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Genetic, physical, and comparative map of the subtelomeric region of mouse Chromosome 4

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Abstract

The subtelomeric region of mouse chromosome (Chr) 4 harbors loci with effects on behavior, development, and disease susceptibility. Regions near the telomeres are more difficult to map and characterize than other areas because of the unique features of subtelomeric DNA. As a result of these problems, the available mapping information for this part of mouse Chr 4 was insufficient to pursue candidate gene evaluation. Therefore, we sought to characterize the area in greater detail by creating a comprehensive genetic, physical, and comparative map. We constructed a genetic map that contained 30 markers and covered 13.3 cM; then we created a 1.2-Mb sequence-ready BAC contig, representing a 5.1-cM area, and sequenced a 246-kb mouse BAC from this contig. The resulting sequence, as well as approximately 40 kb of previously deposited genomic sequence, yielded a total of 284 kb of sequence, which contained over 20 putative genes. These putative genes were confirmed by matching ESTs or cDNA in the public databases to the genomic sequence and/or by direct sequencing of cDNA. Comparative genome sequence analysis demonstrated conserved synteny between the mouse and the human genomes (1p36.3). DNA from two strains of mice (C57BL/6ByJ and 129P3/J) was sequenced to detect single nucleotide polymorphisms (SNPs). The frequency of SNPs in this region was more than threefold higher than the genome-wide average for comparable mouse strains (129/Sv and C57BL/6J). The resulting SNP map, in conjunction with the sequence annotation and with physical and genetic maps, provides a detailed description of this gene-rich region. These data will facilitate genetic and comparative mapping studies and identification of a large number of novel candidate genes for the trait loci mapped to this region.

The subtelomeric region of mouse Chr 4 harbors loci that cause alterations in development, behavior, and disease susceptibility (Table 1) but sparse physical and genetic mapping resources. Identification of candidate genes for these traits has been hampered because genetic and physical maps are difficult to construct accurately near the telomere (Riethman 1997). As a result, the quality of available mapping data is poor in this region. Examples of this include inconsistencies in marker and gene order, both in individual studies and composite maps, low marker density, poor YAC-coverage, lack of a sequence-ready BAC contig, and no available draft or finished genomic sequence.

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The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers: G67732–G67781 (STS *D4Mon1-D4Mon68*), AF311386 (*Tas1r3* gene) and AF389853 (BAC RPCI-23-118E21).

This region contains the saccharin preference locus (*Sac*), which affects the intake of sweeteners (Bachmanov et al. 1997). A *Sac* candidate gene in the area was identified (*Tas1r1*;Hoon et al., 1999), and then excluded (Li et al. 2001). *Sac* has effects on the peripheral sensitivity to sweeteners (Bachmanov et al. 1997;Inoue et al. 2001), and is allelic between the C57BL/6ByJ (B6; high sweetener preferring) and 129P3/J (129) and DBA/2 (low sweetener preferring) strains (Fuller 1974;Lush 1989;Belknap et al. 1992;Lush et al. 1995;Blizard et al. 1999). The *Sac* locus selectively affects sweet and alcohol intake; other taste stimuli, such as sour, salty, and bitter, are unaffected by *Sac* (Bachmanov et al. 1996a;1996b).

To increase the resources available for the identification of *Sac* and other neighboring loci, a detailed characterization of this region was undertaken. First, a dense genetic map was constructed, with markers previously mapped to the region, and updated with new markers generated during the project. The B6 and 129 strains were chosen to create the genetic map because they are allelic for the *Sac* locus and are distantly related. The newly generated genetic map resulted in the construction of a physical map with overlapping BAC clones. A BAC clone covering the *Sac* critical region was sequenced and analyzed for the presence of genes. In addition, comparative mapping was conducted because previous work suggested that significant conserved synteny existed between the telomeric region of mouse Chr 4 and the telomeric region of the p-arm of human Chr 1.

Materials and methods

DNA markers and sequences

Markers on distal Chr 4 used for mouse genotyping and physical mapping were obtained from several sources. The locations of ESTs R75150, M134G01, M136B1, K01599, AA408705, M134C6, D4Xrf497, D4Xrf215, Al225779, and K00231 were found in the radiation hybrid databases of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (http://www-genome.wi.mit.edu/), the Medical Research Council (http://genex.hgu.mrc.ac.uk/), and the Mouse Genome Database (http://www.jax.org) maintained by The Jackson Laboratory. Markers based on BAC end sequences were obtained through The Institute for Genomic Research web site (http://www.tigr.org) or by direct sequencing, when necessary. Some STSs, e.g., R74924, D18402, and U37351 and genes, e.g., BG228522, *Ski*, *Pkcz*, *Gnb1*, *Cdc212*, *DvI1*, *Tnfrsf4* and *Agrn* were available from public databases (National Center for Biotechnology Information [NCBI], Massachusetts Institute of Technology [MIT]). Additional markers, including all *D4Mon* markers, were designed using sequence from the RPCI-23 BAC 118E21.

Primers were purchased from Research Genetics (Huntsville, Ala.) or synthesized at Integrated DNA Technologies, Inc. (Coralville, Iowa). Most primer pairs and their PCR products were tested for polymorphisms between the B6 and 129 strains. Most polymorphic markers were used for genotyping the F_2 mapping panel and to construct the genetic map. Markers located between *D4Mit256* and *K00231* were used to make the BAC contig. Details about markers and their associated primer sequences are given in Table 2.

Genetic mapping and linkage analysis

Mouse genomic DNA was purified from tails by NaOH/Tris (Truett et al. 2000), or phenol/chloroform extraction. Markers that differed in length of the PCR product (simple sequence length polymorphism; SSLP) were tested by using a standard protocol (Dietrich et al. 1992), with minor modifications (Bachmanov et al. 1997;Li et al. 2001). Other markers were tested by using a single-strand conformation polymorphism (SSCP) protocol (Orita et al. 1989), or by sequencing the PCR products of B6 and 129 DNA, to detect single nucleotide or small

insertion/deletion polymorphisms. Linkage analysis and construction of the genetic map were conducted by using MAPMAKER/EXP 1.1 (Lander et al. 1987).

Construction of a BAC contig

The RPCI-23 female (C57BL/6J) mouse BAC library (Osoegawa et al. 2000) was used for library screening. All probes were radioactively labeled by the random hexa-nucleotide method (Feinberg and Vogelstein 1983). Hybridization and washing of membranes followed standard protocols (Church and Gilbert 1984). The library was screened twice, first with a probe generated from YAC 178B3, and second, with pooled probes of markers on the distal end of Chr 4.

To determine the BAC clones corresponding to each probe, positive clones identified by the initial screenings were picked into 384-well microtiter plates and regridded onto 8 × 12 cm Hybond-N^+ membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by using a Biogrid robot (Biorobotics, Haslingfield, Cambridge, UK). Each filter was then hybridized against individual probes, under the same conditions as the primary screening. The secondary screening results were confirmed by PCR STS-content mapping, using overnight cell suspension as templates. BAC end STS markers were used to confirm the BAC physical map and identify polymorphisms.

To determine the size of individual BAC clones, we extracted the DNA using a modified alkaline lysis procedure (Sambrook et al. 1989) and analyzed after digestion with *Not*I (New England Biolabs, Beverley, Mass.) by pulse-field gel electrophoresis (PFG), performed on a Chef Mapper (Bio-Rad, Hercules, Calif.). Samples were run for 16 h on 1% agarose gels using 0.5× TBE buffer, at 14°C; the settings were 200 V (6V/cm) and a linear pulse time ramp from 0.3 to 33 s with a pulse angle of 120°. The gels were subsequently stained with ethidium bromide for photography. Approximate insert fragment sizes were determined by comparison with the low-range PFG size markers (New England Biolabs). Finally, the BAC contig was assembled by using SEGMAP, version 3.48 (University of Washington, Seattle, Wash.).

BAC ends sequencing and STS development

BAC DNA for sequencing was prepared from 200-ml overnight cultures, according to the modified protocol for BACs by using P100 midi-prep columns (Qiagen, Inc., Valencia, Calif.). Unique sequences from BAC ends were identified by analysis with Repeatmasker (Smit and Green 2000), and used to design PCR primers for genotyping F_2 animals and to screen BAC clones to refine STS content mappling.

BAC DNA sequencing and analysis

BAC 118E21 was isolated by using an alkaline lysis protocol, followed by CsCl density gradient centrifugation. Following ethanol precipitation, the CsCl-purified DNA was resuspended at 15 ng/μl in TE. Two hundred microliter of this solution was sonicated for 2 s, with a Misonix XL2015 ultrasonicator (Farmingdale, New York). The sheared DNA was end-filled with T4 DNA polymerase, cloned into pUC18, and cut with *Sma*I. Twenty-five hundred transformants were grown, plasmid DNA was isolated, sequenced forward and reverse with Big Dye Terminators (PE Biosystems, Foster City, Calif.), and data were collected on an ABI3700 DNA sequencer. Sequence data were processed and assembled with the Phred, Phrap, and Consed package of programs on an SGI 02 workstation.

After BAC 118E21 was sequenced, the STS content of this BAC and overlapping BACs was confirmed by aligning the STS and BAC end sequences with the 118E21 sequence (Sequencher, Gene Codes Corporation, Ann Arbor, Mich.). Another genomic clone, AF185591, overlapped with 118E21, and their sequences were assembled by Sequencher.

Repeat sequences were identified with RepeatMasker. Genscan (Burge and Karlin 1998) was used to predict gene content and intron/exon organization. The predicted proteins were submitted to a tBLASTn search through the *nr* and the mouse EST database at NCBI.

For identifying genes through their similarity to ESTs or gene sequences, portions of the 284-kb genomic sequence were submitted to iterative BLAST searches to find matching genes and ESTs, and the cDNAs were aligned with genomic sequence to identify intron/exon organization.

Analysis of gene expression and detection of SNPs

Primers were designed to span intron/exon junctions suggested by Genscan (Tables 4 and 5) and were used to amplify genomic and tongue cDNA. Genomic DNA was either extracted as described above or purchased from The Jackson Laboratory. Tongue cDNA was prepared as follows: total RNA was extracted by using TRIZol Reagent (Life Technologies Inc., Rockville, Md.) from enzymatically separated mouse lingual epithelium (Ruiz et al. 1995;Spielman and Brand 1995), which included fungiform, foliate, and circumvallate taste papillae. The RNA was reverse-transcribed (Superscript reverse transcriptase, Life Technologies). The cDNA samples were amplified by using AmpliTaq DNA Polymerase with GeneAmp (Perkin Elmer Corporation, Branchburg, N.J.). Single bands of expected sizes were excised from the gel, purified, and sequenced to determine the intron/exon junctions. The resulting sequence was used to confirm that the product was from the expected sequence, and to find polymorphism between strains. Sequences were aligned and polymorphisms were identified with Sequencher.

Comparative analysis of mouse and human sequences

To compare the mouse and human sequences over the entire contig, we used BLAST searches to identify conserved genes between mouse and human. Homologous human genes were identified and used as queries against the human *htgs* and *gss* databases. BAC or PAC clones positive for these human sequences were scanned by electronic PCR (http://www.ncbi.nlm.nih.gov/genome/sts/epcr.cgi) to determine the map location. Those BACs or PACs that mapped to human Chr 1p36 were identified and then were assembled by overlapping BLAST alignment to determine a minimum tiling path (Fig. 2).

From the minimum tiling path of ~1.2 Mb of human DNA, the sequence which corresponded to the 284-kb mouse sequence was extracted. A computer algorithm, Pipmaker (http://nog.cse.psu.edu/pipmaker), was used to analyze the sequenced mouse and human region (284 kb). The intron/exon organization used as input to Pipmaker was produced from alignment of the identified cDNAs and genes with genomic DNA (Table 4). In an initial analysis, all mouse cDNA from Table 4 was included; however, for ease of presentation, only those sequences with human–mouse similarity were retained in the final analysis.

Results and Discussion

Creation of a high-resolution linkage map

A genetic map of the subtelomeric region of mouse Chr 4 was produced by using an F_2 generation of mice (N = 628) originating from the C57BL/6ByJ (B6) and 129P3/J (formerly 129/J; abbreviated here as 129) strains (Bachmanov et al. 1997). Thirty markers, which were polymorphic between the 129 and B6 strains, were genotyped, and the constructed genetic map spanned a 13.3-cM region from *D4Mit33* (proximal) to *D4Ertd296e* (distal; Fig. 1a). Marker details are provided in Table 2.

Construction of a BAC contig

The RPCI-23 BAC library (Osoegawa et al. 2000) was screened twice to identify BAC clones covering the *Sac* locus. A probe prepared from a YAC clone (178B3) was used for the first screening. This YAC was mapped to the subtelomeric region of Chr 4, and it appeared to be nonchimeric. Pooled probes including all markers mapped to the subtelomeric region of Chr 4 were used for the second round of screening. To determine the BAC clones positive for each probe, we arrayed all putative clones identified from both screenings to prepare small high-density filters. Each small filter was then hybridized against individual probes. Only positive clones confirmed by both hybridization and PCR were used to construct a contig. The BAC end sequences were either obtained from databases or generated by direct sequencing. Consequently, STS markers were designed from the unique sequences of BAC ends and used to perform STS content mapping against all positive clones.

The BAC-insert sizes were determined by pulse-field gel electrophoresis. Analysis of the 42 BAC clones was conducted with the SEGMAP computer program (Green et al. 1991) and resulted in the assembly of an estimated ~1.2-Mb sequence-ready map representing a 5.1-cM region between *D4Mit256* and *K00231* (Fig. 1b). Since BAC clone 118E21 was the largest (246 kb) clone located within the *Sac* critical region, we chose it to prepare shotgun clones to generate a sequence contig with 5–6 × coverage (Accession #AF389853). Consequently, we generated 50 new STS markers (Table 2), fourteen (28%) of which were polymorphic between 129 and B6 mouse strains. These new markers were developed to fill gaps between previously described polymorphic markers, and were selected to amplify simple repeats where possible (Table 3).

Annotation of raw sequence

BLAST searches using all STSs in the region were conducted to identify any other large genomic clones that overlapped with the sequenced BAC 118E21, but only one was found (Accession #AF185591). These two clones were assembled and yielded a total sequence of 284 kb. After the sequence of the 284 kb was complete, exact STS positions within the region were identified (Table 3).

Two methods were used to identify genes within the 284-kb sequence: predicting genes by computer algorithm (the ab initio method), and by the examination of sequence similarly with other genes and ESTs. Initially, the 284-kb sequence was analyzed by masking the repetitive elements, and a computer algorithm (Gen-scan) was used to predict genes. These predicted genes and the raw sequences were submitted to iterative BLAST searches to determine their similarity to known genes or ESTs. For a predicted gene or genomic sequence to be considered an exact match to a gene or EST deposited in GenBank, the sequence similarity had to be >92% for at least 50% of the sequence (Venter et al. 2001). This degree of similarity allowed for sequencing errors or stain differences, but minimized the possibility that closely related genes were misidentified as exact matches or that alternatively spliced forms were discarded inadvertently.

Of the genes in this region, seven had locus designations and were considered known genes (Fig. 2 and 3): dishevelled, Drosphila *dsh* homolog (*Dvl*, GenBank Accession number NM_010091), Paneth cell enhanced expression gene (*Pcee*, Accession #NM_018856), sweet taste receptor family 1 member 3 gene (*Tas1r3*, Accession #AF311386), calcium-binding protein (*Cab45a*, Accession #U45977), a mammalian homolog of yeast *UBC7* (*Ubc6p*, Accession #U93242), and two members of the tumor necrosis factor receptor superfamily, *Tnfrsf4* (Accession #X85214; aliases *Txgp1* and *ox40*) and *Tnfrsf18* (Accession #NM_009400). Dishevelled (*Dvl*) is one of the best-characterized genes in the region. It was originally discovered because it is an ortholog of the Drosophila dishevelled (*dsh*) gene, a gene that

influences the development of the fly body plan. In the mouse, *Dvl* influences complex social and sensorimotor behavior (Lijam et al. 1997;Lee et al. 1999). Several *Dvl* homologs are located on other mouse chromosomes. The tumor necrosis factor receptors *Tnfrsf4* and *Tnfrsf18* are involved in immune function and cancer biology. *Ubc6p* is a homolog of the ubiquitin-conjugating enzyme, but to the best of our knowledge, functional studies with this gene and associated enzyme have not been conducted. The calcium-binding protein *Cab45a* is present in the lumen of the Golgi apparatus (Scherer et al. 1996). *Tas1r3* belongs to a small family of taste receptors (Hoon et al. 1999), is expressed in taste receptor cells, and has been suggested as a candidate gene for the *Sac* locus (Bachmanov et al. 2001;Kitagawa et al. 2001;Max et al. 2001;Montmayeur et al. 2001;Nelson et al. 2001;Sainz et al. 2001).

In addition to the known genes, other putative genes were identified because sequences within this region matched 15 existing EST clusters (Mm.23825, Mm.23492, NM_025338, Mm. 28978, NM_024472, Mm.22021, Mm.56803, Mm.182484, Mm.133906, Mm.172945, Mm. 32248, NM_026125, Mm.102764, Mm.42006, and Mm.159514) and 11 single cDNA clones (AA546397, AA435261, AA967911, BG243209, BF181401, BF120370, AA218417, AW743506, AI225779, BF319334, AW987045; Table 4). The function of these genes, other than that suggested by expression patterns inferred from tissue libraries from which they were derived, are unknown.

To further confirm that putative genes were actual genes, the EST sequences were aligned with genomic DNA and the intron/exon junctions identified (Table 4). The location of each exon for each gene or EST was determined, as well as the acceptor and donor sites. If we assume that all exons identified by alignment of cDNA with genomic DNA are true exons, then Genscan accurately predicted 58% of the exons. If we assume that all EST clusters or single, non-overlapping ESTs are genes, Genscan predicted at least one exon correctly for 41% of genes.

Characterization of genes within this region

Several genes were selected for further study because they were located within a critical region for the taste-related QTL, *Sac* (Bachmanov et al. 2001). The intron/exon structure of these genes was determined by PCR amplification of genomic and tongue cDNA, using primers that spanned intronic sequence. The PCR experiments were designed to confirm that the predicted genes were present as mRNA, to assess the accuracy of predicted intron/exon junctions, and to determine whether these genes were expressed in tongue. Tongue is an un-derrepresented tissue as a source of template for publicly available EST sequencing data.

Of the seven genes tested, all were expressed in mouse tongue (primers are given in Table 5; results are given in Tables 4 and 5). Of the 16 intron/exon junctions tested by PCR, two junctions predicted by the computer algorithm did not match the junction obtained from sequencing of the cDNA (12.5%; Table 5). The results of this experiment confirm gene expression in tongue cDNA.

Analysis of single nucleotide polymorphisms within genomic and cDNA

Genes within the critical *Sac* region were sequenced by using genomic and cDNA derived from the B6 and 129 strains. The purpose of this experiment was two-fold: to discover allelic genes that could be considered candidate genes for the *Sac* locus, and to identify single nucleotide polymorphisms (SNPs) useful for genetic mapping.

SNPs were detected, on average, every 100–200 bp. Most genes were polymorphic between the two strains, and the frequency of nucleotide variants did not differ appreciably from gene to gene. In addition to nucleotide substitutions, small insertions and deletions were a frequent

source of polymorphisms between strains. All sequence variants detected in coding regions were either silent or missense mutations (Table 5); no nonsense or frameshift mutations were found, nor were any variants detected that would alter an intron/exon splice junction.

There were several sequence variants within the 129 strain. The possibility that these variants were due to sequencing artifacts was excluded by re-sequencing of the areas, which confirmed the heterozygosity of the 129 strain in all cases.

Comparative map between mouse and human

To construct a human–mouse comparative map (Fig. 2), the sequences in the human that align with the 1.2-Mb mouse contig were identified. To do this, mouse genes were submitted to multiple BLAST searches of the human *nr*, *est*, *htgs*, and *gss* databases. For comparative mapping, we used human sequences that fit all three of the following criteria: (1) they were highly homologous to mouse genes; (2) they were located on human Chr 1p36; and (3) they were contained within a BAC or PAC clone.

The selected positive human BACs were then assembled, a minimum tiling path was selected, and the mouse genes and their human homologs were aligned (Fig. 2). This human sequence was compared with the mouse sequence, by using the PIP algorithm that plots percentage identity between two sequences (Schwartz et al. 2000). Almost all named genes in mouse were present in the human sequence, and they were arranged in the same order on the chromosome (Fig. 3). In many areas outside of the predicted exons, there were highly conserved sequences between mouse and humans (e.g., 6–8 kb, 121–126 kb; Fig. 3). These highly conserved regions may represent undetected exons or important and highly conserved regulatory elements.

Conclusions

We have described the genetic and physical map of the subtelomeric region of mouse Chr 4, and a comparative map of the region of conserved synteny in humans. Several interesting features of this region were found. First, gene densities near the mouse and human telomeres are high. More than 20 genes were confirmed within the 284-kb sequence in the mouse. High gene density of the human telomeric region has been demonstrated (Saccone et al. 1992), and the current report suggests this is true in mice, to the extent that Chr 4 is representative.

Second, the human-mouse conservation between gene order and number was strong. Almost all named genes were present in both mouse and human, and in the same order along the chromosome. There is, however, a caveat to the interpretation of the mouse-human conserved synteny map: the human BAC clones were derived from the *htgs* database, and are unfinished. Although the PIP algorithm we used takes into account the possibility of broken clones and creates the most likely alignment, some adjustment of gene order may occur as finished sequence is available. The region of strict conserved synteny extends over at least 1.2 Mb (the size of the mouse–human contig and homology map). Mapping data from other sources indicate that the homology may extend as far as 1p31 (http://genome.ucsc.edu).

Third, the recombination frequency in this region was very high. The 1.2-Mb region represented in the BAC contig corresponded to a genetic distance of over 5 cM as measured in an F_2 mapping panel. This high rate of recombination has been observed in mouse telomeric regions (de Boer and Groen 1974). This terminal map expansion is fortunate for those mapping quantitative traits or genes, because more informative meioses are obtained than in less recombinogenic regions of the genome. The high rate of recombination, and its beneficial effects on gene mapping, partially offset the difficulties encountered in sequencing telomeric regions.

The rate of polymorphisms detected between the two mouse strains, B6 and 129, suggests that many of the genes may have altered function because of numerous amino acid substitutions. The B6 and 129 strains are only distantly related, and the large number of polymorphisms makes these strains good choices for gene mapping studies. Compared with data collected in a large-scale effort to map SNPs among mouse strains (Lindblad-Toh et al. 2000), the frequency of SNPs in distal Chr 4 was more than threefold higher than elsewhere in the mouse genome for comparable strains.

Polymophisms *between* substrains of the 129 strains have been well characterized (Simpson et al. 1997), but the rate of heterozygosity *within* the 129P3/J strain was surprising: inbred strains are supposed to be homozygous at all loci. This finding is consistent with our observations during genotyping with microsatellite markers, which identified some polymorphisms within the 129P3/J substrain (Bachmanov et al., unpublished observations). The rate of *within* substrain polymorphisms could account for some of the phenotypical within-strain variation of the 129 substrain, such as variable agenesis of the corpus callosum (Wahlsten 1982). Several explanations are possible for this variability within this inbred strain. The 129P3/J substrain is maintained using a forced heterozygosity mating scheme: in each generation, heterozygotes at the albino locus $Tyr^{c}/Tyr^{c\text{-}ch}$ are mated with homozygotes, Tyr^{c}/Tyr^{c}. This mating scheme has been conducted at The Jackson Laboratory since 1948 (Withham 1990). The forced heterozygosity may account for variation in the albino region on Chr7, but should not increase the level of heterozygosity of other regions. Alternatively, the increased heterozygosity might be due to the later initiation of inbreeding of the 129P3/J strain compared with other inbred strains. Another possibility might be that the strains could have been contaminated, like other substrains of the 129 strain (Simpson et al. 1997). Finally, the 129P3/J strain could be unusually prone to de novo mutations. Further study of the heterogeneity of the 129P3/J strain should be conducted, because this heterozygosity could have impact upon QTL mapping or other phenotypic studies.

There are two ways to detect genes within genomic DNA sequence: prediction of genes using computer algorithms, and similarity searches. Both work well at identifying most genes, but neither method identifies all genes with certainty. Ab initio methods, implemented with Genscan, detected more than half of the genes in our study, but the genes detected were not necessarily assembled with the same intron/exon boundaries found in the cDNA. These Genscan assemblies could be errors, or they could reflect alternatively spliced forms of a gene that are undetected because they are expressed in tissues not represented in cDNA libraries, or have a low abundance. Comparisons of the genomic sequence using sequence similarity methods also provided additional information about the presence of genes and their structures, many of which were not identified through the Genscan analysis. Still, other genes could exist in this region, and the regions of high sequence similarity identified through the PIP analysis would be good candidates for the undetected genes. The publicly available human draft sequence was poorly annotated in this region and reflected less than half of the gene content of the current analysis (http://genome.ucsc.edu/). Therefore, it is still important for investigators to annotate genomic regions rather than to rely on the annotation available through public and private databases.

Annotation of genomic sequence and predicting genes through areas of conserved synteny with other species should be approached cautiously. For example, regions of high human–mouse homology existed, and in some cases the sequence encompassed a known gene in one species, but not in the other species, and no cDNAs in the other species were found. A case in point is the *Ubc6p* gene in the mouse: there is a high sequence homology between mouse and human genomic sequence, but without the presence of human cDNAs, further work would be needed to name the human sequence appropriately. Another challenge was the large number of orphan

mouse cDNAs in the region; many of these cDNAs could represent novel genes, but it was difficult to determine this accurately without additional work.

Examination of this region led to the identification of many genes that were polymorphic between B6 and 129 mice, near the peak linkage interval for *Sac*, and that were expressed in the tongue. Of these genes, *Tas1r3* is the best candidate for the *Sac* locus (Bachmanov et al. 2001;Kitagawa et al. 2001;Max et al. 2001;Montmayeur et al. 2001;Nelson et al. 2001;Sainz et al. 2001).

The detailed genetic, physical, and comparative maps created here will assist in identification of loci that influence development, behavior, and disease susceptibility, and the sequence-ready BAC contig will be useful for the public Mouse Sequencing consortium. While drafts of the homologous regions are now available from human DNA (Human Genome Sequencing Consortium 2001; Venter et al. 2001), comparison of the maps described herein with the recently released human draft suggests that gaps still remain in the human sequence, particularly in areas closest to the telomere. Identification of all genes in human and mouse subtelomeric regions and the closing of telomeric gaps will involve more effort than that taken to close gaps in other regions of the genome.

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References

Bachmanov AA, Reed DR, Tordoff MG, Price RA, Beauchamp GK. Intake of ethanol, sodium chloride, sucrose, citric acid, and quinine hydrochloride solutions by mice: a genetic analysis. Behav Genet 1996a;26:563–573. [PubMed: 8990535]

Bachmanov AA, Tordoff MG, Beauchamp GK. Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin Exp Res 1996b;20:201–206. [PubMed: 8730208]

Bachmanov AA, Reed DR, Ninomiya Y, Inoue M, Tordoff MG, et al. Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses. Mamm Genome 1997;8:545–548. [PubMed: 9250857]

Bachmanov AA, Li X, Reed DR, Ohmen JD, Li S, et al. Positional cloning of the mouse saccharin preference (*Sac*) locus. Chem Senses 2001;26:925–933. [PubMed: 11555487]

Belknap JK, Crabbe JC, Plomin R, McClearn GE, Sampson KE, et al. Single-locus control of saccharin intake in BXD/Ty recombinant inbred (RI) mice: some methodological implications for RI strain analysis. Behav Genet 1992;22:81–100. [PubMed: 1590732]

Blizard DA, Kotlus B, Frank ME. Quantitative trait loci associated with short-term intake of sucrose, saccharin and quinine solutions in laboratory mice. Chem Senses 1999;24:373–385. [PubMed: 10480673]

Burge CB, Karlin S. Finding the genes in genomic DNA. Curr Opin Struct Biol 1998;8:346–354. [PubMed: 9666331]

Church G, Gilbert W. Genomic sequencing. Proc Natl Acad Sci USA 1984;81:1991–1995. [PubMed: 6326095]

de Boer P, Groen A. Fertility and meiotic behavior of male *T70H* tertiary trisomics of the mouse (*Mus musculus*). Cytogenet Cell Genet 1974;13:489–510. [PubMed: 4461187]

Dietrich W, Katz H, Lincoln SE, Shin H-S, Friedman J, et al. A genetic map of the mouse suitable for typing intraspecific crosses. Genetics 1992;131:423–447. [PubMed: 1353738]

Eicher EM, Washburn LL, Schork NJ, Lee BK, Shown EP, et al. Sex-determining genes on mouse autosomes identified by linkage analysis of C57BL/6J-YPOS sex reversal. Nat Genet 1996;14:206–209. [PubMed: 8841197]

Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983;132:6–13. [PubMed: 6312838]

Fuller JL. Single-locus control of saccharin preference in mice. J Hered 1974;65:33–36. [PubMed: 4847746]

Green ED, Mohr RM, Idol JR, Jones M, Buckingham JM, et al. Systematic generation of sequence-tagged sites for physical mapping of human chromosomes: application to the mapping of human chromosome 7 using yeast artificial chromosomes. Genomics 1991;11:548–564. [PubMed: 1837788]

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, et al. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 1999;96:541–551. [PubMed: 10052456]

Inoue M, McCaughey S, Bachmanov AA, Beauchamp GK. Whole nerve chorda tympani responses to sweeteners in C57BL6/ByJ and 129P3/J mice. Chem Senses 2001;26:915–923. [PubMed: 11555486]

International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921. [PubMed: 11237011]

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochem Biophys Res Commun 2001;283:236–242. [PubMed: 11322794]

Lander E, Green P, Abrahamson J, Barlow A, Daley M, et al. MAPMAKER: An interactive complex package for constructing primary linkage maps of experimental and natural populations. Genomics 1987;1:174–181. [PubMed: 3692487]

Leamy L, Routman E, Cheverud J. A search for quantitative trait loci affecting asymmetry of mandibular characters in mice. Evolution 1997;51:957–969.

Lee JS, Ishimoto A, Yanagawa S. Characterization of mouse dishevelled (Dvl) proteins in Wnt/Wingless signaling pathway. J Biol Chem 1999;274:21464–21470. [PubMed: 10409711]

Li X, Inoue M, Reed DR, Huque T, Puchalski RB, et al. High-resolution genetic mapping of the saccharin preference locus (*Sac*) and the putative sweet taste receptor (T1R1) gene (*Gpr70*) to mouse distal Chromosome 4. Mamm Genome 2001;12:13–16. [PubMed: 11178737]

Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, et al. Social interaction and sensorimotor gating abnormalities in mice lacking DvI1. Cell 1997;90:895–905. [PubMed: 9298901]

Lindblad-Toh K, Winchester E, Daly MJ, Wang DG, Hirschhorn JN, et al. Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. Nat Genet 2000;24:381–386. [PubMed: 10742102]

Lush IE. The genetics of tasting in mice. VI. Saccharin, acesulfame, dulcin and sucrose. Genet Res 1989;53:95–99. [PubMed: 2744455]

Lush IE, Hornigold N, King P, Stoye JP. The genetics of tasting in mice. VII. Glycine revisted, and the chromosomal location of *Sac* and Soa. Genet Res 1995;66:167–174. [PubMed: 8522158]

Martin B, Clement Y, Venault P, Chapouthier G. Mouse chromosomes 4 and 13 are involved in beta-carboline-induced seizures. J Hered 1995;86:274–279. [PubMed: 7657995]

Max M, Shanker YG, Huang L, Rong M, Liu Z, et al. *Tas1r3*, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus *Sac*. Nat Genet 2001;28:58–63. [PubMed: 11326277]

Montmayeur JP, Liberles SD, Matsunami H, Buck LB. A candidate taste receptor gene near a sweet taste locus. Nat Neurosci 2001;4:492–498. [PubMed: 11319557]

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, et al. Mammalian sweet taste receptors. Cell 2001;106:381–390. [PubMed: 11509186]

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proc Natl Acad Sci USA 1989;86:2766–2770. [PubMed: 2565038]

Osoegawa K, Tateno M, Woon PY, Frengen E, Mammoser AG, et al. Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. Genome Res 2000;10:116–128. [PubMed: 10645956]

Riethman H. Closing in on telomeric closure. Genome Res 1997;7:853–855. [PubMed: 9314489]

Rodrigues NR, Cornall RJ, Chandler P, Simpson E, Wicker LS, et al. Mapping of an insulin-dependent diabetes locus, *Idd9*, in NOD mice to chromosome 4. Mamm Genome 1994;5:167–170. [PubMed: 8199406]

Ruiz, C.; McPheeters, M.; Kinnamon, S. Tissue culture of rat taste buds. In: Brand, J., editor. Experimental and Cell Biology of Taste and Olfaction: Current Techniques and Protocols. Boca Raton: CRC Press, Inc; 1995. p. 79-84.

Saccone S, De Sario A, Della Valle G, Bernardi G. The highest gene concentrations in the human genome are in telomeric bands of meta-phase chromosomes. Proc Natl Acad Sci USA 1992;89:4913–4917. [PubMed: 1594593]

Sainz E, Korley JN, Battey JF, Sullivan SL. Identification of a novel member of the T1R family of putative taste receptors. J Neurochem 2001;77:896–903. [PubMed: 11331418]

Sambrook, J.; Fritsch, E.; Maniatis, T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor University Press; 1989.

Scherer PE, Lederkremer GZ, Williams S, Fogliano M, Baldini G, et al. *Cab45*, a novel ($Ca2^{+}$)-binding protein localized to the Golgi lumen. J Cell Biol 1996;133:257–268. [PubMed: 8609160]

Schwartz S, Zhang Z, Frazer KA, Smit A, Riemer C, et al. Pip-Maker—a web server for aligning two genomic DNA sequences. Genome Res 2000;10:577–586. [PubMed: 10779500]

Silveira PA, Baxter AG, Cain WE, van Driel IR. A major linkage region on distal chromosome 4 confers susceptibility to mouse autoimmune gastritis. J Immunol 1999;162:5106–5111. [PubMed: 10227980]

Simpson EM, Linder CC, Sargent EE, Davisson MT, Mobraaten LE, et al. Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. Nat Genet 1997;16:19–27. [PubMed: 9140391]

Smit, F.; Green, P. Repeatmasker. 2000. http://ftp.genome.washington.edu/cgi-bin/RepeatMasker, Computer program

Spielman, A.; Brand, J. Collection of taste tissue from mammals. In: Brand, JG., editor. Experimental Cell Biology of Taste and Olfaction: Current Techniques and Protocols. Boca Raton: CRC Press, Inc; 1995. p. 25-32.

Stoye JP, Kaushik N, Jeremiah S, Best S. Genetic map of the region surrounding the retrovirus restriction locus, *Fv1*, on mouse chromosome 4. Mamm Genome 1995;6:31–36. [PubMed: 7719023]

Taylor B. Epistatic circling gene of C57L/J. Mouse News Lett 1976;55:17.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, et al. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques 2000;29:52–54. [PubMed: 10907076]

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al. The sequence of the human genome. Science 2001;291:1304–1351. [PubMed: 11181995]

Wahlsten D. Deficiency of corpus callosum varies with strain and supplier of the mice. Brain Res 1982;239:329–347. [PubMed: 7093694]

Withham B (1990) Coat colors of sublines of 129 mice: JAX notes, no. 441

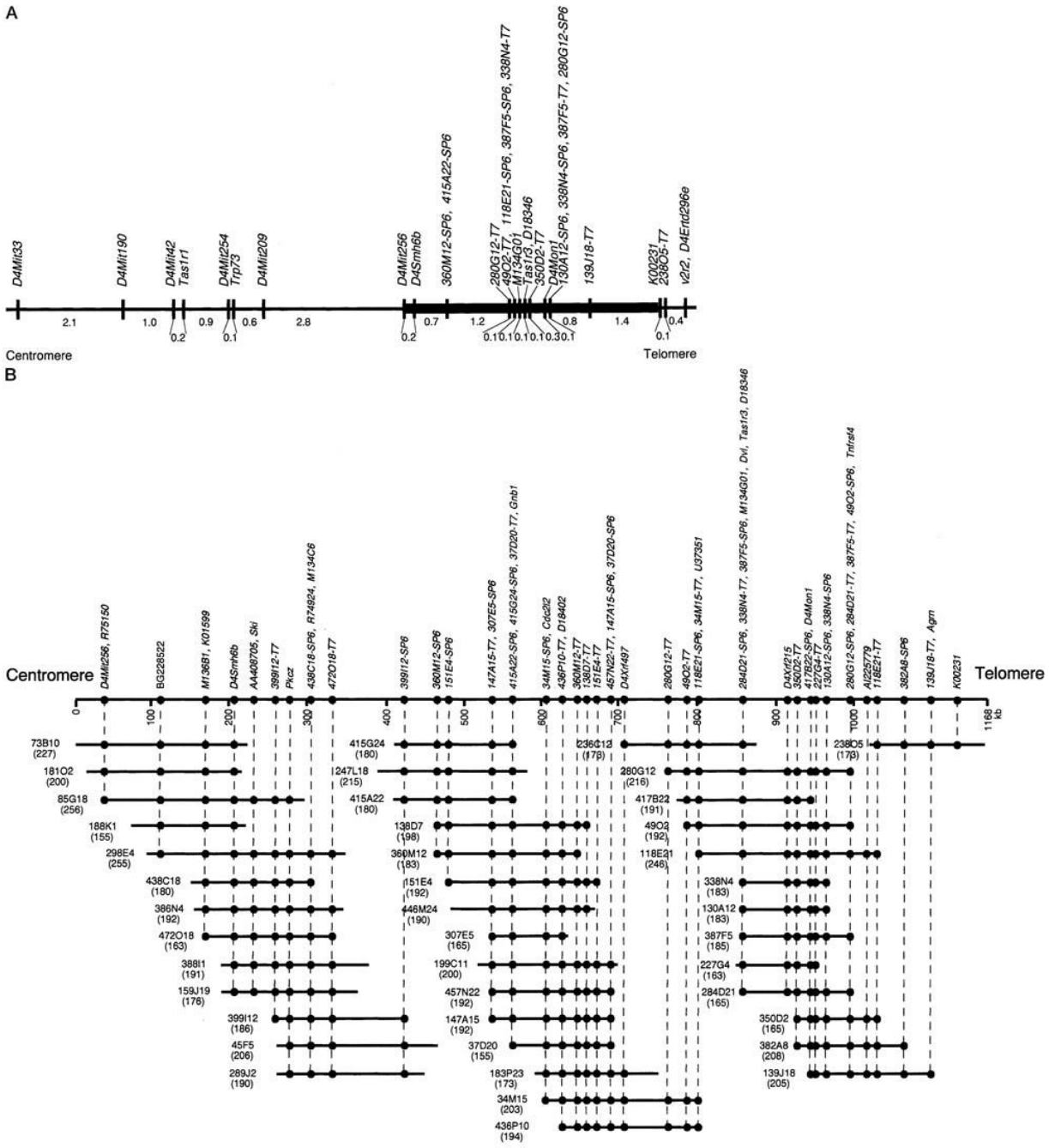


Fig 1.

(A) Linkage map of the distal part of mouse Chr 4. Distances between markers were estimated in cM based on data from the B6 × 129 F_2 intercross (n = 628). The genetic interval from *D4Mit33* to *D4Ertd296e* is 13.3 cM. Thick line indicates region that was physically mapped. **(B)** Physical map and the BAC contig of the subtelomeric region of mouse Chr 4 between *D4Mit256* and *K00231*. BAC sizes (kb) are shown in parentheses. Dots indicate the presence of markers detected by hybridization and PCR, and in some cases, by direct sequencing.

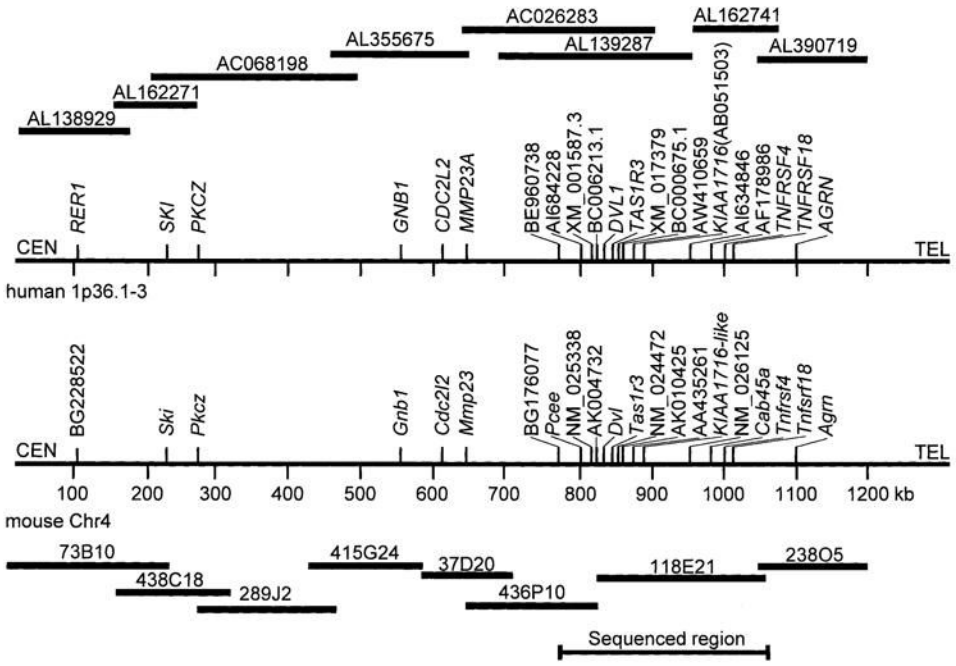
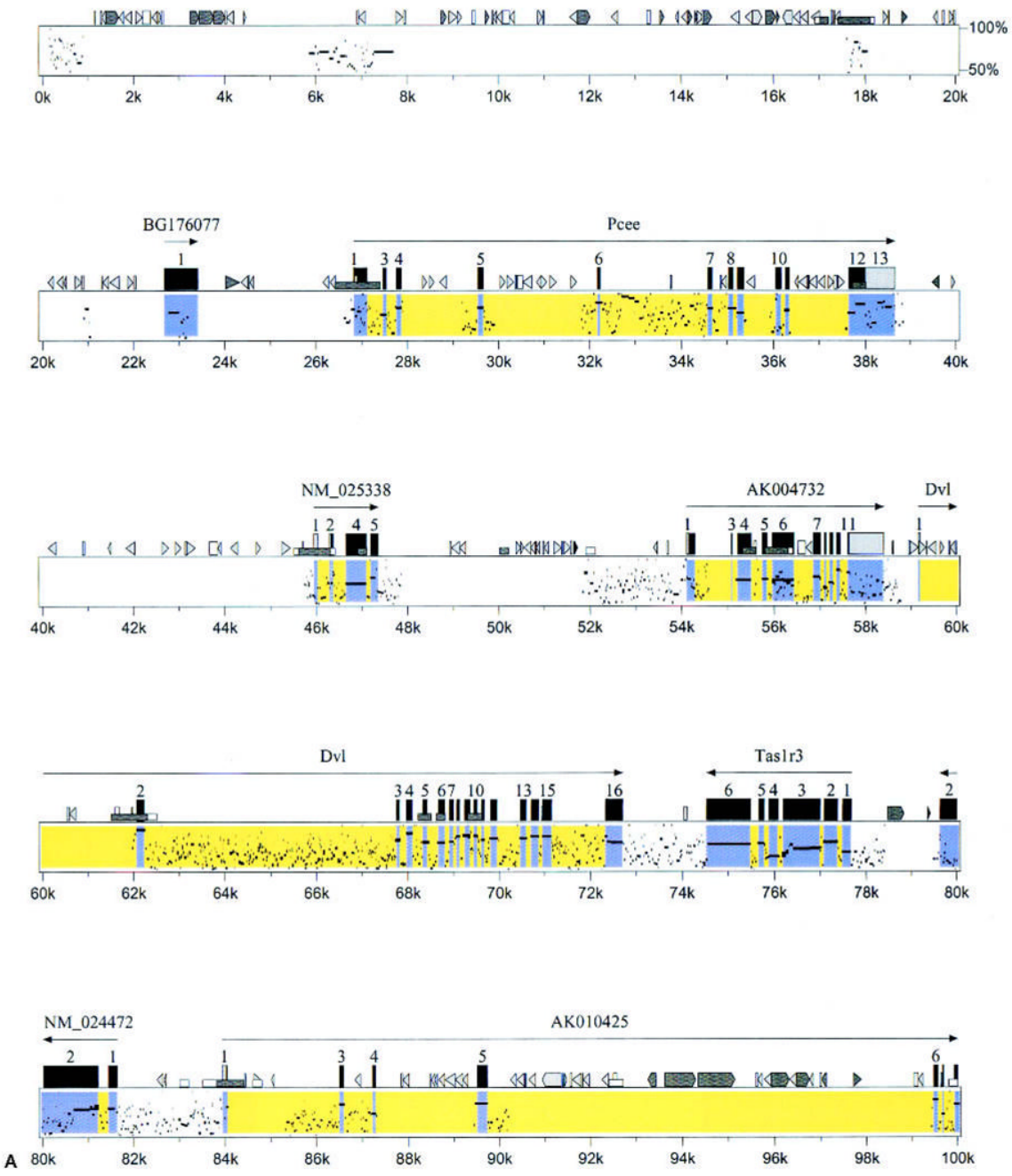
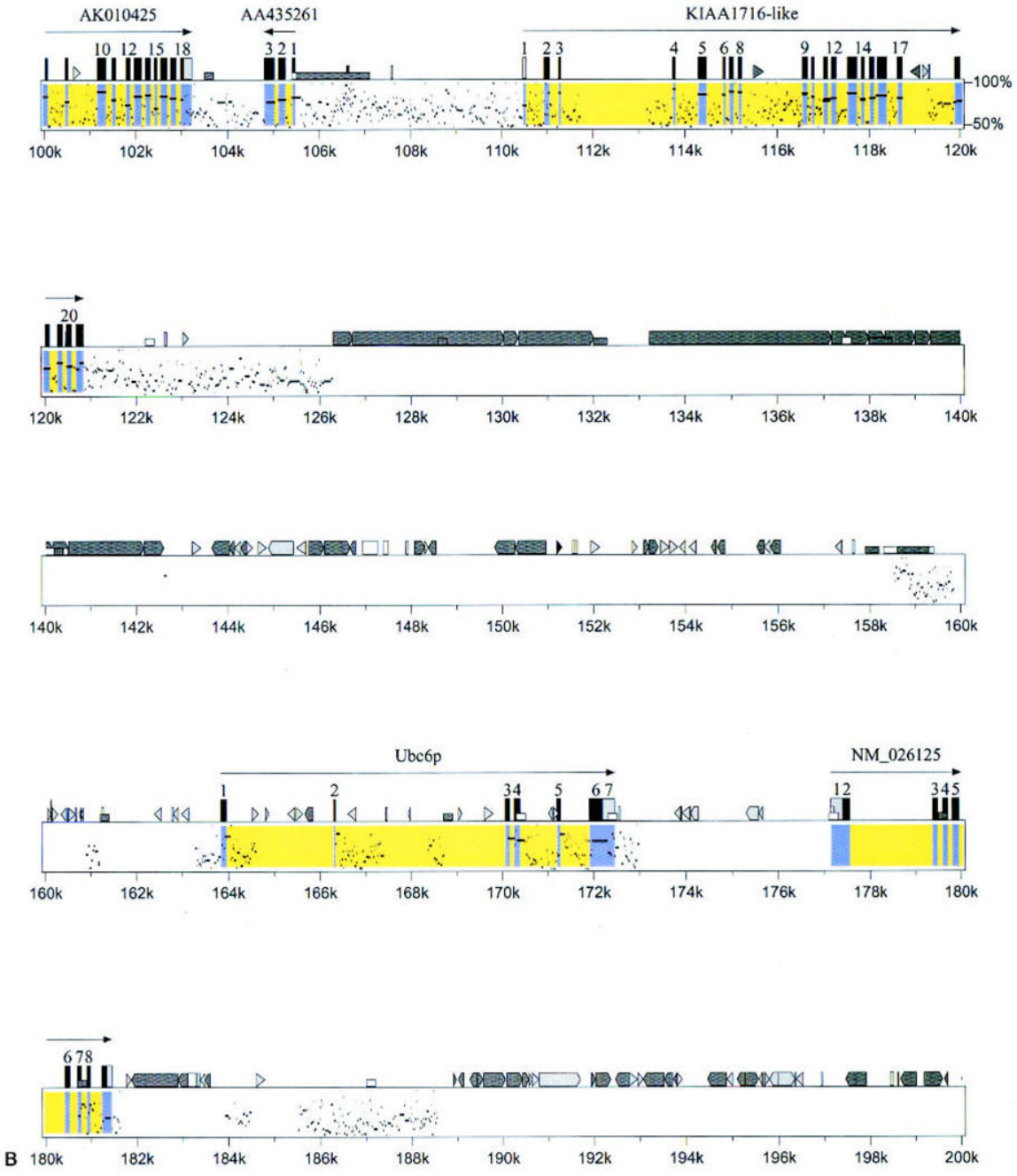


Fig 2.

Mouse and human comparative 1.2-Mb map. The minimum tiling paths of the human BACs (upper) and mouse BACs (lower) are shown. Human BACs are identified by Accession number, and mouse BACs are identified by clone name. Mouse and human genes are denoted by approved gene symbols, if available. If no approved symbol was available in either species, Accession numbers were given. If no Accession number was found, but there was a sequence identity (see Fig. 3) the “like” suffix was added to the human gene name (*KIAA1716*-like).





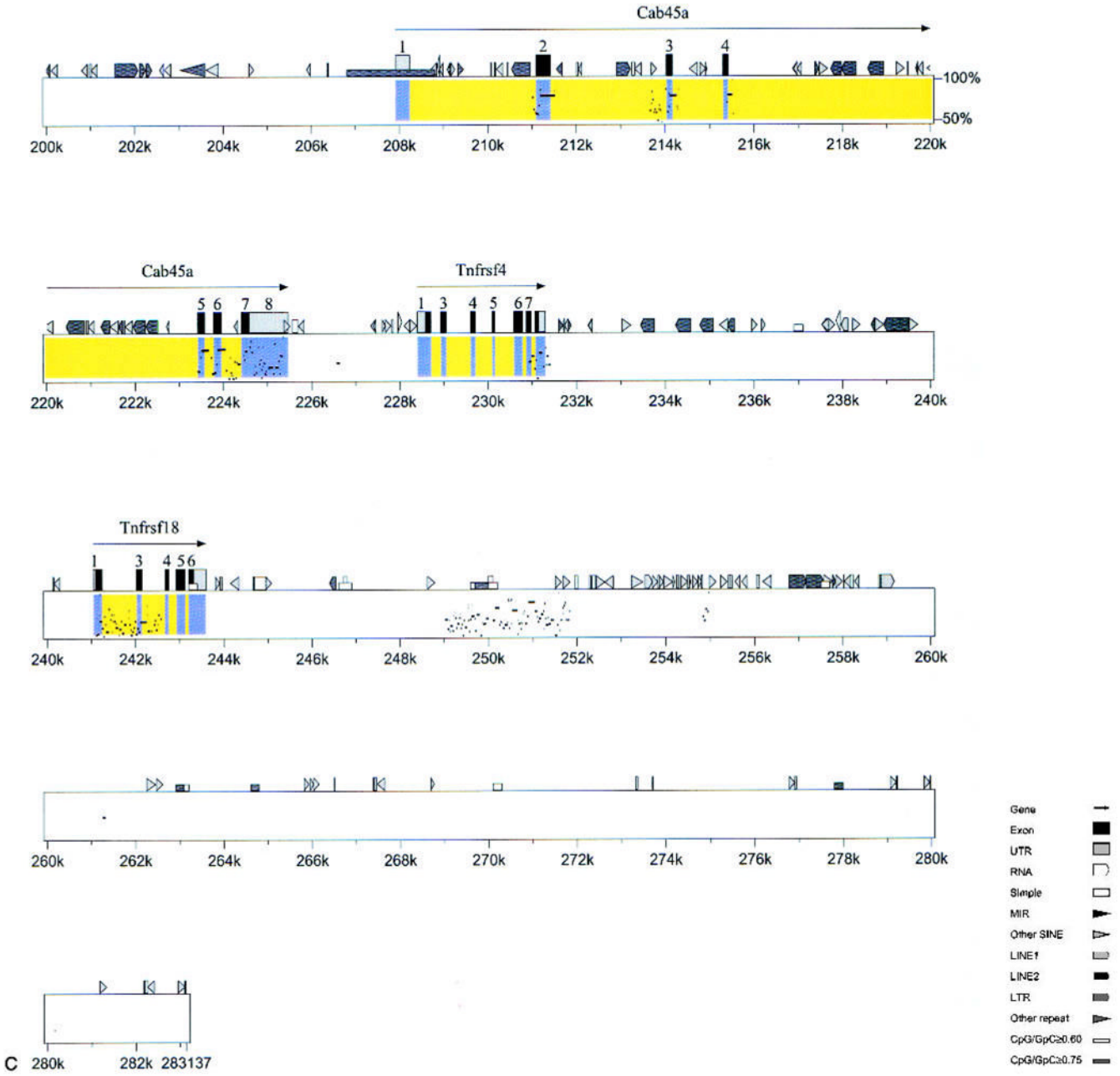


Fig 3.

Percentage identity plot-based comparative map of mouse and human genomes. The mouse genomic sequence is upper, and the human genomic draft sequence is lower, assembled from three overlapping human BAC clones (Accession # AC026283, AL139287, and AL162741). Both the mouse and human sequences are oriented from the centromere (left) to telomere (right), and the percentage identity is shown on the vertical axis (50–100%). The portions of the figure corresponding to exons with exact matches to known genes are yellow; those with exact matches to cDNAs are blue. Mouse cDNA sequences with no homology to human genomic sequence are not included. Mouse genes are denoted as described in Fig. 2.

Table 1

Loci with developmental, behavioral, or disease susceptibility phenotypes mapped to the subtelomeric region of mouse Chr 4.

Name	Symbol	cM	Reference
Friend virus susceptibility 1	*Fv1*	76.5	(Stoye et al. 1995)
Gastritis type A susceptibility locus 2	*Gasa2*	77.5	(Silveira et al. 1999)
Epistatic circling gene of C57L/J	*ecl*	78.4	(Taylor 1976)
Fluctuating asymmetry QTL 1	*Faq1*	79.1	(Leamy et al. 1997)
Beta-carboline-induced seizures 1	*Bis1*	80.0	(Martin et al. 1995)
Insulin-dependent diabetes susceptibility 9	*Idd9*	82.0	(Rodrigues et al. 1994)
Testis-determining autosomal 1	*tda1*	82.0	(Eicher et al. 1996)
Saccharin preference	*Sac*	83.0	(Fuller 1974)

Gene names and symbols are from the Mouse Genome Database (MGD), maintained at The Jackson Laboratory. Distances from the centromere (cM) are from the integrated MGD map.

Table 2

Sequence-tagged sites (STS) in the subtelomeric region of mouse Chr 4.

Locus	Accession[a]	Forward primer 5′ → 3′	Reverse primer 5′ → 3′	Product size (bp) [b]	Polymorphic B6/129[c]	Detection method[d]
D4Mit33	MGI92868	GGAGGTGTCAGGAGCCCT	CTCCTGACTGAGGTACCAAACC	126	Yes	SSLP
D4Mit190	MGI92820	CAGTGAAGCAAAAAGATTCGG	TTCCAGTCTTGGGAATGTATCC	144	Yes	SSLP
D4Mit42	MGI92875	CATGTTTGCCACCCTGAAAC	CCTCACTTAGGCAGGTGACTC	100	Yes	SSLP
Tas1r1	AF301161	GGAGTTGCAGCCTCTACAGC	CAGGAGAGGAAAATGGCAAA	100	Yes	SSCP
D4Mit254	MGI100505	AATACAATTATCATTTGCATTGTGG	TCGTGGTGGACCTTATCTCC	135	Yes	SSLP
Trp73	AF138873	TGAGATCTGGTGCCCTCTCT	GCCTGATCTAGGCTGGAAAA	229	Yes	Both
D4Mit209	MGI92840	CCTTGCTGGTTCTGGCTAAG	CTATCTCCTGAGTTGCTCTGAGC	122	Yes	SSLP
D4Mit256	MGI100503	CTGGAGAGTTAGAATGGGGTACC	ACAGAGGCGCTTCCTAAC	132	Yes	SSLP
R75150	R75150	ACAGGACAAATGCTGGGTTG	GTGGTAAAGAACGCTTGGCT	217	Un	NA
BG228522	BE137821	GCCGATCCTGGTGATGTACT	ACAATGGCTCAAAACCGTTC	200	Un	NA
M136B1	AU042805	TCCTTTATGTCCAACAGCCA	CATGGTCTGTGATGTGACCA	164	No	SSCP
K01599	AA062172	GGAAAAGGGAGTCGCCATA	GAGCCGCCTAACTCTCACAC	166	No	SSCP
D4Smh6b	X58261	CTGTAGGCTGCTTTTATCTTTTG	TGCCCCTTCAGCACATGCCA	102	Yes	SSLP
AA408705	TC87041	GCTTCAGAAAATCGAGGCAC	GCATGGGCTATGATAGGTGG	232	No	SSCP
Ski	U14173	ATCTCTTGTTTCGTGGTGGG	AGGATGCCCATGACTTTGAG	173	No	SSCP
399I12-T7	AZ052540	TGGTGGTGTAATACTATTCCTTTG	TCTTTAATTTTTGGCTTTTTGATACA	102	No	SSCP
Pkcz	NM_008860	ACACATTAAGCTGACGGACT	CAAACATAAGGACACCCAGT	164	Yes	SSCP
R74924	R74924	AGTGCCACCAACCTGGTAAG	AAGTGCCTGCAGGGATGC	165	No	SSCP
M134C6	AI098070	CAAAACCACATGGTTACCGA	GCCCTATTGCCAAATGACTT	264	No	SSCP
438C18-SP6	AZ077160	CGGGACCTAAAACTGGACAA	TGGGGACAGTTACCAGGAAG	254	Yes	SSCP
472O18-T7	AZ114562	CTTTCCATTCTCCACCCTCA	AGGTCCTAGGGAGAGGTCCA	260	No	SSCP
399I12-SP6	AZ052537	CAGCTGTGTGCATGTTGACC	CATCATGAAGACTCAGGGCA	106	No	SSCP
360M12-SP6	AZ027787	AATGATGAAGTGTCAGCCTCAG	CAACAGAACTCAAAGCCTGG	100	Yes	SSCP
151E4-SP6	AZ263331	GACCTTCGGAAGAGCAGTTG	AGTGTGTGTCGCCATATCCA	223	No	SSCP
147A15-T7	AZ273113	GTGGTTGCTGGGATTTGAAC	CAAGCAACCAAACAACCAAA	101	No	SSCP
307E5-SP6	AQ982924	GCTAGTTGGGGAACAAACCA	ACTGCAAATGTCCAACTCCA	149	No	SSCP
Gnb1	NM_008142	GGGCATCTGGCAAAGATTTA	AGATAACCTGTGTGCCCGC	281	No	SSCP
37D20-T7	AZ088686	GAAAACAATCGGGGAGAAGTC	TGAAATTATCACACGCCAGG	109	No	SSCP
415A22-SP6	AZ057087	GTCCACACCTGGCTTTTGTT	CAGCACTCAGTGAGGTTCCA	199	Yes	SSCP
415G24-SP6	AZ059760	ATGTAATGGAAGGGCTGCTG	CAGCACTCAGTGAGGTTCCA	113	No	SSCP
34M15-SP6	AZ238889	CGCTACTTCGCTTTTATCCG	ATGATGACGTACGACGACGA	150	No	SSCP
Cdc2L2	NM_00766	GGCTTCAGCCTCAAGTTCTG	AAAACAACCAAGTTGCCCTG	101	No	SSCP
436P10-T7	NA	CACAGGCCAAGTTGTTGTTG	CAGGGGACCTTCTGAATGAT	115	Un	NA
D18402	D18402	GGAGTTCTCCTACCCTGGCT	GAGGCTCTGAGCAGTGTCAA	167	No	SSCP
360M12-T7	AZ027785	CGGTCAGGAGTAGTGTGGGT	CAGCAGCTGATATTGAGGCA	123	No	SSCP
138D7-T7	AZ281648	ACCTCTAGGGTTTACGGGGA	CCTCAGGTAGTGCAAGCTCC	199	No	SSCP
151E4-T7	AZ263335	GTCCCAAAAGCTAGCACAGG	TCATGAGCCAACCATGTGATT	240	No	SSCP
457N22-T7	AZ089282	CCGGAGGACCATAAATCTGA	CCTCAAAAACAAGCCTGAGC	129	No	SSCP

Locus	Accession[a]	Forward primer 5′ → 3′	Reverse primer 5′ → 3′	Product size (bp) [b]	Polymorphic B6/129[c]	Detection method[d]
37D20-SP6	AZ088681	GCTAGCCTTGAAGCCAACAC	TGAACAGCATGCTTACCCAG	122	No	SSCP
147A15-SP6	AZ273111	TCCGGAGGACCATAAATCTG	CACAGTCCCAGTCATTCCCT	249	Yes	SSCP
D4Xrf497	AA117049	GCGAGACGAGTGGGTAGTTC	ACACTGAAACCTCGCTTGCT	129	No	SSCP
280G12-T7	AZ986251	GGGCTGGGAATTGAACCTAT	TGAATCCCTTACAGCCTTGC	420	Yes	SSCP
49O2-T7	AZ253249	TCCCTAGAGGCCTGTCTGTC	TCGTCTCGGAGCCTCTTCTA	169	Yes	SSCP
34M15-T7	AZ238896	GGGTTTATGTGGCAAGCACT	ACTCCATTTGCCTTTTGTGG	118	No	SSCP
118E21-SP6	AZ274152	GGGGCAGGTGGGTAATAAGT	CAAAAGCCCAACTCCTTGAG	271	Yes	SSCP
U37351	U37351	GTGGCTTGGTGCTATTGACA	GGGGCTATTAAGGCCATTTT	160	No	SSCP
338N4-T7	AQ998700	AGTTACACAGCTGGGACGA	GCAAGAGCCTAGCAATCCAC	245	Yes	SSCP
284D21-SP6	AQ932576	ACAGAAATCCCTCATGCGA	TCAGTGTGGACCAGAAAGTCC	105	No	SSCP
387F5-SP6	AQ998225	CAGTTACACAGCTGGGACGA	GCAAGAGCCTAGCAATCCAC	245	Yes	SSCP
D4Mon7	G67778	CCCACTATGGTCCCAGAGAA	GCGCTGACATCCTCCTATGT	187	No	SSLP
M134G01	AI31686	CACATTAGCCATTGTCCTGG	GGCAGAAAGGAATCAGAAGC	161	Yes	SSCP
D4Mon4	G67775	ACATGCCTGCCTATCTTTGC	GGAACCTGTTTTCCATGGTG	197	No	SSLP
Dvl	NM_010091	AAGTTCATGGGCCTCACCACCTGTG	TACTAGCTACCCTTCACATACC	238	No	SSLP
D4Mon6	G67777	TGAGTGTCCTCTGCCTGATG	CCATGGGAGACCAGAAGGTA	206	Un	SSLP
Tas1r3	AF311386	GCAACAGAGTCACAACTGCC	ACACACAGTACCAACCCCGT	293	Yes	SSCP
D4Mon3	G67774	GCTTACGATGGTCGTGAGGT	GCAAGCAACCTGAACATGAA	188	No	SSLP
D18346	D18346	ATGTCCAGGGTAGAGAGCCC	TGTCCGCAGTGTGGAAACTA	165	Yes	SSCP
D4Xrf215	F07942	TTCCAAGCTCACACATCAGC	GTGCTGCTCTGCATTGAGTG	124	No	SSCP
350D2-T7	AZ032147	TGTAGGGAATGTTTCTGCACC	ACATGGAACAGGATTCTGGC	295	Yes	SSCP
D4Mon41	G67754	TGCATCACTATTAAGCCTCAACC	AAGAATTTGCAAAGACTGTGAGA	260	No	Both
D4Mon43	G67755	GGTGGCTCAAACCATCCATA	GAGGGCAATGAGCAAAATGT	203	Yes	SSCP
D4Mon44	G67756	GGTCCTGTCTCTGGTTCAGG	TAACACCCACATCAGGCAAC	201	Yes	Both
D4Mon48	G67757	CAGCAGGCAAGATGACCTC	GTCCCTCACCAGCCATGTTA	205	Yes	Both
D4Mon49	G67758	AGCCTGGGCTAAGTTGTGTG	TATGGGCCAATGTTGTTCCT	204	Yes	Both
D4Mon50	G67759	ATGGTGGCTCACAACCATCT	AT TTGTCCTCTGATTGCAGC	193	Yes	SSCP
D4Mon52	G67760	ATGCTCAGCCTGCTTTGTTT	GCTGATAGCCCTGGGTTCTA	198	No	Both
D4Mon53	G67761	TGTACGCACAAATTGACTTGC	GAATCCACATTGCAAAGCCTA	222	Yes	SSCP
D4Mon54	G67762	CACAGGCAAATGAAGGGAAG	CCAGACTTCTCCAGCTCTCC	187	Yes	Both
D4Mon31	G67747	CAGATTCTCCAGCTGTCAGG	CTGTGTTTCCGCACCAAGT	229	No	SSCP
D4Mon56	G67763	CCTGTGGTTGACTAGGCAGAA	GCCTGATAGCCTGGAATACA	406	Yes	SSCP
D4Mon5	G67776	ACCTTGTTCCTGGTGTGAGC	TAGCTGGGACGTGGTATGGT	200	No	SSLP
D4Mon58	G67764	TGCACTGACCGTGATAGAGG	CGGTGTAGCTCTGGCTGTCT	200	No	SSCP
D4Mon59	G67765	CATCTCACCAACTCGCACTT	TTTCTGGGAACAAAGAGGCTA	418	No	Both
D4Mon60	G67766	GAACCCAAGTGTTGGGGTAA	TGGAAGCCCATCTGTCTCTT	222	Yes	Both
D4Mon33	G67748	CAGCAGAGGTGATGGGTTCT	TTGTCACACAGTGGTTAAATGC	203	No	SSCP
D4Mon62	G67767	GGTGTGCACCACCATATTCA	GGGAATTATCAGCCAAAAAGC	201	Yes	Both
D4Mon34	G67749	TAGAACCGTGGCTGAGGACT	CCGTAAGATATGAAAGAACTTGGA	201	No	SSCP
D4Mon63	G67768	GCCCAACTGAAAGCTCAACT	GGAAGGGGGATAACAATTGAA	263	Yes	Both
D4Mon64	G67769	TGCTAATTTCAAGCACAGTGAGA	AGCTTGACACCTTGACAGCA	369	Yes	SSCP
D4Mon36	G67750	CCTTCCTCGTCTGAGCTGTT	TTGGGACGTGACCTGAGAAT	232	Yes	SSCP
D4Mon66	G67770	TTCAATTGAGTTTCTCTCCTCTGA	TGCAGGACCAAGAAGTAGGC	200	Yes	SSCP
D4Mon37	G67751	TATGTGTCTGGCCGTTGTTC	GATGTGGGTGCAGGTGAAG	206	No	SSCP
D4Mon39	G67752	GCTGAGCAGCCTCTAGCAA	ACCATGGCTTTTCCCAGTAA	241	No	SSCP
139J18-T7	AZ260477	ACCACTTGGGGGCTACTTCT	GCTGATCCCCCTGTGATTTA	232	Yes	SSCP
D4Mon40	G67753	CTGTGCCTTTGGTGATCAGA	TGTGGCACTCTACGGCATAA	261	No	Both
D4Mon1	G67772	GCAGTGAGCTGCAGAGTTTG	AGGCCTACCCAAGGACATCT	201	Yes	SSCP

Locus	Accession[a]	Forward primer 5′ → 3′	Reverse primer 5′ → 3′	Product size (bp) [b]	Polymorphic B6/129[c]	Detection method[d]
417B22-SP6	AZ061836	AAACAGGCATGAAACTCAGGA	GGGTATCATTGTCACCTCCA	116	No	SSCP
227G4-T7	N/A	GGAAGCAAATGCTCCACTAAA	TATCCCTAGCCCCTTGTGTG	243	No	SSCP
D4Mon28	G67746	GACCTTTGGAAGAGCAGTCG	TGGCAGCTCACAATGTCTTT	296	No	SSLP
D4Mon27	G67745	TGCCTTTTTCTCACATTGTCTC	TTAGAAGCAGAGGCAGAGGC	250	No	SSLP
D4Mon26	G67744	TCAGACATCTCTGGCCTCCT	TTCACTAAGTTGCCCAGGCT	160	No	SSLP
284D21-T7	AQ977902	GGTTTGGGAGTGTTAGGCAA	ACTCAGTTGGCCTCTCCTCA	138	No	SSCP
280G12-SP6	AZ001739	GCCCCATAAAATCCACTCCT	GCTCCGGAAGGCTAGAAGAT	233	Yes	SSCP
387F5-T7	AQ998226	GCCCCATAAAATCCACTCCT	TTGCCTAACACTCCCAAACC	214	Yes	SSCP
49O2-SP6	AZ253245	GATAGTCCCTTAGCCAGCCC	GCCATAGCTCCTCACTGCTC	218	No	SSCP
338N4-SP6	AQ998697	CAGTTTAGCACCCCACCCTA	TCTGCACCTCTGTTCACCTG	115	No	SSCP
130A12-SP6	AZ266745	CAGTTAGCACCCCACCCTAA	TCTGCACCTCTGTTCACCTG	114	Yes	SSCP
D4Mon25	G67743	GTTTCACATGTTGTGGTGGC	GGGACCTTTGGGATAGCATT	131	No	SSLP
Tnfrsf4	NM 011659	AGGCAGAAAGCAGACAAGGA	CGACAGCACTTGTGACCACT	138	Yes	SSCP
D4Mon24	G67742	CAGCAGCAAATGACCTTTCA	GAGGCAGGCAGATTTCTGAG	147	No	SSLP
D4Mon22	G67741	GTTGGGGCTGCTCATAGAAA	GCTGTGGCTCTCTTGGAGTT	422	Yes	Both
D4Mon21	G67740	CAGTTCTTCCCGAAAACCAC	TTTCTGGGAACTGAGATGGC	174	No	Both
A1225779	AI225779	GCTGTTAGTGAGGTCAGGGC	CGTAGGTGGCACAGTTGAGA	104	Yes	SSCP
D4Mon20	G67739	TTAGCGTTAGGGTGAGGGTG	GGAGACTACGGACTTGTGGC	150	No	Both
D4Mon19	G67738	GGAAGGTAGGGCCTGGTAAT	GCTCCAAGATCTGTGCGATT	277	No	Both
D4Mon18	G67737	GCTCAGCCCCTGAATCAATA	GGGATCTGCCTGTCTTACCA	111	No	Both
D4Mon17	G67736	GCTCAAGGAAGGACACACCT	TGCTCTTAACATTTTGAGCCAT	201	No	SSLP
D4Mon16	G67735	ATCAGACAGCCCACAACCTC	TATGTGCCACCACACCTGTC	206	No	SSLP
D4Mon68	G67771	ACATGTCCACTGTGGCAAAA	TGTCATGAGTTTGAGGCCAG	246	No	SSLP
D4Mon2	G67773	TGATCTTTCCAAACGCATAAGA	AGACACCCTAGGTCCTGCTG	151	No	SSLP
D4Mon15	G67734	TGTTCCTGAGTTCACAACGC	ATTCCCAGCAACTACATGGC	269	No	SSLP
D4Mon9	G67779	GGACAGGTAGCTCACCCAAC	CCAAGAGTCAGCCTTGGAGT	200	No	SSLP
D4Mon14	G67733	TACTTCCCCTTTCCCGAACT	TCCTTGGTGCTTACCCTCAC	232	Yes	SSLP
D4Mon13	G67732	CTAGGGGACTCTGCCAAGTG	CAAGACACCCAGTCCCAACT	195	No	SSLP
D4Mon12	G67781	GCAAGTTTCAGGAGCTAGGG	CCCCAGAACCAGAGACCATA	166	Yes	SSLP
D4Mon11	G67780	AGCCAGGGCTACACAGAGAA	ACCCTGCTACAACGCAGACT	153	No	SSLP
118E21-T7	AZ274183	CCCAGAACTCCATCCTCAAA	CCCAACCTGTGGTCAGCTAT	185	No	SSCP
382A8-SP6	AZ018016	AGCAGGCACAGGTCTCTTGT	AAGAACAGGACAGTGGTGGG	202	No	SSCP
139J18-T7	AZ260480	TCAGTTACCAAGGGTTTCGG	ATAGGTTGTCACAGGCCAGG	122	Yes	SSCP
Agrn	M92654	TGTGACTTCCTCTTCCCCAC	TGAGCCACTCCAGATGTCAG	156	No	SSCP
28.MMHAP7FLB4.seq	M92657	CACTAGAGCTGCCACCTTCC	CCCTCAGCACCACTTTTTGT	162	No	SSCP
K00231	AA410003	GTGCTCTGCAGACAAACCAA	GAGCCATTTTGACCCTTAAA	154	Yes	SSCP
V2r2	AF053986	CAATTGAGGAATGGCTACCAA	TGGCTTCATGTCCATTGTGT	170	Yes	SSCP
238O5-T7	NA	TATAAGCAGCCCCTCATTGG	CAGGCCAGACACTGCTTACA	244	Yes	SSCP
D4Ertd296e	C80036	AGGCATATTGTATAATAAATTTGTAGT	CCGGATGACTCTACTTGAC	201	Yes	SSCP

[a] Accession numbers beginning with MGI are from the Mouse Genome Database and all other Accession numbers are from GenBank.

[b] Product size is obtained from B6 genomic DNA.

[c] Un = Unknown, not attempted.

[d] N/A = not available.

Methods of detection: SSLP = simple sequence length polymorphisms; SSCP = single-strand conformation polymorphisms. Several markers were within known genes, e.g., *R74924 (Pkcz); D18402 (Mmp23); U37351 (Pcee)* and *28.MMHAP7FLB4.seq (Agrn).* The STSs are ordered from the centromeric end to the telomeric end.

Table 3

Marker positions within the 284-kb sequenced region of distal Chr 4.

Gene/Marker	Beginning	End
280G12-T7	11715	11858
49O2-T7	27642	27810
118E21-SP6	29363	29633
U37351	38244	38403
34M15-T7	44601	44218
338N4-T7	44905	45148
387F5-SP6	44904	45148
284D21-SP6	45018	45122
D4Mon7	53569	53755
M134G01	58000	58160
D4Mon4	58522	58718
D4Mon6	73982	74187
D4Mon3	84280	84467
D18346	103341	103506
D4Xrf215	117690	118021
350D2-T7	143131	143431
D4Mon41	144063	144322
D4Mon43	144734	144936
D4Mon44	145429	145629
D4Mon48	151165	151369
D4Mon49	151468	151671
D4Mon50	153730	153925
D4Mon52	155679	155876
D4Mon53	155857	157434
D4Mon54	157579	157765
D4Mon31	157787	158016
D4Mon56	160305	160710
D4Mon5	161128	161327
D4Mon58	164439	164638
D4Mon59	165441	165858
D4Mon60	167377	167598
D4Mon33	167809	168011
D4Mon62	171062	171262
D4Mon34	172821	173022
D4Mon63	173883	174145
D4Mon64	175196	175564
D4Mon36	182757	182988
D4Mon66	183512	183711
D4Mon37	187705	187955
D4Mon39	197739	197979
139J18-SP6	199176	199407
D4Mon40	202605	202865
D4Mon1	206251	206451
417B22-SP6	213552	213669
227G4-T7	214230	214473
D4Mon28	221574	221869
D4Mon27	225540	225789
D4Mon26	227773	227932
284D21-T7	230924	231061
280G12-SP6	231023	231255
387F5-T7	231042	231255
49O2-SP6	231113	231331
338N4-SP6	231321	231436
130A12-SP6	231328	231443
D4Mon25	231647	231777
D4Mon24	240095	240241
D4Mon22	244624	245045
D4Mon21	246689	246862
AI225779	247955	248055
D4Mon20	250004	250153
D4Mon19	252161	252437
D4Mon18	253757	253867
D4Mon17	254169	254369
D4Mon16	254415	254620
D4Mon68	255365	255610
D4Mon2	256018	256168
D4Mon15	267313	267581
D4Mon9	268606	268805
D4Mon14	273253	273484
D4Mon13	273608	273802
D4Mon12	276854	277019
D4Mon11	279935	228277

Gene/Marker	Beginning	End
118E21-T7	282595	282779

Marker locations are given as bp within the sequenced region (1–284,000 bp; see Fig 3). Accession numbers for the markers are in Table 2.

Table 4

EST or cDNA matches and exon/intron junctions of genes in the distal region of Chr 4.

Gene/ EST Exon/CD	Exon Position Begin-End	Exon length (bp)	Detected by Genscan	Intron length (bp)	Acceptor site (intron/ EXON)	Donor site (EXON/intron)
BG176077						
E1	22670–23413	744	No	N/A	N/A	N/A
AA546397						
E1	23756–24249	494	No	N/A	N/A	N/A
AI428898						
E1	26304–26696	393	No	N/A	N/A	N/A
Pcee						
E1a	26817–26832	16	No	N/A	N/A	N/A
E1b (CD1)	26833–27114	282	No	N/A	N/A	CTGCCGCAGgtaagggcc
E2 (CD2)	27460–27534	75	Yes	345	tgtccctagGTGGCCATG	TCCATGGAGgtaggcttc
E3 (CD3)	27750–27859	110	Yes	215	acatttcagCATGTGTCC	AGAGAAAAAgtgagtcct
E4 (CD4)	29533–29653	121	Yes	1673	tccctgcagGAAGCCTGT	CCTCACAAGgtgggtttg
E5 (CD5)	32160–32224	65	Yes	2506	atcttccagATAATCGTT	GACTGCATGgtgagttga
E6 (CD6)	34565–34664	100	Yes	2340	tggtttcagGAACTACAT	ACACTGGAGgtaagtatg
E7 (CD7)	35017–35121	105	No	352	tgttcccagATCCCTTTA	CGGAAAAAGgttcttttg
E8 (CD8)	35206–35347	142	Yes	84	ggttctcagGTTGATTTG	CCAAGCTGGgtgagtgtc
E9 (CD9)	36039–36159	121	No	691	gattcagtaGAATCCCCA	TGTAAATGGgtaaggtgc
E10 (CD10)	36243–36336	94	No	83	ctgtttgcaGCTTGCTGA	AAAGAGAAGgtctgtgtc
E11a (CD11)	37631–37985	355	No	1294	tctctacagGAAAAGTGA	33UTR after stop codon
E11b	37986–38638	653	No	N/A	33UTR	33UTR
NM 025338						
E1	45939–46028	90	No	N/A	N/A	GTCTGTTTGgtgcgtgcg
E2a	46276–46308	33	No	247	gtgttctagCTTTGAGCG	N/A
E2b (CD1)	46309–46363	55	No	N/A	N/A	CCTGGGCAGgtaagacgc
E3 (CD2)	46645–47090	446	Yes	281	tttccccagGTTTCAGTC	AAGAAGCAGgtgaatatg
E4 (DC3)	47184–47350	167	No	93	cccttgcagATCAAATTT	N/A
AK004732						
E1a	54090–54134	45	No	N/A	N/A	N/A
E1b (CD1)	54135–54283	149	Yes	N/A	N/A	TTCTTCAGAgtgagtgtg
E2 (CD2)	55071–55091	21	Yes	787	ttcttccagGCTCTGCAG	TGTCCTCAGgtcagtatc
E3 (CD3)	55199–55501	303	Yes	107	cccgacaagGGCCTTCAG	TCATTCGCGgtacactgt
E4 (CD4)	55738–55839	102	Yes	236	gccaagcagCTGTGGACA	CAGAGGATCgtgagtgtg
E5 (CD5)	55955–56425	471	Yes	115	catccgcagCCCTATTAA	CTAGCCCAGgtgtgtgtg
E6 (CD6)	56846–57004	159	Yes	420	tacatctagATCCCACCC	ACAGCGGAGgtgaggctg
E7 (CD7)	57082–57121	40	Yes	77	tcttcacagGATGCAAGA	GTCAAAGGGgtgagtttg
E8 (CD8)	57194–57270	77	Yes	72	tccctttagGAAGGATGT	TCCAGCTAGgtaggggtg
E9 (CD9)	57348–57428	81	Yes	77	cccaaccagATTACAAAA	TGGATAAAGgtgagcact
E10a (CD10)	57594–57619	26	No	165	ggcttccagAGTTCAGGA	33UTR after stop codon
E10b	57620–58382	763	No	N/A	33UTR	33UTR
Dvl						
Promoter	59138–59177	40	No	N/A	N/A	N/A
E1 (CD1)	62060–62229	170	No	N/A	N/A	GGACTTCGGgtcagtggg
E2 (CD2)	67743–67812	70	Yes	5513	tgaccacagGGTGGTGAA	GTTTCCTGGgtgagtcta
E3 (CD3)	67962–68083	122	Yes	149	ccttcacagCTGGTCCTG	CTCCTTCCAgtaaggccc
E4 (CD4)	68308–68411	104	Yes	224	tctatgcagTCCAAATG	GCGATGAGGgtactatgg
E5 (CD5)	68655–68793	139	Yes	243	tccccacagCTGCCCGG	TACGAGCCGgtgggtgac
E6 (CD6)	68891–68984	94	Yes	97	ctgcctcagGCTGAGCAG	ACAGACCGGgtaggcagc
E7 (CD7)	69056–69125	70	Yes	71	cctccgcagGCATCCTCC	CTCTCAACAtgggtgagg
E8 (CD8)	69231–69350	120	Yes	105	ctcctgcagAGAGGCACC	TTGCTGCAGgtgggagcc
E9 (CD9)	69429–69505	77	Yes	78	accccgtagGTGAACGAT	CCAGACAGGgtgaggtgg
E10 (CD10)	69597–69664	68	Yes	91	actctgtagGCCCATCAG	TCCCAAGGGgtgagttac
E11 (CD11)	69804–69944	141	Yes	139	cccacacagCAGCACTGA	GCGCCCCACgtaagtggc
E12 (CD12)	70457–70588	132	Yes	512	gctctgcagAGCTTGAGG	CTGTCATTGgtgagtccc

Gene/ EST Exon/CD	Exon Position Begin-End	Exon length (bp)	Detected by Genscan	Intron length (bp)	Acceptor site (intron/ EXON)	Donor site (EXON/intron)
E13 (CD13)	70687–70854	168	Yes	98	tgtgcccagGGGCGGATG	TGTGCAGTAgtgagtagg
E14 (CD14)	70939–71145	207	Yes	84	tcctgccagACCTCGCAT	AGAGTGAAGgtgagaggt
E15 (CD15)	72318–72691	374	Yes	1172	tccccctagGGAGCAAGA	33UTR after stop codon
Tas1r3						
CD1	77659–77469	191	Yes	UN	53UTR	GTGCAACAG/gtatggagg
CD2	77360–77060	301	Yes	108	tggcctcag/GTTCTCACC	ATGCCACAG/gtgagccca
CD3	76979–76176	804	Yes	80	tcaccacag/GTCAGCATA	CCCTGGCAG/gtaagggta
CD4	76064–75867	198	Yes	111	actccacag/CTCCTGGAG	GGCAACCAG/gtaaggaca
CD5	75768–75648	121	Yes	98	catgtacag/GTGCCAGTC	AGCATCCAG/gtgaaccgt
CD6	75472–74511	962	No	175	tcactacag/ATGACTTCA	33UTR after stop codon
NM_024472						
E1a	81628–81560	69	No	N/A	N/A	N/A
E1b (CD1)	81628–81438	122	Yes	N/A	N/A	ACTTGTCAGgtgcgtcta
CD2	81210–79608	1603	No	227	ctttcgcagGTTTCTAAA	N/A
AK010425						
E1a	83931–84017	87	No	N/A	N/A	N/A
E1b (CD1)	84018–84045	28	No	N/A	N/A	CTCCCTTGGgtgagtccg
E2 (CD2)	86487–86584	98	Yes	2421	tttgagtagGGGCTGGCC	CAATGATGACgtgagtact
E3 (CD3)	87210–87283	74	Yes	625	tctcatcagAGGCGCTTT	GATCATCAGgtaggttgt
E4 (CD4)	89498–89726	229	Yes	2214	ttcccccagCCACTTCCA	ACAGTTCAGgtcaggatc
E5 (CD5)	99458–99556	99	No	9731	cctctgcagGTGGATGAT	GTCTACACGgtcagtgga
E6 (CD6)	99647–99681	35	No	90	tttggccagGGTGACTAT	GCATTTGGGgtaagtagg
E7 (CD7)	99925–100063	139	Yes	243	ctgttctagGGCTGCGTG	GGAGGAAAGgtattgact
E8 (CD8)	100452–100516	65	Yes	388	cgctcccagGTGCTGATT	GACCTTCTGgtaggtgca
E9 (CD9)	101158–101347	190	Yes	641	gacacacagGGAGCGCAT	GGTCCCATGgtaaggccc
E10 (CD10)	101476–101559	84	Yes	128	accctgcagGTTGTGTTT	AAGAACATGgtaagggtt
E11 (CD11)	101792–101881	90	Yes	232	tgcttgcagGTCATCATG	CGCCAGATGgtgagtgcc
E12 (CD12)	101965–102127	163	Yes	83	atgccacagCTGGAGGTA	AGGAATTCCgtaggcagc
E13 (CD13)	102215–102322	108	Yes	87	tttcctcagGGGTCAGCT	TGGTGCAGGgtatgaagg
E14 (CD14)	102408–102469	62	Yes	85	tcctcacagGACTGTTGC	AAAGACAGTgtaagtgta
E15 (CD15)	102544–102686	143	No	74	gccccacagAATTTCCGG	TCTCAAGAGgtgcgccct
E16 (CD16)	102756–102885	130	Yes	69	tctccttagCACCCTCAA	ACTTACCAGgtaaggagc
E17a (CD17)	102980–103045	66	No	94	tccttacagGATGAAGAG	33UTR after stop codon
E17b	103046–103228	183	No	N/A	33UTR	33UTR
AA435261						
E1	105481–105412	70	No	N/A	N/A	GGCCATTCGgtgagcccg
E2	105262–105113	150	Yes	149	ttgccacagTGTACTGAA	CAGATCTGCgtgagctaa
E3	105029–104802	228	No	83	gctctgcagGTACCTGGA	N/A
KIAA1716-like						
E1 (CD1)	110459–110528	70	No	N/A	N/A	CTGGACAAGgtaagagac
E2 (CD2)	110927–111046	120	Yes	398	cacttgtagCTGGTCAAA	GTCATCTCGgtgaggcac
E3 (CD3)	111244–111297	54	Yes	197	tttcttaagGAATGTCTG	TATCACACGgtgagcccc
E4 (CD4)	113735–113793	59	Yes	2437	ctatcccagATCCTGTTT	GTCAAAGAgtaagtgac
E5 (CD5)	114281–114464	184	Yes	487	ggcacccagGGATGTGCG	GTGCTGCAGgtcagctgc
E6 (CD6)	114823–114867	45	Yes	358	ctctggcagATCAATGTC	TTAGATTCTgtgagtgct
E7 (CD7)	114953–115048	96	Yes	85	cactcgcagATGCTGTCC	GCAGCTGAGgtgagtcta
E8 (CD8)	115151–115225	75	Yes	102	tccccacagTTGGACCAG	CAGCAACGGgtgaggccc
E9 (CD9)	116539–116651	113	Yes	1313	cttcctcagGACTTTTCC	ATGGAACCGgtaagggcc
E10 (CD10)	116740–116791	52	Yes	88	ctcactcagACGGTGGTT	AAACTCAAGgtattctgg
E11 (CD11)	116996–117096	101	Yes	204	tgcccacagGATGCACTC	ACCTACCAAgtgaggagg
E12 (CD12)	117169–117280	112	Yes	72	tccctacagGAGCTGTAT	TACAGTGAGgtgcactgc
E13 (CD13)	117522–117730	209	Yes	241	atccaccagAGGCTGGAC	CATCCACAGgtagtcagt
E14 (CD14)	117824–117893	70	Yes	93	gtctaggagCTTGGGCGT	CTGCTAAAGgtgtgtgag
E15 (CD15)	118003–118097	95	Yes	109	gccccgcagCTGATGTGT	CAGTTCCAGgtgaggttg
E16 (CD16)	118175–118377	203	Yes	77	ctggtccagGCAGGACAA	TGTCCTCAGgtgggaggg

Gene/ EST Exon/CD	Exon Position Begin-End	Exon length (bp)	Detected by Genscan	Intron length (bp)	Acceptor site (intron/ EXON)	Donor site (EXON/intron)
E17 (CD17)	118615–118722	108	Yes	237	cccttgcagCTGCTACTA	GTCCACGCAgtgagtgct
E18 (CD18)	119877–120097	221	Yes	1154	ctcccacagAGGGTGCCG	GTACTAGGGgtgagcaca
E19 (CD19)	120274–120383	110	No	176	gctgtccagGGTTCCTTG	CCGTACCGGgtgagttct
E20 (CD20)	120476–120582	107	Yes	92	cccagccagGTCTGTCTG	TGTCACATTgtgagtctg
E21a (CD21)	120677–120825	149	No	94	caaccccagGCTTCGCCT	33UTR after stop codon
E21b	120826–120847	22	No	N/A	33UTR	33UTR
AA967911						
E1	116539–116651	113	Yes	N/A	N/A	ATGGAACCGgtaagggcc
E2	116740–116791	52	Yes	88	ctcactcagACGGTGGTT	AAACTCAAGgtattctgg
E3	116996–117096	101	Yes	204	tgcccacagGATGCACTC	ACCTACCAAgtgaggagg
E4	117169–117280	112	Yes	72	tccctacagGAGCTGTAT	N/A
BG243209						
E1	116996–117096	101	Yes	N/A	N/A	ACCTACCAAgtgaggagg
E2	117169–117280	112	Yes	72	tccctacagGAGCTGTAT	TACAGTGAGgtgcactgc
E3	117522–117730	209	Yes	241	atccaccagAGGCTGGAC	CATCCACAGgtagtcagt
E4	117824–117893	70	Yes	93	gtctaggagCTTGGGCGT	CTGCTAAAGgtgtgtgag
E5	118003–118097	95	Yes	109	gccccgcagCTGATGTGT	N/A
BF181401						
E1	117824–117893	70	Yes	N/A	N/A	CTGCTAAAGgtgtgtgag
E2	118003–118097	95	Yes	109	gccccgcagCTGATGTGT	CAGTTCCAGgtgaggttg
E3	118175–118377	203	Yes	77	ctggtccagGCAGGACAA	TGTCCTCAGgtgggaggg
E4	118615–118722	108	Yes	237	cccttgcagCTGCTACTA	GTCCACGCAgtgagtgct
E5	119692–119782	91	No	969	gtcctacagGTCTAAGCA	N/A
BF120370						
E1	120274–120383	110	No	N/A	N/A	CCGTACCGGgtgagttct
E2	120476–120582	107	Yes	92	cccagccagGTCTGTCTG	TGTCACATTgtgagtctg
E3	120677–121189	513	No	94	caaccccagGCTTCGCCT	33UTR after stop codon
AA547414						
E1	126572–127112	541	No	N/A	N/A	N/A
BG085299						
E1	127208–127796	589	No	N/A	N/A	N/A
AI550648						
E1	127744–128382	639	No	N/A	N/A	N/A
BG070843						
E1	129539–128723	817	No	N/A	N/A	N/A
AA763221						
E1	129440–130032	593	No	N/A	N/A	N/A
AA218417						
E1	131044–130398	647	No	N/A	N/A	N/A
BG066449						
E1	136781–135944	838	No	N/A	N/A	N/A
AA199518						
E1	136706–137369	664	No	N/A	N/A	N/A
BF140854						
E1	139653–140275	623	No	N/A	N/A	N/A
AA823914						
E1	141472–142259	788	No	N/A	N/A	N/A
BE956856						
E1	158678–159251	574	No	N/A	N/A	N/A
AI530700						
E1	160031–159473	559	No	N/A	N/A	N/A
Ubc6p						
E1 (CD1)	163829–163959	131	Yes	N/A	N/A	TCTTGAATGgtaagacta
E2 (CD2)	166294–166334	41	Yes	2334	ctccgttagGCATTATGT	CTTATGAAGgttagtaga
E3 (CD3)	170056–170158	103	Yes	3721	ttggtgtagGTGGCTATT	CAACACAAGgtaagcttc

Gene/ EST Exon/CD	Exon Position Begin-End	Exon length (bp)	Detected by Genscan	Intron length (bp)	Acceptor site (intron/ EXON)	Donor site (EXON/intron)
E4 (CD4)	170244–170382	139	Yes	85	ttctttcagGCTGTGTCT	GACTTCACGgtgaggtgt
E5 (CD5)	171190–171270	81	Yes	807	tcttcctagAAAAAACAG	GTTGTGGAGgtaagaagg
E6a (CD6)	171903–172187	285	Yes	632	ttctctcagGAAATTAAA	33UTR after stop codon
E6b	172188–172454	267	No	N/A	33UTR	33UTR
NM_026125						
E1a	177140–177400	261	No	N/A	N/A	N/A
E1b (CD1)	177401–177559	159	Yes	N/A	N/A	TCCGTCAAGgtaggcaga
E2 (CD2)	179365–179481	117	Yes	1805	actttcagCCCCCGGAA	AAGAAGTCGgtaaggccc
E3 (CD3)	179590–179710	121	Yes	108	ctcttttagCGAGGCCTC	TACTGAAAGgtaagattg
E4 (CD4)	179802–179953	152	Yes	91	tctccacagAGGCCACAG	TTCCAAGCTgtgagtggg
E5 (CD5)	180416–180524	109	Yes	462	tttccacagCCTACTACT	TGCACGTGGgtgagccag
E6 (CD6)	180688–180778	91	Yes	163	tacccgtagACCACAGTG	TCGTCATACgtgagtccc
E7 (CD7)	180904–180982	79	Yes	125	tatgcccagGTCCCTGGA	CATCTACAGgtaagcaac
E8a (CD8)	181226–181334	109	No	243	tgtctttagTCTGGACAG	33UTR after stop codon
E8b	181335–181451	117	No	N/A	33UTR	33UTR
AW743506						
E1	206936–206461	476	No	N/A	N/A	N/A
Cab45a						
E1	207908–208231	324	No	N/A	N/A	GAAGCCGGGgtgagtgag
E2 (CD1)	211086–211415	330	No	2854	accttgcagGAGTCCCAT	CTTTTCCAAgtaagtgtc
E3 (CD2)	214029–214165	137	Yes	2613	tgctactagGGTAGACGT	ACGGTGACGgtacagtgg
E4 (CD3)	215301–215414	114	Yes	1135	tcttttagGCCATGTTT	ATGAGGAGAgtgagtgct
E5 (CD4)	223418–223576	159	Yes	8003	ctcttgcagCACAGGAAG	GGGACTTGGgtaagacct
E6 (CD5)	223775–223950	176	Yes	198	ttatctgcagATCAGGATG	GAGCTAGAGgtgagcaag
E7a (CD6)	224405–224581	177	Yes	454	gtctcacagAACTACATG	33UTR after stop codon
E7b	224582–225468	887	No		33UTR	33UTR
Tnfrsf4						
E1a	228399–228577	179	No	N/A	N/A	N/A
E1b (CD1)	228578–228710	133	Yes	N/A	N/A	GCCAGCCAGgtgagtagc
E2 (CD2)	228932–229054	123	Yes	221	ccactacagGCCATGGTA	GCAACCATCgtgagtcc
E3 (CD3)	229606–229713	108	Yes	551	cttccctagGAAGTGGAA	TTGGAGTTGgtgagcctc
E4 (CD4)	230090–230156	67	No	376	ttgctgcagACTGTGTTC	CTGGACCAAgtgagtact
E5 (CD5)	230582–230778	197	Yes	425	gtcccacagTTGTACCTT	CTCCTGAGGgtaagggac
E6 (CD6)	230858–230977	120	No	79	tcctcatagGCCCTGCAT	AACCTTGTTgtgagtatc
E7a (CD7)	231066–231136	71	Yes	88	ttctcttagGGGGAAACA	33UTR after stop codon
E7b	231137–231293	157	No	N/A	33UTR	33UTR
AW544835						
E1	235140–235698	559	No	N/A	N/A	N/A
Tnfrsf18						
E1a	241046–241090	45	No	N/A	N/A	N/A
E1b (CD1)	241091–241241	151	Yes	N/A	N/A	ATGCTCCAGgtaaggcaa
E2 (CD2)	242020–242142	123	Yes	778	cttgtccagGCAAGGAGG	AGTCTCAAGgtatgttcc
E3 (CD3)	242666–242753	88	Yes	523	tatccacagGGGATATTG	TTGGACCAAgtaagtcct
E4 (CD4)	242925–243127	203	Yes	171	gtgtcccagCTGTTCTCA	GTCCTCGAGgtcagttgt
E5a (CD5)	243199–243320	122	Yes	71	ttactacagAGACCCAGC	33UTR after stop codon
E5b	243321–243593	273	No	N/A	33UTR	33UTR
AI225779						
E1	248278–247905	374	No	N/A	N/A	N/A
BF319334						
E1	249905–249544	362	No	N/A	N/A	N/A
AI132502						
E1	259999–259859	141	No	N/A	N/A	ACCAATCAGgtaagaacc
E2	259420–259280	141	Yes	438	gtgttgtagTTCATGCAG	ACCTTCACGgtcagtcga
E3	252725–252550	176	No	6554	attttacagGGCTGGTGA	N/A
AI595135						

Gene/ EST Exon/CD	Exon Position Begin-End	Exon length (bp)	Detected by Genscan	Intron length (bp)	Acceptor site (intron/ EXON)	Donor site (EXON/intron)
E1	264205–264135	71	No	N/A	N/A	TTCCTACTGgtaaggagg
E2	263876–263796	81	Yes	256	ggtcttcagGCTGGACCC	AAGATGCAGgtgggggctt
E3	263715–263583	133	Yes	80	tatccacagACGACCTGG	AGCATCAATgtgagtaga
E4	263438–263320	119	Yes	144	accctgcagAATCAGCAA	TTCGGGAAGgtaggagac
E5	263243–263020	224	No	76	tctgcccagGCCAGCAGT	CCAAACCAAgtgagccca
E6	261643–261605	39	No	1376	tctcccaagGCTGGACCC	N/A
AK014686						
E1	275577–275229	349	No	N/A	N/A	GCCTCAGAGgtgggggtgca
E2	266501–266434	68	No	8727	cttcttcagGTTTAAACG	TCTGATCAGgtaaacatg
E3	264937–264525	413	No	1496	tctttgcagCAGACACAG	AGGTGCCAGgtgaatgca
E4	263715–263583	133	Yes	809	tatccacagACGACCTGG	AGCATCAATgtgagtaga
E5	263438–263320	119	Yes	144	accctgcagAATCAGCAA	TTCGGGAAGgtaggagac
E6	263243–263117	127	Yes	76	tctcgccagGCCAGCAGT	CCAAACCAAgtgagccca
E7	261643–261558	86	Yes	1473	tctcccaagGCTGGACCC	AAGATGCAGgtgagaagc
E8	259420–258537	884	No	2137	gtgttgtagTTCATGCAG	N/A
AI425800						
E1	275441–275352	90	No	N/A	N/A	TTAGAGCAGgtgggttca
E2	266501–266434	68	No	8850	cttcttcagGTTTAAACG	TCTGATCAGgtaaacatg
E3	264937–264618	320	No	1496	tctttgcagCAGACACAG	GTCCAAGATgtccaagat
E4	260993–260952	42	No	3624	taccgcaagATGCCCTTC	GGTGCAGAGgtgtgggcg
E5	260030–259995	36	No	921	acccccctagATATGTCCA	N/A
AW987045						
E1	270132–269542	591	No	N/A	N/A	N/A

Positions are given from the start of the 284-kb sequence, with the first base pair = +1. E = Exon, CD = Coding region, UN = Unknown, N/A = Not Applicable. The region of 1–40143 bp is from a genomic sequence identified as Cyclin ania 6b (Accession #AF185591), but because only a small part of mRNA for Cyclin ania 6b is known (Accession #AF246633), and because AF246633 is almost fully overlapped by *Pcee*, we denote this sequence as *Pcee*. For most of the *KIAA1716*-like gene, no mouse EST or cDNA was available, but the human sequence was a close match to the mouse genomic DNA, so the intron-exon structure of the human *KIAA1716* was used to show the intron-exon structure in its mouse ortholog. This was true for all but the first exon, which was not available in human, but there was a mouse EST, which was used to predict the boundary. When there was a Unigene cluster associated with a given EST, the longest EST within the cluster was used to align genomic and cNDA sequence (BG176077 = Mm.23825, AI428898 = Mm.23492, AK004732 = Mm.28978, BF 140854 = Mm.172945, AW544835 = Mm.102764, AI595135 = Mm.159514). Several ESTs, shown above, group with Unigene clusters and therefore are likely to represent the same gene. These instances are as follows: AA547414, BG085299 and AI550648 are part of Unigene cluster Mm.56803; BG070843, AA823914 and AA763221 are part of Mm.182484; BG066449 and AA199518 are part of Mm.133906; BE956856 and AI530700 are part of Mm.322248, and AI132502, AK014686 and AI425800 are part of Mm.42006. Gene and cNDA names follow the rules outlined in Figs. 2 and 3.

Table 5

Polymorphisms between the 129 and B6 strains for genes on mouse distal Chr 4

Gene or EST	Source	P[#]	Forward Primer	Reverse Primer	Size (bp)[a]	Pos (bp)	Nt B6	Nt 129	AA B6	AA 129
Pcee	CDNA	1	GCCTCACCGATCGCTATT	GGGCCTTCGTACACTTAACG	132	N/A	Iden	Iden	Iden	Iden
Pcee	CDNA	2	TACAGCTGCCTCCCAGGAAC	ACCTTCAGGCTCCTTTCCAT	205	66	A	A or G	Q	Q or R
Pcee	CDNA	2	TACAGCTGCCTCCCAGGAAC	ACCTTCAGGCTCCTTTCCAT	205	69	T	T or C	V	V or A
Pcee	CDNA	2	TACAGCTGCCTCCCAGGAAC	ACCTTCAGGCTCCTTTCCAT	205	77	A	A or G	N	N or D
Pcee	Gen	3	ATCGGCGACAGGCTCTACT	CTCCATGGAATGTTTCACGA	577	103	A	A or C	N/A	N/A
Pcee	Gen	3	ATCGGCGACAGGCTCTACT	CTCCATGGAATGTTTCACGA	577	179	A or G	A	N/A	N/A
Pcee	Gen	3	ATCGGCGACAGGCTCTACT	CTCCATGGAATGTTTCACGA	577	428	A or DEL	A or DEL	N/A	N/A
NM_025338	CDNA	1	GGGGTGATTGGCAGCTATG	GGTAGATCTTCGGGGTCTGC	508	95	C	T	S	L
NM_025338	CDNA	1	GGGGTGATTGGCAGCTATG	GGTAGATCTTCGGGGTCTGC	508	344	A	A or G	H	H or R
NM_025338	CDNA	1	GGGGTGATTGGCAGCTATG	GGTAGATCTTCGGGGTCTGC	508	392	T or A	T	D or V	V
AK004732	CDNA	1	GCCGAGTCTTCCACCTACAG	CAGCAAGATGAAGAGCAGCA	215	127	T	T or C	I	I or T
AK004732	CDNA	1	GCCGAGTCTTCCACCTACAG	CAGCAAGATGAAGAGCAGCA	215	182	T	C	Y	Y
Dvl1	CDNA	1	GATGTGGTGGACTGGCTGTA	GAAGCCAGGGTCCTGGTAAG	330	73	A	A or G	S	S or G
Dvl1	CDNA	1	GATGTGGTGGACTGGCTGTA	GAAGCCAGGGTCCTGGTAAG	330	306	G	A	P	P
Tas1r3	Gen	1	CACCCATTGTTAGTGCTGGA	ACGGGGTTGGTACTGTGTGT	553	169	A	G	S	S
Tas1r3	Gen	1	CACCCATTGTTAGTGCTGGA	ACGGGGTTGGTACTGTGTGT	553	197	A	A or G	T	T or A
Tas1r3	Gen	1	CACCCATTGTTAGTGCTGGA	ACGGGGTTGGTACTGTGTGT	553	213	T	C	I	T
Tas1r3	Gen	1	CACCCATTGTTAGTGCTGGA	ACGGGGTTGGTACTGTGTGT	553	216	C	T	P	L
Tas1r3	Gen	2	CGGCTGGGCTATGACCTAT	TGCATTGGCCAGACTAGAAA	476	265	T	C	N/A	N/A
Tas1r3	Gen	2	CGGCTGGGCTATGACCTAT	TGCATTGGCCAGACTAGAAA	476	306	T	C	S	S
Tas1r3	Gen	3	GGCAGTTGTGACTCTGTTGC	CACTCCTCCTGCTGCTTTG	1018	589	A	C	N/A	N/A
NM_024472	CDNA	1	GGATGACTCAGAGAAAGATTTCAA	GGTCTAGCAGGGAATGCTCA	641	142	C	T	A	A
NM_024472	CDNA	1	GGATGACTCAGAGAAAGATTTCAA	GGTCTAGCAGGGAATGCTCA	641	227	G	A	A	T
NM_024472	CDNA	1	GGATGACTCAGAGAAAGATTTCAA	GGTCTAGCAGGGAATGCTCA	641	373	C	T	D	D
NM_024472	CDNA	1	GGATGACTCAGAGAAAGATTTCAA	GGTCTAGCAGGGAATGCTCA	641	379	G	A	L	L
AK010425	CDNA	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	228	N/A	Iden	Iden	Iden	Iden
AK010425	Gen	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	560	174	T	G	N/A	N/A
AK010425	Gen	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	560	177	TTT	DEL	N/A	N/A
AK010425	Gen	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	560	180	A	DEL	N/A	N/A
AK010425	Gen	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	560	181	TTTT	DEL	N/A	N/A
AK010425	Gen	1	AGGTGGATGATGAGCTGGAA	CTCTCTGCAGCGTTTGGAAT	560	490	A or G	G	V	V
AK010425	Gen	2	AACTTCGCTTCACCTGTCGT	TCCACAGTCACAGAGCCATC	208	N/A	Iden	Iden	Iden	Iden
AK010425	CDNA	2	AACTTCGCTTCACCTGTCGT	TCCACAGTCACAGAGCCATC	137	N/A	Iden	Iden	Iden	Iden
AA435261	Gen	1	AAATCATGTGTCCCGAGGAG	CTGCTCCTGGCCTAATCAAC	206	N/A	Iden	Iden	Iden	Iden
KIAA1716-like	Gen	1	CATGTGAGGACATTGAACGG	GAGTCAGCCTGCAGCATACA	148	N/A	Iden	Iden	Iden	Iden
NM_026125	CDNA	1	TACTACTCAGGGCGCCTTCC	CGTATGACGATGACACAAGGA	198	N/A	Iden	Iden	Iden	Iden

Gen = genomic DNA. Pos = the nucleotide number, where the first nucleotide of the Forward primer is considered nucleotide 1. Iden = B6 and 129 DNA do not differ. N/A = not applicable; because nucleotide substitution is within an intron, there is no amino acid substitution. The 129 strain has many within strain polymorphism, e.g. A to A or G, means that the nucleotide is A for the B6 strain, and it is polymorphic (A or G) within the 129 strain.

[a] Size in cDNA is the size suggested by aligning an EST with genomic DNA; where no EST was available, the Genscan prediction was used.

[#] P = primer pair.