATTCTTCGTCCCGC	59.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
enx5-4	AAGGCAGTGGAGTCCCG	59.80
enx5-5	TCAGATTTCTTCCCAGTCTGG	59.30
enxh1-f	cccaacatagatggaccaa	56.70
enxh1-r	gaagcagtcataatataaacatcgcc	63.10
enxmus_a	GCGTCCGACACCCAGT	59.10
enxmus_ar	actgggtgtcggacgc	59.10
enxmus_b	GAACTGAAACCTTAAACCAAGAGTG	59.60
enxmus_br	cactcttggttaaggttcagttc	59.60
enxmus_c	ACTAATAAAAAATTATGATGGAAAAGTGC	59.60
enxmus_cr	gcactttccatcataatattttattag	59.60
enxmus_d	TCACGGAGCAGCAGCTC	59.90
enxmus_dr	gagctgctgctccgtga	59.90
enxmus_e	CTGAGCGTATAAAGACACCACCT	59.70
enxmus_er	aggtgggtgtctttatacgctcag	59.70
enxmus_f	AAGCCTCCATGTTTAGAGTCCTC	60.10
enxmus_fr	gaggactctaacatggaggctt	60.10
enxmus_g	TTCGTGCCCTTGTGTGATAG	59.70
enxmus_gr	ctatcacacaagggcacgaa	59.70
enxmus_h	TCAAAGATCCTGTACAGAAAAATGA	59.20
enxmus_hr	tcattttctgtacaggatcttga	59.20
enxmus_i	TTGATTACAGATACAGCCAGGC	59.30
enxmus_ir	gcctggctgtatctgtaataa	59.30
ex01-f	agaacactttggacgggtgctc	64.90
ex01-r	accgttctaccagactccggaag	65.00
ex01b-f	tgaagactgtcacctgtgtctgtg	64.90
ex01b-r	cagagacggcggagcttcatta	64.90
ex03-f	tgttatgtctggtgctcctggaaa	65.00
ex03-r	tggagttgaagagctgggtcg	65.00
ex03b-f	gaatgtttgcacatgtatgtctggtg	65.50
ex03b-r	aagccccaggttcggttgta	64.90
ex10-f	ttttccatgtatccagggttacc	65.00
ex10-r	ggagaactttgtgtccaacctct	65.00
ex10b-f	ttttccatgtatccagggttacc	65.00
ex10b-r	agcaaattatcatggagaactttgtgtg	64.50
h3907-f	GCTCATCGATCTCCCCAAT	60.00
h3907-r	GAGGCAGGTCAACACTATTTCC	60.00
h3907b-f	TCCGGAAATAGTGTTGACCTG	60.00
h3907b-r	CTGAGGTAGATGCAAGAGTACGAA	60.00
h3907c-f	GGAAATAGTGTTGACCTGCCTC	60.00
h3907c-r	AGCGTTACCGGAAGGTTTTT	60.00
h3907d-f	AATCTGGCGGTCCAACC	59.40
h3907d-r	GGACGAATTCCAAAAAGCC	59.50
h3916-f	TGAGGAAGAAGACGACTTGGA	60.00
h3916-r	CTGTGTCTTTGTGACCCACAA	59.90
h5705-f	ACGTTCAACCCCAAGCAC	59.50
h5705-r	ACTCGGGGCACTTGTAAGG	60.10

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
h5733-f	GCTCGCCTGTGTGAGTCC	60.60
h5733-r	GCAAGCATGAGGTCTCCTTC	60.00
h5733b-f	CACTCGGGGCACTTGAAG	60.40
h5733b-r	CATGAGGTCTCCTTCATCTGC	59.80
h5733c-f	TGTGTGAGTCCGCTGGTG	60.50
h5733c-r	GTCTCCTTCATCTGCAGCCT	59.60
h6006-f	ATTTCTCCATGCCCAGGAG	60.00
h6006-r	GATGGCTGTATTCTGATCCACA	60.00
h6124-f	GAGCAGCAGCTAGCCAGG	60.00
h6124-r	CTTCCAGGTACCCAGACCAA	60.00
h6124b-f	CTGGAAGAGTGAGCCTGGTC	60.00
h6124b-r	CATTGCCGTAGATAGCAGCA	60.00
h6124c-f	AAAGCAGCTGTTAAGTATGCCC	59.80
h6124c-r	CTCCTTCAGGGCCTCCC	60.70
h6124d-f	TGCTGCTATCTACGGCAATG	60.00
h6124d-r	GTGGTGCTTGGTGTTCAC	60.00
hPApos-f	AATGATACCTTAATAAAGCTAAGGGGA	60.00
hPApos-r	TTGTCGAAATGTAAGTTTTATTCTTCC	60.20
hTX2-f	nn	
hTX2-r	nn	
hj0105-f	TGGTCGATTCAGTTTGTCTGA	60.20
hj0105-r	TTTTTCAGGTCTAACCCCGC	60.10
hj0127-f	ATCATCTGGTGGCCGATATC	59.70
hj0127-r	AGAAGTAGCGCCATGCCTT	60.00
hj0127b-f	CCACGGGGGATGAAAGTT	60.70
hj0127b-r	AGACGATCGTTAACTCTCACCG	60.70
hj0236-f	ACCTGAGCGAGAACCGC	60.10
hj0236-r	TTCAGGCGGAAGCTCTTG	60.20
hj0236b-f	CAGTGAAAAGGCTGGCG	59.00
hj0236b-r	ATGTGAAGGGCGCTCG	59.40
hox1.11-f	ccagccatccaagattct	
hox1.11-r	gccaataacataccctagatcac	
jln0103-f	gctgttcaggcagtggaaa	60.00
jln0103-r	atggccgtcttctcgaagt	59.80
jln0103b-f	AGTGGAAGAGAAAACAGAGTCCC	60.00
jln0103b-r	CCCAGAACAGCCCCACTTG	60.20
jln0104-f	tggtggctctaagtaccta	60.00
jln0104-r	aagaggggggtgtgcttagt	60.00
jln0105-f	gtgtatgtcatcgccggtc	59.90
jln0105-r	attaagcggatgtcccagg	59.90
jln0105b-f	CGAAGAGGTTTCTAGCGGG	60.00
jln0105b-r	TCACCCAGACTGGCCATT	60.00
jln0108-f	ggcttacttgactgagatgc	60.00
jln0108-r	cgtgagaccgatcactgct	60.00
jln0109-f	caaggacatcacctggact	60.00
jln0109-r	tctaccatttttggtgtctcaga	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
jln0110-f	taaggcctagttacctaataagga	60.00
jln0110-r	gaagaaattcccaaggaggc	60.00
jln0111-f	gttggtgacaatggcattttactg	60.00
jln0111-r	gctacgataaccagaatgcaattc	60.00
jln0115-f	ataacagattatgtgttccaggga	60.00
jln0115-r	agatgcaaacagcacaggc	60.00
jln0117-f	cacatgctgtacttcttagctgaa	60.00
jln0117-r	ccagtagagttgtactggtccctt	60.00
jln0118-f	tctctgcccaccgctac	59.90
jln0118-r	tcttctactctgttctctctgaa	60.10
jln0122-f	catgtcctatgtggacctgct	60.00
jln0122-r	aaaccattcgagaccctac	60.10
jln0127-f	gctgaatttctggcatccat	60.00
jln0127-r	tgaatattgccgccagt	60.00
jln0127b-f	CAGATTAATCGCCGGCTTAG	59.80
jln0127b-r	GCCGAAATGTTCTCTCAAAGA	60.20
jln0128-f	atcaaatactgaggctgggaa	59.90
jln0128-r	tctactgcaggcccagtg	60.00
jln0129-f	tcagctctcgctctgctcaa	60.00
jln0129-r	catcaactgttgaaggagatcc	60.00
jln0132-f	ggttgagaaatagccaccctc	60.00
jln0132-r	tgcaaggctcatgagtatccttg	60.10
jln0132b-f	TGGTTGAGAAATAGCCACCC	59.90
jln0132b-r	ACCAAGGGAAGGAGAGCAAT	60.10
jln0201-f	ctcttctcatgccgagac	60.00
jln0201-r	aaattcgtgagacctccg	60.10
jln0202-f	ctgagcaggatgcaccact	60.00
jln0202-r	gcacacaaagtgcacccac	60.10
jln0207-f	cttctggcaagcagatctc	60.10
jln0207-r	tctggacttctgtttgataaca	60.00
jln0208-f	ggaaaaataatcccagcgt	60.00
jln0208-r	atcgcatgggcaaggata	60.00
jln0210-f	attcatggcagggcagag	59.70
jln0210-r	gtgccactgtcggtagc	60.30
jln0213-f	ttctcagcatcttctatgacttg	60.00
jln0213-r	tctaggcttggccactataatca	60.20
jln0214-f	tacttgagcagcccagtg	60.00
jln0214-r	accctaggtgctggagtaaa	60.00
jln0217-f	catcaccaaggtctgtgaaaaa	60.00
jln0217-r	gcactctgtacctcagtgtaa	60.20
jln0219-f	acaccgtgccacctcat	60.00
jln0219-r	attacaggactcaacgttgatagc	60.00
jln0223-f	gtaccactgcctggtgg	60.00
jln0223-r	agttttagttccatgccaga	60.00
jln0225-f	atgagtgtggaaagccttaa	60.00
jln0225-r	gtccgagtgagatgagta	60.00

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
jln0226-f	agtccaacctgtgtcccaac	59.90
jln0226-r	atgacgaggactctgactgaag	59.90
jln0227-f	atctccccatccactctgtct	59.90
jln0227-r	agtcattctcagccacatgaga	59.90
jln0230-f	gaggggaattcttactctccaagtc	60.00
jln0230-r	actgaagcacaggggctg	60.00
jln0231-f	agccttgaagatctagagacactga	60.10
jln0231-r	ccacactggagctgagatca	60.00
jln0235-f	atgctggaagatctgctg	60.10
jln0235-r	ccacagattgttgaaggacgtt	60.00
jln0236-f	cagcctaaagcttttggccac	60.00
jln0236-r	ggtgagaaccgtgggaact	60.00
jln0236b-f	ACACTCGTAGGGCCCCCTC	60.10
jln0236b-r	CTTGCATGGAGTGTGGCA	60.40
m13-f	tgtaaaacgacggccagt	57.70
m13-r	caggaaacagctatgacc	51.20
m13fa1804f-1	tgtaaaacgacggccagtCTCAAGATGTTGTGGATG	49.80
m13fa1804f-2	tgtaaaacgacggccagtTTCCCATCTCCACATC	49.60
m13fa1804f-2b	tgtaaaacgacggccagtTTTCCATCTCACATCTTG	49.50
m13fa2028f-1	tgtaaaacgacggccagtTATGCACTACGCCTCAT	50.40
m13fa2028f-2	tgtaaaacgacggccagtAACCTCATTATCATGCCT	50.00
m13fa2312f-1	tgtaaaacgacggccagtGTTCCAAACTCCCCTT	50.00
m13fa2312f-2	tgtaaaacgacggccagtTTTCCCAGAAGAGAACTC	49.80
m13fm434f-1	tgtaaaacgacggccagtGGACATTTGCCTATGG	49.80
m13fm434f-2	tgtaaaacgacggccagtTGAACTTTTTGTGAGGAA	49.50
m13fm434f-3	tgtaaaacgacggccagtGTAGCGCTTCCAGTGT	49.70
m13fsp6shlox2	tgtaaaacgacggccagtCTATTAGAATATGCATCAAGC	49.70
m13ft7shlox2	tgtaaaacgacggccagtCGACTCACTATAGGGAGC	50.40
m13ra1804r-1	caggaaacagctatgaccCACTTTGAGTGGAAACAAA	49.80
m13ra1804r-2	caggaaacagctatgaccTTCTGGGGTAAATCCA	49.70
m13ra2028r-1	caggaaacagctatgaccTAGCCAATCAGATGAGG	49.60
m13ra2028r-2	caggaaacagctatgaccAGGCATGATAATGAGGTT	50.00
m13ra2312r-1	caggaaacagctatgaccTTATGCTGTTGGAATCC	49.60
m13ra2312r-2	caggaaacagctatgaccGAAAGAAGGAAGTGTGGT	49.50
m13rm434r-1	caggaaacagctatgaccAAAATCTGCCATTTGC	49.80
m13rm434r-2	caggaaacagctatgaccCCGAGAGAACAAGACAG	49.10
m13rm434r-3	caggaaacagctatgaccTTTGGGTAACTATGCAC	49.20
m13rsp6shlox2	caggaaacagctatgaccCTATTAGAATATGCATCAAGC	49.70
m13rt7shlox2	caggaaacagctatgaccCGACTCACTATAGGGAGC	50.40
mel425-f	CTACTGAGCGGAATTCGTGAG	60.00
mel425-r	GAGCCTGTTTTGACCTCAGC	60.00
mel426-f	CCGTGCATTTCACTGCTG	60.00
mel426-r	GGCTTTCTTCAGGGAAGAGAA	59.90
mel427-f	CCAATTATGTGACGTGCTGG	60.00
mel427-r	AGAGCTGACAGAAGTGTCAATTCC	59.90
mel427-r(2)	CCCTGCTTTCTTGAGGCTC	60.10

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
mel428-f	CTACTGAGCGGAATTCGTGAG	60.00
mel428-r	GGACCATAGAAACAGGCTGTTC	60.00
mel429-f	TCAATCCTTCCACGACTTCC	60.00
mel429-r	CGGTGTGTCTCAAAAGTTGGT	60.10
mel430-f	GGGGGAAAAAAGGTCCATT	60.00
mel430-r	CCAAGCCAAGTGTTTCATGAGT	60.20
mel431-f	TAAAAGGAAAGGGAGCTAGCG	60.00
mel431-r	CTAGTGACGCTGCCCTCAG	59.70
mel432-f	CCCAATTAAATGCTCTCTTTTCATAA	59.90
mel432-r	TTTTGGCCCCTAAATTTGC	59.90
mel433-f	GTGGTGCTTTATATGAAAAATGTCC	60.00
mel433-r	CTTTAAACTCTCTCAGCCACTTCC	60.00
mel434-f	TGCATAATATAACCACATTTTGGC	60.00
mel434-r	CTGGAAGCGCTACATCTACCTT	59.90
mel434f-1	TTTGAATAACTTGTTCTTCGGACA	60.00
mel434r-1	AGACACTGGAAGCGCTACATCT	60.50
mel435-f	ACCCTCCTTTCCAGACGG	60.00
mel435-r	GGGAACTCTTAGTGGCCCA	60.10
mel436-f	GTACAGCATCTAATGGGTTCTGTG	60.00
mel436-r	GCAAACCTCCTGCCTTGTAG	59.90
npv1-f	AAAGGGAGTGGCAGCATTTA	59.70
npv1-r	CAGAAGAAACCCATGGTTCCG	60.50
npv2-f	GAGGAAGGGGTATGTGGGAG	
npv2-r	GCAGAACCCAAAGAGGAGAG	
pBM13for	CGTTGTAAAACGACGGCC	60.10
pBM13rev	CACACAGGAAACAGCTATGACC	59.70
ratca3027-1-f	AACAATTCCACAGTTTATTGTTTTAGG	60.00
ratca3027-1-r	ATTGAGTCCCACAAGTTGTCCT	59.90
ratca3027-2-f	CTGGTGTGATGCCAACAATT	59.40
ratca3027-2-r	TCCATGTGTGCCATGCAT	60.50
ratca3031-1-f	GTGCTCACCCAAGCAGTGT	59.90
ratca3031-1-r	CACCAGGAGTAGTTCTGCCTCT	59.90
ratca3105-1-f	ACTTCTGGGCCATTAGCATTT	60.00
ratca3105-1-r	CAGTGTGCATGCATATACTCATACA	60.00
rmo0115-f	cttgatccaggacaaaatgtca	60.00
rmo0115-r	agtgtgtatgggatcagcagg	60.00
rmo0115b-f	CCTGCTGATCCCATACACACT	60.00
rmo0115b-r	ACGAAACTTCTCAAAGCGGA	60.00
rmo0124-f	agtgaaccacaaaagctattagaga	59.80
rmo0124-r	ctgggggtgacactgagg	60.10
rmo103a	CACCTTTAATCCCAGCACTTG	
rmo103b	AACATACACCTTCTTTCTAGGTGTCT	
rmo103c	CCAGGATTGTTAGTGAGAACAGG	
rmo103d	AAATGGGCATATGTGTGCCT	
rmo106a	CCTGTGTTTCAGATTAAGTGTGTTGT	
rmo106b	ATGAGTTCTGGGGACGGAC	

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
rmo107a	TTTGTGGTCATTGTAAAGGGTG	
rmo107b	GGGTTGGCGGTCATTCAG	
rmo107c	TGAGAACAGGTCAAGTCAAGTCC	
rmo107d	CTGCCATAACAGCTCAGGGT	
rmo116a	AGGTGTAGGCTCTGCTGAGG	
rmo116b	GCAGTGGTGGCGCAC	
rmo116c	CAGGGAGGGATCCCCAG	
rmo116d	GAGCAGCCTCAGCAGAGC	
rmo118a	GGAGGAAGAGCAACTGACTGA	
rmo118b	AAAGATTTACAGAAAACAGATTT	
rmo118c	GCGCACACGCCAGCC	
rmo118d	AAAACATGCTCATTCTGTGA	
rmo118e	TCTGTTCTCTTCAATCCCCTGT	
rmo118for-f	TGGAAGAAAGAAACCACACATG	60.00
rmo118for-r	ATCCAGCCTCACCAGAAATG	60.10
rmo118rev-f	ATAGACCAGGCTCTCCCTAAATG	60.00
rmo118rev-r	CACAGAGCCAGCTGCAGA	60.00
rmo183a	TCTCTGAGAGAGTGTACATGTGACAG	
rmo183b	TCTGTCACATGTACACTCTCTCAGAG	
rmo183c	TACAATCAGAGATGCTGT	
rmo183d	CGACAGAGCAAGTTCCTGG	
rmo183e	TCCTGAGTGCTAGCGTATAAAGG	
rmo183f	CCTTTATACGCTAGCACTCAGGA	59.90
rmo183g	TCTCCCCTCCTCCACTAATAATATAG	59.40
rmo183h	CTATATTATTAGTGGAGGAGGGGAGA	59.40
rmo185a	AAAGTGCCCTAGCCCCTG	
rmo185b	CAATGCCTCGTGGGAGCT	
ryac0309-f	GAGATCAGAGGGAAGTGTGTTGTAAA	
ryac0309-r	TTCAATCCATGCAGCTTCTG	
ryac0327-f	AGTAAGACACATACCAGACCTCTGG	
ryac0327-r	GGAAGTGGTTTCCAAGGTGA	
ryac0330-f	ACCAAATAACCAGGACCCC	
ryac0330-r	TGGATACATGTGTGCGCAG	
ryac0335-f	GATCACAGTTGGTCCCTATGAAG	
ryac0335-r	CTATCAGGTACAAGGCTCCAGG	
ryac2a01-f	TAAGGGTCCCAAGGCCA	
ryac2a01-r	TTGATGGTCCGCCATTG	
ryac2a11-f	ACAACTTTTCAGGCAAAATTGA	
ryac2a11-r	GGGAATGAACATGTGATCCC	
ryac2b01-f	ACATCCAAAGTCACTTGAGTAGACC	
ryac2b01-r	TGGCTGGTGGTCTGCTAAG	
ryac2b06-f	GTGTTTGTGTTTTATGACTATGTGATGTTT	
ryac2b06-r	AGCAAAAGCAGCCTGACAAT	
ryac2c02-f	GGGTGGATCTTTGAGTGAGATC	
ryac2c02-r	AAACACTCCACCCCAGAGG	
ryac2c06-f	TGACCTAGAGTTCCACCAGTT	

Table A-1:

Primer Name	Primer Sequence	Melting Temp.
ryac2c06-r	CCATTTGCAGATGACACCAC	
ryac2c07-f	CTTGTGTATGTAGGAACAAGTGTGTG	
ryac2c07-r	AGGCAGATGAGATTAGCAGCA	
ryac2e01-f	GTTTGTCTACCAACCTTCACACTG	
ryac2e01-r	CCTAGGACATCACTGTCACTTCC	
ryac2e03-f	AGGGTGGGCCCTTAGTTCTA	
ryac2e03-r	CACAGATGATGTAGTGGGGCT	
ryac2e04-f	ACTTCCTTCAGGACTCTGTTTATGTAA	
ryac2e04-r	TCCTCTCCATTCTAAAGGACA	
ryac2e05-f	TCTCAGAGCAGTGAGTAATTGAATAGA	
ryac2e05-r	CTGCTCTGTCAATAGCCTTGG	
ryac2f01-f	GTGCAGAACTAGTGATAGAGGCTC	
ryac2f01-r	AGAACATGTGCTTGA CTGTGCT	
ryac2f02-f	GGTTATATGCCCTGCGAAGA	
ryac2f02-r	AGCATCTCTTTCACAGGCTAGG	
ryac2f09-f	TCTGGCTGAGAGGTTGGTCT	
ryac2f09-r	AACGGCTGCAAGGAAGC	
scos-m13f	tgtaaacgacggccagtataggcgatcacgaggcc	60.10
scos-m13r	caggaaacagctatgaccggaagtcaacaaaaagcagagc	60.40
t3bs	accctcactaaaggaacaaaag	
t3bs ext	gctcgaaattaaccctcactaaag	
t7bs	tgaatacgactcactatagggcg	
t7bs ext	tgaattgtaatacgactcactatagg	
t7bs int	tcactatagggcggaattggg	

Table A-2: The following table lists the STSs and SSR PCR assays used in this work. For some, the predicted PCR product length is also listed.

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
101f4sr1	101f4sr1-f	101f4sr1-r	
11c2sr2	11c2sr2-f	11c2sr2-r	
207con1a	207con1a-f	207con1a-r	137
207con1b	207con1b-f	207con1a-r	147
207rpt1	207rpt1-f	207rpt1-r	137
28e053f05	28e053f05-f	28e053f05-r	
28e05e09	28e05e09-f	28e05e09-r	
3027con1a	3027con1a-f	3027con1a-r	174
3027con1b	3027con1b-f	3027con1b-r	119
3031con1a	3031con1a-f	3031con1a-r	146
3105con1a	3105con1a-f	3105con1a-r	140
320f9sr1	320f9sr1-f	320f9sr1-r	
34 R2A	34 R2A-f	34 R2A-r	
4011_2t3	4011_2t3-f	4011_2t3-r	91
4011_2t3-2	4011_2t3-2f	4011_2t3-2r	160
4011_2t7	4011_2t7-f	4011_2t7-r	94
4011_2t7-2	4011_2t7-2f	4011_2t7-2r	177
4011ssr1a	4011ssr1a-f	4011ssr1a-r	128
4372GI	4372GI-f	4372GI-r	215
4372Gr	4372Gr-f	4372Gr-r	144
45 R2H	45 R2H-f	45 R2H-r	185
4825GI	4825GI-f	4825GI-r	149
4825Gr	4825Gr-f	4825Gr-r	122
4829GI	4829GI-f	4829GI-r	101
4829Gr	4829Gr-f	4829Gr-r	118
4866Gr	4866Gr-f	4866Gr-r	105
5578CA_d	5578CA_d-f	5578CA_d-r	135
5578CAAd-1	5578CAAd-f1	5578CAAd-r1	168
5578CAAd-2	5578CAAd-f2	5578CAAd-r1	135
5578GA1	5578GA1-f	5578GA1-r	263
5578GA2	5578GA2-f	5578GA2-r	117
5578GC	5578GC-f	5578GC-r	189
5579CA1b	5579CA1b-f	5579CA1b-r	114

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
5579CA2	5579CA2-f	5579CA2-r	109
5621CA1	5621CA1-f	5621CA1-r	120
5621CA1b	5621CA1-f2	5621CA1-r	97
5631CA3b	5631CA3b-f	5631CA3b-r	164
5631CA3c	5631CA3b-f	5631CA3c-r	114
5807GI	5807GI-f	5807GI-r	119
5984/85ca_b	5984/85ca_b-f	5984/85ca_b-r	162
5984/85ca_c	5984/85ca_c-f	5984/85ca_c-r	131
5984/85ca_f	5984/85ca_f-f	5984/85ca_f-r	94
5985Gr	5985Gr-f	5985Gr-r	91
6239Gr	6239Gr-f	6239Gr-r	115
6241GI	6241GI-f	6241GI-r	102
6241Gr	6241Gr-f	6241Gr-r	116
6241ca_f	6241ca_f-f	6241ca_f-r	158
6241ca_h	6241ca_h-f	6241ca_h-r	181
6350GI	6350GI-f	6350GI-r	113
6350Gr	6350Gr-f	6350Gr-r	118
6450GI	6450GI-f	6450GI-r	124
7134GC1	7134GC1-f	7134GC1-r	177
7425GD2	7425GD2-f	7425GD2-r	162
D6Mit43	D6Mit43-f	D6Mit43-r	
D6Mit74	D6Mit74-f	D6Mit74-r	
D6Mit76	D6Mit76-f	D6Mit76-r	
R236	R236-f	R236-r	184
ajm1502	ajm1502-f	ajm1502-r	181
ajm1505	ajm1505-f	ajm1505-r	183
ajm1506	ajm1506-f	ajm1506-r	143
ajm1515	ajm1515-f	ajm1515-r	195
ajm1516	ajm1516-f	ajm1516-r	152
ajm1517	ajm1517-f	ajm1517-r	147
ajm1518	ajm1518-f	ajm1518-r	172
ajm1521	ajm1521-f	ajm1521-r	184
ajm1701	ajm1701-f	ajm1701-r	161
ajm1705	ajm1705-f	ajm1705-r	193
ajm1708	ajm1708-f	ajm1708-r	157
ajm1711	ajm1711-f	ajm1711-r	171

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm1802	ajm1802-f	ajm1802-r	113
ajm1803	ajm1803-f	ajm1803-r	152
ajm1805	ajm1805-f	ajm1805-r	123
ajm1806	ajm1806-f	ajm1806-r	158
ajm1807	1807-f	1807-r	114
ajm1808	1808-f	1808-r	112
ajm1809	1809-f	1809-r	116
ajm1810	1810-f	1810-r	116
ajm1811	1811-f	1811-r	136
ajm1812	1812-f	1812-r	118
ajm1813	1813-f	1813-r	97
ajm1816	1816-f	1816-r	130
ajm2028	ajm2028-f	ajm2028-r	226
ajm203	ajm203-f	ajm203-r	140
ajm203b	ajm203b-f	ajm203-r	116
ajm207	ajm207-f	ajm207-r	224
ajm207b	ajm207b-f	ajm207b-r	194
ajm210	ajm210-f	ajm210-r	155
ajm210b	ajm210b-f	ajm210b-r	78
ajm214	ajm214-f	ajm214-r	64
ajm221	ajm221-f	ajm221-r	40
ajm224	ajm224-f	ajm224-r	94
ajm224b	ajm224b-f	ajm224b-r	59
ajm2313	2313-f	2313-r	154
ajm2316	2316-f	2316-r	132
ajm2317	2317-f	2317-r	145
ajm2318	2318-f	2318-r	153
ajm2319	2319-f	2319-r	114
ajm2320	2320-f	2320-r	142
ajm2321	2321-f	2321-r	144
ajm2322	2322-f	2322-r	110
ajm2324	ajm2324-f	ajm2324-r	122
ajm2501	ajm2501-f	ajm2501-r	168
ajm2502	ajm2502-f	ajm2502-r	110
ajm2504	ajm2504-f	ajm2504-r	198
ajm2505	ajm2505-f	ajm2505-r	121

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm2506	ajm2506-f	ajm2506-r	181
ajm2508	ajm2508-f	ajm2508-r	195
ajm2510	ajm2510-f	ajm2510-r	186
ajm2511	ajm2511-f	ajm2511-r	180
ajm2513	ajm2513-f	ajm2513-r	162
ajm2514	ajm2514-f	ajm2514-r	199
ajm2515	ajm2515-f	ajm2515-r	185
ajm2516	ajm2516-f	ajm2516-r	166
ajm2519	ajm2519-f	ajm2519-r	151
ajm2523	ajm2523-f	ajm2523-r	154
ajm2524	ajm2524-f	ajm2524-r	196
ajm2619	ajm2619-f	ajm2619-r	149
ajm2620	ajm2620-f	ajm2620-r	172
ajm2621	ajm2621-f	ajm2621-r	173
ajm2622	ajm2622-f	ajm2622-r	175
ajm2623	ajm2623-f	ajm2623-r	187
ajm2624	ajm2624-f	ajm2624-r	197
ajm2625	ajm2625-f	ajm2625-r	180
ajm2626	ajm2626-f	ajm2626-r	193
ajm2627	ajm2627-f	ajm2627-r	131
ajm2628	ajm2628-f	ajm2628-r	189
ajm2629	ajm2629-f	ajm2629-r	192
ajm2630	ajm2630-f	ajm2630-r	189
ajm2701	ajm2701-f	ajm2701-r	111
ajm2702	ajm2702-f	ajm2702-r	125
ajm2703	ajm2703-f	ajm2703-r	110
ajm2705	ajm2705-f	ajm2705-r	139
ajm2706	ajm2706-f	ajm2706-r	110
ajm2707	ajm2707-f	ajm2707-r	140
ajm2708	ajm2708-f	ajm2708-r	135
ajm2709	2709-f	2709-r	155
ajm2710	2710-f	2710-r	110
ajm2711	2711-f	2711-r	111
ajm2712	2712-f	2712-r	95
ajm2713	2713-f	2713-r	119
ajm2714	2714-f	2714-r	126

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm2715	2715-f	2715-r	137
ajm2716	2716-f	2716-r	117
ajm2717	2717-f	2717-r	130
ajm2718	2718-f	2718-r	156
ajm2719	2719-f	2719-r	124
ajm2720	2720-f	2720-r	122
ajm2721	2721-f	2721-r	128
ajm2722	2722-f	2722-r	153
ajm2723	2723-f	2723-r	120
ajm2724	2724-f	2724-r	126
ajm3027	ajm3027-f	ajm3027-r	139
ajm3031	ajm3031-f	ajm3031-r	111
ajm3036	ajm3036-f	ajm3036-r	128
ajm305	ajm305-f	ajm305-r	206
ajm305b	ajm305b-f	ajm305b-r	152
ajm306	ajm306-f	ajm306-r	112
ajm306b	ajm306-f	ajm306b-r	82
ajm307	ajm307-f	ajm307-r	110
ajm3105	ajm3105-f	ajm3105-r	116
ajm3109	ajm3109-f	ajm3109-r	116
ajm3119	ajm3119-f	ajm3119-r	104
ajm315	ajm315-f	ajm315-r	203
ajm315b	ajm315b-f	ajm315b-r	156
ajm3201	ajm3201-f	ajm3201-r	201
ajm3202	ajm3202-f	ajm3202-r	151
ajm3206	ajm3206-f	ajm3206-r	166
ajm3301	ajm3301-f	ajm3301-r	219
ajm3302	ajm3302-f	ajm3302-r	158
ajm3303	ajm3303-f	ajm3303-r	150
ajm3304	ajm3304-f	ajm3304-r	159
ajm3305	ajm3305-f	ajm3305-r	207
ajm3308	ajm3308-f	ajm3308-r	181
ajm3311	ajm3311-f	ajm3311-r	188
ajm3312	ajm3312-f	ajm3312-r	165
ajm3313	ajm3313-f	ajm3313-r	201
ajm3314	ajm3314-f	ajm3314-r	235

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm3315	ajm3315-f	ajm3315-r	221
ajm3316	ajm3316-f	ajm3316-r	156
ajm3317	ajm3317-f	ajm3317-r	159
ajm3320	ajm3320-f	ajm3320-r	180
ajm3322	ajm3322-f	ajm3322-r	127
ajm3327	ajm3327-f	ajm3327-r	182
ajm3329	ajm3329-f	ajm3329-r	104
ajm3331	ajm3331-f	ajm3331-r	195
ajm3335	ajm3335-f	ajm3335-r	127
ajm3336	ajm3336-f	ajm3336-r	152
ajm3701	ajm3701-f	ajm3701-r	153
ajm3702	ajm3702-f	ajm3702-r	155
ajm3703	ajm3703-f	ajm3703-r	178
ajm3704	ajm3704-f	ajm3704-r	121
ajm3705	ajm3705-f	ajm3705-r	143
ajm3706	ajm3706-f	ajm3706-r	142
ajm3707	ajm3707-f	ajm3707-r	130
ajm3708	ajm3708-f	ajm3708-r	130
ajm3709	ajm3709-f	ajm3709-r	135
ajm3710	ajm3710-f	ajm3710-r	134
ajm3711	ajm3711-f	ajm3711-r	136
ajm3712	ajm3712-f	ajm3712-r	154
ajm3713	ajm3713-f	ajm3713-r	163
ajm3714	ajm3714-f	ajm3714-r	151
ajm3715	ajm3715-f	ajm3715-r	167
ajm3716	ajm3716-f	ajm3716-r	172
ajm3717	ajm3717-f	ajm3717-r	93
ajm3718	ajm3718-f	ajm3718-r	93
ajm3719	ajm3719-f	ajm3719-r	93
ajm3721	ajm3721-f	ajm3721-r	90
ajm3722	ajm3722-f	ajm3722-r	120
ajm3723	ajm3723-f	ajm3723-r	107
ajm3728	ajm3728-f	ajm3728-r	123
ajm3729	ajm3729-f	ajm3729-r	128
ajm3801	ajm3801-f	ajm3801-r	129
ajm3802	ajm3802-f	ajm3802-r	143

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm3803	ajm3803-f	ajm3803-r	160
ajm3804	ajm3804-f	ajm3804-r	150
ajm3805	ajm3805-f	ajm3805-r	169
ajm3805b	ajm3805b-f	ajm3805-r	110
ajm3805c	ajm3805-f	ajm3805b-r	127
ajm3806	ajm3806-f	ajm3806-r	150
ajm3806i1	ajm3806i1-f	ajm3806i1-r	434
ajm3806i4	ajm3806i4-f	ajm3806i4-r	274
ajm3807	ajm3807-f	ajm3807-r	141
ajm3808	ajm3808-f	ajm3808-r	164
ajm3809	ajm3809-f	ajm3809-r	165
ajm3810	ajm3810-f	ajm3810-r	165
ajm3811	ajm3811-f	ajm3811-r	165
ajm3812	ajm3812-f	ajm3812-r	104
ajm3901	ajm3901-f	ajm3901-r	129
ajm3902	ajm3902-f	ajm3902-r	149
ajm3902b	ajm3902b-f	ajm3902b-r	125
ajm3903	ajm3903-f	ajm3903-r	129
ajm3904	ajm3904-f	ajm3904-r	161
ajm3905	ajm3905-f	ajm3905-r	166
ajm3906	ajm3906-f	ajm3906-r	153
ajm3907	ajm3907-f	ajm3907-r	139
ajm3908	ajm3908-f	ajm3908-r	120
ajm3909	ajm3909-f	ajm3909-r	124
ajm3910	ajm3910-f	ajm3910-r	198
ajm3913	ajm3913-f	ajm3913-r	173
ajm3913b	ajm3913b-f	ajm3913b-r	184
ajm3914	ajm3914-f	ajm3914-r	149
ajm3915	ajm3915-f	ajm3915-r	150
ajm3916	ajm3916-f	ajm3916-r	166
ajm3916-b	ajm3916b-f	ajm3916b-r	101
ajm3917	ajm3917-f	ajm3917-r	154
ajm3918	ajm3918-f	ajm3918-r	148
ajm3919	ajm3919-f	ajm3919-r	174
ajm3920	ajm3920-f	ajm3920-r	93
ajm3921	ajm3921-f	ajm3921-r	139

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm3921i1a	ajm3921i1a-f	ajm3921i1a-r	196
ajm3921i1b	ajm3921i1b-f	ajm3921i1b-r	190
ajm3921i2a	ajm3921i1a-f	ajm3921i1a-r2	456
ajm3921i2b	ajm3921i1b-f	ajm3921i1b-r2	386
ajm3921i5a	ajm3921i5a-f	ajm3921i5a-r	
ajm3922	ajm3922-f	ajm3922-r	174
ajm3923	ajm3923-f	ajm3923-r	140
ajm3924	ajm3924-f	ajm3924-r	141
ajm3927	ajm3927-f	ajm3927-r	153
ajm3928	ajm3928-f	ajm3928-r	120
ajm3929	ajm3929-f	ajm3929-r	151
ajm3930	ajm3930-f	ajm3930-r	153
ajm3931	ajm3931-f	ajm3931-r	154
ajm3933	ajm3933-f	ajm3933-r	154
ajm3934	ajm3934-f	ajm3934-r	222
ajm3935	ajm3935-f	ajm3935-r	150
ajm401	ajm401-f	ajm401-r	153
ajm4011	ajm4011-f	ajm4011-r	259
ajm4013	ajm4013-f	ajm4013-r	113
ajm4018	ajm4018-f	ajm4018-r	162
ajm401b	ajm401b-f	ajm401b-r	83
ajm4020	ajm4020-f	ajm4020-r	131
ajm4021	ajm4021-f	ajm4021-r	131
ajm4022	ajm4022-f	ajm4022-r	122
ajm4023	ajm4023-f	ajm4023-r	135
ajm4025	ajm4025-f	ajm4025-r	135
ajm4026	ajm4026-f	ajm4026-r	121
ajm4027	ajm4027-f	ajm4027-r	135
ajm4028	ajm4028-f	ajm4028-r	149
ajm4029	ajm4029-f	ajm4029-r	155
ajm4030	ajm4030-f	ajm4030-r	124
ajm4031	ajm4031-f	ajm4031-r	134
ajm4033	ajm4033-f	ajm4033-r	149
ajm4034	ajm4034-f	ajm4034-r	126
ajm4036	ajm4036-f	ajm4036-r	135
ajm409	ajm409-f	ajm409-r	112

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm409b	ajm409b-f	ajm409b-r	58
ajm4119	ajm4119-f	ajm4119-r	121
ajm4121	ajm4121-f	ajm4121-r	119
ajm4122	ajm4122-f	ajm4122-r	122
ajm4123	ajm4123-f	ajm4123-r	129
ajm4124	ajm4124-f	ajm4124-r	101
ajm4125	ajm4125-f	ajm4125-r	129
ajm4126	ajm4126-f	ajm4126-r	117
ajm4127	ajm4127-f	ajm4127-r	92
ajm4128	ajm4128-f	ajm4128-r	139
ajm4129	ajm4129-f	ajm4129-r	182
ajm4131	ajm4131-f	ajm4131-r	166
ajm4132	ajm4132-f	ajm4132-r	153
ajm4133	ajm4133-f	ajm4133-r	143
ajm4134	ajm4134-f	ajm4134-r	158
ajm4135	ajm4135-f	ajm4135-r	128
ajm4136	ajm4136-f	ajm4136-r	151
ajm419	ajm419-f	ajm419-r	196
ajm419b	ajm419b-f	ajm419b-r	136
ajm4221	ajm4221-f	ajm4221-r	180
ajm4227	ajm4227-f	ajm4227-r	141
ajm4301	ajm4301-f	ajm4301-r	166
ajm4303	ajm4303-f	ajm4303-r	146
ajm4306	ajm4306-f	ajm4306-r	154
ajm4306b	ajm4306b-f	ajm4306b-r	184
ajm4306c	ajm4306c-f	ajm4306c-r	96
ajm4307	ajm4307-f	ajm4307-r	166
ajm4308	ajm4308-f	ajm4308-r	127
ajm4308b	ajm4308b-f	ajm4308b-r	98
ajm4309	ajm4309-f	ajm4309-r	130
ajm4313	ajm4313-f	ajm4313-r	147
ajm4315	ajm4315-f	ajm4315-r	129
ajm4315b	ajm4315b-f	ajm4315b-r	176
ajm4317	ajm4317-f	ajm4317-r	180
ajm4318	ajm4318-f	ajm4318-r	91
ajm4319	ajm4319-f	ajm4319-r	116

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm4320	ajm4320-f	ajm4320-r	129
ajm4321	ajm4321-f	ajm4321-r	160
ajm5005L2/R1	ajm5005L2	ajm5005R1	131
ajm5012	ajm5012-f	ajm5012-r	163
ajm5015	ajm5015-f	ajm5015-r	163
ajm5018	ajm5018-f	ajm5018-r	127
ajm5024	ajm5024-f	ajm5024-r	110
ajm5029	ajm5029-f	ajm5029-r	122
ajm5030	ajm5030-f	ajm5030-r	125
ajm518	ajm518-f	ajm518-r	182
ajm5201	ajm5201-f	ajm5201-r	139
ajm5202	ajm5202-f	ajm5202-r	145
ajm5203	ajm5203-f	ajm5203-r	112
ajm5206	ajm5206-f	ajm5206-r	99
ajm5207	ajm5207-f	ajm5207-r	124
ajm5209	ajm5209-f	ajm5209-r	110
ajm5212	ajm5212-f	ajm5212-r	96
ajm5213	ajm5213-f	ajm5213-r	120
ajm5218	ajm5218-f	ajm5218-r	100
ajm5220	ajm5220-f	ajm5220-r	99
ajm5221	ajm5221-f	ajm5221-r	93
ajm5222	ajm5222-f	ajm5222-r	156
ajm5222i4	ajm5222i4-f	ajm5222i4-r	429
ajm5223	ajm5223-f	ajm5223-r	91
ajm5224	ajm5224-f	ajm5224-r	120
ajm5225	ajm5225-f	ajm5225-r	134
ajm5227	ajm5227-f	ajm5227-r	101
ajm523	ajm523-f	ajm523-r	180
ajm524	ajm524-f	ajm524-r	141
ajm5401	ajm5401-f	ajm5401-r	150
ajm5402	ajm5402-f	ajm5402-r	154
ajm5403	ajm5403-f	ajm5403-r	132
ajm5404	ajm5404-f	ajm5404-r	120
ajm5404b	ajm5404-f2	ajm5404-r	102
ajm5406	ajm5406-f	ajm5406-r	176
ajm5406b	ajm5406-f	ajm5406b-r	104

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm5408	ajm5408-f	ajm5408-r	148
ajm5411	ajm5411-f	ajm5411-r	144
ajm5413	ajm5413-f	ajm5413-r	121
ajm5414	ajm5414-f	ajm5414-r	130
ajm5415	ajm5415-f	ajm5415-r	145
ajm5416	ajm5416-f	ajm5416-r	147
ajm5416b	ajm5416b-f	ajm5416b-r	91
ajm5417	ajm5417-f	ajm5417-r	97
ajm5418	ajm5418-f	ajm5418-r	100
ajm5419	ajm5419-f	ajm5419-r	143
ajm5421	ajm5421-f	ajm5421-r	140
ajm5422	ajm5422-f	ajm5422-r	182
ajm5424	ajm5424-f	ajm5424-r	145
ajm5425	ajm5425-f	ajm5425-r	97
ajm5427	ajm5427-f	ajm5427-r	133
ajm5429	ajm5429-f	ajm5429-r	148
ajm5429b	ajm5429-f	ajm5429b-r	117
ajm5431	ajm5431-f	ajm5431-r	132
ajm5432	ajm5432-f	ajm5432-r	136
ajm5433	ajm5433-f	ajm5433-r	124
ajm5434	ajm5434-f	ajm5434-r	138
ajm5436	ajm5436-f	ajm5436-r	123
ajm5506	ajm5506-f	ajm5506-r	166
ajm5507	ajm5507-f	ajm5507-r	131
ajm5507b	ajm5507b-f	ajm5507b-r	89
ajm5513	ajm5513-f	ajm5513-r	105
ajm5515	ajm5515-f	ajm5515-r	104
ajm5518	ajm5518-f	ajm5518-r	153
ajm5602	ajm5602-f	ajm5602-r	148
ajm5602b	ajm5602b-f	ajm5602b-r	139
ajm5610	ajm5610-f	ajm5610-r	151
ajm5611	ajm5611-f	ajm5611-r	141
ajm5614	ajm5614-f	ajm5614-r	126
ajm5614b	ajm5614b-f	ajm5614b-r	82
ajm5618	ajm5618-f	ajm5618-r	140
ajm5618b	ajm5618b-r	ajm5618b-r	94

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm5704	ajm5704-f	ajm5704-r	135
ajm5704b	ajm5704b-f	ajm5704b-r	112
ajm5705	ajm5705-f	ajm5705-r	122
ajm5708	ajm5708-f	ajm5708-r	131
ajm5709	ajm5709-f	ajm5709-r	149
ajm5710	ajm5710-f	ajm5710-r	122
ajm5711	ajm5711-f	ajm5711-r	126
ajm5712	ajm5712-f	ajm5712-r	131
ajm5715	ajm5715-f	ajm5715-r	125
ajm5718	ajm5718-f	ajm5718-r	132
ajm5720	ajm5720-f	ajm5720-r	139
ajm5722	ajm5722-f	ajm5722-r	128
ajm5723	ajm5723-f	ajm5723-r	172
ajm5724	ajm5724-f	ajm5724-r	129
ajm5728	ajm5728-f	ajm5728-r	119
ajm5729	ajm5729-f	ajm5729-r	119
ajm5731	ajm5731-f	ajm5731-r	127
ajm5732	ajm5732-f	ajm5732-r	109
ajm5733	ajm5733-f	ajm5733-r	142
ajm5734	ajm5734-f	ajm5734-r	129
ajm5805	ajm5805-f	ajm5805-r	96
ajm5806	ajm5806-f	ajm5806-r	147
ajm5807	ajm5807-f	ajm5807-r	105
ajm5808	ajm5808-f	ajm5808-r	147
ajm5809	ajm5809-f	ajm5809-r	113
ajm5810	ajm5810-f	ajm5810-r	126
ajm5811	ajm5811-f	ajm5811-r	121
ajm5813	ajm5813-f	ajm5813-r	121
ajm5813i1	ajm5813i1-f	ajm5813i1-r	559
ajm5813i4	ajm5813i4-f	ajm5813i4-r	385
ajm5814	ajm5814-f	ajm5814-r	114
ajm5815	ajm5815-f	ajm5815-r	114
ajm5820	ajm5820-f	ajm5820-r	128
ajm5821	ajm5821-f	ajm5821-r	146
ajm5823	ajm5823-f	ajm5823-r	134
ajm5925	ajm5925-f	ajm5925-r	111

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
ajm5926	ajm5926-f	ajm5926-r	124
ajm5928	ajm5928-f	ajm5928-r	105
ajm5929	ajm5929-f	ajm5929-r	105
ajm5932	ajm5932-f	ajm5932-r	108
ajm5934	ajm5934-f	ajm5934-r	120
ajm5935	ajm5935-f	ajm5935-r	116
ajm5936	ajm5936-f	ajm5936-r	119
ajm6003	ajm6003-f	ajm6003-r	118
ajm6004	ajm6004-f	ajm6004-r	93
ajm6004b	ajm6004b-f	ajm6004b-r	99
ajm6005	ajm6005-f	ajm6005-r	101
ajm6006	ajm6006-f	ajm6006-r	99
ajm6110	ajm6110-f	ajm6110-r	122
ajm6124	ajm6124-f	ajm6124-r	101
ajm623	ajm623-f	ajm623-r	161
ajm701	ajm701-f	ajm701-r	153
ajm703	ajm703-f	ajm703-r	93
ajm706	ajm706-f	ajm706-r	202
ajm707	ajm707-f	ajm707-r	98
ajm709	ajm709-f	ajm709-r	122
ajm711	ajm711-f	ajm711-r	229
ajm713	ajm713-f	ajm713-r	155
ajm716	ajm716-f	ajm716-r	174
ajm719	ajm719-f	ajm719-r	153
am20719	am20719-f	am20719-r	124
am20732	am20732-f	am20732-r	98
am20741	am20741-f	am20741-r	130
am20806	am20806-f	am20806-r	144
am20821	am20821-f	am20821-r	118
am20824	am20824-f	am20824-r	115
am20830	am20830-f	am20830-r	107
belbacl	belbacl-f	belbacl-r	129
belbacr	belbacr-f	belbacr-r	138
bjr16	bjr16-f	bjr16-r	100
bjr17	bjr17-f	bjr17-r	140
bjr18	bjr18-f	bjr18-r	126

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
bjr24	bjr24-f	bjr24-r	148
ex01	ex01-f	ex01-r	129
ex01b	ex01b-f	ex01b-r	102
ex03	ex03-f	ex03-r	160
ex03b	ex03b-f	ex03b-r	123
ex10	ex10-f	ex10-r	149
ex10b	ex10b-f	ex10b-r	162
h3907	h3907-f	h3907-r	90
h3907b	h3907b-f	h3907b-r	107
h3907c	h3907c-f	h3907c-r	133
h3907d	h3907d-f	h3907d-r	95
h3916	h3916-f	h3916-r	95
h5705	h5705-f	h5705-r	96
h5733	h5733-f	h5733-r	96
h5733b	h5733b-f	h5733b-r	113
h5733c	h5733c-f	h5733c-r	78
h6006	h6006-f	h6006-r	94
h6124	h6124-f	h6124-r	98
h6124b	h6124b-f	h6124b-r	94
h6124c	h6124c-f	h6124c-r	98
h6124d	h6124d-f	h6124d-r	123
hPApos	hPApos-f	hPApos-r	119
hTX2	hTX2-f	hTX2-r	
hj0105	hj0105-f	hj0105-r	100
hj0127	hj0127-f	hj0127-r	97
hj0127b	hj0127b-f	hj0127b-r	100
hj0236	hj0236-f	hj0236-r	100
hj0236b	hj0236b-f	hj0236b-r	110
jln0103	jln0103-f	jln0103-r	113
jln0103b	jln0103b-f	jln0103b-r	144
jln0104	jln0104-f	jln0104-r	122
jln0105	jln0105-f	jln0105-r	131
jln0105b	jln0105b-f	jln0105b-r	92
jln0108	jln0108-f	jln0108-r	110
jln0109	jln0109-f	jln0109-r	143
jln0110	jln0110-f	jln0110-r	107

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
jln0111	jln0111-f	jln0111-r	123
jln0115	jln0115-f	jln0115-r	110
jln0117	jln0117-f	jln0117-r	104
jln0118	jln0118-f	jln0118-r	111
jln0122	jln0122-f	jln0122-r	126
jln0127	jln0127-f	jln0127-r	93
jln0127b	jln0127b-f	jln0127b-r	99
jln0128	jln0128-f	jln0128-r	100
jln0129	jln0129-f	jln0129-r	111
jln0132	jln0132-f	jln0132-r	124
jln0132b	jln0132b-f	jln0132b-r	90
jln0201	jln0201-f	jln0201-r	124
jln0202	jln0202-f	jln0202-r	95
jln0207	jln0207-f	jln0207-r	121
jln0208	jln0208-f	jln0208-r	124
jln0210	jln0210-f	jln0210-r	93
jln0213	jln0213-f	jln0213-r	128
jln0214	jln0214-f	jln0214-r	127
jln0217	jln0217-f	jln0217-r	99
jln0219	jln0219-f	jln0219-r	96
jln0223	jln0223-f	jln0223-r	127
jln0225	jln0225-f	jln0225-r	131
jln0226	jln0226-f	jln0226-r	95
jln0227	jln0227-f	jln0227-r	120
jln0230	jln0230-f	jln0230-r	122
jln0231	jln0231-f	jln0231-r	116
jln0235	jln0235-f	jln0235-r	120
jln0236	jln0236-f	jln0236-r	93
jln0236b	jln0236b-f	jln0236b-r	89
mel425	mel425-f	mel425-r	148
mel426	mel426-f	mel426-r	122
mel427	mel427-f	mel427-r	148
mel427(2)	mel427-f	mel427-r(2)	116
mel428	mel428-f	mel428-r	140
mel429	mel429-f	mel429-r	101
mel430	mel430-f	mel430-r	120

Table A-2:

Assay Name	Primer 1 Name	Primer 2 Name	Predicted Product Length
mel431	mel431-f	mel431-r	119
mel432	mel432-f	mel432-r	168
mel433	mel433-f	mel433-r	124
mel434	mel434-f	mel434-r	154
mel435	mel435-f	mel435-r	160
mel436	mel436-f	mel436-r	118
npv1	npv1-f	npv1-r	128
ratca3027-1	ratca3027-1-f	ratca3027-1-r	174
ratca3027-2	ratca3027-2-f	ratca3027-2-r	159
ratca3031-1	ratca3031-1-f	ratca3031-1-r	141
ratca3105-1	ratca3105-1-f	ratca3105-1-r	123
rmo0115	rmo0115-f	rmo0115-r	101
rmo0115b	rmo0115b-f	rmo0115b-r	135
rmo0124	rmo0124-f	rmo0124-r	99
rmo118for	rmo118for-f	rmo118for-r	107
rmo118rev	rmo118rev-f	rmo118rev-r	132
ryac0309	ryac0309-f	ryac0309-r	
ryac0327	ryac0327-f	ryac0327-r	
ryac0330	ryac0330-f	ryac0330-r	
ryac0335	ryac0335-f	ryac0335-r	
ryac2a01	ryac2a01-f	ryac2a01-r	
ryac2a11	ryac2a11-f	ryac2a11-r	
ryac2b01	ryac2b01-f	ryac2b01-r	
ryac2b06	ryac2b06-f	ryac2b06-r	
ryac2c02	ryac2c02-f	ryac2c02-r	
ryac2c06	ryac2c06-f	ryac2c06-r	
ryac2c07	ryac2c07-f	ryac2c07-r	
ryac2e01	ryac2e01-f	ryac2e01-r	
ryac2e03	ryac2e03-f	ryac2e03-r	
ryac2e04	ryac2e04-f	ryac2e04-r	
ryac2e05	ryac2e05-f	ryac2e05-r	
ryac2f01	ryac2f01-f	ryac2f01-r	
ryac2f02	ryac2f02-f	ryac2f02-r	
ryac2f09	ryac2f09-f	ryac2f09-r	

Table A-3: B6-ob x cast cross full typings (see Chapter II)

B6-ob x DNA	D6Nds4	D6Mit42	R236	MPC1305 D6Mit76	MPC1683 D6Mit75	D6Mit43-x	MPC1322 D6Mit74	17 L2H	17 R2H	D6Mit33	MPC I32 D6Mit72
136	3	3	3	3	3	3	3	3	3	3	1
138	1	1	1	1	1	1	1	1	1	1	3
139	1	1	1	1	1	1	1	1	1	1	3
140	3	3	-	3	3	3	3	3	3	3	2
146	1	1	1	1	1	1	1	1	1	1	3
157	1	3	3	3	3	3	3	3	3	3	3
169	1	1	1	1	1	1	1	1	1	1	3
181	1	1	1	1	1	1	1	1	1	1	3
183	1	1	1	1	1	1	3	3	3	3	3
194	1	1	-	1	1	1	3	3	3	3	3
195	1	1	-	1	1	1	1	1	1	1	3
208	1	1	-	1	1	1	1	1	1	3	3
214	1	1	-	1	1	1	1	1	1	1	3
215	1	1	3	3	3	3	-	3	3	3	3
218	1	1	3	3	3	3	3	3	3	3	2
239	1	1	-	1	1	1	1	3	3	3	3
255	1	1	-	1	1	1	1	1	1	1	3
256	1	1	-	1	1	1	?	1	1	1	3
267	1	1	-	3	3	3	3	3	3	3	3
273	1	1	1	-	1	1	1	1	1	1	3
288	1	1	-	1	1	1	1	1	1	1	3
289	1	3	-	3	3	3	3	3	3	3	3
297	1	1	-	1	1	1	1	1	1	1	3
305	1	1	-	1	1	1	3	3	3	3	3
306	1	1	-	3	3	3	3	3	3	3	3
309	1	3	-	3	3	3	3	3	3	3	3
314	1	1	-	1	1	1	1	1	1	1	3
322	1	1	-	1	1	1	1	1	1	1	3
334	1	1	-	1	1	1	1	1	1	1	3
340	1	1	-	1	1	1	1	1	-	1	3
119	1	1	-	1	1	1	1	3	3	3	3
121	1	1	-	1	1	1	1	3	3	3	3
126	1	1	-	1	1	1	1	1	1	1	3
144	1	1	-	3	3	3	3	3	3	3	3
188	1	1	1	1	1	1	1	1	1	1	3
210	3	3	3	3	3	3	2	2	2	2	2
233	1	1	-	1	1	1	1	1	1	1	3
251	1	3	3	3	3	3	3	3	3	3	3
339	1	1	-	1	1	1	3	3	3	3	3
344	1	1	-	1	1	1	1	-	1	1	3

121-453000

Table B-1: Genomic clones used in project.

Genomic Clone Name	Clone Type	Species	Strain	Source Clone	Source PCR Assay
1	cosmid	mouse	B6	YAC 45	
10	cosmid	mouse	B6	YAC 45	
100	cosmid	mouse	B6	YAC 45	
102	cosmid	mouse	B6	YAC 45	
104	cosmid	mouse	B6	YAC 45	
105	cosmid	mouse	B6	YAC 45	
106	cosmid	mouse	B6	YAC 45	
107	cosmid	mouse	B6	YAC 45	
108	cosmid	mouse	B6	YAC 45	
109	cosmid	mouse	B6	YAC 45	
11	cosmid	mouse	B6	YAC 45	
110	cosmid	mouse	B6	YAC 45	
111	cosmid	mouse	B6	YAC 45	
112	cosmid	mouse	B6	YAC 45	
113	cosmid	mouse	B6	YAC 45	
114	cosmid	mouse	B6	YAC 45	
115	cosmid	mouse	B6	YAC 45	
116	cosmid	mouse	B6	YAC 45	
117	cosmid	mouse	B6	YAC 45	
118	cosmid	mouse	B6	YAC 45	
119	cosmid	mouse	B6	YAC 45	
12	cosmid	mouse	B6	YAC 45	
120	cosmid	mouse	B6	YAC 45	
121	cosmid	mouse	B6	YAC 45	
122	cosmid	mouse	B6	YAC 45	
123	cosmid	mouse	B6	YAC 45	
124	cosmid	mouse	B6	YAC 45	
125	cosmid	mouse	B6	YAC 45	
126	cosmid	mouse	B6	YAC 45	
127	cosmid	mouse	B6	YAC 45	
129	cosmid	mouse	B6	YAC 45	
131	cosmid	mouse	B6	YAC 45	
135	cosmid	mouse	B6	YAC 45	
137	cosmid	mouse	B6	YAC 45	
14	cosmid	mouse	B6	YAC 45	

Table B-1: Genomic clones used in project.

Genomic Clone Name	Clone Type	Species	Strain	Source Clone	Source PCR Assay
142	cosmid	mouse	B6	YAC 45	
143	cosmid	mouse	B6	YAC 45	
144	cosmid	mouse	B6	YAC 45	
15	cosmid	mouse	B6	YAC 45	
16	cosmid	mouse	B6	YAC 45	
17	cosmid	mouse	B6	YAC 45	
18	cosmid	mouse	B6	YAC 45	
19	cosmid	mouse	B6	YAC 45	
20	cosmid	mouse	B6	YAC 45	
22	cosmid	mouse	B6	YAC 45	
4011-2	cosmid	rat	WKY		
4342G	P1	mouse	ES		ajm2705
4343G	P1	mouse	ES		ajm2705
4344G	P1	mouse	ES		ajm2705
4372G	P1	mouse	ES		ajm3302
4373G	P1	mouse	ES		ajm3302
4374G	P1	mouse	ES		ajm3302
4746G	P1	rat			4011_2t3
4747G	P1	rat			4011_2t3
4748G	P1	rat			4011_2t3
4749G	P1	rat			4011_2t3
4779G	P1	mouse	ES		ajm4029
4825G	P1	mouse	ES		ajm3935
4826G	P1	mouse	ES		ajm3935
4827G	P1	mouse	ES		ajm4133
4828G	P1	mouse	ES		ajm4133
4829G	P1	mouse	ES		ajm4133
4866G	P1	mouse	ES		ajm4313
4997G	P1	mouse	ES		ajm4313
5	cosmid	mouse	B6	YAC 45	
5171G	P1	mouse	ES		ajm3935
5578G	P1	rat			ajm5518
5579G	P1	rat			ajm5506
5580G	P1	rat			ajm5506
5621G	P1	rat			ajm5610

Table B-1: Genomic clones used in project.

Genomic Clone Name	Clone Type	Species	Strain	Source Clone	Source PCR Assay
5622G	P1	rat			ajm5610
5631G	P1	rat			ajm5513
5632G	P1	rat			ajm5513
5633G	P1	rat			ajm5515
5634G	P1	rat			ajm5515
5791G	P1	rat			ajm5507
5792G	P1	rat			ajm5507
5793G	P1	rat			ajm5507
5807G	P1	mouse	ES		ajm5414
5808G	P1	mouse	ES		ajm5414
5883G	P1	mouse	ES		ajm3809
5884G	P1	mouse	ES		ajm3809
5885G	P1	mouse	ES		ajm3809
5984G	P1	mouse	ES		ajm5403
5985G	P1	mouse	ES		ajm5403
6112G	P1	mouse	ES		ajm3804
6113G	P1	mouse	ES		ajm3804
6114G	P1	mouse	ES		ajm3808
6115G	P1	mouse	ES		ajm3808
6116G	P1	mouse	ES		ajm3808
6117G	P1	mouse	ES		ajm3810
6118G	P1	mouse	ES		ajm3810
6119G	P1	mouse	ES		ajm3810
6120G	P1	mouse	ES		34 R2A
6121G	P1	mouse	ES		34 R2A
6122G	P1	mouse	ES		34 R2A
6123G	P1	mouse	ES		34 R2A
6239G	P1	mouse	ES		ajm6124
6240G	P1	mouse	ES		ajm6124
6241G	P1	mouse	ES		ajm6006
6242G	P1	mouse	ES		ajm6006
6243G	P1	mouse	ES		ajm6006
6350G	P1	mouse	ES		D6Mit74
6351G	P1	mouse	ES		D6Mit76
6450G	P1	mouse	non-ES		D6Mit43

Table B-1: Genomic clones used in project.

Genomic Clone Name	Clone Type	Species	Strain	Source Clone	Source PCR Assay
6451G	P1	mouse	non-ES	YAC 45	D6Mit43
7	cosmid	mouse	B6		
7134G	P1	rat			ajm5611
7155G	P1	rat			rmo0124
7234G	P1	mouse	?		ajm3913
7235G	P1	mouse	?		ajm3913
7236G	P1	mouse	?		ajm3913
7237G	P1	mouse	?		ajm3913
7425G	P1	rat			ajm5602b
7527G	P1	rat			ajm5602b
8	cosmid	mouse	B6	YAC 45	
9	cosmid	mouse	B6	YAC 45	
97	cosmid	mouse	B6	YAC 45	
98	cosmid	mouse	B6	YAC 45	
99	cosmid	mouse	B6	YAC 45	
YAC 31	YAC	mouse	B6		
YAC 34	YAC	mouse	B6		
YAC 35	YAC	mouse	B6		
YAC 36	YAC	mouse	B6		
YAC 43	YAC	mouse	B6		
YAC 44	YAC	mouse	B6		
YAC 45	YAC	mouse	B6		
YAC 56	YAC	mouse	B6		
YAC 68	YAC	mouse	B6		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
0-13-3	PCR Product	Phage Vector<->Vector PCR	Mouse		
0-33-2	PCR Product	Phage Vector<->Vector PCR	Mouse		
1-a1	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-a7	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-b2	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-b7	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-c2	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-c8	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-d11	PCR Product	Phage Vector<->Internal PCR	Mouse		
1-d2	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-a2	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-a3	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-d1	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-f1	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-g2	PCR Product	Phage Vector<->Internal PCR	Mouse		
10-g3	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-a10	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-a8	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-b2	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-b7	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-e3	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-e5	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-e8	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-f4	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-f7	PCR Product	Phage Vector<->Internal PCR	Mouse		
11-f8	PCR Product	Phage Vector<->Internal PCR	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
11-f9	PCR Product	Phage Vector<->Internal PCR	Mouse		
12-a3	PCR Product	Phage Vector<->Internal PCR	Rat		
12-c1	PCR Product	Phage Vector<->Internal PCR	Rat		
12-c2	PCR Product	Phage Vector<->Internal PCR	Rat		
12-c3	PCR Product	Phage Vector<->Internal PCR	Rat		
13-a1	PCR Product	Phage Vector<->Internal PCR	Rat		
13-a4	PCR Product	Phage Vector<->Internal PCR	Rat		
13-b1	PCR Product	Phage Vector<->Internal PCR	Rat		
13-b4	PCR Product	Phage Vector<->Internal PCR	Rat		
13-c1	PCR Product	Phage Vector<->Internal PCR	Rat		
13-c4	PCR Product	Phage Vector<->Internal PCR	Rat		
13-d4	PCR Product	Phage Vector<->Internal PCR	Mouse		
13-e1	PCR Product	Phage Vector<->Internal PCR	Mouse		
13-e4	PCR Product	Phage Vector<->Internal PCR	Mouse		
13-f4	PCR Product	Phage Vector<->Internal PCR	Mouse		
14-a2	PCR Product	Phage Vector<->Internal PCR	Rat		
14-a3	PCR Product	Phage Vector<->Internal PCR	Rat		
2-a10	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-a11	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-b11	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-c10	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-d12	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-e12	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-g11	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-g12	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-h10	PCR Product	Phage Vector<->Internal PCR	Mouse		
2-h11	PCR Product	Phage Vector<->Internal PCR	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
3-a1	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-a4	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-b2	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-c1	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-c4	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-d1	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-d4	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-e1	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-e4	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-f2	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-f3	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-g2	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-g3	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-h1	PCR Product	Phage Vector<->Internal PCR	Mouse		
3-h4	PCR Product	Phage Vector<->Internal PCR	Mouse		
311408	cDNA Clone	Preexisting	Mouse		
331315	cDNA Clone	Preexisting	Mouse		
333406	cDNA Clone	Preexisting	Mouse		
350742	cDNA Clone	Preexisting	Mouse		
353492	cDNA Clone	Preexisting	Mouse		
354434	cDNA Clone	Preexisting	Mouse		
367880	cDNA Clone	Preexisting	Mouse		
368840	cDNA Clone	Preexisting	Mouse		
368958	Clone	Preexisting	Mouse		
374288	cDNA Clone	Preexisting	Mouse		
374606	cDNA Clone	Preexisting	Mouse		
374676	cDNA Clone	Preexisting	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
391537	cDNA Clone	Preexisting	Mouse		
4-a1	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-a12	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-a5	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-a7	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-b1	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-b4	PCR Product	Phage Vector<->Internal PCR	Mouse		
4-b9	PCR Product	Phage Vector<->Internal PCR	Mouse		
4011_2	Clone	Rat Cosmid Library Screening	Rat	r cDNA 4011	
406890	cDNA Clone	Preexisting	Mouse		
4372GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4372G	
4372GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4372G	
439918	Clone	Preexisting	Mouse		
442394	cDNA Clone	Preexisting	Mouse		
444010	cDNA Clone	Preexisting	Mouse		
444796	cDNA Clone	Preexisting	Mouse		
45 r2h	PCR Product	YAC End IPCR	Mouse	Whole YAC 45	
464649	cDNA Clone	Preexisting	Mouse		
4825GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4825G	
4825GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4825G	
4827GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4827G	
4829GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4829G	
4829GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4829G	
4866GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 4866G	
5-a1	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-b1	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-b4	PCR Product	Phage Vector<->Internal PCR	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
5-c2	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-c3	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-d4	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-e1	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-e4	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-f2	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-f3	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-g1	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-g3	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-h1	PCR Product	Phage Vector<->Internal PCR	Mouse		
5-h3	PCR Product	Phage Vector<->Internal PCR	Mouse		
554253	cDNA Clone	Preexisting	Mouse		
554569	cDNA Clone	Preexisting	Mouse		
5578ca-a	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-b	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-c	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-d	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-e	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-f	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
5578ca-h	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole P1 5578G	
575512	cDNA Clone	Preexisting	Mouse		
577485	cDNA Clone	Preexisting	Mouse		
577692	cDNA Clone	Preexisting	Mouse		
577842	cDNA Clone	Preexisting	Mouse		
5807GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 5807G	
5984/85ca-a	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	
5984/85ca-b	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
5984/85ca-c	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	
5984/85ca-d	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	
5984/85ca-e	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	
5984/85ca-f	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 5984G/5985G	
5985GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 5985G	
6-a2	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-a5	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-b3	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-b8	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-c4	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-c7	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-d2	PCR Product	Phage Vector<->Internal PCR	Mouse		
6-d5	PCR Product	Phage Vector<->Internal PCR	Mouse		
617642	cDNA Clone	Preexisting	Mouse		
6239GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6239G	
6241/42ca-b	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 6241G/6242G	
6241/42ca-c	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 6241G/6242G	
6241/42ca-f	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 6241G/6242G	
6241/42ca-g	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 6241G/6242G	
6241/42ca-h	Clone	Cosmid/P1 SSR Hybridization Subcloning	Mouse	Whole P1s 6241G/6242G	
6241GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6241G	
6241GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6241G	
6350GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6350G	
6350GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6350G	
6450GL	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6450G	
6450GR	PCR Product	P1 End IPCR (w/chimeric M13 primers)	Mouse	Whole P1 6450G	
7-a2	PCR Product	Phage Vector<->Internal PCR	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
7-a5	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-b2	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-b5	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-c2	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-c5	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-d2	PCR Product	Phage Vector<->Internal PCR	Mouse		
7-d5	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-a1	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-a11	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-a4	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-a9	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-c10	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-c4	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-d7	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-d9	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-e10	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-e4	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-f2	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-f7	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-g1	PCR Product	Phage Vector<->Internal PCR	Mouse		
8-g7	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-a4	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-b12	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-c11	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-c4	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-c6	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-d11	PCR Product	Phage Vector<->Internal PCR	Mouse		

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
9-f11	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-f6	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-g6	PCR Product	Phage Vector<->Internal PCR	Mouse		
9-h12	PCR Product	Phage Vector<->Internal PCR	Mouse		
R236	Clone	Preexisting	Rat		
R300	Clone	Preexisting	Rat		
ajm1501	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1502	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1503	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1504	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1505	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1506	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1507	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1508	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1509	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1510	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1511	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1512	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1513	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1514	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1515	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1516	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1517	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1518	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1519	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1520	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1521	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm1522	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1523	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1524	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1525	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1526	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1527	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1528	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1529	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1530	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1531	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1532	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1533	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1534	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1535	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1536	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1601	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1602	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1603	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1604	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1605	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1606	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1607	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1608	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1609	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1610	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1611	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1612	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm1613	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1614	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1615	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1616	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1617	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1618	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1619	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1620	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1621	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1622	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1623	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1624	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1701	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1702	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1703	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1704	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1705	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1706	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1707	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1708	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1709	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1710	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1711	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1712	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1713	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1714	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1715	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm1716	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1717	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1718	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1719	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1720	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1721	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1722	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1723	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1724	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 B	
ajm1725	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1726	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1727	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1728	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1729	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1730	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1731	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1732	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1733	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1734	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1735	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1736	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1801	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm1802	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1803	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1804	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1805	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1806	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm1807	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1808	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1809	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1810	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1811	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1812	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1813	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1814	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1815	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1816	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1817	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1818	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1819	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1820	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1821	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1822	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1823	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm1824	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2001	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2002	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2003	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2004	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2005	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2006	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2007	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2008	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2009	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm2010	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2011	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2012	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2013	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2014	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2015	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2016	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2017	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2018	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2019	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2020	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2021	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2022	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2023	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2024	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2025	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2026	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2027	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2028	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2029	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm203	Clone	Exon Trapping	Mouse	45 A	
ajm2030	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2031	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2032	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2033	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm207	Clone	Exon Trapping	Mouse	E1	
ajm210	Clone	Exon Trapping	Mouse	E1	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm2101	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2102	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2103	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2104	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2105	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2106	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2107	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2108	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2109	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2110	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2111	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2112	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2113	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm2114	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	45 A	
ajm214	Clone	Exon Trapping	Mouse	E1	
ajm221	Clone	Exon Trapping	Mouse	E1	
ajm2213	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2214	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2215	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2216	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2217	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2218	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2219	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2220	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2221	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2222	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2223	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm2224	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm224	Clone	Exon Trapping	Mouse	E1	
ajm2301	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2302	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2303	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2304	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2305	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2306	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2307	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2308	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2309	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2310	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2311	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2312	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm2313	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2314	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2315	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2316	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2317	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2318	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2319	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2320	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2321	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2322	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2323	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2324	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2325	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm2326	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2327	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2328	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2329	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2330	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2331	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2332	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2333	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2334	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2335	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2336	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2401	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2403	Clone	Exon Trapping	Mouse	LA	
ajm2410	Clone	Exon Trapping	Mouse	LA	
ajm2501	Clone	Exon Trapping	Mouse	LA	
ajm2502	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2503	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2504	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2505	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2506	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2507	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2508	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2509	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2510	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2511	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2512	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2513	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm2514	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2515	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2516	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2517	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2518	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2519	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2520	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2521	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2522	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2523	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2524	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2606	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2619	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2620	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2621	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2622	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2623	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2624	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2625	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2626	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2627	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2628	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2629	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2630	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2701	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2702	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2703	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm2704	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2705	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2706	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2707	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2708	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2709	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2710	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2711	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2712	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2713	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2714	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2715	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2716	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2717	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2718	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2719	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2720	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2721	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2722	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2723	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2724	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm2801	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2802	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2803	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2804	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2805	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2806	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm2807	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2808	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2809	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2810	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2811	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2812	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2813	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2814	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2815	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2816	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2817	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2818	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2819	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2820	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2821	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2822	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2823	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm2824	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm3001	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 45	
ajm3002	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3003	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3004	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3005	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3006	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3007	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3008	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3009	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3010	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3011	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3012	Clone	Mouse->Rat cDNA/cDNA Selection, 65C	Rat	m cDNA 1	
ajm3013	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3014	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3015	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3016	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3017	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3018	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3019	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3020	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3021	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3022	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3023	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3024	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3025	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3026	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3027	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3028	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3029	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm303	Clone	Exon Trapping	Mouse	F	
ajm3030	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3031	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3032	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3033	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3034	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3035	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3036	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm305	Clone	Exon Trapping	Mouse	F	
ajm306	Clone	Exon Trapping	Mouse	F	
ajm307	Clone	Exon Trapping	Mouse	F	
ajm308	Clone	Exon Trapping	Mouse	F	
ajm310	Clone	Exon Trapping	Mouse	F	
ajm3101	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3102	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3103	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3104	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3105	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3106	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3107	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3108	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3109	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3110	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3111	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3112	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3113	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3114	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3115	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3116	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3117	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3118	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3119	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3120	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3121	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3122	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3123	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm3124	Clone	Mouse->Rat cDNA/cDNA Selection, 55C	Rat	m cDNA 1	
ajm315	Clone	Exon Trapping	Mouse	F	
ajm3201	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3202	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3203	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3204	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3205	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3206	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3207	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 1	
ajm3208	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm3209	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm3210	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm3211	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm3212	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
ajm3301	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3302	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3303	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3304	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3305	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3306	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3307	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3308	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3309	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3310	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3311	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3312	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3313	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3314	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3315	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3316	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3317	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3318	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3319	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3320	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3321	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3322	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3323	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3324	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3325	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3326	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3327	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3328	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3329	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3330	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3331	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3332	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3333	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3334	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3335	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3336	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3701	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3702	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm3703	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3704	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3705	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3706	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3707	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3708	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3709	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3710	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3711	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3712	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3713	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3714	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3715	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3716	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3717	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3718	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3719	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3720	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3721	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3722	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3723	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3724	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3725	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3726	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3727	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3728	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
ajm3729	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3801	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3802	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3803	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3804	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3805	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3806	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3807	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3808	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3809	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3810	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3811	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3812	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3901	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3902	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3903	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3904	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3905	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3906	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3907	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3908	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3909	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3910	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3911	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3912	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm3913	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31, 35	
ajm3914	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31, 35	
ajm3915	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31, 35	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm3916	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3917	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3918	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3919	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3920	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3921	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3922	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3923	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3924	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3925	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3926	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3927	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3928	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3929	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3930	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3931	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3932	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3933	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3934	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3935	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm3936	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4001	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4002	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4003	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4004	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4005	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4006	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm4007	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4008	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4009	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm401	Clone	Exon Trapping	Mouse	F	
ajm4010	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4011	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4012	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4013	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4014	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4015	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4016	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4017	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4018	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm4019	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 2	
ajm402	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4020	Clone	Exon Trapping	Mouse	F	
ajm4021	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4022	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4023	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4024	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4025	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4026	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4027	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4028	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4029	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4030	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4031	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm4032	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4033	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4034	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4035	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4036	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm409	Clone	Exon Trapping	Mouse	F	
ajm410	Clone	Exon Trapping	Mouse	F	
ajm4101	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4102	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4103	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4104	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4105	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4106	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4107	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4108	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4109	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4110	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4111	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4112	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4113	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4114	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4115	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4116	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4117	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4118	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 68	
ajm4119	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm412	Clone	Exon Trapping	Mouse	F	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm4120	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4121	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4122	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4123	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4124	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4125	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4126	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4127	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4128	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4129	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm413	Clone	Exon Trapping	Mouse	F	
ajm4130	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4131	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4132	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4133	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4134	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4135	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4136	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm419	Clone	Exon Trapping	Mouse	F	
ajm422	Clone	Exon Trapping	Mouse	F	
ajm4221	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4222	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4223	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4224	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4225	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4226	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4227	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm4228	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4229	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 3	
ajm4301	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4302	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4303	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4304	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4305	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4306	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4307	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4308	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4309	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4310	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4311	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4312	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm4313	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole rat cosmid 4011-2	
ajm4314	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole rat cosmid 4011-2	
ajm4315	Clone	Cosmid/P1 SSR Hybridization Subcloning	Rat	Whole rat cosmid 4011-2	
ajm4316	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4317	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4318	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4319	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4320	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm4321	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm501	Clone	Exon Trapping	Mouse	G	
ajm5012	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm5015	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm5018	Clone	YAC random subclone	Mouse	Whole YAC 68	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm502	Clone	Exon Trapping	Mouse	G	
ajm5024	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm5029	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm503	Clone	Exon Trapping	Mouse	G	
ajm5030	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm5031	Clone	YAC random subclone	Mouse	Whole YAC 68	
ajm504	Clone	Exon Trapping	Mouse	G	
ajm505	Clone	Exon Trapping	Mouse	G	
ajm506	Clone	Exon Trapping	Mouse	G	
ajm507	Clone	Exon Trapping	Mouse	G	
ajm508	Clone	Exon Trapping	Mouse	G	
ajm509	Clone	Exon Trapping	Mouse	G	
ajm510	Clone	Exon Trapping	Mouse	G	
ajm511	Clone	Exon Trapping	Mouse	H	
ajm512	Clone	Exon Trapping	Mouse	H	
ajm513	Clone	Exon Trapping	Mouse	H	
ajm514	Clone	Exon Trapping	Mouse	H	
ajm515	Clone	Exon Trapping	Mouse	H	
ajm516	Clone	Exon Trapping	Mouse	H	
ajm517	Clone	Exon Trapping	Mouse	H	
ajm518	Clone	Exon Trapping	Mouse	H	
ajm519	Clone	Exon Trapping	Mouse	H	
ajm520	Clone	Exon Trapping	Mouse	H	
ajm5201	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm5202	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm5203	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm5204	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm5205	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5206	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5207	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5209	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm521	Clone	Exon Trapping	Mouse	H	
ajm5212	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5213	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5214	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5215	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5218	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5219	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm522	Clone	Exon Trapping	Mouse	H	
ajm5220	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5221	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5222	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5223	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5224	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5225	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5226	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5227	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5228	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5229	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm523	Clone	Exon Trapping	Mouse	H	
ajm524	Clone	Exon Trapping	Mouse	H	
ajm525	Clone	Exon Trapping	Mouse	E2	
ajm526	Clone	Exon Trapping	Mouse	E2	
ajm527	Clone	Exon Trapping	Mouse	E2	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm528	Clone	Exon Trapping	Mouse	E2	
ajm529	Clone	Exon Trapping	Mouse	E2	
ajm530	Clone	Exon Trapping	Mouse	E2	
ajm531	Clone	Exon Trapping	Mouse	E2	
ajm532	Clone	Exon Trapping	Mouse	E2	
ajm533	Clone	Exon Trapping	Mouse	E2	
ajm534	Clone	Exon Trapping	Mouse	E2	
ajm535	Clone	Exon Trapping	Mouse	E2	
ajm536	Clone	Exon Trapping	Mouse	E2	
ajm5401	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5402	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5403	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5404	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5405	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5406	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5407	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5408	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5409	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5410	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5411	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5412	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5413	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5414	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5415	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5416	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5417	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5418	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm5419	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5420	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5421	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5422	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5423	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5424	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5425	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5426	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5427	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5428	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5429	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5430	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5431	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5432	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5433	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5434	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5435	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5436	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5501	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5502	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5503	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5504	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5505	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5506	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5507	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5508	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5509	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm5510	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5511	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5512	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5513	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5514	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5515	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5516	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5517	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5518	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5519	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5520	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5521	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5522	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5523	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5524	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5601	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5602	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5603	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5604	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5605	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5606	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5607	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5608	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5609	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5610	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5611	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5612	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm5613	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5614	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5615	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5616	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5617	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5618	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5619	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5620	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5621	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5622	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5623	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5624	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5701	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5702	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5703	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm5704	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5705	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5706	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5707	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5708	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5709	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5710	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5711	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5712	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5713	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5714	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5715	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm5716	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5717	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5718	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5719	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5720	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5721	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5722	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5723	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5724	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5725	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5726	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5727	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5728	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5729	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5730	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5731	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5732	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5733	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5734	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5735	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5736	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5801	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5802	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5803	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5804	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5805	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5806	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm5807	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5808	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5809	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5810	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5811	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5812	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5813	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5814	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5815	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5816	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5817	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5818	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5819	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5820	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5821	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5822	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5823	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5824	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm5925	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5926	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5927	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5928	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5929	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5930	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5931	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5932	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5933	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm5934	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5935	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm5936	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
ajm6001	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6002	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6003	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6004	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6005	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6006	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6007	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6008	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6009	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm601	Clone	Exon Trapping	Mouse	F	
ajm6010	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm602	Clone	Exon Trapping	Mouse	F	
ajm603	Clone	Exon Trapping	Mouse	F	
ajm604	Clone	Exon Trapping	Mouse	F	
ajm605	Clone	Exon Trapping	Mouse	F	
ajm606	Clone	Exon Trapping	Mouse	F	
ajm607	Clone	Exon Trapping	Mouse	F	
ajm608	Clone	Exon Trapping	Mouse	E2	
ajm609	Clone	Exon Trapping	Mouse	E2	
ajm610	Clone	Exon Trapping	Mouse	E2	
ajm6109	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm611	Clone	Exon Trapping	Mouse	E2	
ajm6110	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
ajm6111	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ajm6112	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm612	Clone	Exon Trapping	Mouse	E2	
ajm6123	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm6124	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
ajm613	Clone	Exon Trapping	Mouse	E2	
ajm614	Clone	Exon Trapping	Mouse	E2	
ajm615	Clone	Exon Trapping	Mouse	E2	
ajm616	Clone	Exon Trapping	Mouse	E2	
ajm617	Clone	Exon Trapping	Mouse	E2	
ajm618	Clone	Exon Trapping	Mouse	E2	
ajm619	Clone	Exon Trapping	Mouse	E2	
ajm620	Clone	Exon Trapping	Mouse	E2	
ajm621	Clone	Exon Trapping	Mouse	E2	
ajm622	Clone	Exon Trapping	Mouse	E2	
ajm623	Clone	Exon Trapping	Mouse	E2	
ajm624	Clone	Exon Trapping	Mouse	E2	
ajm625	Clone	Exon Trapping	Mouse	E2	
ajm626	Clone	Exon Trapping	Mouse	E2	
ajm627	Clone	Exon Trapping	Mouse	G	
ajm628	Clone	Exon Trapping	Mouse	H	
ajm629	Clone	Exon Trapping	Mouse	H	
ajm630	Clone	Exon Trapping	Mouse	H	
ajm631	Clone	Exon Trapping	Mouse	H	
ajm632	Clone	Exon Trapping	Mouse	H	
ajm6325	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm6326	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm6327	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm6328	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm6329	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm633	Clone	Exon Trapping	Mouse	H	
ajm6330	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ajm634	Clone	Exon Trapping	Mouse	H	
ajm635	Clone	Exon Trapping	Mouse	H	
ajm636	Clone	Exon Trapping	Mouse	H	
ajm701	Clone	Exon Trapping	Mouse	I	
ajm702	Clone	Exon Trapping	Mouse	I	
ajm703	Clone	Exon Trapping	Mouse	I	
ajm704	Clone	Exon Trapping	Mouse	I	
ajm705	Clone	Exon Trapping	Mouse	I	
ajm706	Clone	Exon Trapping	Mouse	I	
ajm707	Clone	Exon Trapping	Mouse	I	
ajm708	Clone	Exon Trapping	Mouse	J	
ajm709	Clone	Exon Trapping	Mouse	J	
ajm710	Clone	Exon Trapping	Mouse	J	
ajm711	Clone	Exon Trapping	Mouse	J	
ajm712	Clone	Exon Trapping	Mouse	J	
ajm713	Clone	Exon Trapping	Mouse	J	
ajm714	Clone	Exon Trapping	Mouse	J	
ajm715	Clone	Exon Trapping	Mouse	J	
ajm716	Clone	Exon Trapping	Mouse	J	
ajm717	Clone	Exon Trapping	Mouse	J	
ajm718	Clone	Exon Trapping	Mouse	J	
ajm719	Clone	Exon Trapping	Mouse	K	
ajm720	Clone	Exon Trapping	Mouse	K	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ajm721	Clone	Exon Trapping	Mouse	H	
ajm722	Clone	Exon Trapping	Mouse	H	
ajm723	Clone	Exon Trapping	Mouse	H	
ajm724	Clone	Exon Trapping	Mouse	H	
bjr02	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr03	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr04	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr05	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr06	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr07	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr08	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr09	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr10	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr11	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr12	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr13	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr14	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 43	
bjr15	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr16	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr17	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr18	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr19	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr20	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr21	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr22	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr23	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
bjr24	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
bjr25	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
cDNA-a linker	Adapter	Preexisting	Other	None	
cDNA-b linker	Adapter	Preexisting	Other	None	
enxhum	Clone	Preexisting	Human		
enxmus	Clone	Preexisting	Mouse		
jln0101	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0102	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0103	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0104	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0105	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0106	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0107	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0108	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0109	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0110	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0111	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0112	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0113	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0114	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0115	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0116	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0117	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0118	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0119	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0120	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0121	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jln0122	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
jIn0123	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jIn0124	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 31,35	
jIn0125	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0126	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0127	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0128	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0129	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0130	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0131	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0132	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0201	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0202	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0203	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0204	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0205	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0206	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0207	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0208	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0209	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0210	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0211	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0212	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0213	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0214	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0215	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0216	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	
jIn0217	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34,36,56	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
jln0218	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0219	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0220	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0221	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0222	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0223	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0224	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0225	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0226	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0227	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0228	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0229	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0230	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0231	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0232	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0233	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0234	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0235	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
jln0236	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 34, 36, 56	
mel425	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel426	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel427	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel428	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel429	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel430	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel431	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel432	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
mel433	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel434	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel435	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
mel436	Clone	Mouse->Mouse genomic/cDNA Selection	Mouse	Whole YAC 44	
npv1	Clone	Preexisting	Rat		
npv2	Clone	Preexisting	Rat		
rmo-100	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-1000	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	3902/3907 probe pool
rmo-1001	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	3902/3907 probe pool
rmo-1002	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	3902/3907 probe pool
rmo-1003	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	3902/3907 probe pool
rmo-1004	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	3902/3907 probe pool
rmo-101	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-1010	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5606 probe pool
rmo-1011	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	rmo0115 probe pool
rmo-1012	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5518 probe pool
rmo-1013	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5513 probe pool
rmo-1014	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5507 probe pool
rmo-1015	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5513 probe pool
rmo-1016	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5513 probe pool
rmo-1017	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5513 probe pool
rmo-1018	Lambda Clone	Phage Library Screening	Rat	Rat thymus cDNA library	5513 probe pool
rmo-102	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-103	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-104	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-105	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool
rmo-106	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3907/3910/4121/... probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
rmo-107	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-108	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-109	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-110	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-111	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-112	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-113	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-114	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-115	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-116	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-117	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-118	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-120	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-121	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-122	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-123	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-124	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-125	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-126	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-127	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-128	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-129	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-130	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-131	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-132	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-133	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-134	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
rmo-135	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-136	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-137	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-138	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-139	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-140	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-141	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-142	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-143	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-144	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-145	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-146	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-147	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-148	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-149	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-150	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-151	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-152	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-153	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-154	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-155	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-156	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-157	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-158	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-159	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-160	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-161	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
rmo-162	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-163	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-164	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-165	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-166	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-167	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-168	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-169	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-170	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-171	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/3910/4121/... probe pool
rmo-180	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-181	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-182	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-183	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-184	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-185	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-186	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-187	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-188	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-189	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-190	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-191	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3808/3810/5206/... probe pool
rmo-200	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3902/3907 probe pool
rmo-201	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3902/3907 probe pool
rmo-202	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3902/3907 probe pool
rmo-203	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	5207/5221/5813 probe pool
rmo-204	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	5207/5221/5813 probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
rmo-205	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5207/5221/5813 probe pool
rmo-206	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-207	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-208	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-209	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-210	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-211	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5811/6005 probe pool
rmo-212	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	4126/5814 probe pool
rmo-213	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	4126/5814 probe pool
rmo-214	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	4126/5814 probe pool
rmo-215	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-216	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-217	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-218	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-219	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-220	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-221	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3921/4303/5704 probe pool
rmo-222	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3902/3907 probe pool
rmo-223	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5201/5421 probe pool
rmo-224	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5201/5421 probe pool
rmo-225	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5201/5421 probe pool
rmo-226	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5201/5421 probe pool
rmo-230	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3809/4315/4317 probe pool
rmo-231	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3809/4315/4317 probe pool
rmo-232	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5806 probe pool
rmo-233	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	5806 probe pool
rmo-234	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT)	3805 probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
rmo-235	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3805 probe pool
rmo-236	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3805 probe pool
rmo-237	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3804 probe pool
rmo-238	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3804 probe pool
rmo-239	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3804 probe pool
rmo-240	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	jin0202 probe pool
rmo-241	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3910/4121/5222 probe pool
rmo-242	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3910/4121/5222 probe pool
rmo-243	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3910/4121/5222 probe pool
rmo-244	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3910/4121/5222 probe pool
rmo-245	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-246	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-247	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-248	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-249	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-250	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-251	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-252	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3905/5433/5723/5820 probe pool
rmo-253	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-254	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-255	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-256	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-257	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-258	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4133/5415/5705 probe pool
rmo-300	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-301	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-302	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
rmo-303	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-304	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-305	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-306	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-307	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-308	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-309	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-310	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-311	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-312	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-313	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-314	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-315	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-316	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo-317	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3913/6006 probe pool
rmo0101	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0102	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0103	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0104	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0105	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0106	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0107	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0108	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0109	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0110	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0111	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0112	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
rmo0113	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0114	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0115	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0116	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0117	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0118	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0119	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0120	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0121	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0122	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0123	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0124	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0125	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0126	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0127	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0128	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0130	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0131	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0132	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0134	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0135	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
rmo0136	Clone	Mouse->Rat cDNA/cDNA Selection, 50C	Rat	m cDNA 4	
ro-10	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-11	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-12	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-13	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806 probe pool
ro-14	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/ Region/Lib	Probe Pool
ro-15	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-16	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-17	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-18	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-19	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-2	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/4308 probe pool
ro-20	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	4313 probe pool
ro-21	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-22	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-23	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-24	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	5821 probe pool
ro-25	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-26	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-27	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-28	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-29	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-3	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-30	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-31	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-33	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/4308 probe pool
ro-34	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-35	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-36	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	5821 probe pool
ro-4	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-5	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-6	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3806/3915/4128/... probe pool
ro-7	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/4308 probe pool

Table B-2: cDNA, exon, and other clones used in this work.

Fragment Name	Fragment Type	Source Procedure	Species Strain	Source Pool/Region/Lib	Probe Pool
ro-8	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	3907/4308 probe pool 3907/4308 probe pool
ro-9	Lambda Clone	Phage Library Screening	Mouse	Mouse thymus cDNA library (dT	
supercos1	Clone	Preexisting	Mouse	None	

APPENDIX C

Table C-1: Listings of DNA sequences comprising the "state" transcription units

alaska proj
Sequencher™ "alaska proj"

1 item.



ajm3935.psq

arizona proj
Sequencher™ "arizona proj"

1 item.



ajm5436.psq

california proj
Sequencher™ "california proj"

1 item.



ajm5402.psq







colorado proj
Sequencher™ "colorado proj"

Name	Size	Kind	Label	Last Modified	Comments
ajm4303.psq 5' end	112 BPs	DNA Fragment	-	Thu, Apr 16, 1998 3:52:44 AM	
ajm4307.psq 5' end	52 BPs	DNA Fragment	-	Thu, Apr 16, 1998 3:53:13 AM	
ajm5429.psq 5' end...	73 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:30:24 AM	
▼ Contig[0001]	724 BPs	Contig of 14	-	Fri, Apr 24, 1998 9:19:01 AM	
r_aa893270.psq	481 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:28:15 AM	
h_aa805383.psq...	259 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:45:22 AM	
h_aa078157.psq...	142 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:35:50 AM	
h_aa805383.psq...	95 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:48:03 AM	
h_aa358782.psq...	196 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:52:52 AM	
UT ajm3926.psq...	35 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:10:41 AM	
UT ajm3921.psq...	85 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:10:47 AM	
UT ajm7318.psq...	205 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:19:01 AM	
ajm5406.psq	249 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:26:35 AM	
aa013791.psq	344 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:26 PM	
w15896.psq	485 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:59:24 AM	
aa140380.psq	477 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:36 PM	
ajm5429.psq 5' e...	108 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:30:24 AM	
ajm5429.psq 3' e...	86 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:27:40 AM	
▼ Contig[0002]	824 BPs	Contig of 4	-	Thu, Apr 16, 1998 2:54:47 AM	
w50789.psq	315 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:40 PM	
ajm5427.psq	258 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:39 PM	
jln0103.psq	185 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:37 PM	
aa789830.psq	329 BPs	DNA Fragment	-	Thu, Apr 16, 1998 1:38:47 AM	
▼ Contig[0003]	42 BPs	Contig of 2	-	Thu, Apr 16, 1998 2:55:31 AM	
h_aa805383.psq...	42 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:48:03 AM	
h_aa358782.psq...	23 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:51:31 AM	
▼ Contig[0004]	135 BPs	Contig of 3	-	Thu, Apr 16, 1998 4:04:40 AM	
ajm4307.psq 3' e...	135 BPs	DNA Fragment	-	Thu, Apr 16, 1998 3:53:10 AM	
ajm4303.psq 3' e...	97 BPs	DNA Fragment	-	Thu, Apr 16, 1998 3:52:34 AM	
ajm7304.psq	59 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:02 PM	
h_aa078157.psq 5'...	91 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:35:50 AM	
h_aa358782.psq 3'...	139 BPs	DNA Fragment	-	Thu, Apr 16, 1998 2:52:52 AM	
▶ Left(25) Chimeric	1763 BPs	Contig of 27	-	Mon, Jan 13, 1997 11:16:39 AM	
▼ Middle(2)	1274 BPs	Contig of 7	-	Fri, Apr 10, 1998 12:26:53 AM	
ajm7432.psq	257 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:26:00 AM	
ajm3930.psq	256 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:26:53 AM	
ajm5821.psq	295 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:56:48 PM	
ajm7430.psq	525 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:20:18 AM	
ajm7431.psq	533 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:20:07 AM	
ajm7408.psq	398 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:20:30 AM	
ajm4134.psq	258 BPs	DNA Fragment	-	Fri, Apr 10, 1998 12:26:53 AM	
▼ Right(1)	870 BPs	Contig of 24	-	Sun, Apr 12, 1998 5:46:58 PM	
h_aa688229.psq	308 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:29 PM	
h_aa031699.psq	458 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
h_aa569335.psq	323 BPs	DNA Fragment	-	Sun, Apr 12, 1998 4:59:21 PM	






colorado proj
Sequencher™ "colorado proj"

Name	Size	Kind	Label	Last Modified	Comments
h_r72865.psq	430 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
h_r55505.psq	354 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
h_aa401910.psq	417 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:41:55 PM	
h_aa527779.psq	540 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:41:21 PM	
h_aa626717.psq	544 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
h_aa827707.psq	290 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
h_aa741451.psq	365 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:38:27 PM	
h_r55506.psq	368 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:25:04 PM	
h_r73337.psq	432 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:25:09 PM	
h_t23193.psq	89 BPs	DNA Fragment	-	Fri, Apr 10, 1998 1:05:58 AM	
h_r75712.psq	172 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:56:55 PM	
h_aa402070.psq	409 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:46:58 PM	
ajm5415.psq	202 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
w42309.psq	317 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
ajm5733.psq	223 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
aa789386.psq	262 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
aa014885.psq	506 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:33:26 PM	
ajm5705.psq	290 BPs	DNA Fragment	-	Sun, Apr 12, 1998 5:25:04 PM	
jln0236.psq	290 BPs	DNA Fragment	-	Fri, Apr 10, 1998 1:27:38 AM	
ajm6335.psq	350 BPs	DNA Fragment	-	Fri, Apr 10, 1998 1:28:31 AM	
ajm7409.psq	350 BPs	DNA Fragment	-	Fri, Apr 10, 1998 1:28:31 AM	
UT ajm3926.psq 5'...	108 BPs	DNA Fragment	-	Thu, Jan 16, 1997 3:44:32 PM	
UT ajm5414.psq	263 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:21 PM	
UT ajm5709.psq	215 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:20 PM	
UT Contig[0001]	955 BPs	Contig of 6	-	Fri, Apr 24, 1998 9:55:09 AM	
aa717327.psq	518 BPs	DNA Fragment	-	Fri, Apr 17, 1998 9:19:07 AM	
aa915439.psq	333 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:52:11 AM	
aa289466.psq	442 BPs	DNA Fragment	-	Fri, Apr 17, 1998 9:15:41 AM	
w13452.psq	423 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:17 PM	
ajm4306.psq	202 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:18 PM	
ajm7305.psq	307 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:17 PM	
UT Contig[0010]	228 BPs	Contig of 5	-	Fri, Apr 24, 1998 9:12:55 AM	
UT ajm7318.psq...	110 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:12:51 AM	
UT ajm3926.psq...	115 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:12:51 AM	
UT ajm3921.psq...	132 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:12:51 AM	
UT ajm7313.psq	228 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:12:51 AM	
UT ajm7316.psq	218 BPs	DNA Fragment	-	Fri, Apr 24, 1998 9:12:55 AM	







illinois proj
Sequencher™ "illinois proj"

Name	Size	Kind	Label	Last Modified	Comments
 ajm5805.psq 5' end	282 BPs	DNA Fragment	-	Thu, Jan 16, 1997 4:29:56 PM	
 ajm6719.psq	388 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:06:54 AM	
 ajm7111.psq	465 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:07:52 AM	
 ajm7412.psq	407 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:49 AM	
▼  Contig[0002]	783 BPs	Contig of 3	-	Wed, Apr 15, 1998 5:01:45 PM	
ajm7402.psq	344 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:01:45 PM	
ajm7424.psq	356 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:01:45 PM	
ajm7420.psq	515 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:56 AM	
▼  Contig[0011]	1322 BPs	Contig of 12	-	Mon, Oct 27, 1997 1:20:40 PM	
ajm6929.psq	618 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:20:40 PM	
ajm7013.psq	262 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:56 PM	
ajm6708.psq	334 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:54 PM	
ajm6709.psq	349 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:50 PM	
ajm7015.psq	530 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:18:36 PM	
ajm5805.psq 3' e...	54 BPs	DNA Fragment	-	Thu, Jan 16, 1997 4:29:56 PM	
ajm7016.psq	333 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:49 PM	
ajm6927.psq	602 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:50 PM	
ajm4121.psq	309 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:48 PM	
ajm6928.psq	543 BPs	DNA Fragment	-	Thu, Jan 16, 1997 4:37:25 PM	
ajm5823.psq	216 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:46 PM	
ajm7014.psq	416 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:55 PM	







indiana proj
Sequencher™ "indiana proj"

Name	Size	Kind	Label	Last Modified
 ajm3909.psq	223 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:05 PM
 ajm6910.psq	491 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:04 PM
▼  Contig[0001]	999 BPs	Contig of 10	-	Thu, Jul 31, 1997 12:45:18 PM
jams0310.psq 3'...	74 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:44:25 PM
jams0329.psq	367 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:44:25 PM
jams0222.psq	398 BPs	DNA Fragment	-	Thu, Jul 31, 1997 10:45:21 AM
jams0334.psq	298 BPs	DNA Fragment	-	Thu, Jul 31, 1997 10:41:36 AM
aa119529.psq 5'...	188 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:00:09 AM
aa119529.psq	375 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:02 PM
w59190.psq	133 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:03 PM
aa030273.psq	429 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:45:18 PM
aa450817.psq	340 BPs	DNA Fragment	-	Tue, Jul 29, 1997 6:00:39 PM
ajm7019.psq	366 BPs	DNA Fragment	-	Thu, Jul 31, 1997 10:48:13 AM
▼  Contig[0002]	848 BPs	Contig of 19	-	Thu, Jul 31, 1997 1:11:44 PM
jams0312.psq	356 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
jams0314.psq	356 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
ajm6918.psq	350 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
ajm6919.psq 5' e...	268 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:36:35 PM
ajm6110.psq	253 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
ajm6003.psq	198 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
ajm6901.psq	80 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:39:50 AM
ajm6917.psq 5' e...	371 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:40:46 PM
ajm6916.psq 5' e...	385 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:42:35 PM
ajm6915.psq	59 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:41:13 AM
jams0310.psq 5'...	366 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:40:44 PM
ajm6920.psq	65 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:30:00 PM
ajm6902.psq	308 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:41:43 PM
w53903.psq 3' end	65 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:34:06 PM
ajm5225.psq 5' e...	139 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:40:43 PM
ajm6916.psq 3' e...	54 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:05:48 PM
ajm5225.psq 3' e...	89 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:02:56 PM
ajm6917.psq 3' e...	182 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:57:51 AM
aa119529.psq 3'...	184 BPs	DNA Fragment	-	Thu, Jul 31, 1997 11:00:09 AM
▼  Contig[0003]	722 BPs	Contig of 8	-	Tue, Apr 14, 1998 5:44:27 PM
r_aa799859.psq	471 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:44:16 PM
r_aa874817.psq	337 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:44:16 PM
ajm6909.psq	326 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:44:23 PM
aa475218.psq	304 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:44:27 PM
ajm7232.psq	384 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:23:35 PM
ajm7017.psq	386 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:51:57 PM

indiana proj
Sequencher™ "indiana proj"

Name	Size	Kind	Label	Last Modified
aa154382.psq	422 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:08:38 PM
aa727853.psq	309 BPs	DNA Fragment	-	Tue, Apr 14, 1998 5:11:05 PM
▼  Contig[0004] (chim...	579 BPs	Contig of 5	-	Tue, Apr 14, 1998 6:41:15 PM
c87059.psq	497 BPs	DNA Fragment	-	Tue, Apr 14, 1998 6:41:15 PM
ajm7021.psq	430 BPs	DNA Fragment	-	Tue, Apr 14, 1998 6:40:42 PM
ajm6919.psq 3' e...	122 BPs	DNA Fragment	-	Tue, Apr 14, 1998 6:36:54 PM
aa612053.psq	430 BPs	DNA Fragment	-	Tue, Apr 14, 1998 6:37:53 PM
ajm7020.psq	509 BPs	DNA Fragment	-	Tue, Apr 14, 1998 6:41:15 PM
▼  Contig[0005]	496 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:22:40 PM
jams0327.psq	493 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:24 PM
jams0326.psq	496 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:23 PM
▼  Contig[0006] (chim...	473 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:22:40 PM
ajm6903.psq	469 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:01 PM
ajm7231.psq	368 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:13 PM
▼  Contig[0007]	735 BPs	Contig of 5	-	Tue, Apr 14, 1998 7:40:00 PM
jams0311.psq	341 BPs	DNA Fragment	-	Tue, Apr 14, 1998 7:39:26 PM
jams0309.psq	338 BPs	DNA Fragment	-	Tue, Apr 14, 1998 7:39:26 PM
aa245283.psq	439 BPs	DNA Fragment	-	Tue, Apr 14, 1998 7:40:00 PM
jams0313.psq	415 BPs	DNA Fragment	-	Tue, Apr 14, 1998 7:40:00 PM
jams0315.psq	420 BPs	DNA Fragment	-	Tue, Apr 14, 1998 7:40:00 PM
▼  Contig[0008]	569 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:22:40 PM
jams0223.psq	569 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:16 PM
ajm6908.psq	511 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:58:02 PM
▼  w53903.psq 5' end	21 BPs	DNA Fragment	-	Thu, Jul 31, 1997 12:34:06 PM


iowa proj
Sequencher™ "iowa proj"

Name	Size	Kind	Label	Last Modified	Comments
 ajm6735.psq	465 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:06:57 AM	
▼  Contig[0001]	943 BPs	Contig of 5	-	Wed, Apr 15, 1998 3:46:31 PM	
ajmtb7.psq	219 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:19 PM	
ajm6720.psq	227 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:21 PM	
ajm5222.psq	207 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:22 PM	
ajm6930.psq	433 BPs	DNA Fragment	-	Wed, Apr 15, 1998 3:46:31 PM	
ajm7006.psq	523 BPs	DNA Fragment	-	Wed, Apr 15, 1998 3:46:21 PM	
▼  Contig[0002] (chim...	741 BPs	Contig of 2	-	Wed, Apr 15, 1998 3:47:24 PM	
ajm6710.psq	325 BPs	DNA Fragment	-	Wed, Apr 15, 1998 3:47:24 PM	
ajm7005.psq	508 BPs	DNA Fragment	-	Wed, Apr 15, 1998 3:47:24 PM	
▼  Contig[0003]	400 BPs	Contig of 2	-	Thu, Apr 16, 1998 4:28:04 PM	
ajm6707.psq	322 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:06:48 AM	
aa560646.psq	140 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:25:23 PM	
▼  Contig[0004]	571 BPs	Contig of 2	-	Thu, Apr 16, 1998 4:40:18 PM	
ajm6718.psq	356 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:06:53 AM	
aa450576.psq	404 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:37:49 PM	
▼  Contig[0005]	507 BPs	Contig of 3	-	Thu, Apr 16, 1998 4:55:51 PM	
aa111560.psq	232 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:53:20 PM	
aa125270.psq	410 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:51:14 PM	
ajm6734.psq	368 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:50:41 PM	


kansas proj
Sequencher™ "kansas proj"

Name	Size	Kind	Label	Last Modified	Comments
ajm3905.psq (Tope...	205 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:35 PM	
ajm5723.psq (Wichi...	258 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:37 PM	
ajm5820.psq (Salina)	224 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:38 PM	

kentucky proj
Sequencher™ "kentucky proj"

Name	Size	Kind	Label	Last Modified		Comments
▼  Contig[0001]	669 BPs	Contig of 5	-	Wed, Jan 8, 1997	5:26:11 PM	
aa103490.psq	402 BPs	DNA Fragment	-	Wed, Jan 8, 1997	4:59:49 PM	
w64104.psq	451 BPs	DNA Fragment	-	Wed, Jan 8, 1997	4:59:48 PM	
w59428.psq	595 BPs	DNA Fragment	-	Wed, Jan 8, 1997	4:59:47 PM	
ajm4124.psq	275 BPs	DNA Fragment	-	Wed, Jan 8, 1997	4:59:41 PM	
jln0214.psq	239 BPs	DNA Fragment	-	Wed, Jan 8, 1997	4:59:40 PM	

michigan proj
Sequencher™ "michigan proj"

<u>Name</u>	<u>Size</u>	<u>Kind</u>	<u>Label</u>	<u>Last Modified</u>
▼  Contig[0001]	204 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:27:16 PM
ajm4125.psq	188 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:51 PM
ajm4123.psq	204 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:50 PM

mississippi proj
Sequencher™ "mississippi proj"

1 item.








ajm4122.psq


missouri proj
Sequencher™ "missouri proj"

Name	Size	Kind	Label	Last Modified
⌵ A380611-F	20 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:50:10 PM
⌵ ajm3907.psq	259 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:05 PM
⌵ ajm5805.psq 5' end	282 BPs	DNA Fragment	-	Thu, Jan 16, 1997 4:29:56 PM
⌵ ajm7410.psq	402 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:20 PM
⌵ ajm7411.psq (chim...	414 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:19 PM
⌵ ajm7412.psq	407 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:49 A
⌵ ajm7417.psq	520 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:18 PM
⌵ ajm7418.psq 5' end...	16 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:40:25 A
▼ [C] Contig[0001]	256 BPs	Contig of 4	-	Thu, Apr 16, 1998 3:55:20 PM
ajm3902.psq	256 BPs	DNA Fragment	-	Wed, Jan 8, 1997 6:00:04 PM
ajm7224.psq	91 BPs	DNA Fragment	-	Thu, Apr 16, 1998 3:55:20 PM
ajm7125.psq	117 BPs	DNA Fragment	-	Wed, Jan 8, 1997 6:00:00 PM
ajm7501.psq	119 BPs	DNA Fragment	-	Wed, Jan 8, 1997 6:00:02 PM
▼ [C] Contig[0002]	784 BPs	Contig of 3	-	Wed, Apr 15, 1998 5:01:45 PM
ajm7492.psq	344 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:01:45 PM
ajm7424.psq	356 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:01:45 PM
ajm7420.psq	515 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:56 A
▼ [C] Contig[0003] (chim...	1709 BPs	Contig of 26	-	Thu, Apr 16, 1998 12:45:46 A
h_aa329701.psq...	89 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:31:28 A
h_aa393259.psq	456 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
h_t59126.psq	354 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:37:11 A
h_aa310726.psq	316 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:21:55 PM
h_w03104.psq	282 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
aa570958.psq	418 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
aa103043.psq	462 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
w41876.psq	325 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
w42385.psq	431 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:43:09 A
ajm7418.psq 3' e...	484 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:40:25 A
aa591558.psq	238 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:00:49 A
h_n72879.psq	489 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:24 PM
h_aa282388.psq	535 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:37:35 PM
h_aa329701.psq...	242 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:31:28 A
h_aa398517.psq	516 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:29:50 PM
h_aa857091.psq	417 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:41:13 PM
h_t59050.psq	367 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:16:04 A
h_aa227558.psq	406 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:28:32 A
h_aa227697.psq	419 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:45:48 PM
h_t31886.psq	321 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:49:05 PM
h_aa282045.psq	427 BPs	DNA Fragment	-	Wed, Apr 15, 1998 5:48:03 PM
h_aa504428.psq	480 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:26:37 A

missouri proj
Sequencher™ "missouri proj"

Name	Size	Kind	Label	Last Modified
h_aa806462.psq	180 BPs	DNA Fragment	-	Wed, Apr 15, 1998 11:58:10 P
h_aa907813.psq	386 BPs	DNA Fragment	-	Wed, Apr 15, 1998 11:55:26 P
h_z38899.psq	235 BPs	DNA Fragment	-	Wed, Apr 15, 1998 11:58:55 P
h_aa748777.psq	208 BPs	DNA Fragment	-	Thu, Apr 16, 1998 12:00:06 A
▼  Contig[0004]	75 BPs	Contig of 2	-	Wed, Jan 15, 1997 2:10:18 PM
ajm7609.psq	58 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:16 A
ajm7113.psq	75 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:07:54 A
▼  Contig[0005]	342 BPs	Contig of 2	-	Fri, Apr 24, 1998 2:45:23 PM
ajm3803.psq	324 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:45:23 PM
ajm5207.psq	166 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:45:23 PM
▼  Contig[0007]	1335 BPs	Contig of 10	-	Fri, Apr 24, 1998 3:00:18 PM
ajm7109.psq	364 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:46:24 PM
ajm6721.psq	306 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:44:12 PM
jams0324.psq	588 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:48:47 PM
ajm7011.psq	493 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:48:52 PM
ajm5813.psq 5' e...	165 BPs	DNA Fragment	-	Thu, Jan 16, 1997 7:46:17 PM
ajm6736.psq	417 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:59:53 PM
jams0323.psq	385 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:59:51 PM
ajm5813.psq 3' e...	107 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:50:01 PM
ajm7012.psq	439 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:59:51 PM
jams0328.psq	266 BPs	DNA Fragment	-	Fri, Apr 24, 1998 3:00:18 PM
▼  Contig[0012]	681 BPs	Contig of 8	-	Mon, Oct 27, 1997 1:28:49 PM
ajm7406.psq	370 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:25:20 PM
ajm6333.psq	451 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:25:18 PM
ajm7427.psq	378 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:26:10 PM
ajm3806.psq	296 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:27:41 PM
ajm7428.psq	384 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:28:49 PM
ajm3910.psq	269 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:57:22 PM
A380611-R	20 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:50:32 PM
A380612	19 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:50:50 PM
▼  Contig[0013]	435 BPs	Contig of 5	-	Mon, Oct 27, 1997 1:30:04 PM
ajm7415.psq	434 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:52 A
ajm6334.psq	435 BPs	DNA Fragment	-	Mon, Oct 27, 1997 1:30:04 PM
A380614-R	20 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:51:46 PM
A380614-F	20 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:51:30 PM
A380613	20 BPs	DNA Fragment	-	Sun, Oct 12, 1997 5:51:10 PM

montana proj
Sequencher™ "montana proj"

Name	Size	Kind	Label	Last Modified	Comments
 ajm4133.psq	228 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:27 PM	

nebraska proj
Sequencher™ "nebraska proj"

Name	Size	Kind	Label	Last Modified	Comments
ajm5201.psq (Omaha)	280 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:46 PM	
ajm5421.psq (Linco...	265 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:00:47 PM	

nevada proj
Sequencher™ "nevada proj"

1 item.















ajm5708.psq

new mexico proj
Sequencher™ "new mexico proj"

Name	Size	Kind	Label	Last Modified	Comments
ajm5704.psq (Albuq...	342 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:07 PM	
ajm5928.psq (Las C...	253 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:00 PM	
ajm7303.psq (chim...	305 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:26 AM	
ajm7312.psq (chim...	330 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:31 AM	
ajm7320.psq (chim...	135 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:04:40 AM	
ajm7323.psq (chim...	434 BPs	DNA Fragment	-	Wed, Jan 15, 1997 5:09:27 PM	

north carolina proj
Sequencher™ "north carolina proj"

Name	Size	Kind	Label	Last Modified
 ajm3804.psq	325 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:13 PM
 ajm3809.psq	183 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:09 PM
 ajm4315.psq	219 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:11 PM
 ajm4317.psq	235 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:12 PM
 ajm7114.psq	467 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:07:55 A
 ajm7115.psq	439 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:07:56 A
 ajm7116.psq	453 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:07:57 A
 ajm7126.psq	403 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:00 A
 ajm7127.psq	406 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:01 A
 ajm7128.psq	394 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:02 A
 Contig[0001]	581 BPs	Contig of 5	-	Thu, Apr 16, 1998 4:38:46 AM
ajm7219.psq	422 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:24 PM
ajm7220.psq	416 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:37:10 AM
ajm5814.psq	272 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:08 PM
ajm7218.psq	227 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:38:46 AM
ajm7217.psq	220 BPs	DNA Fragment	-	Thu, Apr 16, 1998 4:38:46 AM
 Contig[0002]	743 BPs	Contig of 3	-	Wed, Jan 15, 1997 5:23:21 PM
ajm7222.psq	405 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:26 PM
ajm4126.psq	315 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:10 PM
ajm7221.psq	420 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:17 A

north dakota proj
Sequencher™ "north dakota proj"

1 item.



ajm5223.psq

ohio proj
Sequencher™ "ohio proj"

Name	Size	Kind	Label	Last Modified
ajm3810.psq (Cleve...	235 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:39 PM
ajm5206.psq (Cinci...	197 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:40 PM
ajm5806.psq (Youn...	195 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:41 PM
Contig[0001]	288 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:36:11 PM
ajm5808.psq	216 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:37 PM
ajm3908.psq	169 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:01:36 PM

columbus


oklahoma proj
Sequencher™ "oklahoma proj"

1 item.



ajm4318.psq



oregon proj
Sequencher™ "oregon proj"

Name	Size	Kind	Label	Last Modified
▼  Contig[0001]	1277 BPs	Contig of 9	-	Fri, Apr 17, 1998 7:11:04 AM
ajm5932.psq	275 BPs	DNA Fragment	-	Fri, Apr 17, 1998 6:44:08 AM
aa387280.psq	406 BPs	DNA Fragment	-	Fri, Apr 17, 1998 6:44:18 AM
w64353.psq	316 BPs	DNA Fragment	-	Fri, Apr 17, 1998 6:51:02 AM
w65056.psq	329 BPs	DNA Fragment	-	Fri, Apr 17, 1998 6:47:18 AM
aa008150.psq	489 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:05:09 AM
aa008149.psq	490 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:05:11 AM
aa267666.psq	435 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:06:39 AM
w30278.psq	286 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:07:31 AM
r_d86685.psq	478 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:02:38 AM

pennsylvania proj
Sequencher™ "pennsylvania proj"

Name	Size	Kind	Label	Last Modified
aa755732.psq (mou...	287 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:36:43 AM
ajm77 29.Seq	185 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:04:49 A
ajm7725.Seq 5' end	109 BPs	DNA Fragment	-	Thu, Feb 1, 1996 10:06:11 PI
ajm78 06.Seq 3' end	108 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:25:21 A
ajm78 07.Seq	309 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:34:14 PM
ajm78 08.Seq	285 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:34:36 PM
ajm78 10.Seq	288 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:35:23 PM
ajm78 12.Seq	427 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:36:12 PM
ajm78 14.Seq	404 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:37:01 PM
ajm78 20.Seq	412 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:39:24 PM
ajm78 22.Seq	192 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:06:46 A
ajm78 23.Seq	400 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:40:36 PM
ajm78 24.Seq	425 BPs	DNA Fragment	-	Thu, Feb 1, 1996 8:41:02 PM
Complete gene	1274 BPs	Contig of 60	-	Fri, Apr 17, 1998 7:50:50 AM
aa197670.psq (m...	296 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:43:14 AM
aa672056.psq (m...	527 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:44:13 AM
ajm5513-5	19 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:58:07 PM
ajm78 11	246 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:43:05 AM
ajm77 30	285 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:44:10 AM
ajm78 01	216 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:43:05 AM
ajm5513f1	20 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:43:05 AM
ajm5513r8	21 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:55:36 PM
ajm79 01	474 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:44:10 AM
ajm5513f2	18 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:46:32 PM
ajm5513h	21 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:44:10 AM
ajm5513g	20 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:44:10 AM
ajm5513r7	18 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:55:09 PM
ajm78 25	313 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:56:31 PM
ajm78 13	316 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:56:23 PM
ajm78 16	318 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:56:14 PM
ajm77 31	283 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:55:59 PM
ajm78 15	304 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:55:59 PM
ajm79 02	312 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:55:58 PM
ajm5513f3	18 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:46:53 PM
ajm5513r6	18 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:54:42 PM
ajm77 29.Seq 3'...	98 BPs	DNA Fragment	-	Fri, Feb 2, 1996 2:24:16 AM
AJM7027.PSQ (5...	122 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:07:57 A
AJM7124.PSQ (5...	122 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:11:40 A
AJM5513.PSQ	121 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:11:39 A
ajm5513f4	20 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:47:19 PM

pennsylvania proj
Sequencher™ "pennsylvania proj"

Name	Size	Kind	Label	Last Modified
ajm5513b	21 BPs	DNA Fragment	-	Tue, Feb 13, 1996 11:29:09 A
ajm78 06.Seq 5'...	206 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:25:21 A
ajm78 09.Seq	316 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:41:00 A
ajm78 21.Seq	318 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:37:32 A
ajm78 05.Seq	313 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:41:00 A
ajm79 07	374 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:57:51 PM
ajm7725.Seq 3'...	190 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:08:06 A
ajm79 06	366 BPs	AutoSeq Frag, ABI	-	Wed, Feb 14, 1996 6:58:09 PM
Lyp/wt polymorp...	40 BPs	DNA Fragment	-	Wed, Nov 26, 1997 3:45:38 AM
ajm5513r5	18 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:54:20 PM
ajm5513f5	19 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:47:42 PM
ajm7732.psq (mo...	288 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:30:26 A
ajm5513c	20 BPs	DNA Fragment	-	Tue, Feb 13, 1996 11:29:37 A
ajm79 05	433 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:50:05 AM
ajm79 03	435 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:50:05 AM
ajm5513r4	19 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:53:57 PM
ajm7804.psq (mo...	216 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:30:24 A
ajm5513f6	23 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:48:05 PM
aa183650.psq (m...	345 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:50:08 AM
ajm5513r3	20 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:53:30 PM
ajm7803.psq (mo...	248 BPs	DNA Fragment	-	Fri, Feb 2, 1996 12:30:22 A
ajm77 27.Seq	312 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:50:15 AM
ajm77 26.Seq	304 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:50:17 AM
ajm5513e	26 BPs	DNA Fragment	-	Tue, Feb 13, 1996 11:30:03 A
ajm5513d	25 BPs	DNA Fragment	-	Tue, Feb 13, 1996 11:30:26 A
ajm79 09	204 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:50:19 AM
ajm79 08	269 BPs	AutoSeq Frag, ABI	-	Fri, Apr 17, 1998 7:50:20 AM
ajm5513f7	20 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:48:34 PM
ajm5513r2	20 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:53:01 PM
ajm5513f	21 BPs	DNA Fragment	-	Tue, Feb 13, 1996 11:30:43 A
ajm5513f8	19 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:48:57 PM
ajm5513r1	25 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:52:36 PM
ajm5513-3	24 BPs	DNA Fragment	-	Sun, Apr 21, 1996 8:57:33 PM
poly-A tail	9 BPs	DNA Fragment	-	Wed, Jan 24, 1996 2:21:42 PM
▼  Contig[0014]	322 BPs	Contig of 3	-	Sun, Apr 21, 1996 8:42:12 PM
ajm78 17.Seq (m...	322 BPs	DNA Fragment	-	Thu, Feb 1, 1996 11:56:39 P
ajm78 18.Seq (m...	318 BPs	DNA Fragment	-	Thu, Feb 1, 1996 11:56:41 P
ajm78 19.Seq (m...	321 BPs	DNA Fragment	-	Thu, Feb 1, 1996 11:56:43 P
 h_aa860471.psq	391 BPs	DNA Fragment	-	Fri, Apr 17, 1998 7:35:21 AM




south dakota proj
Sequencher™ "south dakota proj"

1 item.




jln0129.psq



tennessee proj
Sequencher™ "tennessee proj"

Name	Size	Kind	Label	Last Modified
▼  Contig[0001] (Mem...	555 BPs	Contig of 2	-	Fri, Apr 17, 1998 8:05:18 AM
aa016423.psq	555 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:05:18 AM
jln0202.psq	191 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:05:16 AM
▼  Contig[0002] (Nash...	1616 BPs	Contig of 8	-	Fri, Apr 17, 1998 8:44:48 AM
h_r71963.psq	394 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:44:08 AM
h_aa077571.psq	299 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:25:24 AM
h_aa280069.psq	535 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:30:46 AM
h_aa255706.psq	425 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:23:10 AM
jln0210.psq	159 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:21:54 AM
h_aa477916.psq	154 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:23:34 AM
h_aa478214.psq	430 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:44:48 AM
h_aa742283.psq	519 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:36:40 AM
 w08046.psq	795 BPs	DNA Fragment	-	Fri, Apr 17, 1998 8:47:00 AM











texas proj
Sequencher™ "texas proj"

<u>Name</u>	<u>Size</u>	<u>Kind</u>	<u>Label</u>	<u>Last Modified</u>
▼  Contig[0001]	1308 BPs	Contig of 14	-	Fri, Apr 24, 1998 12:47:39 PM
aa137916.psq	576 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:09 PM
w53217.psq	507 BPs	DNA Fragment	-	Fri, Apr 17, 1998 9:08:23 AM
w83896.psq	417 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:10 PM
aa882404.psq	562 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:07:35 P
aa116260.psq	73 BPs	DNA Fragment	-	Wed, Jan 8, 1997 4:59:11 PM
aa116261.psq	469 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:28:36 P
ajm5433.psq	271 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:31:07 P
w75571.psq	473 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:42:21 P
aa286631.psq	311 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:42:21 P
r_aa925494.psq	511 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:42:21 P
r_AA925456.psq	333 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:42:21 P
h_aa257024.psq	311 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:47:39 P
h_AA614290.psq...	132 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:42:56 P
h_AA614290.psq...	170 BPs	DNA Fragment	-	Fri, Apr 24, 1998 12:45:01 P

washington proj
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Name	Size	Kind	Label	Last Modified
▼  Contig[0001]	2365 BPs	Contig of 3	-	Thu, Jan 16, 1997 3:28:57 PM
<i>muspdib.psq</i>	2365 BPs	DNA Fragment	-	Thu, Jan 16, 1997 3:28:57 PM
<i>ajm5729.psq</i>	176 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:28 PM
<i>ajm3916.psq</i>	257 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:27 PM
▼  ratcab.txt	2398 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:29 PM

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Name	Size	Kind	Label	Last Modified
 ajm7506.psq	363 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:12 A
 ajm7507.psq	377 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:13 A
 ajm7605.psq	376 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:14 A
 ajm7607.psq	383 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:14 A
▼  Contig[0001]	666 BPs	Contig of 4	-	Wed, Jan 15, 1997 6:14:06 PM
ajm7502.psq	225 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:49 PM
ajm7503.psq	307 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:50 PM
ajm5221.psq	262 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:45 PM
ajm7608.psq	382 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:09:16 A
▼  Contig[0002]	1489 BPs	Contig of 9	-	Fri, Apr 24, 1998 1:27:17 PM
jams0320.psq	622 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:24:00 PM
jams0319.psq	623 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:24:00 PM
jams0321.psq	617 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:24:00 PM
ajm7026.psq	346 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:24:00 PM
ajm5224.psq	190 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:24:00 PM
ajm6926.psq	469 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:26:49 PM
ajm6931.psq	469 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:26:12 PM
ajm6925.psq	464 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:26:12 PM
ajm7024.psq	502 BPs	DNA Fragment	-	Fri, Apr 24, 1998 1:27:17 PM
▼  Contig[0003]	267 BPs	Contig of 2	-	Wed, Jan 8, 1997 5:42:19 PM
ajm3812.psq	260 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:53 PM
ajm3808.psq	267 BPs	DNA Fragment	-	Wed, Jan 8, 1997 5:02:53 PM
▼  Contig[0008]	500 BPs	Contig of 7	-	Fri, Apr 24, 1998 2:10:10 PM
ajm7225.psq	245 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:20 A
ajm6913.psq	417 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:09:03 PM
ajm7110.psq	421 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:10:10 PM
ajm7112.psq	421 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:10:07 PM
ajm6914.psq	471 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:08:48 PM
jams0227.psq	500 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:08:48 PM
jams0229.psq	500 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:08:49 PM
▼  Contig[0009]	361 BPs	Contig of 2	-	Wed, Jan 15, 1997 6:13:39 PM
ajm7228.psq	357 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:22 A
ajm7227.psq	361 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:08:21 A
▼  Contig[0013]	393 BPs	Contig of 5	-	Fri, Apr 24, 1998 2:17:54 PM
ajm6711.psq	334 BPs	DNA Fragment	-	Mon, Jan 13, 1997 11:06:52 A
ajm7118.psq	393 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:17:54 PM
ajm7119.psq	393 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:17:54 PM
ajm6906.psq	393 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:17:54 PM
ajm6907.psq	393 BPs	DNA Fragment	-	Fri, Apr 24, 1998 2:17:54 PM

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1 item.



ajm5227.psq

