A Genetic Map of the Mouse Suitable for Typing Intraspecific Crosses

William Dietrich,*,† Hillary Katz,* Stephen E. Lincoln,* Hee-Sup Shin,*,† Jeffrey Friedman,‡ Nicholas C. Dracopoli† and Eric S. Lander*,†

**Whitehead Institute for Biomedical Research, Cambridge Massachusetts 02142, †Center for Genome Research and Department of Biology, Massachusetts Institute of Technology, Cambridge Massachusetts 02139, and ‡The Rockefeller University, New York, New York 10021*

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ABSTRACT

We report the construction of a genetic linkage map of the mouse, consisting entirely of genetic markers that can be rapidly typed by polymerase chain reaction and that show a high degree of polymorphism among inbred laboratory strains. Specifically, the map contains 317 simple sequence length polymorphisms at an average spacing of 4.3 cM and is detectably linked to approximately 99% of the mouse genome. In typical crosses between inbred laboratory strains, about 50% of the markers are polymorphic, making it straightforward to follow inheritance in almost any cross.

THE mouse is a powerful genetic system for the study of mammalian biology: a century of work has yielded thousands of mutants defining single gene variation and scores of inbred strains defining polygenic variation affecting physiology, development and behavior (GREEN 1989; FESTING 1979). Because most of these genes are known only by their phenotypic effect, detailed study requires cloning the genes based on their chromosomal position relative to a genetic map. The ideal genetic map for this purpose would consist of genetic markers that were (1) highly abundant and evenly distributed, so that the entire genome could be simultaneously followed in a cross; (2) highly polymorphic, so that one could study any cross between laboratory strains; (3) rapidly typed, so that scoring a cross would be short relative to generation time; and (4) easily disseminated, so that any laboratory would have ready access to them. Such a genetic map would allow initial localization of genes and then provide starting points for chromosomal walks to clone them.

The first genetic map of the mouse was based on visible mutant phenotypes. Given the difficulty of isolating large numbers of mutants and the considerable effort needed to map two mutations relative to one another, this work proceeded slowly. Although the first linkage group in the mouse was found (HALDANE, SPRUNT and HALDANE 1915) soon after the notion of linkage was first elucidated in Drosophila (STURTEVANT 1913), it took more than 60 years before linkage groups were found corresponding to all 20 mouse chromosomes in the mid-1970s (EICHER 1981; DAVISSON, RODERICK and DOOLITTLE 1989). Moreover, this map was tedious to apply in practice because at most a few visible markers could be used simultaneously in a cross.

The situation was transformed by the recognition that minor variations in DNA sequence provide a virtually inexhaustible supply of genetic markers that can be used to follow inheritance (BOTSTEIN *et al.* 1980). At the time, such variations could be most conveniently detected as restriction fragment length polymorphisms (RFLPs). In the mouse, the RFLP approach proved to be extremely powerful in interspecies crosses (ROBERT *et al.* 1985; AVNER *et al.* 1988). Comparing the laboratory mouse *Mus musculus* and the exotic species *Mus spretus*, a typical DNA probe had greater than 90% probability of detecting an RFLP with only a handful of enzymes. Using such interspecific crosses, detailed genetic maps have been constructed showing the positions of hundreds of genes (BUCHBERG *et al.* 1989; KINGSLEY, JENKINS and COPELAND 1989; CECI *et al.* 1989, 1990a,b; JUSTICE *et al.* 1990a,b; SIRACUSA *et al.* 1990; BAHARY *et al.* 1991; COPELAND and JENKINS 1991).

Notwithstanding the great utility of RFLPs, they still have several major limitations. (1) The rate of polymophism is considerably lower among inbred laboratory strains, making it difficult to type crosses between such strains. (2) Typing RFLPs is time-consuming and difficult to automate. (3) Disseminating RFLPs involves managing and distributing large numbers of DNA probes. The first limitation is especially serious. Although interspecies crosses are quite useful, there are many circumstances in which it is preferable to use crosses between two inbred laboratory strains–including mapping of many mutations whose phenotypes are affected by genetic background, mapping of modifier genes, and mapping of polygenic factors underlying physiological differences between strains. Ideally, crosses should be designed according to phe-

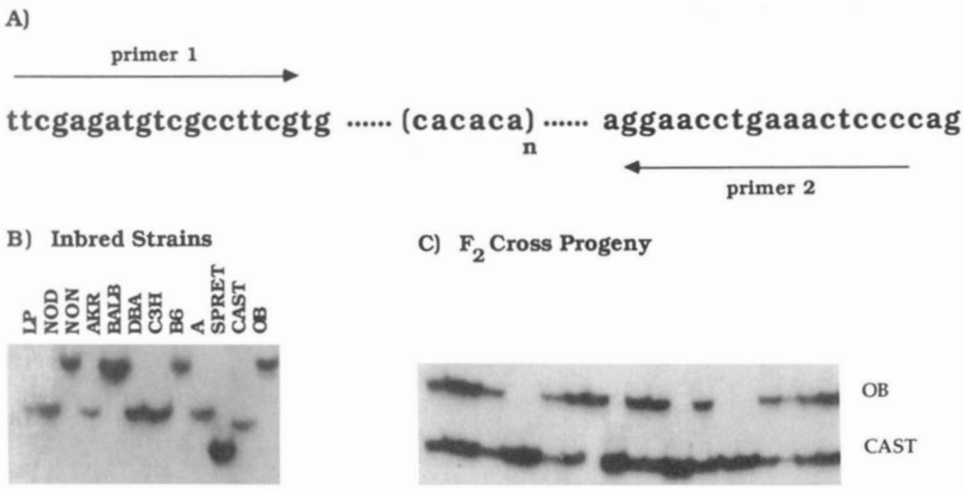


FIGURE 1.—Illustration of a simple sequence length polymorphism, D3Mit21. (A) Diagram of PCR primers flanking region containing CA-repeat; (B) characterization of SSLP alleles in 12 inbred strains (left to right: LP/J, NOD/MrkTacBr, NON/Lt, AKR/J, BALB/cJ, DBA/2J, C3H/HeJ, C57BL/6J, A/J, SPRET/Ei, CAST/Ei, and C57BL/6J-ob/ob), showing four distinct allele sizes; (C) segregation of SSLP alleles in 21 progeny from the OB × CAST intercross used for constructing the genetic map.

notypic and biological considerations, rather than to maximize polymorphism for mapping.

An alternative source of DNA polymorphism has recently been described (WEBER and MAY 1989), based on variation in the length of simple sequence repeats (SSRs) (also called microsatellites) that occur frequently in most eukaryotic genomes (HAMADA, PETRINO and TAKUNAGA 1982; STALLINGS *et al.* 1991). Such simple sequence length polymorphisms (SSLPs) can be easily typed by using the polymerase chain reaction (PCR) with primers flanking the SSR (Figure 1). Recent studies show that SSRs show extraordinarily high rates of polymorphism in both human (WEBER and MAY 1989; WEBER 1990) and mouse (LOVE *et al.* 1990; CORNALL *et al.* 1991; HEARNE *et al.* 1991). Moreover, the typing of SSRs is rapid and automatable and the genetic markers are easily disseminated simply by publishing the primer sequences.

Here, we report the construction of a complete genetic linkage map of the mouse consisting entirely of SSLPs. The map contains 317 SSLPs at an average spacing of 4.3 cM and is detectably linked to about 99% of the mouse genome. In typical crosses between inbred laboratory strains, about 50% of the markers are polymorphic, making it straightforward to follow inheritance throughout the genome in almost any cross.

MATERIALS AND METHODS

Overview: Briefly, the map was constructed as follows. (1) Random clones containing SSRs (specifically, $(CA)_n \cdot (GT)_n$ repeats) were isolated from an M13 library of mouse genomic DNA containing small inserts and their DNA sequences were determined. (2) Public computer databanks were searched to find the sequence of known genes containing SSRs. (3) From each such DNA sequence, PCR primers were selected using a computer program to generate assays designed to work under a single uniform set of experimental conditions. (4) Each PCR assay was tested to determine whether it revealed an SSLP between the two parental strains, OB and CAST (see below), used in the mapping cross and, if so, to measure the allele sizes generated in each of 12 inbred strains. (5) To construct a genetic linkage map, the SSLPs were used to genotype the progeny of an F_2 intercross between OB and CAST and linkage analysis was carried out using a computer program. (6) The newly generated map was then anchored relative to the existing genetic map by two methods: those markers that were polymorphic between the strains C57BL/6J and DBA/2J were typed in the BXD recombinant inbred lines so as to compare them with known strain distribution patterns and those markers that were chosen from the DNA sequences of genes with known chromosomal positions were assigned accordingly.

Isolation of clones containing simple sequence repeats: Random genomic libraries were constructed by digesting male C57BL/6J DNA to completion with *Mbo*I (New England Biolabs), fractionating the DNA on a 4% NuSieve GTG Agarose gel (FMC Bioproducts), and cloning the fragments in the size range 250–500 bp into the *Bam*HI site of M13 mp19 (Boehringer Mannheim). (The use of male DNA was inadvertent; we had intended to use female DNA so that the *X* chromosome would have been equimolar with the autosomes, rather than half-molar.) The libraries were plated at low density of about 500 plaques per 150-mm plate so that individual clones could be picked without the need for secondary purification. Duplicate plaque lifts (Colony/Plaque Screen, Du Pont) were prepared, simultaneously hybridized with end-labeled $(CA)_{15}$ and $(GT)_{15}$ oligonucleotides (T4 polynucleotide kinase, New England Biolabs; [γ-^{32}P]ATP, 5000 Ci/mmol, New England Nuclear) at 65° in hybridization solution as described by CHURCH and GILBERT (1984) and washed in 0.1 × SSC/0.1% SDS at 65° four times for 5 min each. We screened for $(CA)_n \cdot (GT)_n$ repeats because they are the most frequent simple sequence repeat in the mouse genome (HAMADA, PETRINO and TAKUNAGA 1982; J. SEGRE, personal communication). Strongly hybridizing plaques were picked into 1 ml Luria broth (LB).

Length screen of clones: Clones were screened prior to sequencing to determine the length of the insert. Using 5 μl of the supernatant from the plaque picked into LB, phage DNA was amplified in a 50-μl PCR reaction (Amplitaq DNA polymerase, Perkin Elmer Cetus) with the primers flanking the M13 cloning site (5′-TGTAAAACGACGCGGAGT-3′ and 5′-CAGGAAACAGCTATGACC-3′). Phage containing inserts greater than 500 bp were discarded, because they could not be sequenced in a single pass.

Sequencing: Phage DNA was prepared essentially as described (SAMBROOK, FRITSCH and MANIATIS 1989) and the DNA sequencing was carried out according to Applied Biosystem's Taq Cycle Sequencing protocol using an ABI 373A DNA sequencing apparatus. DNA sequences containing SSRs with at least 10 repeat units were used in subsequent steps.

Database searches: GenBank was searched to find DNA sequences containing SSRs, using a variety of computer programs including FASTN and BLAST (ALTSCHUL *et al.* 1990). Specifically, we searched for all occurrences of at least 10 repeats of a dimer, trimer or tetramer.

PCR primer selection: PCR primers flanking the SSRs were selected, using a computer program called PRIMER (M. J. DALY, S. E. LINCOLN and E. S. LANDER, unpublished). The primers were chosen to have a target melting temperature of 60° (BRESLAUER *et al.* 1986; RYCHLIK and RHOADS 1989) and a target length of 20 bases. In addition, primer pairs were chosen to avoid significant homology to one another or to the murine repeat elements L1, B1 and B2 (KRAYEV *et al.* 1980, 1982; LOEB *et al.* 1986). Primer pairs were tested under a single set of PCR conditions; the use of the computer program greatly increased our success in creating PCR assays that satisfied this rigorous requirement. PCR primers were obtained commercially (RESEARCH GENETICS, HUNTSVILLE, ALABAMA).

Mapping cross, recombinant inbred panel and mice: PCR assays were first tested to determine whether they revealed SSLPs between a C57BL/6J-*ob*/*ob* (OB), a congenic line carrying the recessive obese mutation, and an inbred strain of *M. musculus castaneus* (CAST/Ei). If so, allele sizes were determined in female DNA from 12 inbred strains: OB, CAST, C57BL/6J, SPRET/Ei, DBA/2J, A/J, C^3H/HeJ, BALB/cJ, AKR/J, LP/J, NOD/MrkTacBr and NON/Lt. To construct the genetic map, the assays revealing polymorphism between OB and CAST were then genotyped in 46 non-obese F_2 progeny of an OB × CAST cross; this mapping panel provides 92 informative meioses corresponding to about 1 crossover per 1.1 cM. To anchor the map using recombinant inbred (RI) strains, the BXD RI lines 2, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28 and 29 were used. (The remaining four BXD RI lines were omitted in the interests of streamlining procedures: the 22 strains used together with the two parental controls correspond to one-quarter of a microtiter plate and thus permit four markers to be genotyped per microtiter plate.) All DNA was prepared according to standard protocols (SAMBROOK, FRITSCH and MANIATIS 1989).

Genotyping by PCR: To genotype F_2 progeny for SSR polymorphisms, PCR reactions were performed with radioactively labeled primer and products were visualized on acrylamide gels. Primers were end-labeled with [γ-^{32}P]ATP (specific activity 6000 Ci/mmol, Du Pont/NEN) using T4 kinase (NEB) according to standard protocols (SAMBROOK, FRITSCH and MANIATIS 1989). A 20-ng aliquot of genomic DNA was amplified in a 10-μl PCR reaction using AmpliTaq DNA polymerase (Perkin Elmer Cetus) according to manufacturer's specifications. The primer concentrations were: 100 nM of each of the two primers unlabeled and 20 nM of one primer end-labeled. The reactions were overlaid with 40 μl of light mineral oil. Reactions were amplified on either an MJ Research PTC96 Thermal Cycler (MJ Research) or the GeneMachine 2 (USA Scientific Products) using the following thermocycling protocol: initial denaturation at 94° for 3 min, followed by 25 cycles of 94° for 1 min, 55° for 2 min and 72° for 3 min. (Recently, we have successfully used an alternative amplification protocol which may yield cleaner results for some markers: initial denaturation at 94° for 3 min, followed by 25 cycles of 94° for 15 sec, 55° for 2 min and 72° for 2 min, and finally followed by a single cycle of 72° for 7 min.) PCR products were diluted twofold with loading buffer consisting of xylene cyanol and bromophenol blue dyes in 100% formamide, denatured for 5 min on a 100° heating block and electrophoresed on 7% denaturing polyacrylamide gels (SequaGel, National Diagnostics) for 3 hr at 20 V/cm (120 W). Gels were wrapped in Saran Wrap (Dow Chemical) and exposed directly to film for 4–16 hr at −80°. Autoradiographs were independently scored twice.

Streamlining of genotype analysis: Considerable attention was devoted to streamlining the procedures, so that a single person could process some 800 PCR samples each day. Reactions were set up in flexible 96-well plates (Becton Dickinson Labware) using a Biomek 1000 Workstation (Beckman Instruments). In some cases, we coamplified two SSLPs known to yield substantially different product sizes in the same reaction, thereby increasing efficiency. In other cases, we combined two SSLPs that gave substantially different products sizes after amplification but before gel analysis. Loading of the gels was streamlined by using an array of 12 10-μl syringes (Hamilton, Reno, Nevada) spaced to fit into 96-well microtiter plates. The gel combs were handmade sharkstooth combs designed so that the syringe array loaded every other well (G. CHURCH, personal communication).

Linkage analysis: Linkage analysis was performed using the MAPMAKER computer package, essentially as described (LANDER *et al.* 1987; LINCOLN and LANDER 1987; DONIS-KELLER *et al.* 1987; CHANG *et al.* 1988). Markers were assigned into linkage groups based on pairwise LOD scores of at least 5.0. For each linkage group, a "framework" map was constructed consisting of a subset of markers that could be ordered with a LOD score of at least 3.0. Some 66% of the markers easily fell into framework maps. The remaining markers were then mapped relative to the framework maps. Some 92% of the markers could be ordered with a LOD score of at least 2.0.

Error checking: To maximize the accuracy of our data, we developed a new mathematical method for identifying potentially erroneous genotypes. Briefly, the approach is as follows. Rather than assuming that the observed data represents the true genotype, we considered it a phenotype caused by the genotype, according to a penetrance function: phenotype reflected the true genotype with probability $1 - \epsilon$, but differed from it (*i.e.*, was erroneous) with probability ϵ. Genetic linkage analysis was then carried out under this assumption, which explicitly allows for the possibility of error throughout the data. For each typing (*i.e.*, each observation of an individual at a locus), we calculated under this model the LOD score, $\text{LOD}_{\text{error}} = \log_{10}(P_{\text{error}}/P_{\text{correct}})$, where P_{error} is the probability of the overall data set arising if the given typing is erroneous and P_{correct} is the probability of the overall data set arising if the given typing is correct. For the most part, the potential errors correspond to apparent double crossovers in a relatively small region and instances in which a single crossover apparently occurs in a small interval rather than in a much larger adjacent interval. For LOD scores ≥ 1.0, the autoradiograms were independently reread and, if there was any ambiguity, the typing was repeated. In our analysis, we used a value of $\epsilon = 0.007$ based on empirical studies of our error rate (see RESULTS). This method will be described in more detail elsewhere (E. S. LANDER and S. E. LINCOLN, in preparation).

Recombinant inbred analysis: Data from the recombinant inbred strains were analyzed with the RI Manager

computer program (MANLEY and ELLIOT 1991) using the "find" function to detect linkage.

Mathematical analysis of distribution of interval sizes: To test whether the genetic markers were randomly distributed in the genome, we examined the observed distribution L_{obs} of distances between adjacent markers and compared it to the expected distribution L_{exp} under the assumption of random distribution of markers. We calculated the distribution L_{exp} as follows. For a map with an average spacing of d cM and a cross with n informative meioses, the probability $P_{d,n}(k)$ that two adjacent markers will recombine in exactly k meioses was calculated as:

$$p_{d,n}(k) = \int_0^\infty \left[\binom{n}{k} \theta(x)^k (1 - \theta(x))^{n-k}\right] \frac{e^{-x/d}}{d}\, dx$$

where $\theta(x)$ is an appropriate mapping function. We used Kosambi's mapping function for this calculation. (Although no simple mapping function perfectly fits the recombinational data from the mouse, the choice is adequate for the purpose inasmuch as the same mapping function was used in the construction of the map.) To understand the equation, observe that the last term in the integral is the probability density that the two adjacent markers lie at a distance of x cM apart while the preceding bracketed term is the probability that two markers at x cM will recombine in k of n meioses. Here, we have an average spacing of $d = 4.3$ cM and the 46 animal F_2 intercross provides $n = 92$ informative meioses.

Nomenclature: Loci defined by SSLPs are named according to standard convention–*e.g.*, *D1Mit7* refers to a locus on chromosome *1* isolated at the MIT Center for Genome Research, with arbitrary reference number 7. We have used this nomenclature both for SSRs in anonymous sequence and also for SSRs occurring within known gene sequences. By distinguishing between a gene and a particular SSR within the gene, the nomenclature remains unambiguous even for situations in which a single gene contains multiple SSRs, as happens in a number of cases. J. TODD has concurred in this decision and has assigned such designation to SSLPs in genes previously published by his group; these names are given in the tables. Similarly, a single SSR might be studied with various different PCR assays. To avoid ambiguity, we also distinguish between the SSR locus (locus name) and the particular PCR assay (assay name) used to study the locus. This is especially useful in the case of the six SSLPs which were independently identified twice (see below). Several SSRs previously published by J. TODD were renamed, with his permission, based on newly determined or revised chromosomal location: *D0Nds25* was renamed *D2Nds2*, *D8Nds1* was renamed *D4Nds10*, *D0Nds27* was renamed *D6Nds4*, *D0Nds22* was renamed *D10Nds3*, *D4Nds1* was renamed *D6Nds5*, *D0Nds19* was renamed *D12Nds1*, and *D1Nds3* was renamed *D15Nds2*.

Finally, we refer for simplicity to laboratory mouse as *M. musculus*, although these strains represent a combination of genomes from *M. musculus* and *Mus domesticus*.

RESULTS

Screen for polymorphism: Primer pairs flanking SSRs were first tested to determine whether they revealed polymorphism between OB and CAST, the strains used for genetic mapping. These strains were chosen because they belong to different subspecies and thus were likely to show a high rate of polymorphism, but they are sufficiently closely related that F_1 progeny of both sexes are fertile (unlike hybrids with the distinct species *M. spretus*, in which males are sterile). This allowed us to use an F_2 intercross rather than a backcross for genetic mapping–providing twice as many informative meioses per progeny.

Overall, we designed and tested 455 primer pairs, with 394 obtained from sequencing random clones containing CA- or GT-repeats and 61 obtained from searching GenBank for SSRs. Of these, 393 (86%) produced working PCR products of the expected size under the single uniform set of PCR conditions employed. This success rate increased steadily over the course of the project as the PRIMER program was refined, so that the success rate near the end of the project exceeded 90%. Of these 393 assays, 303 (77%) yielded SSLPs between OB and CAST. To this collection, we added 34 SSLPs previously described by J. TODD and colleagues (LOVE *et al.* 1990; CORNALL *et al.* 1991; HEARNE *et al.* 1991) for a total of 337 SSLPs. Of these, 18 produced patterns that we found difficult to interpret reliably. The remaining 319 produced easily scored polymorphisms (accompanied, in some cases, by background bands). These 319 SSLPs were used for genetic mapping; the primers are listed in Table 1.

To facilitate the use of these markers in other crosses, we determined the allele sizes in twelve commonly used inbred laboratory strains (Table 2). The typical rate of polymorphism between an inbred laboratory strain and either *M. musculus castaneus* or *M. spretus* was about 90% and, more remarkably, the typical rate of polymorphism between inbred laboratory strains was about 50% (Table 3). For relatively short CA-repeats, the length of the SSR is known to be correlated with its rate of polymorphism in humans (WEBER 1990); we saw no such correlation in our data, however, probably because the vast majority of the SSRs used were very long (85% had more than 15 repeats).

Genetic map construction: To construct the genetic linkage map, we typed the 319 SSLP markers in 46 progeny from an OB × CAST F_2 intercross. The primary genetic data is available by request from the authors. Based on linkage analysis, 317 of the 319 markers fell into 20 linkage groups. These markers defined a genetic map of the mouse genome, with an average spacing of about 4.3 cM (Figure 2). The remaining two loci show no significant linkage to other markers in the map; these markers were retyped several times to confirm the data but no errors were found. We estimate that the map is linked to some 99% of the mouse genome.

Error checking: Given the large size of our data set (nearly 15,000 genotypes), some errors are bound to occur. Such errors pose problems for the construction of dense genetic maps: they spuriously inflate appar-

TABLE 1

Primer sequences for simple sequence repeats

Locus name	Gene name	Assay name	Left primer	Right primer
D1Mit1		L33	GATCCTCAGATTGAAGAATC	GAGCCACCAGAGATGTAAGA
D1Mit2		A26	TTGAATTCAAACATCATCAGGC	CTATCTGTAACCCCAGCTCCC
D1Mit3		M253	TTTTTGTTTTCTTTTCTTTTCCC	CCCTCTTCTGGTTTCCACAT
D1Mit4		M46	GCTACTGCTTTGGAGTCAGT	ATGACTTGAGCTCAGTCTCTG
D1Mit5		L20	AGATAGCAGAGCCTGAGCCA	CCTGAACTCCACCATTTAGC
D1Mit7		A80	TGGTAGAGGAAGGTGCACG	GCAGGGGAGTAGTACCACCA
D1Mit8		L31	CTGAAAATCGTCCCTTGACC	CAGGAGCATGAAATGGGGAT
D1Mit9		M111	AACTGCAGGCTAGAGACCCA	ATGTGCACATACCAAAGGCA
D1Mit10		A117	AAACCATGCAGGTACTGATATGG	GAAGAAATTAACTGAGAGCAAGGC
D1Mit11		M17	GATCAGATTAAGATGTATATTATAA	GAACCCCAAAAAGAAATCTG
D1Mit12		M93	ACCATATCTCTACATGCTTGTGC	GCATTTGGTTTATTTTTCCACG
D1Mit13		L30	TGATGCTTGCACGTTGAGAT	AAAACTGGTTCCTGGTTCCC
D1Mit14		M193	GCCAGACAGGGCTACATTGT	AGACTGAACTCTGGCCTCCA
D1Mit15		M146	TCCACAGAACTGTCCCTCAA	ATACACTCACACCACCCCGT
D1Mit16		L46	AGAGTTAGCTGCCTAGCTTGAGTG	TGGAAAGATCTAGGGTTGTCAAAA
D1Mit17		M41	GTGTCTGCCTTTGCACCTTT	CTGCTGTCTTTCCATCCACA
D1Mit18		A77	TCTGGTTCCAGGCTTGATTC	TCACAAGTGAGGCTCCAGG
D1Mit19		L86	GATCCCAGCCAATAGAAGTACA	GAAAGGTTTCCTATCCTATGGC
D1Nds2		T17	ACATATATGGACTACATACATAC	AGACACATACAACATAGAATTGTT
D2Mit1		M128	CTTTTTCGTATGTGGTGGGG	AACATTGGGCCTCTATGCAC
D2Mit2		M112	AGTCCTCCTTGGACTTCCATTAG	TGGATTATATTTTCAAGACCAGA
D2Mit3		M116	GGGTATCTTCATGCCAGTGG	GGTGAGGACACGAGGCTATG
D2Mit4		M52	ACACCAACCCAAGCAATTGT	GAGCACGGAACAGGCATAAC
D2Mit5		A41	CCGGGGATCATCTTAGGACT	CCCCCTCTACACACTTGCAT
D2Mit6		L18	AACAAACAAACCCCTTGCCC	CTCTAACACAGCCCCAGGTG
D2Mit7		L44	AAGGCAAGCATTCTGCCACT	CTCCCGGCAAGAACTGTTTT
D2Mit8		M199	CTTCATTGCCAATGCTCTCA	TGAAGGTGAAAACAGAGGCA
D2Mit9		M85	GTCTGCACTCTCACCAGCAA	CAGCTTGAAATGCCTTTGAG
D2Mit10		M39	CATCAGGAAACACAGGACCA	ACCTAACCCTAATGATGGGG
D2Mit11		M134	CAAACCCCCAGCTCTCTCTT	CCATACCCAGGCTCCATCTA
D2Mit12		M179	CTACTTCCCAGGTGCTTGGA	TCCAAAGAACTGAATGGACA
D2Mit13		M130	AGGACCAGAAGGACAGACCTATC	AAATCTCACAGGGAAAAAAAAGC
D2Mit14		M163	CAACACAAATGTGAGCATGC	TGCTCACCTGCCTAGTACTT
D2Mit15		A61	ATGCCTTAGAAGAATTTGTTCCC	CTTGAAAAACACATCAAAATCTGC
D2Mit16		M186	CTTGGATAGGGATGGAGGGT	ACAGACATGTAGGCACACCA
D2Mit17		M246	AGGCAATTACAAGGCCTGG	CACCCATCTCCCTCAGTCAT
D2Mit19		A83	TGCTAAAAGTCTGGCATTTGG	CAAATGTTTGTCTTTCAAAAGCC
D2Mit21		M184	GGCTTAGGCCCAAATTTTCT	TGGAAAGCTCATCTCTTCCT
D2Mit22		M167	GCTCCCTTTCCTCTTGAACC	GGGCCCTTATTCTATCTCCC
D2Mit24		M75	ACTTGGCTTACAGGGGACCT	TACCAGTCCCTTTCCACCTG
D2Mit25		A67	TATGCCACTCAGAAGAGGTCG	ATATGTGCATTGCATGAACTCC
D2Mit26		M37	TGTTCTTTGCTCATCCACCA	AGGCTGATGGTAACAGTGGG
D2Mit27		M106	AGGCTAAGCCTTGCATCAAA	GTCGCAAAATGTGGATGATG
D2Mit28	*Snap*	D25	TGCTTCTCTCATGGTATTACCTAG	ACAAACCACACAGACATTTACAAT
D2Mit29	*Svp-4*	D115	CGGTGACGAAGCTTCTGAG	CTTTGAATATGAACTCTCACCTTCC
D2Mit30	*Trh-1*	D111	CATCCAAGCAGTAACGTAGACG	AAATGTTACACCCTCTGCGG
D2Nds1		T19	CTGCATGCACTGTATTTGTAT	ACTCATGGGTTGTGCATATGG
D2Nds2		T57	AACATTGAGGACATTTGGTGA	CGCTGTATGCATCCTTAAGAA
D2Nds3	*Il-1B*	T15	CCAAGCTTCCTTGTGCAAGTA	AAGCCCAAAGTCCATCAGTGG
D3Mit1		M28	TGTGCACAGGGGTACATACA	TCATTTTCTTCCTCCCCCTC
D3Mit3		M250	CCTTTTGAGGCAAAGCTCC	CTAAGTCCTGCACCTGCCTC
D3Mit4		L40	TGTGCCTGCAAGTTGTTCTT	CTACAGTGGGGGCAGAAGGT
D3Mit5		M123	AGCCCCTTCCAAGTGTCTCT	GGTTTCGGAATGAGATGAGC
D3Mit6		M149	AACTTCAACATGTGAGGGGC	CCTGAAACAAAGCAACAGCA
D3Mit7		M74	ATGCAACTAACTTTATTGAAAATC	TACAATTATCCGGGAGCTA
D3Mit9		A85	AACTTCATTTGCTTGGAAACTACC	TGTTTTATATTGCCCTGTATGTGC
D3Mit10		A34	CTGGCTTGGTGGAAGTCCT	CCTAAGCCAGCTACCACCAC
D3Mit11		L38	CCAACCACAGTAACACATGT	TGGAGACCAATGCGAACAAC
D3Mit12		A60	TAGACCAATCTTGGGAGTGTCC	GGAAAAGCATAAGAAACAACCG
D3Mit13		L37	TTTCTGCATTATGTGGGCTT	AACCACAGATGACAATTGAA
D3Mit13		L8	CCTTTCTGATTATGTGGGCT	CCACTGAAGGATAACCACAG
D3Mit14		M206	ATTGCGGTTAAAGTTTGCTT	TCCTGCAAATTGTCCTCTGA

TABLE 1–Continued

Locus name	Gene name	Assay name	Left primer	Right primer
D3Mit15		A55	AATTTGCATTCCAGGACCAC	AGGAAGTGACGTTGGGTTTG
D3Mit16		M159	TGCTTGTCCTGTGTTAATGA	TGAGAATGGAGGTGAACAGC
D3Mit17		M235	CATGGCTCCATGGTTCTTG	CCACGGAGAACAACTGAAGA
D3Mit18		A96	GAACAGTTCCCAGGTCCTCA	CTGCCTTTAAATTCTGTCACCC
D3Mit19		M141	CAGCCAGAGAGGAGCTGTCT	GAACATTGGGGTGTTTGCTT
D3Mit21	*Il-2*	D31	AAGCTCTACAGCGGAAGCAC	CTGGGGAGTTTCAGGTTCCT
D3Mit22	*Rp132-ps*	D122	AAGGATTGAAGAATGGTTGGG	AATCAGCGATTTCAGCACG
D3Nds2		T21	ACACATTGGAGATGCACAGCG	TCTGCATGCCAGGGTTGTGAT
D4Mit1		A73	ATGATGTACACTTAGGCATTGCA	AGAAATATGGCAAGCAAAATGG
D4Mit2		L67	GCACTCACACACTCACATGC	TGCACCAGTGACTTTACCCC
D4Mit2		L6	GGATTTCTTGGGCACTCACA	GCACCAGTGACTTTACCCCA
D4Mit4		M31	CGGAATAGGCAGCTATGCTC	TCCATAGACCCTGCATGTGA
D4Mit5		A1	CGCCTCTGTCTCTACCTCTCA	CCTAAAAAGTGTCTTCTGACCTCC
D4Mit6		M64	TGTGGGCAGTGTAAGCACTC	CTTTCCTCTGTGCTCGTGTG
D4Mit7		A71	CCGGGGATCATGTTTAGAGA	AGAGGGATAATTTTTGAATTGCC
D4Mit9		M241	GGCTTTGGAATGCTATGCAT	TGGCAGGAGGTATGACAGAA
D4Mit11		M8	GGTTCACCAAAGGACTTCGA	CCTGTGACCCCTTGGAAGTA
D4Mit12		M15	GCTTGCTTTAGGAGTGTGCC	TATTTGCTCTCCATTTCCCC
D4Mit13		M169	GCTGGTAGCTGGCTTTTCTC	CAGATGTTCCTACTGCTTGG
D4Mit14		A69	TACAATAGTTAGCTCAGGCCAGC	GGGGTGAGGAGAGTGACTCA
D4Mit15		A122	AGGAATACTGAATGTGGACTTTCC	TCCCTTGATTAACAGAAGACCTG
D4Mit16		A65	GATCACCCAAGGCTGGC	TCCCCGTGAACTTCCATC
D4Mit17	*Orm-1*	D1	TGGCCAACCTCTGTGCTTCC	ACAGTTGTCCTCTGACATCC
D4Mit205		M205	TGTGTGAACATGTCTACCCC	GGGGACCGAAGTAACAGTGA
D4Wsm1	*Ifa*	F1	TCAGTATGTACATCCATGCC	TAAAAATGATAAGTTGTTTTATGAA
D4Nds2		T24	CTTCTGTCTGCTGAGGATACC	CCATGATGAGCCAAAATGAAT
D4Nds10		T29	TGTAAGCCATTCTAATAGATC	GAGGGAATAGAACTGACTGGT
D5Mit1		A82	AATAAAGCTGTGAGGTAAACCCC	GAAACAAATGATTGTTTTGAGCC
D5Mit3		M197	AAGGGCAAGCCATTTAAGGT	GCCCCAATCTAGGAGGCTAC
D5Mit4		M189	CTAGTCATTGGCTCCAAGGG	ATGCACTGGGAGAGTGAAGG
D5Mit5		A11	TGAGTGAGGTGTGGTGATAACC	TGTGTCTTCCCCTTTCAACC
D5Mit6		L42	CTCCAAATGGAACTATGGAA	CATGATATTAAGCAGCTGTG
D5Mit7		M154	AAAGGGGGTCTTCTTTGGAA	TCTCCTGTAGTGGGTGGTTT
D5Mit9		A9	TTCCTAGCATTTCCCTGGG	ATCTGGAGAGAATTGTAGTCTGGG
D5Mit10		M207	CGAGAAGTTGGAAAGACCCA	GGCACCCATGCCTCTATG
D5Mit11		M97	GATCTTCCTACCTTCTTACCCAC	CATGATTTTATTTGGGGGG
D5Mit12	*Csnb*	D128	TTAGGCAAGTGTTAGACTAAAAGGG	GGAAATCCTCTTAGACCTTAAATGC
D5Nds2		T26	TAATCTATTGTTTGTGGAAAG	GTATCAGGCAAACTGGACC
D5Nds4	*Afp*	T61	AGCAGGGCTACACAGAGAAAC	ATTCCCATATTTGCATCTCCA
D6Mit1		A10	GGCACATTTGCCTTTGTTTT	TCTCCTATCTCTCCACCTTTTCC
D6Mit3		L59	ATGGGTACCACCCTATCATACCTA	TTATACACTGATATCTTGATAGCC
D6Mit4		M239	ACTAGGAAACACACTGATTCATATG	GAGGTGACAAAATTTTCAAAAA
D6Mit5		M161	CACGGAGAGGACCTACATGC	AGCTGCTCGTCTCCACACTT
D6Mit6		M259	TTCTCTCAGTCTTGTCTGTGTACA	GTGAGGCTCAAAGAAAGGGC
D6Mit6		M227	GAGGCTCAAAGAAAGGGCTT	TTCTCTCAGTCTTGTCTGTGTACA
D6Mit8		M240	TGCACAGCAGCTCATTCTCT	GGAAGGAAGGAGTGGGGTAG
D6Mit9		L23	GTCTGTTTTGGCATATGGCA	TCTGGGTANCCAACCATGTT
D6Mit10		M78	TCAGAGGAACAAAGCAGCAT	CCTGTGGCTAACAGGTAAAA
D6Mit11		M170	ACTGGCCTCTTTTATGTGCA	TGTGAGTGTGAGTTCAGGGG
D6Mit12		M11	CCACATCCATGTAAAAGCTG	TGGTTCAATGAAAGTTGCCA
D6Mit13	*Prp*	D34	TTTTGTTTCCTTTCAGCATG	GGGAGCCATTGTCCTATTCA
D6Mit14		M190	ATGCAGAAACATGAGTGGGG	CACAAGGCCTGATGACCTCT
D6Mit15		M148	CACTGACCCTAGCACAGCAG	TCCTGGCTTCCACAGGTACT
D6Mit16	*Ly2*	D11	AGGCTTTGATGCTGTATAGG	CACCAGGAACGTAAGTGAGC
D6Rck1	*Cpa*	F3	CAGCTGAGTCATTAGAGCACTTACC	CTCAGACCTACTAGAGAAGTGCAGAGC
D6Rck2	*Mirp*	F2	GAACACCCCTGGACCGTATTCTCA	GATCGCTGGACACTTCTCTGAGTG
D6Rck3		F103	GACAAGAGGACGCATCTTTTG	CTACGAAAAGTCAACCTCGAGG
D6Nds4		T59	ACCTCAGCGGTTCTTTATGAG	TGGTCCACCCTGAATGAGTCC
D6Nds5		T23	GGAATGTCTTATTTAAGTCAG	AGTGGAGTAATATTTGAACAA
D7Mit1		M208	GTCCCAGTGTGTATATATAATCCAG	GGATTATACACACAGATGTTGGG
D7Mit5		M187	TCGTGTCAAATTGCTTATGC	ACTGTGTGTGCCTGTGTTTG
D7Mit7		L12	ACTCAAAGGTTGTCCTGGCA	TGGTAGTGGTGGCTNCGGTG
D7Mit8		M183	TTGGCCTTTATAGGCACCTG	TAAGGCACCATGATATGGCA
D7Mit9		A89	GACAGGTGGTTCTTTAATAATCCG	GGAGCTTTAAAGGACAATTTTCA
D7Mit10		L72	GTTGTTCGGGAAGGGAAGAT	CCTTGGCACGAGATGAACTG

TABLE 1–Continued

Locus name	Gene name	Assay name	Left primer	Right primer
D7Mit10		L25	GAAGATTGGGCTGTCTGCAC	TGAAGCTGATGGAGCTGATG
D7Mit12		M23	GCTGGGTTTATTCATTGCAA	TCCAGCTCATGGGTAGAAGA
D7Mit13		A113	ATGGGGAAAGTGACTGAGGA	ATTTTTGTAGCTTGAAGGTATGGC
D7Mit14		L79	TCCCTCCTCATGTTTTCATG	GATGATTGGGAGAAGCAAGG
D7Mit15		M47	GTGTGCACCCACATGGATAC	AGGGAAAGCACTTGACCATG
D7Mit16		A13	CTGGTCTCTGTCCTTGGAGC	AAAGAAAATATTCTTGTTGCCAGC
D7Mit17		M91	CTGGCATTTATGTTGCTTCA	AACTTGCCTTCTGTCCTCCA
D7Mit18	*Gas-2*	D117	GGGAGCCCAGCTTCTACTG	TCCTAACACCCTTCCTGGTG
D7Mit19	*Tyr*	D108	GCTGCAGCTCTCTCTGGG	GATGGCTCTGATACAGCAAGC
D7Mit20	*Mb-1*	D103	GTGTAGCAATGGTGTCGGTG	AAGCCTGCCTCCAGATGTAA
D7Nds1		T27	GAGATCTTCCATACTCATATT	TAGATAGTGTTAACAGTGACC
D7Nds2		T28	CAGACTTTCATTTCTTTGGATAC	ATGCCATCATGTGTTGAAGCA
D7Nds4	*Int-2*	T63	GTGACAATACATTCCTGCTGT	CTCAGATCTTATCTCTAGCAC
D7Nds5	*Ngfg*	T62	CTCCACATGTGTATGTGTATG	ATGGAGGCCGAAGAAAGAATC
D8Mit1		M70	TTTTGCTGTCTAGGTCCTGACTC	CAGCCTCATTAGTAAGGGACCTT
D8Mit3		M195	TCCCATTCTCGCATAAGTCC	GATGGGAAGACAGGGTAGCA
D8Mit4		M71	CCAACTCATCCCCAAAGGTA	GTATGTTCAAGGCTGGGCAT
D8Mit5		M176	TCCCTTTTCCCTGTGCTATG	GCCGTTCATTTAACCCTTCA
D8Mit6		M158	CAGGCAGCTTGCTAGGACTT	TACTGCCTTTAGCCCAGTGG
D8Mit7		M138	TTGGTGAACACCAGGTTCAA	ATGATGTTAGTGGTCTGGGG
D8Mit8		M257	GAGGGGCTGGAAGAAAGAAC	AGCCCAGACTGCTTCCTTTT
D8Mit9		A62	ATTTGAATTGTGCAGACCTGG	CTGCTTGTTTTTATCTCCTGGG
D8Mit11		A105	GCAGCAGTGGTAGCAAATAGC	CTTAATCAGCAATCCTTGACACC
D8Mit12		L11	GATCTCTACATCAAAAGGGA	TTCAGTTTTGTTTCTGAAAC
D8Mit13		M77	CCTCTCTCCAGCCCTGTAAG	AACGTTTGTGCTAAGTGGCC
D8Mit14		L34	TTTTCACACTCACGTGTGCG	GTCTCTCCTTCCTGGCGCTG
D8Mit15	*Mt2*	D20	AGCTGAATTTGAGCTAGTCG	AAGCTTACGGTTTAATCCCC
D8Mit16	*Polb*	D100	GCCTGGATTTCCTCATTGAA	AGTTGGTTATCCCTGAAAATATACA
D9Mit1		M88	GAGCTGTAACACTGACAATGTGC	TATCTCAATGCACACTTTTGTGC
D9Mit2		L32	GTGGTCTGCCCTCTTCACAT	CAAAGCCAGTCCAACTCCAA
D9Mit4		M151	TGCTGAGCAAGCTATGAGGA	GACAGCCCATCACAGCTACA
D9Mit6		A78	GTACCCGGGGATCTGGTG	CTGAGAAATGGAAACGTTGTTG
D9Mit8		M211	GATGAAGACAATAAAGAACCTTAAA	AAGAGCTAACCCATTGCTGC
D9Mit9		A72	TACCCGGGGATCTTCTTTCT	AGAGCTTTCCCGCTACACAA
D9Mit10		M86	TAACCAACCCTTCAAGGCAC	AATCCTTGGCTGAAGGGAAT
D9Mit11		L60	GCCTTCATGTGTACCTGAATGCAC	GGCTCTGTAATCACTGAAGCTGGT
D9Mit12		M73	ATTCAAGGGGCAGTACACAT	TGGTCCTGGTAAAACTGCCT
D9Mit14		M236	CCAAAGGACTGCTATTTGCG	GTAATATTGCTACACTCATGCACA
D9Mit15		M160	TTCAGTCCAGTCTGGGGGTA	CCCCCAGTTTTGTTGTTTTG
D9Mit16		A5	TCTGTGCCTCTTGGAGTGTG	AGGATTGGGGCTTTGTTCTT
D9Mit17		L19	GCCAAGGCTGTCTCTTAGCC	GAGAGAAGGGTTCTGGGCAG
D9Mit18		M10	TCACTGTAGCCCAGAGCAGT	CCTGTTGTCAACACCTGATG
D9Mit19		M157	CCAAACACAACCCCTCAGAA	TCATGGCTTCAAGACTGCTT
D9Mit20		L64	CCCTTGCAGCCCATCGCCTA	TAGACACATAGCTGGAGGTTTTCT
D9Mit21	*Cyp1a2*	D15	CAGTCCCTGGTTAATAACAACAAC	TATAGTCCATTGTGGCAGAGGAGT
D9Mit22	*Ncam*	D134	ATTGCATAACACCCCCACAT	CAGTGCTTAACTGCTCAAATGC
D9Mit23	*T3d*	D4	AAGAAGTTTCCATGACATCATGAA	AGAAGAAAATTCTTGACAGCTCTG
D9Mit24	*Trf*	D26	CCTTCTAAACACAGGCTTTTTGAG	CTGATGATCACCTCATTTCCTGAG
D9Nds2		T30	TCCTTGGAGTTAAAACTTGGA	AGATAAATTCAATGAGTCCTA
D10Mit1		M153	GGAGAAAACCAACTCCTGCA	AATGTGAAAATGTGGAGTGG
D10Mit2		M24	CTGCTCACAACCCATTCCTT	GTTCATTTGAGGCACAAGCA
D10Mit3		A114	GTTGATAGTCCCACCTCACTCA	TGAGAAATTCCATCTGTGGC
D10Mit4		M139	TAGGATTACAACCTTGCCCC	CACAAGGGAAAGTCTCCAGC
D10Mit5		M67	AAGTGAAGGTGCTGGTCACC	GGGAATTTCACAAAGACAGC
D10Mit7		L62	GATCTATGTGAGTGCGAGGCTAGC	TCAAACCAGATGGCACTGAAGACT
D10Mit8		M3	AGTGTTAGTGGCTGGGGTTG	TGAACGTTTCAGTTGGTCCA
D10Mit9		A37	ATTTGGAGCACGCATCTTCT	AGGCCCACCTTGTACTTGTG
D10Mit10		M7	CCAGTCTCAAAACAACAACAAAC	TTGCACCTAGATTGCCTGA
D10Mit11		A88	GAGAAGTCACTGGGAGCTGG	TTGCCAGGTTGCTCTTCTTT
D10Mit12		M172	ATGTCCAAAACACCAGCCAG	GGAAGTGATGGAGCTCTGTT
D10Mit13		A63	GATGGAGCTTCTATGTCAACCC	TTATTTCCACTGAACTTCCTTTCC
D10Mit14		M175	AGAGGGGACAAGGAGAGACC	AAGGTTTGGGTTCAGTTCCC
D10Mit15	*Sqr3*	D30	ATGCGTACAGGCAAAACACC	GCTACATTGGTCTGTGACGC
D10Nds1		T31	TGCACACCCACAGCACACATG	AAGGTTTAAGAAGGTCAAATCATA
D10Nds2		T32	CTATTTACTTAACTCACAATT	TGGTCTTTTGCTCCATAAACT
D10Nds3		T54	TGACATTTTGCGATTTTCATTTGT	GACACATGGATCCTCACATGC

TABLE 1–Continued

Locus name	Gene name	Assay name	Left primer	Right primer
D11Mit1		M215	GGGTCTCTGAAGGCTTTGTG	TGAATACAGAAGCCACGGTG
D11Mit2		L14	TCCCAGAGGTCTCCAAGACA	CCACAGTGTGTGATGTCTTC
D11Mit4		A124	CAGTGGGTCATCAGTACAGCA	AAGCCAGCCCAGTCTTCATA
D11Mit5		A2	TTCTGTGAGCCTGGAGGAGT	TACAGGACTAGTTTCCATTTGGG
D11Mit7		M119	AGGGTATTCTCTAGCCTCCACAC	TTTGAGGCAAGATGTCATGTATG
D11Mit8		M212	CTTTTCATGGAGGCACAGGT	TGTGAACAGAGACACACATTCA
D11Mit10		M162	GAACCGCAAGTCATGAATCA	TGGTTTATTCCTGAAGCTGC
D11Mit11		M43	TATTCTCTCCTTCCCCCCAC	TAGAGTTGGGACACCCAAGC
D11Mit12		L3	AGGGTTATGCTCTTGGCTGC	GATTTTCCTAGGCTGGCTGG
D11Mit13	*Ace*	DACE	ATAACACCAACATTACCATAGAGGG	ATACTAAGTTCAGACTTTTCACCAATTTT
D11Mit14	*AntP91a*	D2	CCACTTAGTATATCTTGTCC	GCATGACTTGGCCTATCACC
D11Mit15	*Glut-4*	D5	TGACATTTGGCGGAGCTAAC	ACATGTACTTGCCAGGGTAC
D11Mit16	*Lif*	D133	CAGCTAGAAATGGCAATGAGG	CTTGTTCTACACCCAGCAAGC
D11Nds1		T33	TAAGAACCTTCTGTAGTTATT	ACCTTAGTTAGAGTTGGTCTC
D11Nds7	*Gfap*	T12	AACTGTTCAAAGCCATTTCG	CTATGGACTCACAGCCAGGCT
D11Nds9	*Il-5*	T14	CCTTTCTGAAAGTATTAAGAGT	ACAACCATCTGCATATCCAGC
D12Mit1		M50	TACCCGGGGATCTTTTGTTT	AAGTGGACTGCCAGAGGATG
D12Mit2		M27	ACACAGGCTAAAACATGGGC	GCATCTGTATTCCACAGGCA
D12Mit3		L41	TAAAGGGGTTTGCTTAAACA	ATGCCACTGAATGTCAAATT
D12Mit4		A64	ACATCCCCAGCTCTTGTTTG	AAACCAAACCAAAGAAGCTTAGG
D12Mit5		L58	CACATAGACCAGACAGGCATGCGT	CAAGGTCACGTTGCTAGCTAGGAA
D12Mit6		L16	ATGCTCGACATCAACCTTGG	TATCTGTGTGGCTGGAACGA
D12Mit7		M62	CCGGGGATCTAAAACTACAT	TCTAATCTCAGCCCAATGGT
D12Mit8	*Igh-C*	D7	TTGCCTAACCCACTCACACC	TGGTGACTCCTTACAGAGGC
D12Nds1		T51	AGTGATGTGATTACAGGTTTG	CACTCTATAAACCCACTGCAG
D12Nds2	*Igh-V*	T1	ACATGGTAATTTATGGGCAA	CTGGATACCTGCAATAGTAGA
D12Nds11	*Odc*	T64	CATTTGAGGACAGTCAGGATC	GGAACTTTCATGCAGTACTAG
D13Mit1		A86	TCAACTCTTCTGTAAACCAGATGC	GTCTGTTTGATTCCTGACCTCC
D13Mit3		M79	TCAGGCTCATCCCAGATACC	TTTTGCAGAGAACACACACC
D13Mit4		M231	TGTGGGACAACTGTGACAAA	CACCCAAGGCCCACTTC
D13Mit5		M38	AGAAGCCAGCAGGTGTTTTC	CCAGGAAGTAACCCCAAACA
D13Mit7		A68	CGGTACCCGGGGATCTAC	AGCCCAGCTTGTGAAGTGTT
D13Mit8		M61	GCCCCATTTCTGAAGTTTCA	AATAGACTCTTCAGCCCCCC
D13Mit9		M147	GGGTTCCAGATTGAGTGGAA	TTGCCAAAGTGTCAAAATCA
D13Mit10		L61	AGTCCTGCCATTTGTCCTCTGACC	ATGTCTTAGTCTCACATGCTGGGG
D13Mit11		A91	CATGGCTCCTTTAACCTGTTT	CAATGATTAACCCTTGAAAAAACA
D13Mit13	*Il-9*	D24	CTGTGGTAAGTCCAGATTTG	GGAAAGAGTAGGAAGATGCC
D13Mit14	*Sqr4*	D29	GGAACAGCAAGCTCTAAGGG	CTACCAGGCCTCCCAAGATA
D14Mit1		A103	GATCTATATGTCCCAACTATAAAG	ATTTTGACTAGGATTGTTTGAGGG
D14Mit2		A24	TGTCTGACCCATTGGAATTATG	TGAAGAAGACACCTAACACTGACC
D14Mit3		M32	GCAATTACACCTCCTCGGAG	CACAAGGGCATATGGTACCC
D14Mit4		M228	AGGCACCCCCTCACAGTAC	TTCATTCCTCCTGCTGACCT
D14Mit5		M214	CACATGAACAGAGGGGCAG	GTCATGAAGTGCCCACCTTT
D14Mit6		A119	GACAAACGCTTTCATCTACAAGG	TGTGCACATTCATCCACATG
D14Mit7		L27	AATGTATGGGCATGTGCGTG	GAGATAGTCAACCAAAACAA
D14Mit8		A44	TCACAGGTGCTCTCAGTCATG	GCAAATACTTCCCTTCTTGGG
D14Mit9		A93	AGGGGAAGGGAAGATGAAGA	GGTGTGACCACTGCCTAGGT
D14Nds1	*Plau*	T10	TGCTGGCTAGGAATAAACAGA	AGGGAATTCATGTTCAGGATA
D15Mit1		L29	AACATGGTCCCACAGGTGTC	AGTAGAAGCTGCAGCCCTGG
D15Mit2		L10	AGAGCATGTCCTCACCCCTT	CCTGGAAAGGTCTCAGGGAA
D15Mit3		L78	TTTCCATTTTGGAGCCAGAG	TATCCTTGTCCTGCCATCGT
D15Mit5		L1	CTTCCTAATTCCTGTCAAGCAAAT	GTTTCATTGGTCAATGGAAACTTA
D15Mit6		A59	CCTGGTCTGAAACACTTTTGC	CTTGTGAGTGCTCCATGCC
D15Mit7		M30	TTTGCAGCTGTGTTCTGCAT	GATTAGGCCACGTGAGCTTC
D15Mit8		A79	GGAAAAGGGAAAAAGATGTGC	TATATTACACTTTCCTTTGCTGCA
D15Mit9		M232	CCATGAGTCCTTCATGCCTT	TGTATATGCAGAAGCAGGCA
D15Mit10		M76	GATCTATAACCAGGGCAGGG	TTAATTCACGGAAATGTTTCAATTT
D15Mit11		M237	TGTGAGAAAAATGACAGTAAGGC	TCACAGAAAGACAAGACAAAAGG
D15Mit12		M34	ATGGACACCTGACACTGCAA	AAGGGCTTTTACCTGGGAAT
D15Mit13		A36	GGAGACAAAAATGAACTCCTGG	TTGTAAGACAAGCATAGCTCAACA
D15Mit14	*Gdc-1*	D17	GAGGAAAACCATGTCAATCACTTC	CCTCCTCTTAAACCAAGATCTCTG
D15Mit15	*Hox3.1*	D6	AGCATACACTCTCTTGTTCCTGCT	AATAAATACCAGAGAAGCACCGTG
D15Mit16	*Hoxmaa*	D131	AGACTCAGAGGGCAAAATAAAGC	TCGGCTTTTGTCTGTCTGTC

TABLE 1–Continued

Locus name	Gene name	Assay name	Left primer	Right primer
D15Mit17	*Myc*	D22	GCGTCACTGATAGTAGGGAG	GTACCCCAATCCTGAACCAC
D15Nds1		T35	GAGTAGGTTGGAATTTCTCTC	ACAAATATACACTACTGGACAA
D15Nds2		T18	GCCTATTTATTTCAAAGATATGAC	TGATATCGAGGCATACATGAG
D16Mit1		A70	CGCCCTCTAAGGTGACTCAG	AGAGAGGGGTTATGGGGTTG
D16Mit2		L80	CCAATGCCCTCTTATGACCT	TTCTAGTGCGTCCTACCCAG
D16Mit3		M127	TCTAACGCCCTCTCTCTACC	CCAAATGTGATTGCACAAGG
D16Mit4		M203	AGTTCCAGGCTACTTGGGGT	GAGCCCTCATTGCAAATCAT
D16Mit5		A38	CGGGGATCATCCCTAAAAAC	TCCCCAATTCCTCTTGTGTC
D16Mit6		L7	CAGGTCCAAGAGGAGAACCA	TTTGACCTGTGAGCCTGTGG
D16Mit7		L39	CTGCCACCCCTGAACCATTA	CTACAAGATGTGGGGCATGA
D16Nds2		T37	ATTGGTGAGCTTACAGAATAC	GTGGTCATGATATTCGTAGAT
D17Mit1		M124	TGCTTGAAATCCTGGGTTCA	TGCAAAAATGTATGTGCCTG
D17Mit2		A18	ACAAACATGTTGGCCTAATTCC	TTGAGTTTAAGCCCCTAGAATCC
D17Mit3		L28	GATCTTTTCTTATTCTGGTT	GCAAAGTCATGTACTCTGAG
D17Mit4		M114	GCTGTGCTTCCACACTCAGT	TTTCTGAAAAAGCCTCTCAA
D17Mit5		M92	TGGGAACTTTCCAGACTTCC	CCCTTTCCTCCAAACTCTCA
D17Mit6		M254	GTACATGTAGAGAAATGGAGGTG	GCTTATGTTCTTTAACAAGAATGTG
D17Mit7		L4	ACTCCTTNGGGACCTGCATT	ACCGCTCAGGGAGTGCACTT
D17Mit7		A23	TCTAATCCCATGTATATGTGGTGG	TTCCTCTGGACTCCTTGGG
D17Mit9		A51	TCAGCCCTTAAAAATTACTCTTGG	CCCCACCAACTGTCCTCTAA
D17Mit10		L36	TGCACTTGCATAAGGAAAAC	GACTTTGGGGCCTACTTATG
D17Mit11		M145	TGAATTTATGAGGGGGTCA	TGTCCCATATCTCTCTTTATACACA
D17Mit13		L57	GATCCAGACCACACCCCCTCACCA	TCCTTTGAGAGCCAAGCTTGAAGG
D17Mit16		A25	CCAGAAGACAGCATTCCACA	GTATGTCAGGGCTAGTTGACAGG
D17Mit18		M33	GCAGCTCATTCTTAGTCCCTAAT	TCATGAGTCCCCAAACTAGC
D17Mit19		M44	GAGCTGGTAAATGCTTTGGC	TTGAGTACCCCGTACTTGCC
D17Mit20	*C3*	D129	AGAACAGGACACCGGACATC	TCATAAGTAGGCACACCAATGC
D17Mit21	*Mhcab2*	D21	TAACACCAGACATTGACCTC	AGTCTAGATATGTGTCTCCC
D17Mit22	*Mhceb2*	D16	GGTAAGCATTAGATAGAGAG	TTATGATCTCCACACACGTG
D17Mit23	*Pim1*	D106	TCGAGCTGGTTGAACGAAC	CGGGAAAGCATGGAATTTAA
D17Mit24	*Thy19*	D12	ACCTCTCACCTCTCTCTGTG	TGGAGAGACGTCCTATGATG
D17Nds2	*Hsp68*	T9	GTAATTGCGTTGACTGTTAAAT	AGTGCTGCTCCCAACATTACT
D17Nds3	*Tnfb*	T68	TTCCTGTGGCGGCCTTATCAG	AGACAATGGGTAACAGAGGCA
D18Mit1		M42	TGAGCAAAATACATTGCATG	GGGATACCAGGCCAGACATA
D18Mit2		L9	TTCCCTATCCAGTTGTGTGC	CCCCTGTAGCTCAACCCACT
D18Mit3		L76	TTCCCTATCCAGTTGTGTGC	AGCAGAGAATGCACCACCTC
D18Mit4		M51	ACTGTTGCTGGGGAATGG	CCAAGTTCAAAGCTGCTGG
D18Mit5		M57	TTGTCCACTGATTGCCACAT	CGTATACCCCCACCATGTTC
D18Mit6		A104	GATGAGCTAGGAGGAGATATGAGC	CATACTTACTACAGGGTTTTGGGC
D18Mit7		M108	ACAGGAGAACGGGAACTCAG	GCCAGAGTGGACCAAGATGA
D18Mit8		L24	TTTGGAATCTGGCATGTTAC	GTCTGAAATGAAGTGCCTGC
D18Mit9		M209	AGAGGCATTGCACACACAAG	GCCCCTTGGAGAGTTGGT
D18Mit10		A100	TATCCACCCATTCCAACCTC	GGATTGAGGTTGCTCTTGGA
D18Mit12		A20	TTGTCAGTTTCTTGTGAGGGG	TGTTTAATAAGCCTTTTCCTGAGG
D18Mit14		L13	GAGGTGATGTGGACACACTC	ACACAGCCTAGAATGCACGG
D18Mit15		L87	CAGACTTCATAGCAACACCCTG	TAACATGAAAACAGAAACAGCCA
D18Mit16		A35	TTCCCTTTGGAGACTGTGCT	TGGAATTACAGGGCTTCCTG
D18Mit17	*Grl-1*	D118	TCAGGCAGATTCCAAGCAG	CTGTGGGTAGCCCAAGTCAT
D18Nds1	*Mbp*	T11	CAGTACAGCCAGGACACAGAA	ATGGCTGACCAACTCTCTAGC
D19Mit1		A17	AATCCTTGTTCACTCTATCAAGGC	CATGAAGAGTCCAGTAGAAACCTC
D19Mit2		M109	TGTTGATAGTGCAAGGTGCG	CAAGGGGCCATACCTAGTGA
D19Mit3		M13	CTTCCCCTACTGCAGTGCTC	TTGCATAGTTGGCCAAAGTG
D19Mit4		M230	CGGCTACCCGACACTCTAAA	ATTGGCTTGCCCTAACCC
D19Mit5		A75	TGTTTTGACCTATTTGTTTCATGG	GGTATCTCCTAGTTTTCCTGATTT
DXMit1		L43	CAAGCAACCGAGGAAGACAT	CAGGATGCTAATCACCCTGC
DXMit3		M131	AAAAGGTCATGGCAAAAGGA	AGGAGAAAGTGCAGGGAGGT
DXMit4		M118	TGGACAGTGCTTGAGGAATG	GCAAAACAGCTACATTTGGG
DXMit5		A19	CAACCTCTGAGCTCTCCCAC	TGTTGTCTAATTCCTTCAGGCA
DXMit6	*Zfx*	D28	ACCATTCAAATTGGCAAGGC	GTGGCTCGAGTTGTTTGCAG
DXNds1	*Hprt*	T8	TGACAACTTCTGTCCTCAACA	ATGCCGTCCTTTATCTAGAAC
DXNds2	*Plp*	T4	TAATATAACAGATAACCAACCATT	CATTTTGTAAGATGAGTTTCTA
Unmapped		*A66*	TCAGGGCTCTCTAAGGGACA	ACTATGCAGCCACCAAATCC
Unmapped		*M251*	TTCCTCAACTAAACGCTGGA	CATTTTCCTGTATACCTGAATTTT

The gene name given for SSLPs found in gene sequences from GenBank. The assay name refers to the specific assay used to genotype the locus; formal reference to the assay should be preceded by the symbol "Mit-". The primer sequences are given from 5′ to 3′.

TABLE 2

Allele sizes of simple sequence length polymorphisms in 12 inbred strains

Locus name	Gene name	Assay name	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
D1Mit1		L33	123	118	123	135	126	123	132	123	—	—	—	—
D1Mit2		A26	172	150	172	185	—	—	—	—	—	—	—	—
D1Mit3		M253	160	185	160	200	160	185	185	185	206	185	185	187
D1Mit4		M46	200	168	200	170	200	200	200	200	200	197	195	210
D1Mit5		L20	148	126	148	150	152	152	152	148	150	150	152	152
D1Mit7		A80	108	156	108	125	125	125	125	108	108	125	108	108
D1Mit8		L31	220	190	220	178	220	220	220	220	201	220	201	220
D1Mit9		M111	160	140	160	162	160	160	160	160	147	160	147	160
D1Mit10		A117	140	152	140	125	140	140	147	140	135	140	140	135
D1Mit11		M17	100	111	100	—	106	100	100	100	106	100	106	106
D1Mit12		M93	133	129	133	170	133	133	133	126	133	133	133	—
D1Mit13		L30	202	207	202	—	202	210	211	202	202	202	202	202
D1Mit14		M193	180	200	180	170	215	215	215	205	215	175	175	190
D1Mit15		M146	160	188	160	154	160	186	183	160	183	183	183	183
D1Mit16		L46	190	185	190	195	201	185	190	190	190	164	185	190
D1Mit17		M41	170	190	170	188	174	183	183	176	183	170	183	176
D1Mit18		A77	160	180	160	170	160	160	160	170	170	160	170	205
D1Mit19		L86	113	148	113	123	120	121	121	113	113	113	108	103
D1Nds2		T17	180	167	180	123	180	158	159	158	190	178	159	158
D2Mit1		M128	124	140	124	96	120	120	120	120	120	120	120	124
D2Mit2		M112	147	129	129	138	129	129	129	129	129	135	135	129
D2Mit3		M116	160	194	160	158	160	160	160	160	160	160	160	160
D2Mit4		M52	190	166	190	176	190	190	190	190	190	190	190	190
D2Mit5		A41	141	180	—	—	139	137	139	141	141	141	141	139
D2Mit6		L18	135	147	135	110	126	126	135	135	126	135	135	135
D2Mit7		L44	150	122	150	148	145	147	145	150	147	145	143	145
D2Mit8		M199	188	180	188	212	188	188	188	188	188	188	188	188
D2Mit9		M85	190	195	190	174	195	187	190	190	190	185	185	190
D2Mit10		M39	152	150	152	158	150	156	150	152	152	152	152	152
D2Mit11		M134	226	232	226	264	226	232	226	232	232	226	232	232
D2Mit12		M179	201	194	201	189	189	200	200	189	200	189	201	189
D2Mit13		M130	190	193	190	170	192	192	180	192	192	193	193	193
D2Mit14		M163	142	152	142	198	130	130	130	130	130	142	130	130
D2Mit15		A61	145	178	145	160	145	162	160	145	160	145	145	145
D2Mit16		M186	238	250	238	242	238	238	238	238	238	238	238	238
D2Mit17		M246	205	242	205	420	220	220	220	220	220	214	242	214
D2Mit19		A83	108	124	108	127	108	108	108	108	108	108	108	108
D2Mit21		M184	260	250	258	250	250	260	258	258	258	258	256	256
D2Mit22		M167	190	162	190	112	190	190	190	190	190	147	147	—
D2Mit24		M75	180	183	180	180	180	180	180	180	180	180	180	180
D2Mit25		A67	118	140	118	126	118	118	118	118	118	118	—	118
D2Mit26		M37	195	210	195	190	195	195	195	195	195	210	210	210
D2Mit27		M106	180	238	180	250	180	—	180	—	—	—	—	—
D2Mit28	*Snap*	D25	130	142	130	123	130	130	130	130	130	—	—	—
D2Mit29	*Svp-4*	D115	115	120	115	110	115	115	115	115	115	115	115	115
D2Mit30	*Trh-1*	D111	320	340	320	80	137	137	137	137	137	121	121	121
D2Nds1		T19	178	158	178	182	185	182	182	185	182	152	188	185
D2Nds2		T57	122	88	122	114	122	122	122	122	122	122	122	122
D2Nds3	*Il-1B*	T15	280	190	280	140	280	280	280	400	270	270	280	270
D3Mit1		M28	145	118	120	—	120	120	120	123	120	120	123	143
D3Mit3		M250	108	200	108	88	108	109	104	109	108	104	108	109
D3Mit4		L40	140	150	140	147	140	140	140	140	140	140	140	140
D3Mit5		M123	188	182	182	178	188	188	188	188	188	188	188	182
D3Mit6		M149	145	133	147	125	136	136	136	136	136	136	136	147
D3Mit7		M74	147	142	147	147	147	142	142	142	142	142	142	142
D3Mit9		A85	225	238	225	210	238	214	216	238	238	225	230	216
D3Mit10		A34	145	158	145	132	140	134	—	134	132	136	121	138
D3Mit11		L38	147	204	147	152	147	165	165	165	165	163	147	147
D3Mit12		A60	155	120	155	—	126	157	155	155	157	126	126	157
D3Mit13		L37	220	225	220	236	220	220	220	220	220	220	235	237
D3Mit13		L8	220	238	220	240	220	—	220	220	220	220	238	240
D3Mit14		M206	170	127	170	132	198	198	198	198	198	198	198	170

TABLE 2—Continued

Locus name	Gene name	Assay name	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
D3Mit15		A55	145	185	145	175	212	145	145	145	145	145	145	—
D3Mit16		M159	188	194	188	220	—	186	186	186	—	186	186	186
D3Mit17		M235	208	200	208	—	180	180	180	180	180	180	208	188
D3Mit18		A96	235	242	235	192	235	214	214	214	214	214	235	214
D3Mit19		M141	160	176	160	147	176	176	176	176	176	160	176	158
D3Mit21	*Il-2*	D31	236	216	236	208	218	218	218	236	218	236	218	218
D3Mit22	*Rp132-ps*	D122	240	265	240	207	238	255	240	220	238	240	245	220
D3Nds2		T21	115	147	115	133	122	115	115	115	115	115	115	115
D4Mit1		A73	120	93	120	120	120	115	120	112	112	120	112	112
D4Mit2		L67	178	172	178	178	178	172	178	172	172	172	172	172
D4Mit2		L6	195	185	195	195	195	185	195	185	185	185	185	185
D4Mit4		M31	165	169	165	158	165	163	167	163	163	165	163	163
D4Mit5		A1	138	115	138	120	148	138	138	138	138	138	138	138
D4Mit6		M64	80	60	80	58	78	62	62	62	80	80	62	83
D4Mit7		A71	151	147	151	160	151	151	151	151	151	151	151	149
D4Mit9		M241	206	212	206	238	208	210	210	210	195	200	210	200
D4Mit11		M8	144	170	144	183	144	178	144	144	144	144	149	144
D4Mit12		M15	198	190	198	185	168	168	168	168	170	169	167	170
D4Mit13		M169	92	88	92	106	97	92	108	92	111	108	108	92
D4Mit14		A69	133	130	133	145	140	140	133	140	133	133	133	140
D4Mit15		A122	280	315	280	—	280	330	330	330	280	330	330	318
D4Mit16		A65	220	245	220	226	239	239	239	239	220	239	220	239
D4Mit17	*Orm-1*	D1	147	145	147	105	141	147	147	147	138	136	136	136
D4Mit205		M205	195	197	—	190	197	204	204	204	202	200	201	202
D4Wsm1	*Ifa*	F1	160	185	—	—	—	—	—	—	—	—	—	—
D4Nds2		T24	97	95	97	98	97	91	97	97	93	89	93	97
D4Nds10		T29	90	80	90	—	90	90	90	90	90	—	90	90
D5Mit1		A82	137	145	137	149	129	137	137	137	129	137	137	129
D5Mit3		M197	165	147	165	—	165	165	165	165	165	165	165	165
D5Mit4		M189	195	238	195	250	195	195	195	195	195	195	195	195
D5Mit5		A11	145	166	145	163	145	145	145	145	145	160	145	145
D5Mit6		L42	135	125	135	108	135	135	135	135	135	135	135	131
D5Mit7		M154	160	147	160	160	147	160	147	147	147	147	147	147
D5Mit9		A9	149	180	149	138	149	149	149	149	149	149	149	149
D5Mit10		M207	196	209	196	205	203	188	194	190	201	192	200	196
D5Mit11		M97	203	195	206	210	188	188	188	199	188	188	188	203
D5Mit12	*Csnb*	D128	120	85	120	115	115	120	115	120	120	115	115	110
D5Nds2		T26	168	175	168	122	178	168	178	168	168	178	178	168
D5Nds4	*Afp*	T61	90	85	90	—	97	90	97	97	90	86	97	85
D6Mit1		A10	217	239	217	280	217	217	245	245	217	245	217	217
D6Mit3		L59	308	236	308	—	308	300	308	300	239	308	308	236
D6Mit4		M239	102	107	102	121	102	90	102	90	95	90	90	108
D6Mit5		M161	168	158	168	168	168	168	168	168	168	168	168	168
D6Mit6		M259	100	109	100	96	100	110	100	100	100	100	110	100
D6Mit6		M227	100	110	100	—	100	113	100	100	100	100	113	100
D6Mit8		M240	164	180	164	182	164	190	164	190	170	190	188	178
D6Mit9		L23	143	138	143	152	123	123	123	143	143	123	123	123
D6Mit10		M78	198	210	198	212	206	198	191	198	198	207	191	198
D6Mit11		M170	94	127	94	98	94	94	94	94	94	94	94	94
D6Mit12		M11	123	147	123	170	123	123	123	123	123	123	123	123
D6Mit13	*Prp*	D34	158	170	158	—	152	152	156	152	152	160	155	152
D6Mit14		M190	160	172	160	174	149	156	149	152	149	174	174	168
D6Mit15		M148	260	220	260	260	195	195	195	195	260	260	150	195
D6Mit16	*Ly2*	D11	155	167	155	152	147	157	147	155	130	155	130	155
D6Rck1	*Cpa*	F3	250	230	—	234	250	—	—	—	—	—	—	—
D6Rck2	*Mirp*	F2	170	155	—	147	174	—	—	—	—	—	—	—
D6Rck3		F103	110	90	—	112	—	—	—	—	—	—	—	—
D6Nds4		T59	91	114	91	112	91	91	91	91	91	91	91	91
D6Nds5		T23	98	108	98	118	—	105	—	105	—	105	98	105
D7Mit1		M208	298	309	298	298	298	298	298	298	298	298	301	298
D7Mit5		M187	215	182	215	215	215	215	215	215	215	215	215	215
D7Mit7		L12	80	90	80	—	90	77	90	77	80	80	80	90
D7Mit8		M183	150	153	150	165	148	151	148	148	146	146	146	—
D7Mit9		A89	130	145	130	—	130	130	130	130	128	128	128	130
D7Mit10		L72	180	190	180	180	180	180	180	180	180	180	—	180

TABLE 2—Continued

Locus name	Gene name	Assay name	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
D7Mit10		L25	150	158	150	150	150	150	150	150	150	150	150	150
D7Mit12		M23	197	208	197	220	206	197	197	197	206	205	197	199
D7Mit13		A113	195	200	195	210	195	195	195	195	200	195	195	195
D7Mit14		L79	147	142	147	147	147	147	147	137	147	147	147	147
D7Mit15		M47	138	127	138	129	138	138	138	123	123	138	138	134
D7Mit16		A13	245	248	248	—	248	248	248	248	248	248	248	248
D7Mit17		M91	160	144	160	170	162	160	162	160	160	162	144	162
D7Mit18	*Gas-2*	D117	120	109	120	112	120	120	120	120	120	120	120	120
D7Mit19	*Tyr*	D108	135	131	135	127	135	135	135	135	135	135	135	135
D7Mit20	*Mb-1*	D103	107	100	107	80	107	107	107	107	107	107	95	107
D7Nds1		T27	238	301	238	270	260	260	265	270	270	247	247	270
D7Nds2		T28	118	114	118	97	112	116	112	119	119	114	114	114
D7Nds4	*Int-2*	T63	168	145	166	175	160	160	160	166	166	166	166	160
D7Nds5	*Ngfg*	T62	145	150	145	—	157	142	143	140	145	143	143	143
D8Mit1		M70	215	255	215	215	215	215	215	215	215	215	215	215
D8Mit3		M195	178	185	178	160	187	187	187	187	187	187	187	187
D8Mit4		M71	157	191	157	170	195	200	195	200	195	195	200	160
D8Mit5		M176	166	150	166	100	166	166	166	166	166	166	166	166
D8Mit6		M158	170	201	170	195	170	170	170	170	170	170	170	170
D8Mit7		M138	178	226	178	347	178	178	178	178	178	178	—	178
D8Mit8		M257	125	93	125	110	116	118	116	116	116	116	116	116
D8Mit9		A62	153	119	153	116	151	153	151	151	140	153	153	—
D8Mit11		A105	215	203	215	195	213	215	213	217	215	214	213	215
D8Mit12		L11	120	127	120	125	120	120	120	120	120	120	117	120
D8Mit13		M77	98	114	98	114	98	98	86	105	94	98	98	108
D8Mit14		L34	145	158	145	132	145	170	140	170	140	145	140	170
D8Mit15	*Mt2*	D20	180	187	180	160	180	180	180	180	178	180	185	178
D8Mit16	*Polb*	D100	310	315	310	325	300	310	300	310	300	310	310	310
D9Mit1		M88	110	132	110	110	110	110	110	110	110	110	110	110
D9Mit2		L32	177	161	177	161	177	185	185	185	176	170	185	160
D9Mit4		M151	124	131	124	120	138	138	140	138	124	138	138	136
D9Mit6		A78	144	136	142	—	140	140	140	142	140	140	140	140
D9Mit8		M211	185	180	185	210	193	195	—	193	193	193	193	178
D9Mit9		A72	126	116	126	112	126	138	138	138	126	138	126	130
D9Mit10		M86	150	178	150	156	147	150	150	150	150	147	150	150
D9Mit11		L60	76	100	76	145	108	122	122	122	115	110	112	100
D9Mit12		M73	93	100	93	—	88	82	82	82	88	91	91	93
D9Mit14		M236	78	92	78	95	78	—	—	—	80	70	—	—
D9Mit15		M160	160	166	160	138	155	155	155	155	157	155	155	155
D9Mit16		A5	180	196	180	200	180	167	167	167	176	176	180	180
D9Mit17		L19	157	130	157	145	157	161	161	161	145	143	145	140
D9Mit18		M10	180	210	180	180	204	210	210	213	204	204	180	180
D9Mit19		M157	102	92	102	108	89	108	108	108	89	89	102	102
D9Mit20		L64	114	108	114	106	106	117	117	117	114	106	106	123
D9Mit21	*Cypla2*	D15	187	210	187	168	180	187	187	187	189	187	180	180
D9Mit22	*Ncam*	D134	220	230	220	208	230	230	230	225	210	210	—	—
D9Mit23	*T3d*	D4	210	290	210	320	210	210	210	210	214	212	211	210
D9Mit24	*Trf*	D26	127	149	127	145	127	136	136	136	136	132	136	136
D9Nds2		T30	121	130	121	110	125	125	125	125	125	127	125	130
D10Mit1		M153	100	112	100	—	100	—	—	87	87	110	—	60
D10Mit2		M24	124	121	124	116	124	132	124	132	124	120	132	132
D10Mit3		A114	245	210	245	205	215	245	215	245	215	245	245	245
D10Mit4		M139	134	147	134	134	134	134	134	134	134	134	134	134
D10Mit5		M67	190	201	190	210	190	190	190	190	190	190	190	190
D10Mit7		L62	147	137	147	176	147	147	147	147	147	147	147	147
D10Mit8		M3	208	188	201	215	201	201	201	201	201	201	201	206
D10Mit9		A37	159	155	159	155	159	159	159	159	159	159	—	159
D10Mit10		M7	180	136	180	160	128	128	128	128	128	180	128	128
D10Mit11		A88	201	172	201	175	172	172	172	172	172	201	201	172
D10Mit12		M172	242	236	242	—	242	242	212	242	212	242	212	242
D10Mit13		A63	130	113	130	—	130	130	130	130	130	130	130	130
D10Mit14		M175	192	174	192	199	182	182	194	182	188	192	182	182

TABLE 2—Continued

Locus name	Gene name	Assay name	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
D10Mit15	*Sqr3*	D30	185	140	185	124	189	185	185	175	185	185	187	—
D10Nds1		T31	130	132	130	—	130	152	152	152	152	130	145	152
D10Nds2		T32	145	127	145	138	150	145	145	145	145	145	145	145
D10Nds3		T54	94	89	94	94	94	94	94	94	94	94	94	94
D11Mit1		M215	153	110	153	126	153	153	153	162	162	153	153	153
D11Mit2		L14	124	118	124	111	126	115	140	115	140	115	140	115
D11Mit4		A124	250	246	246	238	300	307	242	242	307	242	244	306
D11Mit5		A2	220	144	220	—	189	213	188	188	213	178	185	185
D11Mit7		M119	144	148	144	172	144	144	144	144	144	144	144	144
D11Mit8		M212	155	170	155	—	155	155	155	133	133	155	155	155
D11Mit10		M162	100	125	100	116	100	132	100	100	100	100	100	100
D11Mit11		M43	238	216	238	210	238	244	238	238	238	238	238	238
D11Mit12		L3	140	150	140	140	140	150	147	145	140	140	142	140
D11Mit13	*Ace*	DACE	161	165	—	—	—	—	—	—	—	—	—	—
D11Mit14	*AntP91A*	D2	158	148	158	146	161	158	167	158	139	161	158	161
D11Mit15	*Glut-4*	D5	147	143	147	143	143	147	147	147	147	151	147	151
D11Mit16	*Lif*	D133	120	135	120	113	120	120	120	120	113	113	120	—
D11Nds1		T33	102	132	102	100	108	102	108	108	108	108	108	108
D11Nds7	*Gfap*	T12	163	181	163	163	153	153	163	153	153	153	163	—
D11Nds9	*Il-5*	T14	306	309	306	—	306	306	306	306	306	302	302	306
D12Mit1		M50	255	230	255	250	244	244	244	244	244	244	244	270
D12Mit2		M27	132	178	132	132	149	132	132	132	132	149	149	132
D12Mit3		L41	123	112	123	130	127	123	123	123	127	123	123	127
D12Mit4		A64	203	270	206	214	208	208	208	196	184	184	199	208
D12Mit5		L58	180	163	180	144	163	163	163	163	163	182	180	163
D12Mit6		L16	108	125	108	110	108	108	108	108	108	108	108	121
D12Mit7		M62	108	130	108	—	121	108	108	123	123	106	123	123
D12Mit8	*Igh-C*	D7	172	180	172	148	181	148	174	174	185	174	170	170
D12Nds1		T51	93	112	93	—	93	93	93	93	93	—	93	93
D12Nds2	*Igh-V*	T1	155	159	195	195	162	193	178	165	170	183	195	165
D12Nds11	*Odc*	T64	170	178	170	158	175	178	178	178	178	—	178	—
D13Mit1		A86	149	151	149	153	149	149	149	149	140	153	149	153
D13Mit3		M79	159	196	159	178	196	188	196	188	164	188	164	163
D13Mit4		M231	185	209	185	209	185	185	185	185	185	185	185	185
D13Mit5		M38	194	190	194	—	194	194	194	194	194	194	194	194
D13Mit7		A68	140	137	140	121	140	145	140	142	140	142	142	142
D13Mit8		M61	190	200	190	250	190	190	182	190	182	190	184	182
D13Mit9		M147	126	116	126	132	145	126	145	126	126	126	145	126
D13Mit10		L61	152	144	152	105	152	160	160	160	149	160	160	160
D13Mit11		A91	147	162	147	162	158	158	158	158	158	158	160	162
D13Mit13	*Il-9*	D24	151	142	151	145	145	140	140	140	151	151	145	140
D13Mit14	*Sqr4*	D29	150	120	150	156	150	146	146	146	150	143	150	146
D14Mit1		A103	108	104	108	142	98	108	98	108	108	108	104	98
D14Mit2		A24	144	146	144	153	146	144	144	140	144	144	146	146
D14Mit3		M32	236	225	236	245	236	236	236	236	236	236	236	240
D14Mit4		M228	196	200	196	186	194	196	196	196	196	196	200	198
D14Mit5		M214	178	182	178	156	164	178	164	178	164	178	178	178
D14Mit6		A119	150	157	150	185	155	155	155	155	155	150	155	155
D14Mit7		L27	109	91	109	107	99	99	99	99	99	112	109	112
D14Mit8		A44	203	210	203	190	203	203	203	203	203	205	203	195
D14Mit9		A93	238	245	238	—	238	238	238	245	245	238	238	238
D14Nds1	*Plau*	T10	182	201	182	190	201	182	182	190	182	190	188	190
D15Mit1		L29	185	180	185	—	190	190	190	190	190	—	183	190
D15Mit2		L10	94	109	94	—	89	89	89	89	89	89	89	89
D15Mit3		L78	140	152	140	154	142	142	137	138	140	140	137	140
D15Mit5		L1	100	123	100	—	118	118	118	100	118	123	118	132
D15Mit6		A59	130	104	130	106	134	132	127	130	130	128	128	104
D15Mit7		M30	109	115	109	126	100	109	100	109	109	109	109	129
D15Mit8		A79	117	123	117	119	125	117	125	117	117	117	117	120
D15Mit9		M232	138	153	138	300	138	138	138	138	138	138	138	138
D15Mit10		M76	222	242	222	178	222	220	—	222	222	222	236	236
D15Mit11		M237	106	126	106	110	106	94	106	106	121	106	106	106
D15Mit12		M34	150	123	150	144	160	150	150	150	144	150	150	161
D15Mit13		A36	140	165	140	190	120	140	140	140	125	—	110	120
D15Mit14	*Gdc-1*	D17	190	270	190	188	190	183	183	195	190	188	190	230

TABLE 2—Continued

Locus name	Gene name	Assay name	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
D15Mit15	*Hox3.1*	D6	159	164	159	168	145	159	166	159	145	—	—	159
D15Mit16	*Hoxmaa*	D131	120	145	120	155	145	126	120	126	145	126	—	123
D15Mit17	*Myc*	D22	145	143	145	143	145	145	138	138	145	143	140	145
D15Nds1		T35	100	146	100	—	98	98	98	98	105	98	96	100
D15Nds2		T18	122	115	122	—	115	111	122	115	122	—	122	120
D16Mit1		A70	106	94	106	140	106	106	106	106	106	106	106	106
D16Mit2		L80	189	193	189	177	189	189	189	189	189	189	189	189
D16Mit3		M127	102	76	102	97	100	104	100	104	104	104	104	100
D16Mit4		M203	132	130	132	145	123	147	123	149	126	149	149	149
D16Mit5		A38	158	163	158	163	134	134	160	134	160	158	160	160
D16Mit6		L7	190	175	190	212	195	190	190	190	195	190	190	185
D16Mit7		L39	162	175	162	165	162	162	162	165	165	162	162	162
D16Nds2		T37	98	88	98	—	103	90	103	90	88	88	88	103
D17Mit1		M124	201	208	201	—	201	195	195	195	193	193	201	201
D17Mit2		A18	230	250	230	—	230	220	230	230	225	230	230	230
D17Mit3		L28	130	128	130	120	123	132	123	130	130	128	130	—
D17Mit4		M114	95	98	—	140	95	95	95	95	95	95	95	95
D17Mit5		M92	260	250	260	242	260	260	260	260	260	260	260	260
D17Mit6		M254	106	88	106	104	102	102	102	102	102	102	102	102
D17Mit7		L4	200	214	200	178	204	204	204	204	204	200	204	204
D17Mit7		A23	145	170	145	—	152	152	152	152	154	146	154	152
D17Mit9		A51	117	134	117	100	117	117	117	117	117	117	117	115
D17Mit10		L36	159	133	159	165	150	150	150	159	159	150	148	150
D17Mit11		M145	176	192	176	178	150	160	176	150	176	150	178	160
D17Mit13		L57	149	144	149	146	144	144	142	144	142	149	149	149
D17Mit16		A25	123	92	122	98	109	94	94	109	94	110	90	122
D17Mit18		M33	246	256	246	238	241	242	241	241	246	241	241	246
D17Mit19		M44	185	158	185	180	185	185	185	185	185	180	174	185
D17Mit20	*C3*	D129	180	198	185	212	178	178	178	178	178	185	185	185
D17Mit21	*Mhcab2*	D21	140	108	140	140	158	124	124	158	124	126	124	136
D17Mit22	*Mhceb2*	D16	160	178	160	164	185	162	162	185	160	—	160	158
D17Mit23	*Pim1*	D106	138	140	138	—	140	145	145	140	145	138	138	140
D17Mit24	*Thy19*	D12	145	140	145	120	130	145	147	130	147	145	145	130
D17Nds2	*Hsp68*	T9	110	105	110	80	105	105	—	105	105	110	125	110
D17Nds3	*Tnfb*	T68	145	120	145	90	126	126	160	126	160	132	132	145
D18Mit1		M42	154	140	154	147	154	154	154	154	154	154	154	136
D18Mit1		A104	145	130	145	143	145	145	145	145	145	145	145	126
D18Mit2		L9	130	163	130	148	130	130	130	130	130	130	130	132
D18Mit3		L76	216	158	189	213	207	207	189	218	189	—	216	218
D18Mit4		M51	212	180	210	188	195	188	195	195	195	170	180	175
D18Mit5		M57	189	200	189	208	189	189	189	189	189	189	189	200
D18Mit7		M108	93	123	93	152	123	93	123	93	93	100	93	132
D18Mit8		L24	77	90	77	88	80	74	80	74	80	—	—	80
D18Mit9		M209	170	172	170	145	160	160	160	160	170	168	160	160
D18Mit10		A100	108	117	109	109	108	108	108	108	108	108	108	108
D18Mit12		A20	122	110	122	132	122	122	132	122	122	132	132	122
D18Mit14		L13	108	130	108	103	103	103	110	103	103	110	110	108
D18Mit15		L87	162	147	162	—	164	164	173	162	160	173	173	158
D18Mit16		A35	207	201	207	199	207	207	207	207	207	207	207	207
D18Mit17	*Grl-1*	D118	212	203	214	210	190	190	190	190	190	190	190	190
D18Nds1	*Mbp*	T11	146	190	146	162	146	146	146	146	146	146	146	—
D19Mit1		A17	123	138	123	162	145	145	145	145	145	143	145	147
D19Mit2		M109	185	163	—	188	—	185	185	185	185	—	196	185
D19Mit3		M13	200	218	200	206	200	200	200	200	205	200	200	215
D19Mit4		M230	200	242	200	190	200	200	200	200	200	200	200	200
D19Mit5		A75	214	195	214	205	214	214	214	214	214	214	214	214
DXMit1		L43	100	108	100	96	86	86	86	86	86	86	86	100
DXMit3		M131	178	182	178	187	178	178	178	178	178	178	178	178
DXMit4		M118	108	100	108	102	108	108	108	108	108	108	108	108
DXMit5		A19	150	145	150	150	150	150	150	140	140	150	150	150
DXMit6	*Zfx*	D28	208	204	208	204	208	208	208	208	208	—	—	208
DXNds1	*Hprt*	T8	108	120	108	110	108	108	108	108	110	—	110	110
DXNds2	*Plp*	T4	178	181	178	—	178	178	178	178	178	—	178	—
Unmapped		M251	95	120	95	160	95	100	95	95	95	95	—	—
Unmapped		A66	242	206	242	245	202	230	206	202	230	206	206	242

The strain designations are: OB = C57 BL/6J-*ob/ob*, CAST = CAST/Ei, B6 = C57BL/6J, SPR = SPRET/Ei, DBA = DBA/2J, A = A/J, C3H = C3H/HeJ, BALB = BALB/cJ, AKR = AKR/J, NON = NON/Lt, NOD = NOD/MrkTacBr, LP = LP/J. All allele sizes are given in base pairs. Dashes indicate missing data. Allele sizes are determined relative to molecular weight standards run in another lane, and thus should be considered approximate.

TABLE 3

Polymorphism rates of simple sequence repeats

	OB	CAST	B6	SPR	DBA	A	C3H	BALB	AKR	NON	NOD	LP
OB	—											
CAST	100.0	—										
B6	6.5	98.6	—									
SPR	90.7	95.9	90.2	—								
DBA	52.4	92.5	51.4	90.2	—							
A	53.2	94.4	52.7	92.8	45.8	—						
C3H	52.1	95	50.5	91.2	34.8	35.1	—					
BALB	50.6	94.1	49.3	93.2	45.2	31.6	38	—				
AKR	53.8	94.4	52.4	90.5	48.3	46.2	43.9	42.9	—			
NON	50.5	95.5	49.3	88.9	50.8	51.2	46.7	47.1	53.6	—		
NOD	55.4	92.9	54.5	90.9	53.6	51.2	48.1	51.0	51.0	43.9	—	
LP	58.7	92.1	57.4	91.3	53.4	54.5	53.8	49.3	56.7	55.4	55.9	—

The polymorphism rates were determined for those SSRs that were variant between OB and CAST, thus the rate for that strain combination is necessarily 100% for the markers reported. Strain designations are: OB = C57 BL/6J-*ob*/*ob*, CAST = CAST/Ei, B6 = C57BL/6J, SPR = SPRET/Ei, DBA = DBA/2J, A = A/J, C3H = C3H/HeJ, BALB = BALB/cJ, AKR = AKR/J, NON = NON/Lt, NOD = NOD/MrkTacBr, LP = LP/J.

ent map distances and can interfere with the ability to resolve genetic order accurately (BUETOW 1991). Accordingly, we developed a novel mathematical approach (see MATERIALS AND METHODS) for identifying the potentially erroneous data, so that they could be checked with special care.

We first obtained an empirical estimate of the error rate in our data, by independently repeating the genotyping of about 10% of the loci. Comparing the duplicate typings, we found a discrepancy rate of 1.4% corresponding to an error rate of 0.7 ± 0.2%. Using this estimate, we used a computer program to identify all typings that were at least 10-fold more likely to have arisen if erroneous than if correct (*i.e.*, $LOD_{error} \geq 1.0$). Each such typing was checked by reinspecting the autoradiogram and, if there was any ambiguity, by repeating the typing from scratch. From among the typings identified as potential errors, actual errors were found in 72 cases or about 0.5% of the data. Simulation studies (not shown) showed that the expected number of actual errors that would fail to give rise to a $LOD_{error} \geq 1.0$ was about 20. About half of these errors would be expected to occur at markers that were either at the ends of linkage groups or adjacent to large intervals (since the power to detect error by virtue of double crossovers is least in these cases). Accordingly, we retyped all such markers from scratch. Overall, we estimate that approximately 10 errors may remain in the data–corresponding to a residual error rate of about 0.1%. These data should provide a firm foundation on which to build an even denser map.

Anchoring of the map: It was important to anchor our map relative to the existing mouse genetic map, in order to increase its utility for genetic studies. We used two methods. (1) Because 157 of the genetic markers are polymorphic in the BXD crosses, these markers could be mapped in the BXD recombinant inbred lines (BAILEY 1971; TAYLOR, HEINIGER and MEIER 1973). We typed a well spaced collection of 121 of these markers (Table 4), of which 100 could be unambiguously linked to known strain distribution patterns which then served as anchor points. Most anchors are indicated in Figure 2, although some are omitted when several anchors are present in the same region. (2) Because 32 of our SSLPs came from genes with previously known chromosomal positions, this provided a further collection of anchor points. [Conversely, our map provided chromosomal locations for 10 genes which were previously unmapped or incorrectly mapped (Table 5).]

Further confirmation of our anchoring came from two sources: (1) W. FRANKEL and J. COFFIN (personal communication) mapped a number of RFLPs corresponding to endogenous retroviruses segregating in our cross, six of which are shown; and (2) our map included 30 SSLPs whose positions had been previously determined in crosses by J. TODD and colleagues (LOVE *et al.* 1990; CORNALL *et al.* 1991; HEARNE *et al.* 1991).

Mutation rate: Studying the BXD recombinant inbred lines provided an excellent opportunity to measure the average mutation rate of SSLPs per generation, by looking for the occurrence of individual RI lines fixed for a nonparental allele. We observed nine such events, indicated in Table 4. Since we have typed 22 RI strains for 121 genetic markers and since the RI lines have been separated for about 75 generations (TAYLOR 1989), we estimate that there were about 200,000 opportunities for mutational

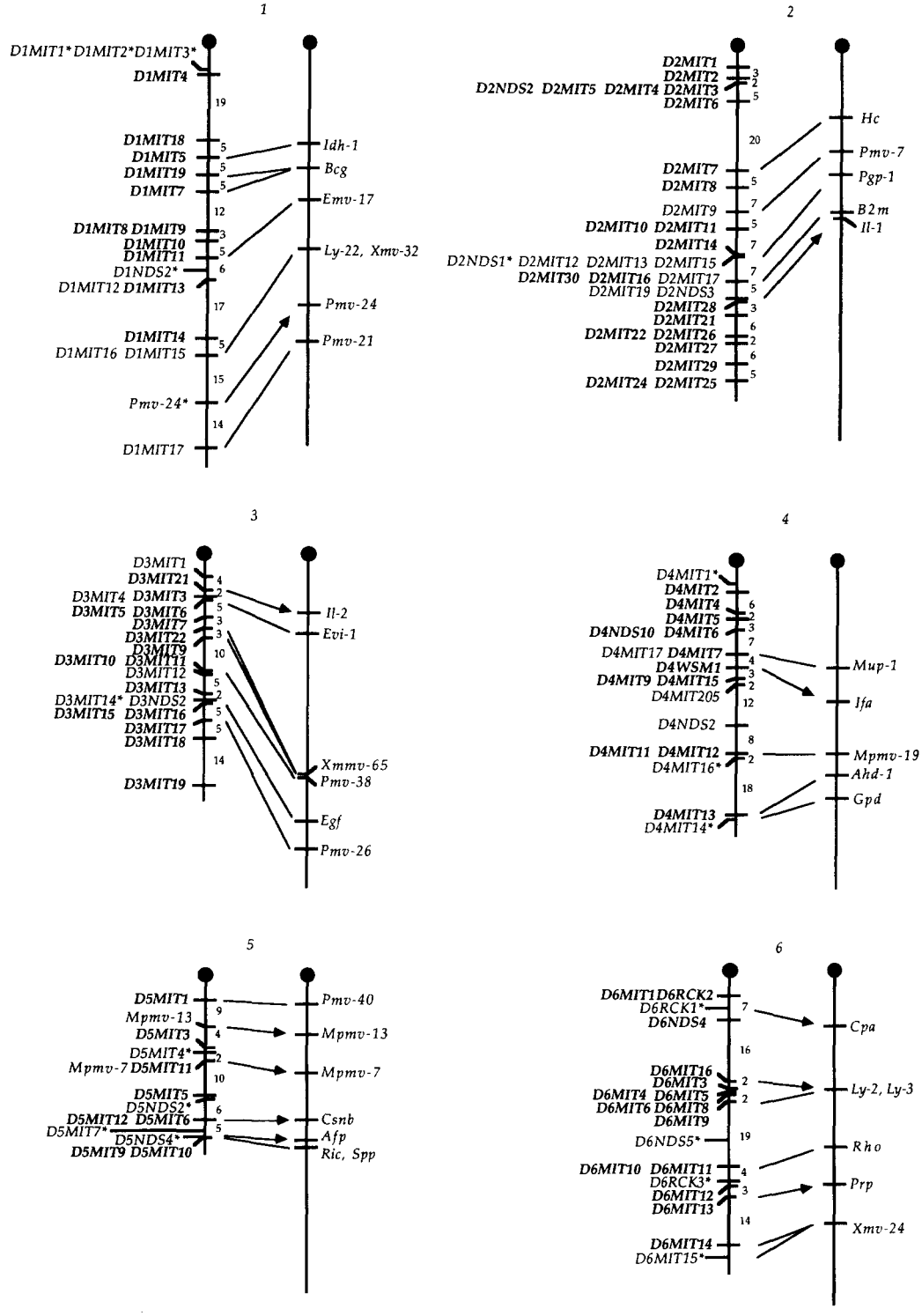
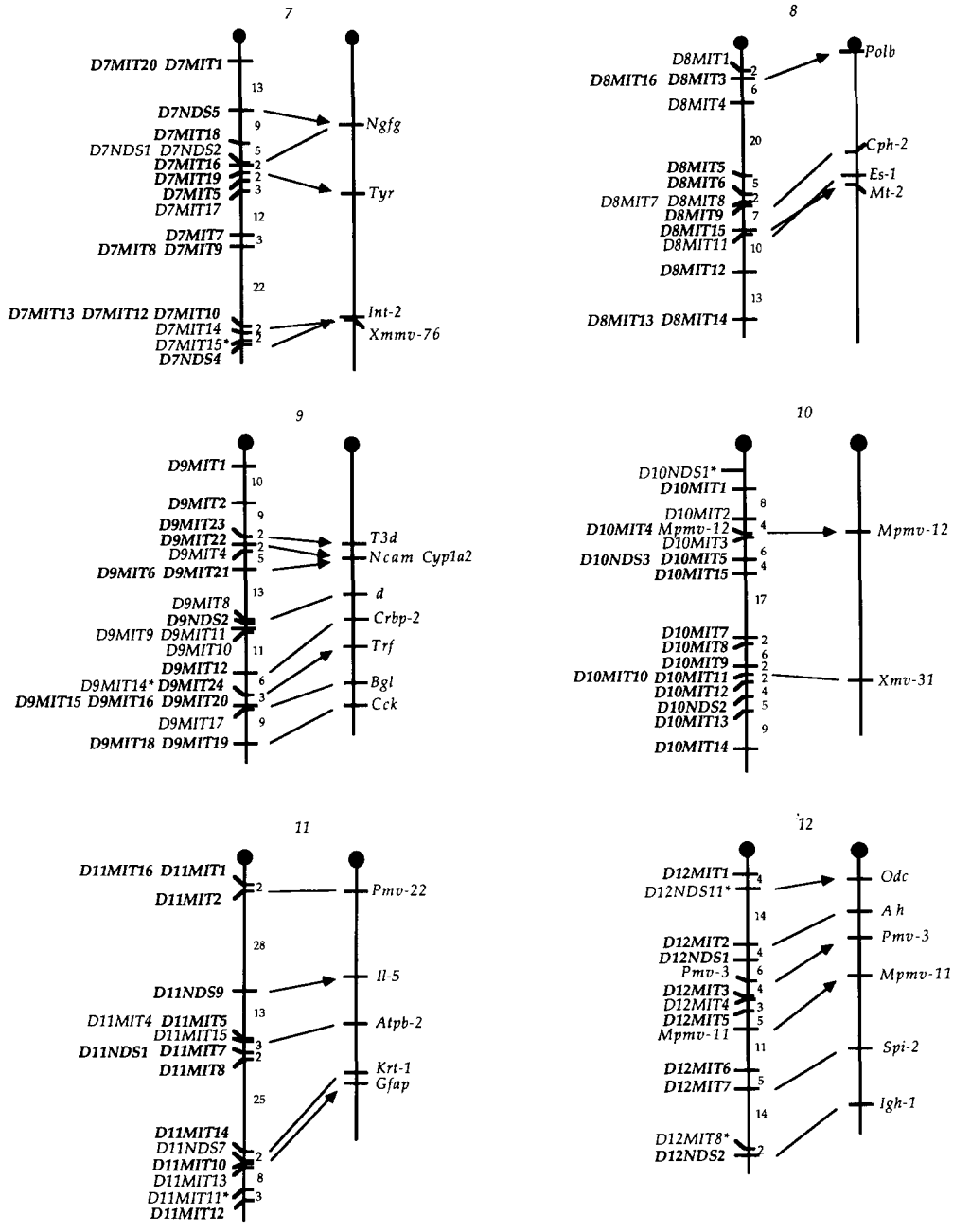


FIGURE 2.—Genetic linkage map of the mouse. Chromosomes are represented by two diagrams, the left side being the map reported in this paper, and right side being taken from the consensus map reported in the October 1990 edition of the GBASE database. For the SSLP map, a length of five cM has been arbitrarily added to each end. For the GBASE map, map lengths are equal to the fractional cytogenetic length for the chromosome multiplied by 1600 cM (the estimated genetic length of the mouse genome). Centromeres are indicated by filled circles. SSLPs are defined in Table 2. Six retroviral markers (denoted by their usual locus names) were scored in the cross and are shown on the map. Symbols indicate the degree of support for the indicated genetic order. Markers whose order relative to the map is supported by a LOD score of at least 3 are shown in bold type; by a LOD score of between 2 and 3 in plain face type; and by a LOD score of between 1 and 2 are marked with an asterisk (see MATERIALS AND METHODS). Markers listed on the same horizontal line did not recombine in the 46 animal F_2 intercross studied here. Centimorgan distances between markers are indicated, except for those less than 2 cM. Centimorgans are based on Kosambi's map function. Although the appropriate mapping function for the mouse genome is not precisely known, this function should be adequate for the present purposes. In any case, the choice of mapping function only has a significant effect on the large intervals,



whose distances should necessarily be considered to be approximate. Maximum likelihood order for LOD 1 markers relative to flanking markers is indicated, but exact distances are not. Such markers are indicated by a horizontal tick mark that does not cross the chromosome. The lines connecting the two maps indicate anchor points: Lines with arrowheads indicate that identity between markers on the two maps. Lines without arrowheads indicate that an SSLP is genetically linked to the marker shown on the GBASE map, based on analysis of BXD recombinant inbred strains. Because lines with arrowheads indicate identity and lines without arrowheads simply imply linkage, two such lines may cross one another without implying inversion of gene order (as occurs on chromosome *8*). (Figure 2 is concluded on page 440.)

events. This corresponds to an average mutation rate of about 1/22,000 per locus per generation.

Repeat occurrence of SSRs: In selecting SSRs from the genome, it is possible that some loci will be sampled more than once. Specifically, suppose that k objects are randomly chosen with replacement from a set of size N. For $k^2 > N > k^{3/2}$, a simple Poisson approximation shows that about $k(k-1)/2N$ objects will be chosen twice and few or no objects will be chosen three times. In the present case, our 319 clones containing CA-repeats were not selected at random from the genome, but rather from the set of CA-repeats contained in *Mbo*I fragments of between 250 and 500 bp and positioned within the fragment so that we would have sufficient flanking sequence to choose PCR primers. The proportion of such CA-repeats can be estimated to be about 17% (calculations not shown). If the total number of CA-repeats in the genome is M, we would expect to see about $(319 \times 318)/(2 \times 0.17\ M) \approx 300{,}000/M$ duplicate clones

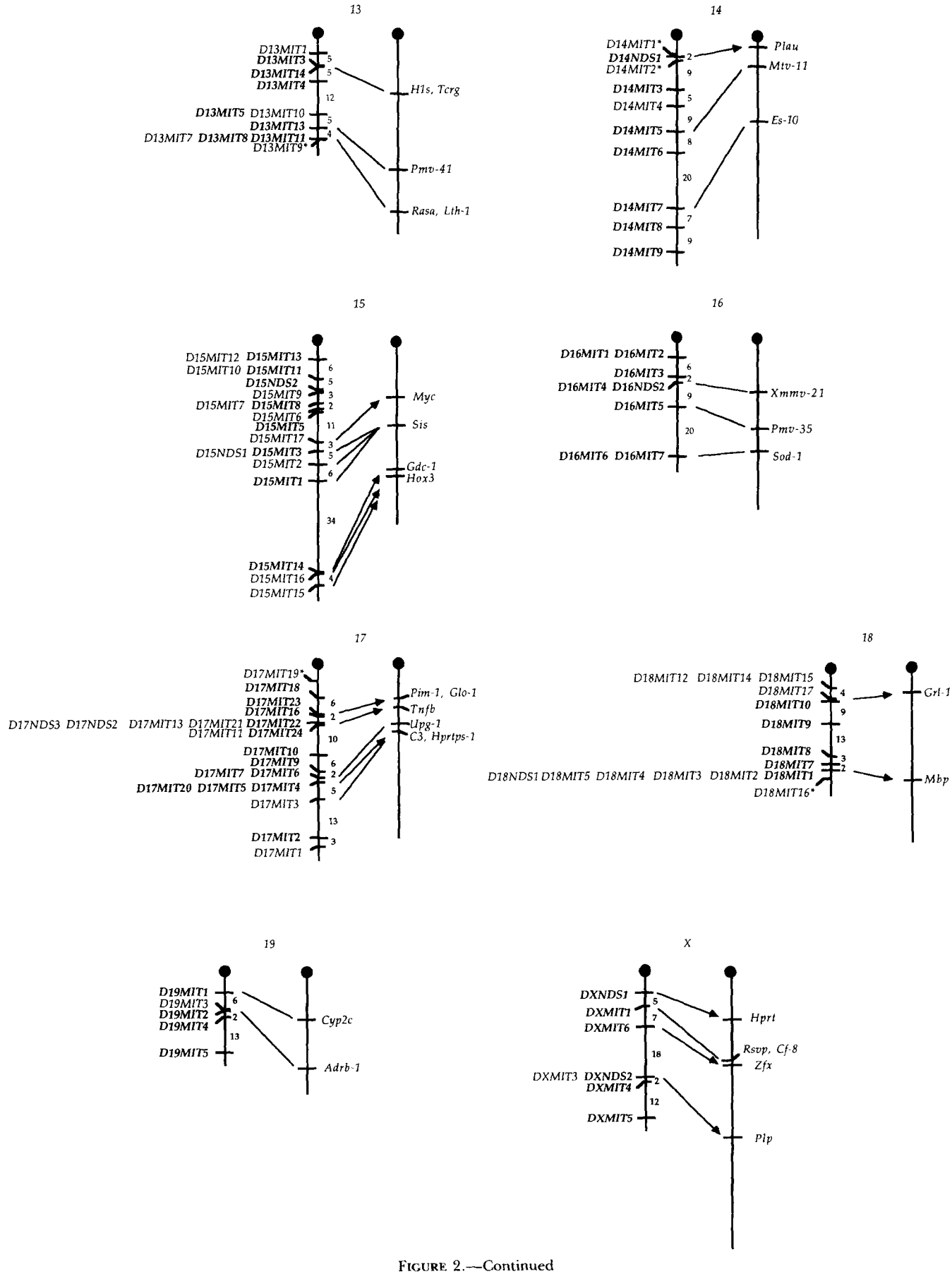


FIGURE 2.—Continued

TABLE 4

BXD strain distribution patterns

Locus name	Assay name	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
D1Mit1	L33	U	U	U	D	D	B	D	D	B	B	D	D	B	D	D	D	D	B	B	D	B	D	D	U	U	U
D1Mit5	L20	U	B	B	D	D	B	B	B	B	B	D	D	B	D	B	B	B	B	B	D	D	D	D	U	U	U
D1Mit7	A80	U	B	B	D	D	B	U	B	D	B	D	B	B	B	U	B	B	D	B	D	D	B	U	U	U	U
D1Mit11	M17	U	B	D	D	B	B	D	B	D	B	D	B	D	B	D	B	D	D	D	D	B	D	B	U	U	U
D1Mit16	L46	U	B	D	B	B	**	D	B	D	B	D	B	B	B	B	B	D	D	D	D	D	D	D	U	U	U
D1Mit17	M41	U	B	D	D	D	D	B	B	D	D	D	B	B	B	B	D	D	D	D	B	D	B	D	U	U	U
D1Mit19	L86	U	B	B	D	D	B	B	B	D	B	D	B	B	B	B	B	D	D	B	D	D	D	B	U	U	U
D2Mit6	L18	U	B	U	B	D	D	B	D	D	D	B	B	D	B	B	D	D	B	D	D	B	B	B	U	U	U
D2Mit7	L44	U	B	D	B	B	D	B	B	D	D	B	B	B	D	B	D	D	B	D	D	B	B	B	U	U	U
D2Mit9	M85	U	D	D	B	B	D	B	B	D	D	B	B	B	D	D	D	B	D	B	B	B	B	B	U	U	U
D2Mit12	M179	U	D	D	B	D	B	D	B	D	D	B	D	D	D	D	D	B	D	D	B	D	D	D	U	U	U
D2Mit14	M163	U	B	D	B	B	**	D	D	D	D	B	D	D	D	D	D	B	D	D	B	B	B	D	U	U	U
D2Mit17	M246	U	B	B	B	D	B	D	D	B	B	D	D	D	D	D	D	B	D	D	B	D	D	D	U	U	U
D2Mit30	D111	U	B	B	B	D	B	D	D	B	B	D	D	D	D	D	D	U	D	D	B	D	U	D	U	U	U
D2Nds1	T19	U	D	D	B	B	D	D	D	D	D	B	B	D	D	D	D	B	D	D	B	D	B	D	U	U	U
D3Mit5	M123	U	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	B	D	D	B	U	U	U
D3Mit9	A85	U	B	D	B	B	B	D	B	B	B	D	B	B	B	B	D	B	B	B	D	B	D	B	U	U	U
D3Mit10	A34	U	B	B	B	D	B	D	B	B	B	D	B	B	B	B	D	D	B	B	D	B	D	B	U	U	U
D3Mit12	A60	U	D	B	B	D	B	D	B	B	B	D	**	B	B	B	D	D	B	B	D	B	D	B	U	U	U
D3Mit15	A55	U	D	B	B	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	D	B	U	U	U
D3Mit17	M235	U	B	B	D	D	D	D	B	D	B	B	B	D	D	D	D	B	B	B	D	B	D	B	U	U	U
D3Mit19	M141	U	D	B	D	B	B	D	D	D	B	D	B	D	B	D	B	D	B	B	D	B	D	B	U	U	U
D3Mit21	D31	U	D	B	B	D	B	D	D	D	D	D	B	D	D	B	D	B	B	B	B	D	D	B	U	U	U
D3Mit22	D122	U	B	D	B	B	B	D	D	B	B	D	B	B	B	B	D	B	B	B	D	B	D	B	U	U	U
D3Nds2	T21	U	D	B	B	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	D	B	U	U	U
D4Mit12	M15	U	B	D	B	D	D	B	D	D	D	D	D	D	D	B	D	**	D	B	D	D	D	D	U	U	U
D4Mit13	M169	U	B	D	B	D	B	B	D	D	D	D	D	D	B	B	D	B	D	B	B	B	D	B	U	U	U
D4Mit14	A69	U	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	B	D	B	U	U	U
D4Mit16	A65	U	B	D	B	D	D	B	D	D	D	B	D	D	B	B	D	B	D	B	D	D	D	B	U	U	U
D4Mit17	D1	U	B	B	B	B	B	B	B	D	B	D	B	B	B	B	D	D	B	D	D	D	B	B	U	U	U
D5Mit1	A82	U	D	D	D	B	D	U	D	B	B	B	D	B	D	D	B	B	B	B	B	D	B	D	U	U	U
D5Mit7	M154	U	D	D	B	B	B	B	D	B	D	B	D	B	B	B	D	D	B	D	B	B	B	D	U	U	U
D5Mit10	M207	U	D	D	B	B	B	D	D	B	D	B	D	D	B	B	D	D	B	D	U	B	B	D	U	U	U
D5Mit11	M97	U	U	D	B	B	B	B	B	B	B	B	D	B	D	D	D	D	B	D	B	B	D	B	U	U	U
D6Mit9	L23	U	B	D	B	D	D	D	B	D	B	B	B	B	D	B	D	D	B	D	B	D	B	B	U	U	U
D6Mit10	M78	U	B	D	B	B	B	D	B	D	B	D	B	B	D	B	B	B	B	D	D	D	B	D	U	U	U
D6Mit13	D34	U	B	D	B	B	D	D	B	D	B	D	B	B	D	B	B	B	B	D	D	B	B	D	U	U	U
D6Mit14	M190	U	B	D	B	D	D	B	B	D	B	D	B	D	D	B	B	B	D	B	D	D	B	D	U	U	U
D6Mit15	M148	U	B	D	B	D	D	B	B	D	B	D	B	D	D	B	B	B	D	B	D	D	B	D	U	U	U
D6Mit16	D11	U	B	D	D	D	B	D	B	D	B	B	U	B	U	B	D	D	B	D	D	D	B	B	U	U	U
D7Mit7	L12	U	B	D	B	B	D	B	B	D	D	B	B	D	B	D	B	D	B	B	B	B	B	D	U	U	U
D7Mit12	M23	U	D	D	B	B	B	B	D	D	B	D	B	D	D	D	B	B	B	B	B	D	D	D	U	U	U
D7Mit17	M91	U	B	D	U	B	D	B	D	D	D	D	B	B	B	B	B	B	U	U	D	U	D	U	U	U	U
D7Nds2	T28	U	**	D	B	B	D	B	D	**	D	B	B	B	B	B	B	D	B	B	D	B	B	B	U	U	U
D8Mit4	M71	U	B	B	B	B	B	D	B	D	B	B	D	D	B	B	B	D	B	D	B	B	B	D	U	U	U
D8Mit8	M257	U	B	D	B	B	D	D	B	D	B	B	D	D	D	B	B	B	B	D	B	B	B	B	U	U	U
D8Mit9	A62	U	B	B	B	D	D	D	B	D	B	B	D	D	D	B	B	D	B	D	B	U	B	B	U	U	U
D8Mit11	A105	U	B	D	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	D	B	D	D	B	U	U	U
D9Mit4	M151	U	D	B	D	D	D	B	D	B	D	B	D	D	D	B	D	B	D	D	D	B	B	D	U	U	U
D9Mit8	M211	U	B	D	D	D	D	B	D	B	B	B	D	D	D	B	D	D	B	B	D	B	B	D	U	U	U
D9Mit11	L60	U	B	B	D	D	B	B	D	B	B	B	D	D	B	B	D	D	B	B	D	B	B	B	U	U	U
D9Mit12	M73	U	B	B	D	D	B	B	D	B	B	B	B	D	B	D	D	B	B	B	D	B	B	B	U	U	U
D9Mit15	M160	U	D	B	B	D	B	B	B	B	B	B	B	D	B	D	B	D	D	B	D	D	B	B	U	U	U
D9Mit18	M10	U	D	B	D	B	B	B	B	B	B	B	D	B	B	B	D	D	D	B	B	B	B	B	U	U	U
D9Mit19	M157	U	D	B	D	B	B	B	B	B	B	B	D	B	B	B	D	D	D	B	B	B	B	B	U	U	U
D9Mit20	L64	U	D	B	B	D	B	B	B	B	B	B	B	D	B	D	D	D	B	B	D	B	B	B	U	U	U
D9Mit21	D15	U	D	D	D	D	D	B	D	B	D	B	D	D	D	B	D	B	D	B	D	B	B	D	U	U	U
D9Nds2	T30	U	B	B	D	D	D	B	D	B	B	B	D	D	D	B	D	D	B	B	D	B	B	D	U	U	U
D10Mit3	A114	U	D	D	B	B	B	B	B	B	D	D	B	B	B	B	D	B	D	D	D	B	D	B	U	U	U
D10Mit10	M7	U	D	D	D	D	B	D	B	B	D	B	D	D	B	B	D	B	D	D	D	D	D	B	U	U	U
D10Mit11	A88	U	D	D	D	D	B	D	B	B	D	B	D	D	B	B	D	B	D	D	D	D	D	B	U	U	U
D10Mit14	M175	U	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	B	D	D	D	D	D	B	U	U	U
D10Mit15	D30	U	D	D	B	D	B	B	B	**	D	B	D	D	B	B	D	B	D	D	B	B	D	B	U	U	U
D11Mit2	L14	U	B	B	D	B	D	B	B	D	D	B	U	D	B	D	B	B	D	D	B	D	D	D	U	U	U
D11Mit4	A124	U	D	B	D	B	B	B	B	D	B	B	D	B	D	B	D	B	B	B	B	B	D	D	U	U	U
D11Mit14	D2	U	D	D	D	D	D	B	B	B	D	D	D	B	D	D	D	D	B	D	B	B	B	D	U	U	U
D11Mit15	D5	U	D	B	D	B	B	B	B	D	B	D	D	B	D	U	D	B	B	B	B	B	D	D	U	U	U
D12Mit1	M50	U	D	B	B	D	D	B	D	D	D	B	B	B	B	D	D	D	D	D	B	D	B	B	U	U	U
D12Mit2	M27	U	D	B	B	B	D	B	B	B	B	D	B	B	D	D	D	D	D	D	B	D	B	D	U	U	U
D12Mit3	L41	U	B	B	B	D	D	B	D	B	B	D	B	B	B	D	B	B	D	D	D	D	D	D	U	U	U
D12Mit5	L58	U	B	B	B	D	B	B	D	D	B	D	B	B	B	D	B	B	B	D	D	D	D	B	U	U	U
D12Mit7	M62	U	B	B	D	D	D	D	B	B	B	D	D	B	D	D	B	B	B	D	D	D	D	B	U	U	U
D12Mit8	D7	U	B	B	D	B	D	D	D	B	B	D	D	D	B	D	B	B	B	D	B	D	D	B	U	U	U
D12Nds2	T1	U	B	B	D	B	D	D	D	B	B	D	D	D	B	D	B	B	B	D	B	B	D	B	U	U	U
D13Mit3	M79	U	B	B	B	B	B	B	D	D	D	B	B	D	B	U	B	B	D	B	D	D	B	D	U	U	U
D13Mit9	M147	U	D	B	B	B	D	D	D	D	D	D	D	B	B	D	D	B	D	D	B	D	D	B	U	U	U
D13Mit11	A91	U	D	B	D	B	D	D	D	D	D	D	D	B	B	D	D	B	D	D	B	D	B	B	U	U	U
D13Mit13	D24	U	D	B	D	B	D	B	D	D	D	B	D	B	B	D	D	B	B	D	D	D	D	B	U	U	U
D14Mit1	A103	U	D	B	D	B	D	D	B	B	B	D	D	D	D	B	D	D	B	D	B	B	B	D	U	U	U
D14Mit2	A24	U	D	B	D	B	D	D	B	B	B	D	B	D	D	B	D	D	B	B	B	B	B	D	U	U	U
D14Mit5	M214	U	D	B	D	D	D	D	B	B	D	D	D	D	D	B	B	D	B	B	B	B	B	D	U	U	U
D14Mit6	A119	U	D	B	D	D	D	D	B	B	D	D	D	B	D	B	B	D	B	B	D	B	B	D	U	U	U

TABLE 4—Continued

Locus name	Assay name	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
D14Mit7	L27	U	D	B	D	D	D	D	B	B	D	D	U	B	D	B	B	B	D	B	D	B	B	D	U	U	U
D14Nds1	T10	U	D	B	D	B	D	D	B	B	B	D	B	D	D	B	D	D	B	B	B	B	B	D	U	U	U
D15Mit1	L29	U	B	D	B	D	D	B	B	D	D	D	B	D	B	D	D	D	B	D	B	D	D	B	U	U	U
D15Mit2	L10	U	D	D	B	D	D	B	B	D	D	D	B	D	D	D	D	D	B	D	B	D	D	B	U	U	U
D15Mit3	L78	U	B	B	B	D	D	B	B	D	D	B	B	D	D	B	D	B	B	D	B	D	D	B	U	U	U
D15Mit5	L1	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	B	D	D	B	U	U	U
D15Mit6	A59	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	**	B	D	D	B	U	U	U
D15Mit7	M30	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	B	D	D	B	U	U	U
D15Mit8	A79	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	B	D	D	B	U	U	U
D15Mit12	M34	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	D	D	**	B	U	U	U
D15Mit13	A36	U	D	B	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	D	D	D	B	U	U	U
D15Mit15	D6	U	B	D	B	D	D	B	D	D	B	B	B	D	B	D	D	B	B	D	B	D	B	D	U	U	U
D15Nds2	T18	U	D	D	B	B	D	B	B	B	D	B	B	D	B	B	D	B	B	D	D	D	D	B	U	U	U
D16Mit3	M127	U	B	B	B	D	B	D	B	B	B	B	D	B	D	D	B	B	U	U	D	U	B	D	U	U	U
D16Mit4	M203	U	B	B	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	D	D	B	D	U	U	U
D16Mit5	A38	U	B	B	B	D	B	D	B	B	B	D	D	B	B	D	D	B	B	D	D	D	B	U	U	U	U
D16Mit6	L7	U	B	B	B	B	D	D	B	B	D	D	B	B	D	D	B	B	B	D	D	D	B	D	U	U	U
D17Mit3	L28	U	U	D	B	B	B	D	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	B	U	U	U
D17Mit6	M254	U	D	D	D	B	B	D	B	D	B	B	D	D	B	B	B	D	B	D	D	D	D	B	U	U	U
D17Mit7	L4	U	D	D	D	B	B	D	B	D	B	B	D	D	B	B	B	D	D	D	B	D	D	B	U	U	U
D17Mit7	A23	U	D	D	D	B	B	D	B	D	B	B	D	D	B	B	B	D	D	D	B	D	D	B	U	U	U
D17Mit10	L36	U	D	D	D	B	B	D	B	D	B	B	D	D	B	B	B	D	B	D	D	D	D	B	U	U	U
D17Mit11	M145	U	B	D	D	B	U	D	B	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Mit13	L57	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Mit16	A25	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Mit21	D21	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Mit22	D16	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Mit24	D12	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D17Nds2	T9	U	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	U	U	U
D18Mit4	M51	U	B	D	D	B	U	B	D	B	B	D	D	D	B	B	B	D	B	B	B	B	D	B	U	U	U
D18Mit7	M108	U	B	B	D	B	D	B	D	B	B	D	D	D	B	D	B	D	B	D	B	B	B	B	U	U	U
D18Mit8	L24	U	D	B	D	B	D	B	D	B	B	D	D	D	B	D	B	D	D	D	B	B	B	B	U	U	U
D18Mit9	M209	U	D	B	D	B	D	B	D	D	D	D	D	D	B	B	B	D	D	D	B	B	B	B	U	U	U
D18Mit10	A100	U	D	D	D	D	D	D	D	B	D	D	D	D	B	D	D	D	B	B	B	B	B	B	U	U	U
D18Mit14	L13	U	D	B	D	B	D	D	D	D	D	B	B	D	B	D	B	D	B	B	B	B	B	B	U	U	U
D18Mit15	L87	U	D	B	D	B	D	D	D	D	B	D	B	B	B	D	B	D	B	B	B	B	B	B	U	U	U
D18Mit17	D118	U	D	B	D	B	D	D	D	D	D	B	D	D	B	D	B	D	B	B	B	B	B	B	U	U	U
D19Mit1	A17	U	B	B	D	D	B	D	B	B	B	B	B	B	D	B	B	B	D	B	B	B	B	B	U	U	U
DXMit1	L43	U	B	B	D	B	D	B	B	B	B	B	B	B	B	D	B	B	B	B	B	B	D	B	U	U	U

The strains carrying the C57BL/6J allele are denoted by B and those carrying the DBA/2J allele are denoted by D. Strains whose allele was not determined are denoted by U. Mutant alleles, differing from both B and D, are denoted by **.

TABLE 5

Locations for previously unmapped genes

Name	Sequence	Chromosome	Reference
Trh-1 (*D2Mit30*)	His-t-RNA	*2*	MORRY and HARDING (1986)
Ace (*D11Mit13*)	Angiotensin converting enzyme	*11*	BERNSTEIN *et al.* (1989); HOWARD *et al.* (1990)
Snap (*D2Mit28*)	Synaptosomal associated protein 25	*2*	OYLER *et al.* (1991)
Rpl-32ps (*D3Mit22*)	Ribosomal protein L32′ (pseudogene)	*3*	JACKS, POWASER and HACKETT (1988)
Sqr-3 (*D10Mit15*)	Simple quadruplet repeat. pmlc3	*10*	SCHAFER *et al.* (1986)
Lif (*D11Mit16*)	Leukemia inhibitory factor	*11*	STAHL *et al.* (1990)
Antp91a (*D11Mit14*)	Tum⁻P91A antigen	*11*	LURQUIN *et al.* (1989)
Sqr-4 (*D13Mit14*)	Simple quadruplet repeat. pmlc4	*13*	SCHAFER *et al.* (1986)
Svp-4 (*D2Mit29*)	Seminal vesicle secretory protein IV	*2*	CHEN *et al.* (1991)
Mb-1 (*D7Mit20*)	Murine b-cell 1	*7*	KASHIWAMURA *et al.* (1990)

arising. (Actually, a small proportion of the clones were selected from GenBank and thus could not duplicate one another. However, this affects the estimate only slightly.)

After completing the map, we examined our data and found, in fact, six duplicate SSRs, defining the loci: *D3Mit13*, *D4Mit2*, *D6Mit6*, *D7Mit10*, *D17Mit7* and *D18Mit1*. In at least three of these cases, we can be certain that the clones were independent–either because they arose in libraries constructed at different times or because their sequences were from complementary strands. As should be the case, the independent typings of the duplicate loci showed no recombination. The number of duplicates is consistent with the genome containing about 50,000 distinct CA-repeat-containing SSLPs, which broadly agrees with previous estimates of the total number of CA-repeats in the genome (HAMADA and TAKUNAGA 1982). Although the number of duplicates is quite small, we plan to adjust our protocol in further work to check for duplicates immediately after sequencing and to use randomly sheared DNA inserts to decrease their frequency.

DISCUSSION

Utility of maps based on SSLPs: Simple sequence length polymorphisms are rapidly becoming a method

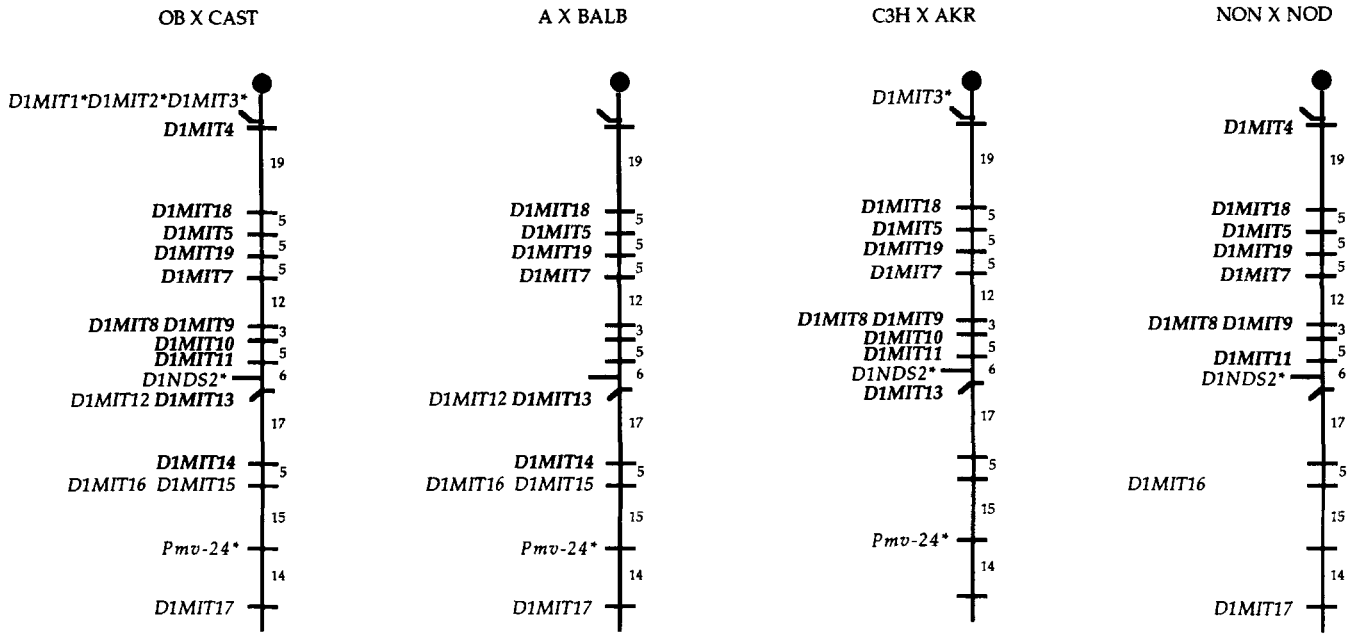


FIGURE 3.—Illustration of high polymorphism rate in different crosses. For chromosome *1*, the diagrams shows those SSLPs that are polymorphic in four typical crosses.

of choice for genetic mapping in human, mouse and rat, due to their exceptionally high rate of polymorphism and their relative ease of use. In humans, the high degree of polymorphism helps overcome the difficulties inherent in studying families in randomly breeding populations. In mouse and rat, the markers make it feasible to map the entire genome in any cross between laboratory strains; this has begun to allow genetic dissection of polygenic traits such as type I diabetes (TODD *et al.* 1991) and hypertension (JACOB *et al.* 1991; HILBERT *et al.* 1991).

We have developed a genetic map of the mouse consisting of 317 SSLP markers, with an average spacing of about 4.3 cM. Although the map was constructed in a cross between two divergent subspecies of *M. musculus* (OB × CAST), it can now be applied to map genes in most intraspecific crosses. Some 50% of the markers are polymorphic in a typical cross between two inbred laboratory strains, providing a genetic map with an average spacing of less than 9 cM. This is illustrated in Figure 3, showing the coverage of chromosome *1* in various crosses.

We hope that the map will prove useful to mouse geneticists. Because our map is anchored relative to the existing mouse map, it should be straightforward to identify the SSLPs in specific regions of interest. Additional anchor points will be added over time, by our laboratory and others. Because we have developed a dense collection of highly polymorphic SSLPs that work under a single set of PCR conditions, it should be possible to choose a relatively small subset of markers that are informative in any cross of interest and span the genome. In this fashion, it should be feasible for mouse geneticists rapidly to map any monogenic trait, as well as to undertake genetic dissection of polygenic traits. Indeed, all the laboratory work involved in constructing the map reported here was accomplished by two of us (W. DIETRICH and H. KATZ) in less than 18 months, and we have been able to apply it to genotype new crosses for the entire genome in a few weeks per cross (W. DIETRICH, unpublished results).

In addition to their utility in genetic mapping, the SSLPs should be valuable for studies of loss of heterozygosity (LOH) in murine tumors. Apart from the fact that DNA polymorphisms are generally useful in recognizing LOH, SSLPs offer the advantage that only a small tissue sample is required for PCR typing. This may be especially valuable in the case of tumors that must be dissected carefully from surrounding tissue.

Also, SSLPs may be useful in population genetic and evolutionary studies. For example, we note that the rate of polymorphism ranges from a low of about 32% for closely related strains such as DBA/2J and C3H/HeJ, A/J and C3H/HeJ, or A/J and Balb/cJ, to more than 50% for more distantly related strains such as C57BL/6J and AKR/J, or LP/J and A/J, to about 90% for intersubspecific and interspecific comparisons. This suggests that SSLPs may offer considerable power in tracing gene flow in closely related populations and may also offer advantages over simple nucleotide substitutions in reconstructing phylogenies (ATCHLEY and FITCH 1991) because they mutate more rapidly.

Coverage of the genome: The map appears to cover the vast majority of the mouse genome. One way to assess the coverage of the map is simply to observe

TABLE 6

Genetic length of mouse chromosomes

Chromosome	Consensus genetic length (cM)	Map reported in this paper (cM)
1	98	111
2	101	90
3	100	61
4	81	67
5	84	38
6	74	71
7	89	77
8	81	67
9	70	73
10	78	71
11	78	89
12	73	73
13	72	65
14	49	69
15	56	82
16	58	37
17	36	50
18	57	32
19	36	22
X	88	33
Total	1459	1267

The lengths represent genetic distance between most proximal and most distal markers. The consensus genetic length is from consensus map in Encyclopedia of the Mouse Genome, 1990. See text for description.

that only 2 of 319 markers failed to show linkage to our map. Another way is to compare our map to the consensus map reported in the GBASE database (Table 6). Of course, the two maps would not be expected to agree perfectly because genetic distance is known to be affected by strain background: our map is constructed in a single cross between two subspecies, while the GBASE consensus map represents a complex weighted average of a variety of different crosses. Nonetheless, the maps are colinear and the correspondence between them is good: our map shows a genetic length of 1267 cM contained between the most terminal markers, compared to a length of 1459 cM between the most terminal markers in the GBASE map. The difference amounts to an average of 5 cM lying beyond the most terminal marker at each end of the 20 mouse chromosomes.

A few specific features deserve mention.

The map has a few large intervals. Chromosome *15* contains the largest interval, of about 34 cM. Interestingly, the genetic length of this interval in the GBASE map appears to be only about 17 cM, suggesting enhanced recombination in this interval in our cross. The next largest interval is about 28 cM on chromosome *11*. Mathematically, an interval of this size would be expected by chance assuming a random distribution of markers.

Comparison with the GBASE map suggests that the terminal regions of most chromosomes are well covered, with the exception of the distal 20–25 cM on chromosome 5 and the distal 15–20 cM on chromosome 13. These intervals are not significantly larger than would be expected by chance.

Although the total length of chromosome 3 agrees well between our map and the GBASE consensus map, the region from Il-2 to Xmmv-65 seems to be compressed. Our map shows about 15 cM between these markers, compared to 40 cM on the GBASE map. This might be due to structural heterogeneity between OB and CAST chromosomes such as one or more inversions, although there is no large block of recombinationally inseparable markers as might be expected from a single large inversion. Additional anchors will be needed to resolve this.

Chromosome 18 shows an unusually large cluster of recombinationally unseparated markers. This might be due to an inversion or to a heterogeneity in the distribution of SSRs. The anchoring information suggests that the entire chromosome is represented in the map.

Random distribution of markers: Broadly speaking, the genetic markers appear to be randomly distributed throughout the genome. One way to assess this is to compare to the number of markers that would be expected to fall on each chromosome based on its physical size (estimated by cytogenetic length) to the number actually observed. (In this calculation, we must account for the fact that the genomic library used to isolated SSRs was made from a male mouse. We thus expect a twofold underrepresentation of the *X* chromosome.) The agreement is excellent (Table 7). Only chromosome *17* shows a significant deviation from expectation. In fact, the deviation is explained by the disproportionate number of SSLPs derived from cloned genes in GenBank on chromosome *17* (specifically, 7 of the 54 SSLPs derived from GenBank sequences in our map) owing to the extensive study of this chromosome, which is the site of the *major histocompatibility complex* and the *t complex*.

Another way to assess whether the markers are randomly distributed is to compare the observed distribution of distances between adjacent markers to that expected under the assumption that SSRs are randomly distributed across the genetic map (see MATERIALS AND METHODS). The distributions agree quite well (Figure 4). There appears to be a slight excess of zero distances–the proportion of pairs of adjacent loci that showed no recombination in our cross was 25.1% compared to an expectation of 20.3%±2.4%–but the deviation is just at the edge of statistical significance. This might hint at slight clustering of SSRs with respect to genetic distance, which could be due to uneven spacing of either SSRs or recombination along the physical map.

In short, the assumption of random distribution of

TABLE 7

Number of markers on each chromosome

Chromosome	Percent of genome based on physical map[a]	No. markers expected ±1 SD[b]	Markers in this paper	Z-score[c]
1	7.20	23.6 ± 4.7	19	−0.98
2	6.95	22.8 ± 4.6	30	1.58
3	5.99	19.6 ± 4.3	21	0.32
4	5.89	19.3 ± 4.3	19	−0.07
5	5.68	18.6 ± 4.2	12	−1.58
6	5.53	18.1 ± 4.1	20	0.46
7	5.19	17.0 ± 4.0	20	0.75
8	4.97	16.3 ± 3.9	14	−0.58
9	4.79	15.7 ± 3.9	21	1.38
10	4.74	15.5 ± 3.8	17	0.39
11	4.72	15.5 ± 3.8	16	0.14
12	4.88	16.0 ± 3.9	11	−1.28
13	4.38	14.3 ± 3.7	11	−0.90
14	4.46	14.6 ± 3.7	10	−1.23
15	4.05	13.3 ± 3.6	18	1.33
16	3.81	12.5 ± 3.5	8	−1.29
17	3.86	12.6 ± 3.5	22	2.69
18	3.88	12.7 ± 3.5	16	0.94
19	2.73	8.9 ± 2.9	5	−1.34
X	6.23	10.2 ± 3.1	7	−1.02

[a] Based on cytogenetic length EVANS (1989).

[b] Based on proportional size of each chromosome, adjusted for the *X* chromosome being at half-molar representation (since the vast majority of markers were isolated from a genomic library from male DNA).

[c] *Z*-score = (observed-expected)/standard deviation.

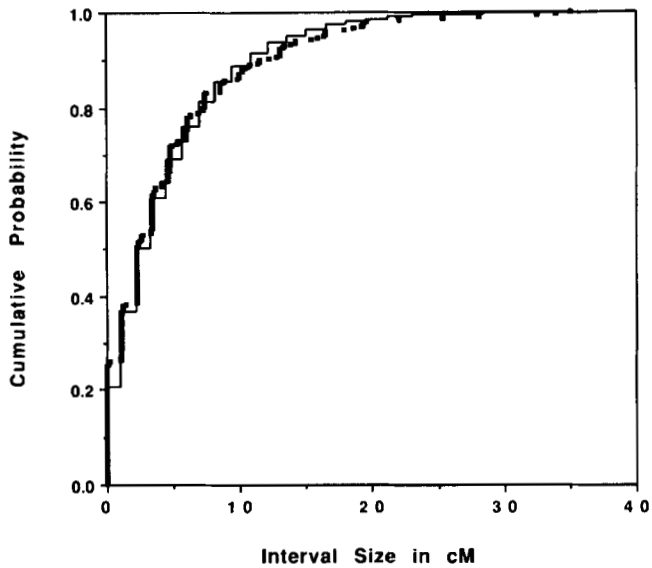


FIGURE 4.—Cumulative probability distribution of interval sizes in the genetic map. Points show observed cumulative distribution for intervals in our map. The solid line represents the expected distribution, assuming that SSLPs are randomly distributed with respect to centimorgans (see MATERIAL AND METHODS for formula). Note that the distributions of interval sizes is expected to show discrete jumps, because only a finite number N of meioses are studied and thus recombination fractions will be approximately integral multiples of $1/N$.

SSRs fits the data reasonably well at this level of resolution, although there may hints of clustering. Of course, significant inhomogeneity may become apparent at higher resolution. These findings bode well for the general usefulness of SSRs in the construction of genetic maps in other organisms, including the human.

Toward a dense genetic map of the mouse: The approach described here should allow the construction of much denser maps consisting of thousands of SSLPs. Indeed, SSLPs appear to be in abundant supply and to be randomly distributed throughout the genome–at least at the level of resolution examined here. With a genetic linkage map of 3000 SSLPs, one would have genetic landmarks at an average spacing of 1 million basepairs. Coupled with high quality yeast artificial chromosome libraries, such a dense collection of landmarks would permit rapid and straightforward cloning of the region containing any gene of interest and should greatly advance the genetic understanding of mammalian biology.

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Note added in proof: The locus *D18Mit6* was omitted in Figure 2. It did not recombine with *D18Mit1*.

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