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A Genetic Linkage Map of the Mouse Using Restriction Landmark Genomic Scanning (RLGS)

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Manuscript received January 18, 1994
Accepted for publication August 6, 1994

ABSTRACT

We have developed a multiplex method of genome analysis, restriction landmark genomic scanning (RLGS) that has been used to construct genetic maps in mice. Restriction landmarks are end-labeled restriction fragments of genomic DNA that are separated by using high resolution, two-dimensional gel electrophoresis identifying as many as two thousand landmark loci in a single gel. Variation for several hundred of these loci has been identified between laboratory strains and between these strains and *Mus spretus*. The segregation of more than 1100 RLGS loci has been analyzed in recombinant inbred (RI) strains and in two separate interspecific genetic crosses. Genetic maps have been derived that link 1045 RLGS loci to reference loci on all of the autosomes and the *X* chromosome of the mouse genome. The RLGS method can be applied to genome analysis in many different organisms to identify genomic loci because it uses end-labeling of restriction landmarks rather than probe hybridization. Different combinations of restriction enzymes yield different sets of RLGS loci providing expanded power for genetic mapping.

A TOP-DOWN approach for attaining a complete map of the mouse genome uses a high density genetic map as the basis for identifying landmarks in the physical genome. Two approaches can be considered for identifying markers to be used for constructing maps of complex genomes such as mammals. One approach is to simplify the procedure for detecting loci by establishing robotized systems for characterizing sequence tagged sites (STSs) throughout the genome. For example, polymerase chain reaction (PCR) methods for detecting CA-repeat or simple sequence repeat (SSR) polymorphisms have been used to identify several hundred loci in the mouse genome (DIETRICH *et al.* 1992; HEARNE *et al.* 1991). SSRs are abundant in mammalian DNA with each site present throughout the gene pool. The number of tandem repeats at a locus is often variable between common laboratory strains and between *Mus* species and subspecies making these highly useful markers for genome analysis. SSR genetic markers have also been used to establish high resolution genetic maps of the human genome (NIH/CEPH Collaborative Mapping Group, 1992). Overall, the SSR loci have several advantages for genome analysis that lend themselves to the integration of genetic and physical maps. However, the PCR amplification is done separately for each locus, requiring multiple analyses of each individual. In addition, the unique sequence identifiers for each site must be determined for each organism, a procedure that requires a substantial investment of time for sequence analysis before these loci can be used for genome analysis.

As a second, alternative approach to genetic analysis, we have developed restriction landmark genomic scanning (RLGS) as a method of genome analysis which is based on the concept that restriction enzyme sites can be used as landmarks (HATADA *et al.* 1991; HAYASHIZAKI *et al.* 1993). This method provides an alternative, multiplex approach to genome analysis that can be used to map a large number of loci simultaneously.

The characterization and mapping of RLGS loci that have *Not*I or similar landmark sequences that are CpG-rich frequently identifies CpG islands that are associated with functional genes. Thus, there is a high likelihood that cloned probes for these landmarks will identify homologous loci in other species that can be used for comparative mapping (KALCHEVA *et al.* 1995). In addition, many of the landmark restriction enzymes are methylation-sensitive providing additional opportunities for characterizing changes in the DNA methylation of a spot that may be related to developmental regulation of a gene (KAWAI *et al.* 1993) imprinting (HAYASHIZAKI *et al.*

1994), or genomic changes in methylation that accompany malignant transformation of cells (HIROTSUNE *et al.* 1992). Thus, there is a direct opportunity to relate methods of genome analysis to studying the regulation of specific loci or broader changes in genome function during development and in disease.

In this report, we demonstrate that RLGS mapping can be used effectively to identify a large number of loci and that these loci can be efficiently, accurately and rapidly mapped in the mouse genome using analyses of either RI strain or interspecific backcrosses.

MATERIALS AND METHODS

RLGS

The RLGS procedure has three primary features that are critical for identifying restriction landmarks (HATADA *et al.* 1991). These include: (1) blocking, the pretreatment of genomic DNA with ddNTPs to fill nicked or broken sites that would be end-labeled in addition to the landmark enzyme cleavage sites; (2) landmark cleavage, the digestion of genomic DNA with restriction enzymes with overhanging ends that facilitate end-labeling (landmark enzyme, E_L) and (3) labeling the landmark cleavage sites in the genomic DNA. The RLGS profiles for a genotype are determined by digestion with three restriction enzymes. The first enzyme (E_L) is typically a rare site restriction enzyme such as *Not*I that defines the labeled restriction landmark. The end-labeled landmark fragments of genomic DNA are reduced in size by a second restriction enzyme (E_B) before the DNA is separated in the first dimension. The third enzyme (E_C) is used to treat the DNA that is separated in the first dimensional gel and the cleaved fragments are then separated in a second dimension in an acrylamide gel.

Labeling: Genomic DNA (10 μg) was treated with 10 units of DNA polymerase I in 1× high salt buffer [50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 100 mM NaCl, 10 mM dithiothreitol] in the presence of 0.4 μM dGTP[aS], 0.4 μM dCTP[aS], 0.4 μM dCTP[aS], 0.4 μM ddATP[aS]. In place of ddTTP[aS] and ddATP[aS], ddTTP and ddATP of the same concentration can be used, respectively. This reaction mixture was incubated at 37° for 30 min. The DNA polymerase I was inactivated by heating at 65° for 30 min. The DNA was then digested with 100 units of restriction enzyme E_L, such as *Not*I. The reaction mixture was incubated at 37° for 1 hr. A sample of the reaction mixture was incubated with 0.5 μg control DNA, which was then analyzed by electrophoresis to confirm complete digestion. The cleavage ends were then filled in with 20 units of Sequenase Ver. 2.0 (U.S. Biochemical Corp.) in the presence of 0.15 μM [α-^{32}P]dGTP (3,000 Ci/mmol) and 0.33 μM [α-^{32}P]dCTP (6,000 Ci/mol) at 37° for 30 min. The polymerase reaction was stopped by the addition of 9 μl of stopper [3 mM ddGTP and 7 mM ddCTP] reaction mixture. Phenol extraction was not performed at this point because it has been found to cause preferential loss of the largest *Not*I fragments.

Restriction enzyme digestion: The second digestion was performed by restriction enzyme E_B, such as *Eco*RV or *Pvu*II, in 150 μl of 1× high buffer. After phenol extraction, the labeled DNA fragments were recovered by ethanol precipitation. The precipitate was dispersed by 10 μl of TE buffer [10 mM Tris-HCl (pH 7.4), 1 mM EDTA], and the DNA concentration was adjusted to 0.2 μg/μl by dye solution [0.1% bromophenol blue (BPB), 0.1% xylene cyanol, 6% glycerol]. The labeled sample, 10 μl, was loaded onto 14 mm × 1 mm × 0.8 mm slot of horizontal thin layer 0.8% agarose gel (1-mm thickness), and subjected to electrophoresis in 1× TAM [50 mM Tris-HCl (pH 7.5), 0.7 mM magnesium acetate] for 12 hr at 3 V/cm (when the BPB reaches the end of the gel). The DNA-containing portion of the gel was cut out as an 8 mm × 400 mm gel strip. The gel strip was clipped between two glass plates separated by 1-mm spacers. 1.5 ml of the reaction mixture of 1000 units of restriction enzyme E_C, such as *Hin*fI, *Mbo*I or *Pst*I in 1× high buffer was injected into the space between one side of glass plate and the gel top. After sealing the glass strips with tape to avoid drying, the DNA fragments were digested at 37° for 2 hr. The gel strip was soaked in 1× TBE [90 mM Tris-borate, 2 mM EDTA], to exchange the buffer in gel. The digestion of the samples with the first and second restriction enzyme was determined by testing an aliquot of the sample in a test digestion with plasmid. The digested plasmid was examined in agarose gels stained with ethidium bromide. The digestion of the third restriction enzyme was not directly determined but preliminary analyses were carried out using increasing amounts of enzyme and examining the two-dimensional profiles of labeled spots. The minimum units of enzyme required to complete the digestion of mouse genomic DNA were determined and a 10-fold excess of enzyme was used for routine analyses.

TABLE 1

Restriction enzyme combinations

Combination	E_L	E_B	E_C	Crosses analyzed
A	*Not*I	*Pvu*II	*Pst*I	BSS, BXD
B	*Not*I	*Eco*RV	*Hinf*I	HSH
C	*Not*I	*Eco*RV	*Mbo*I	BXD

Restriction enzymes used to create RLGS patterns in different analyses. The landmark enzyme (E_L), the second enzyme (E_B) and the third enzyme (E_C) are listed for each combination and the crosses analyzed using these combinations.

The strip was placed onto the top edge of the 5.0% polyacrylamide gel 45 cm × 46 cm × 1.0 thick and bonded to it with 1.0% SeaKem GTG agarose (FMC Inc.) in 1× high buffer. The DNA fragments in the agarose gel strip were separated in the second dimension using horizontal polyacrylamide gel electrophoresis at approximately 6 V/cm using constant power.

Restriction enzyme combinations: Three different restriction enzyme combinations have been used to derive RLGS patterns (Table 1). Combination A uses *Not*I (GC/GGCCGC) as the landmark cleavage that is end labeled followed by the cleavage with the 6-bp endonuclease *Pvu*II (CGA/TCG). These fragments were separated in the first dimensional agarose gels and cleaved in the gel with the third endonuclease *Pst*I (CTGCA/G). Combination B also used *Not*I for the landmark cleavage followed by digestion with *Eco*RV (GAT/ATC). The third cleavage was done with *Hinf*I (G/ANTC). Combination C consisted of *Not*I, *Eco*RV and *Mbo*I (/GATC).

Mouse resources: Backcross progeny were produced from C57BL/6Ros (B6) and outbred *Mus spretus* (S) using (C57BL/6 × *M. spretus*)F_1 females × *M. spretus* males (BSS). The BSS progeny (n = 72) segregated C57BL/6 (B6) alleles for each locus so that the resulting genetic maps derived from this pedigree are for the B6 genome. The BSS progeny were analyzed using enzyme combination A. A second backcross was produced from C3H/HeJ (H) and *M. spretus* (S) using (C3H × *M. spretus*)F_1 × C3H/HeJ (HSH). The HSH backcross progeny (n = 35) segregated *M. spretus* alleles for each locus so that the resulting genetic maps are for the *M. spretus* genome. The HSH backcross was analyzed using enzyme combination B. Recombinant inbred (RI) strains of the BXD series were used to analyze genetically both B-specific and D2-specific RLGS spots using two separate enzyme combinations A and C.

TABLE 2

Molecular size ranges

Area	1st Dimension separation in agarose		Area	2nd Dimension separation in acrylamide	
A	Upper border	13286	0	Upper border	1620
B	13286	9688	1	1620	723
C	9688	6223	2	723	511
D	6223	4254	3	511	389
E	4254	3472	4	389	260
F	3472	2690	5	260	209
G	2690	1882	6	209	149
H	1882	1268	7	149	88
I	1268	651	8	88	64
J	651	Lower border	9	64	Lower border

Molecular size ranges in the subdivisions of the first and second dimension of the electrophoretic separation of RLGS spots.

TABLE 3

RLGS locus distribution

Cross	Enzyme combination	RLGS profile subsection I	II	III	IV	V	Total
$B6SF_1$	A	83	61	27	97	97	365
$B6D2F_1$	A	89	42	95	65		291
HSF_1	B	59	68	72	87	71	357
$B6D2F_1$	C	48	52	16	60	20	196
Total							1209

RLGS locus distribution in two dimensional profiles separated into subregions (I, II, III, IV and V) for F_1 hybrids of $B6SF_1$, $B6D2F_1$ and HSF_1 for the enzyme combinations A, B and C.

Identification of spots in an RLGS profile: Variant spots were identified by comparing the patterns of F_1 hybrids with those of their parents. In the case of backcross progeny, either B6-specific or S-specific spots were marked and numbered on the RLGS film of the $B6SF_1$ hybrid or the HSF_1 to make the master plate. By comparing the RLGS profiles of backcross animals with the master plate, the presence or absence of the numbered spots was scored and progeny distribution patterns (PDPs) were collected. The segregation of B6-specific and D2-specific RLGS spots were identified for the BDF_1 hybrid in the same manner. The strain-specific spots in this hybrid were analyzed in recombinant inbred (RI) strains and the resulting strain distribution patterns (SDPs) were used to identify linkage relationships in the BXD RI strain series. The spots were arbitrarily numbered on the autoradiogram and identified formally by the restriction enzyme combination used for the analysis, the strain that carries the variant allelic form, the strain background of the other parent, and the gel area codes representing decile regions of the first and second dimension indicated by letter (A–J) and numbers (0–9) respectively. The molecular sizes in the subdivisions of the first and second dimension separation are shown in Table 2. As an example, spot AB(S) 393 in area code D3 would be an RLGS spot in a BSF_1 profile that is produced by enzyme combination A, it would be a B6-specific spot identified in the BSF_1 hybrid and it would be from a *Not*I-*Pvu*II fragment of 4254–6223 bp (area D) that was cleaved by *Pst*I to make a *Not*I-*Pst*I fragment between 389 and 511 bp.

Genetic analysis

BSS backcross: The PDP for each locus was initially compared with the PDPs of centromeric satellite sequences that had been analyzed using *in situ* analysis of the major satellite probe pMR196 on karyotypically analyzed metaphase chromosomes of each backcross progeny (MATSUDA and CHAPMAN 1991; MATSUDA *et al.* 1993). The initial linkage analysis was performed using a modification of the program, RI Manager (Map Manager) (MANLY and ELLIOTT 1991), that was adapted to analyze backcrosses. Our strategy involved the identification of linkages between the centromeric heterochromatin of satellite sequences identified as heterochromatin, centromeric (*Hc*) of specific chromosomes and RLGS spots at the 99.99% confidence level. If linkage of more than one RLGS locus was identified to the *Hc* locus of a specific chromosome, the order of these loci on the chromosome map was determined by haplotype analysis and minimizing multiple recombination events. In using the centromeric heterochromatin as an anchor for

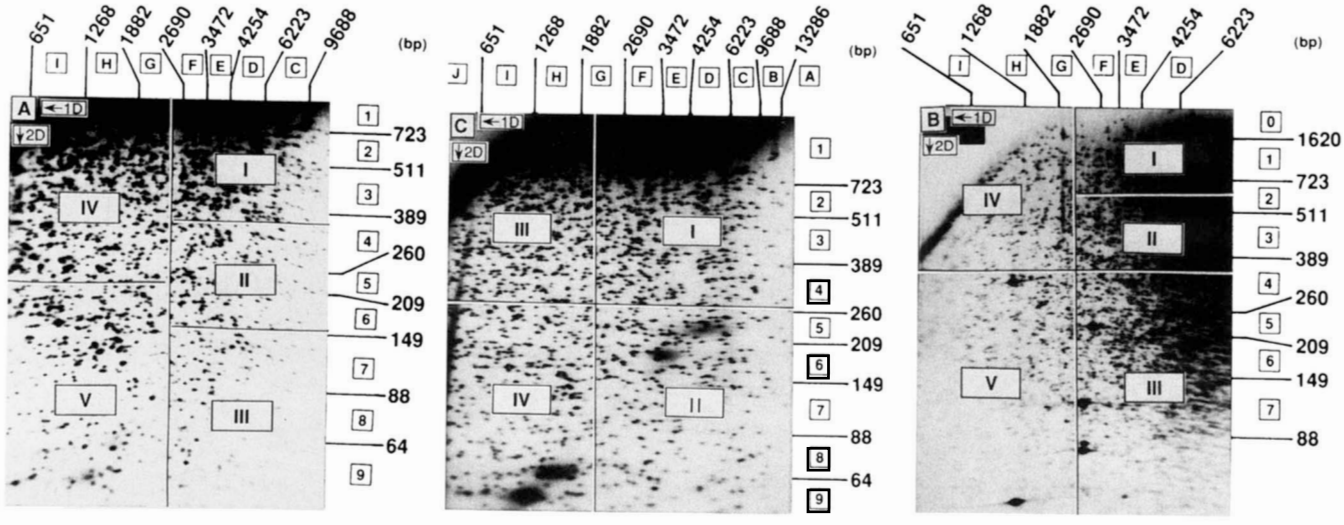


FIGURE 1.—Two-dimensional RLGS profiles for (A) BSF_1 using enzyme combination A; (B) BDF_1 using enzyme combination A; and (C) HSF_1 using enzyme combination B. The direction of the first dimension separation is from right to left and the relative position of the molecular sizes of the area codes is shown at the top of each profile. The area code subdivisions in the acrylamide dimension are indicated on the right side of each profile. Each RLGS profile is also subdivided into subsections (indicated by Roman numerals) that are shown in greater detail in Figure 2.

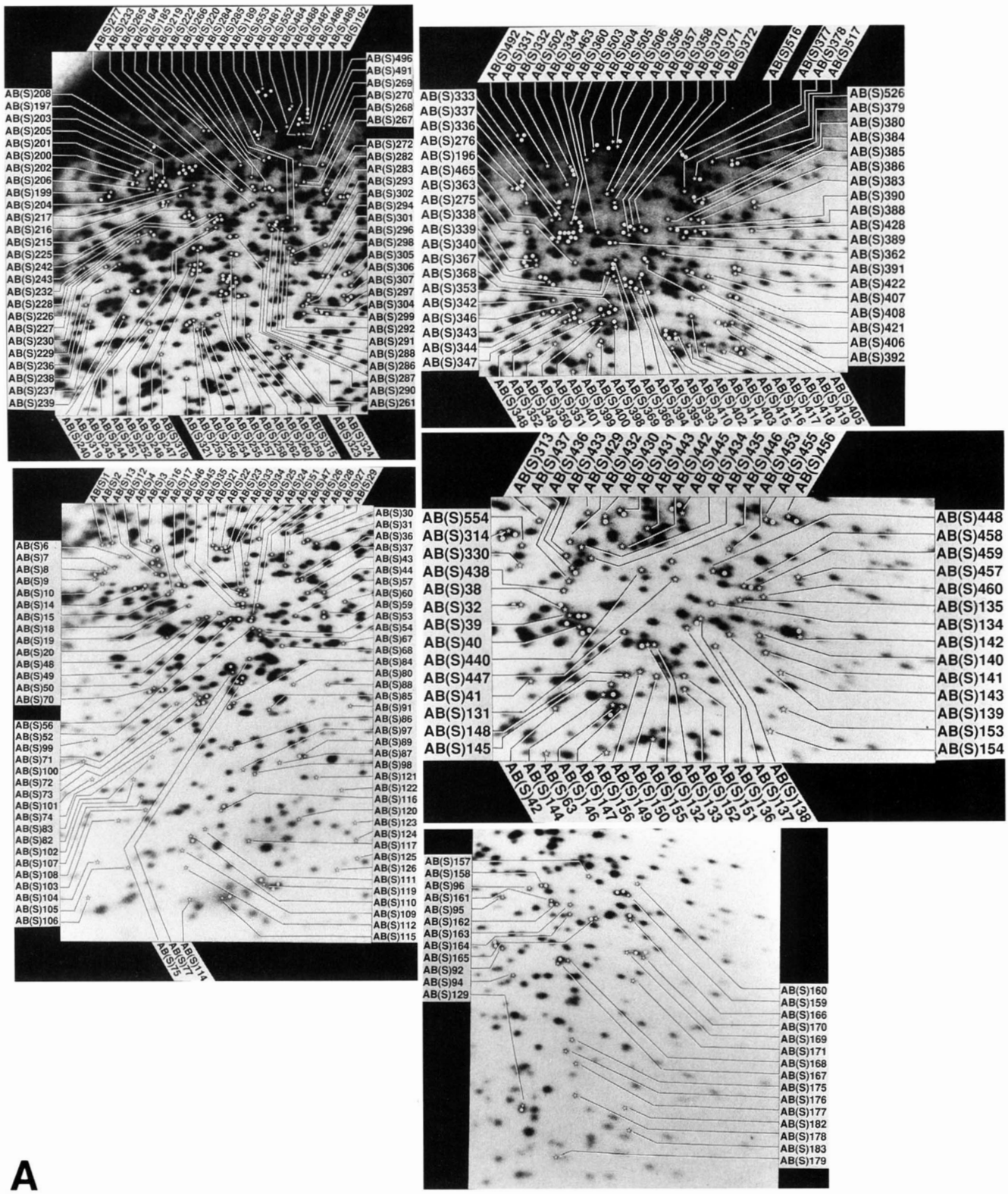


FIGURE 2.—Localization of numbered loci in the RLGS profile subsections indicated in Figure 1 for: (A) the B6-specific loci in the BSF_1 profile using enzyme combination A; (B) the B6- and D2-specific loci of the BDF_1 profile using enzyme combination A; (C) the H-specific loci of the HSF_1 profile using enzyme combination B; and (D) the B6- and D2-specific loci of the BDF_1 profile using enzyme combination C. Each subsection identified in Figure 1 is located in the same position indicated. RLGS spots identity codes are listed on the periphery of the subsection and connected by a line to the spot in the profile. The spot identity codes indicate the enzyme combination used in the first letter, the strain of origin of the variant spot as the second letter, the non-segregating parent strain in the backcross is shown in parenthesis and the specific spot number is the last 1, 2 or 3 digits.

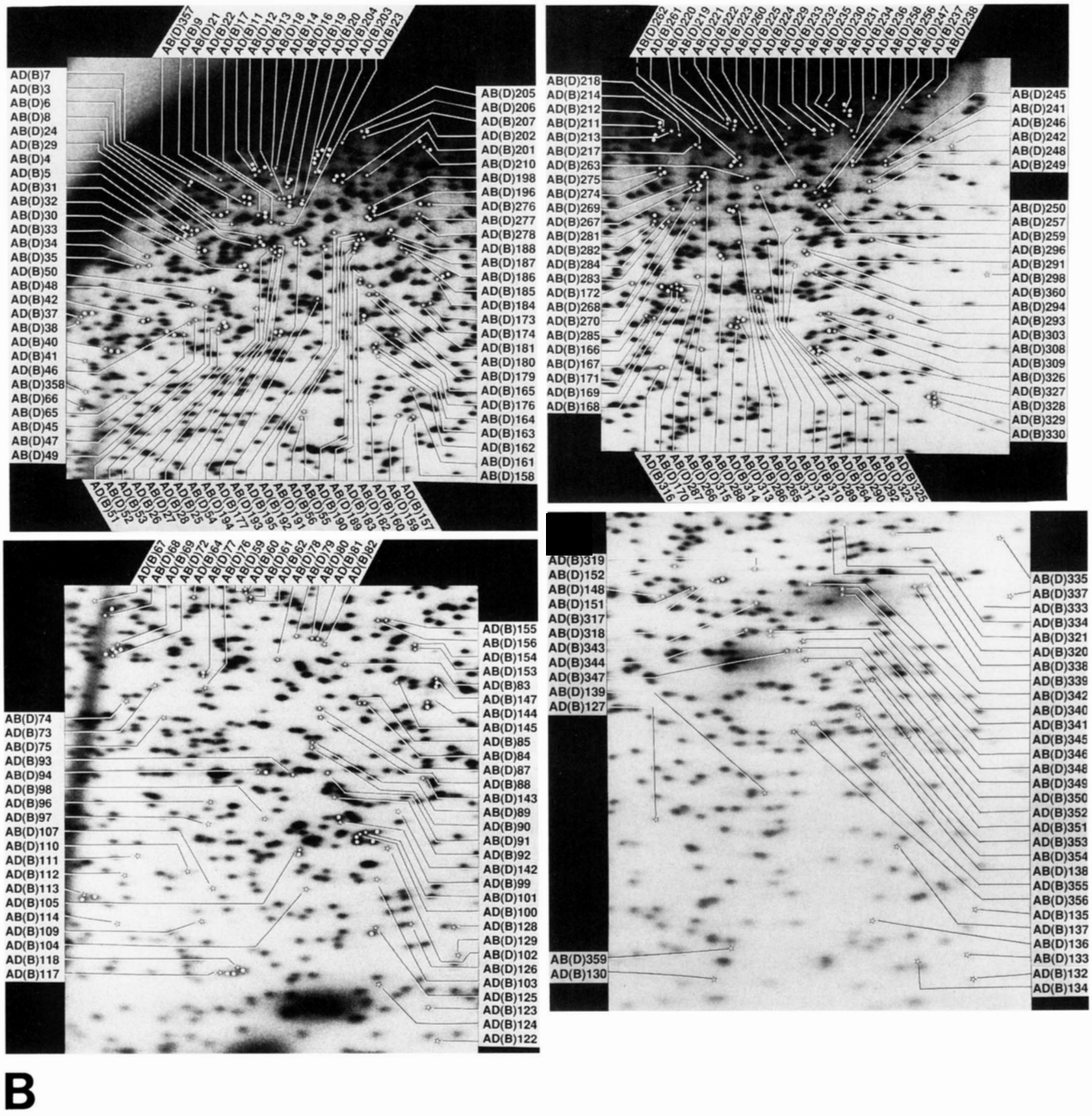


FIGURE 2.—Continued

each chromosome we assumed that the *Hc* locus would be the most proximal marker on each chromosome. Once linkages of some RLGS loci to a specific chromosome were established, PDPs of these were used to identify linkage with additional loci. The procedure was repeated until no additional linkages could be identified for a chromosome. Additional chromosome specific markers were analyzed in the backcross series if the resulting chromosome map was shorter than what was expected from available literature and to provide additional references that would relate the RLGS genetic map to other the genetic maps of the mouse genome. This procedure also demonstrated that the RLGS genetic maps reliably localize to the expected chromosome. Gene order was determined from haplotype analysis and accepting those orders that minimized multiple recombination events across the chromosome. The combined use of a high confidence interval for linkage and haplotype analysis to establish gene orders provide the basis of error checking in the backcross genetic maps. For example, the occurrence of a double recombination that involved only a single locus was considered to be unlikely unless the flanking loci were more than 20 map units apart. When single locus double recombinations occurred, the recombinant locus was re-examined in the original autoradiogram. If the score was not changed upon re-examination, the locus was identified as provisional for a chromosome and placed on a chromosome by use of LOD scores for linkage. The extra recombination events that resulted from these events were not included in the overall chromosome map distance estimate and the order of

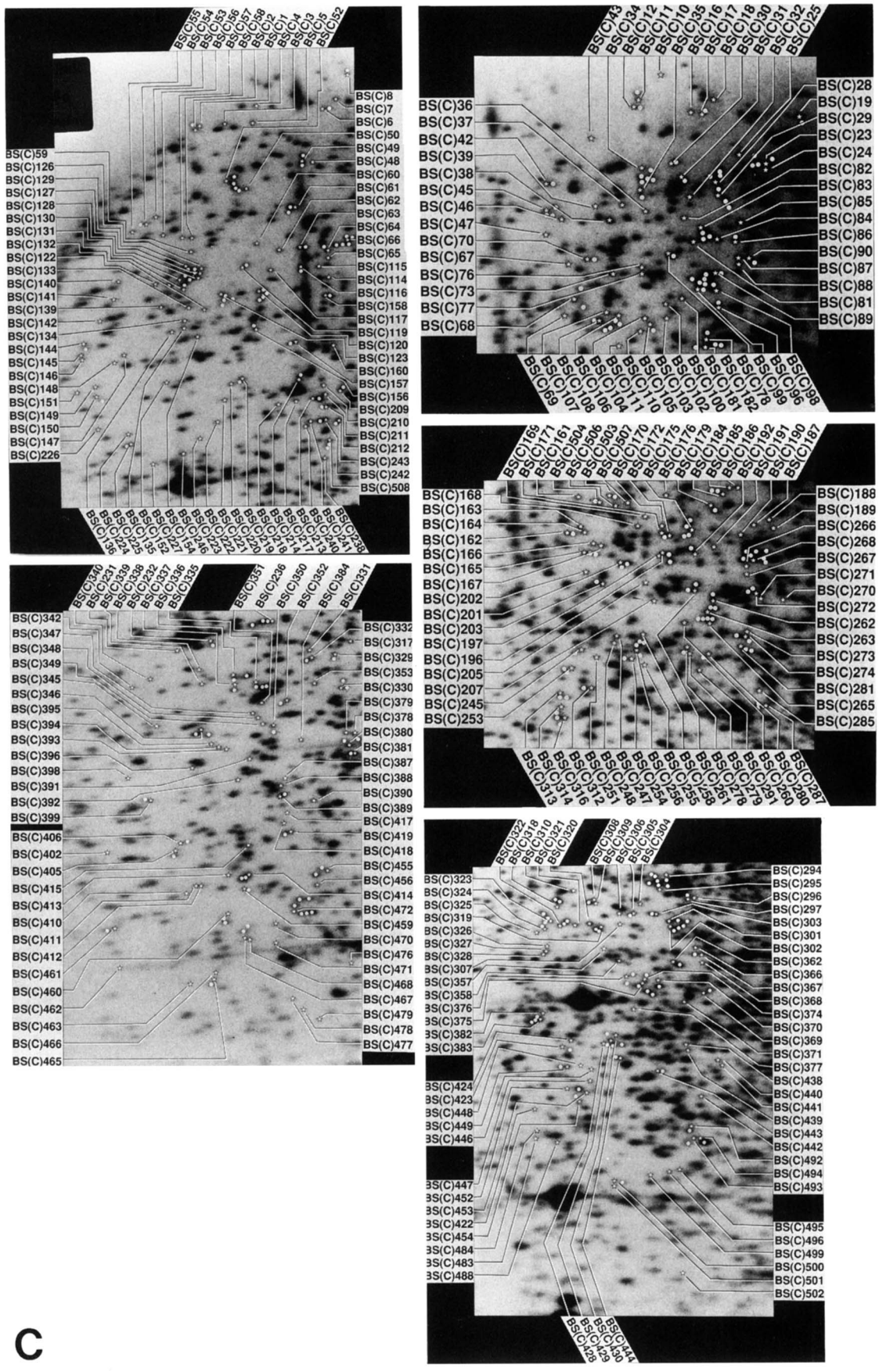


FIGURE 2.—Continued

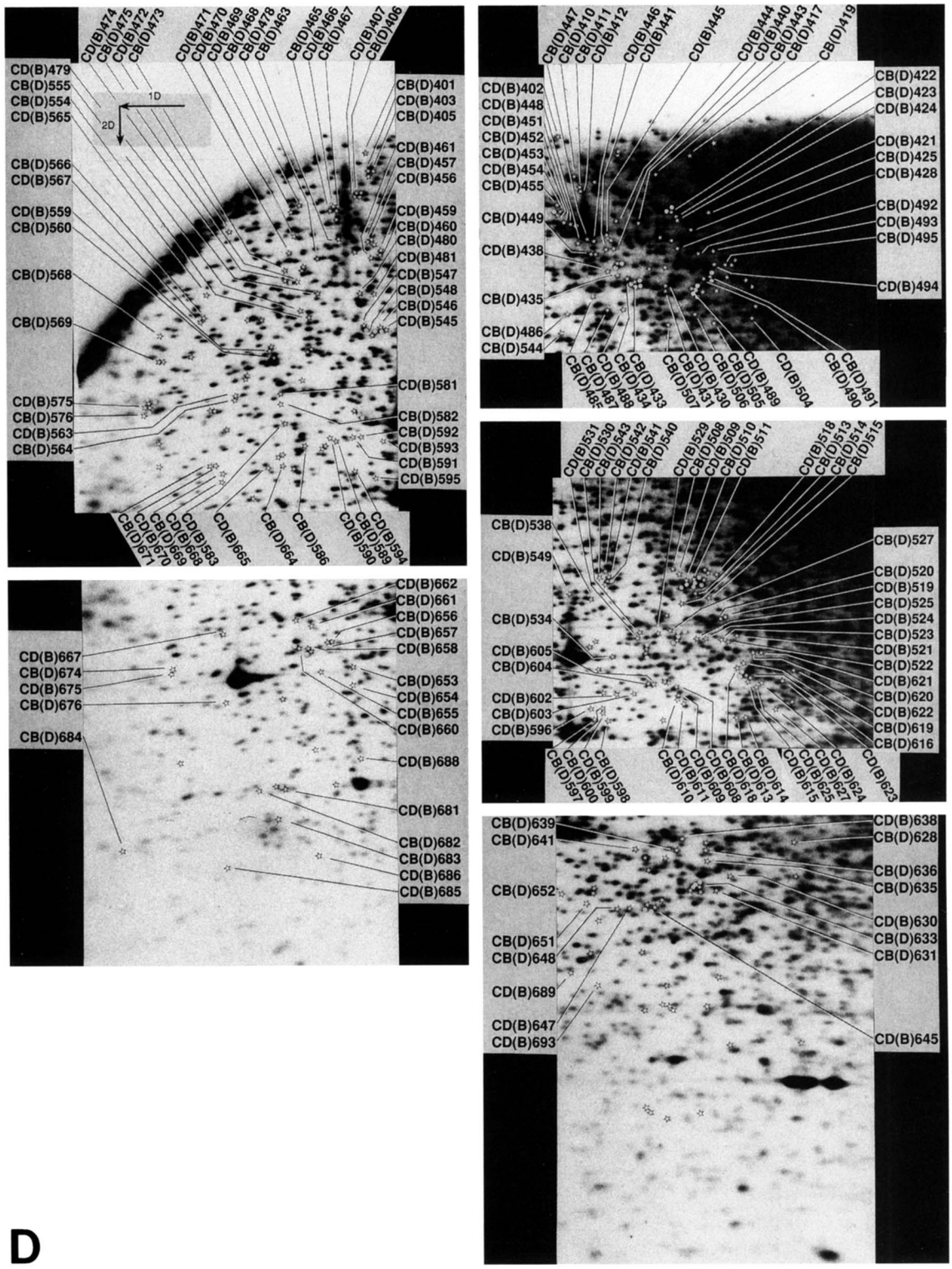


FIGURE 2.—Continued

the locus in the haplotype was considered to be ambiguous.

HSH backcross: A similar strategy of genetic analysis was followed in this backcross except that a series of anchor loci were analyzed for each chromosome. These loci included PCR-based SSR loci and functional genes that were determined by Southern analysis.

BXD RI strain analysis: The segregation of RLGS loci were analyzed in 26 BXD strains as either B6-specific or D2-specific spots. Within the BXD series the B6-specific spots are identified by spot numbers that are either AB(D)# or CB(D)# while D2-specific spots are given the identifying numbers AD(B)# or CD(B)# where the first letter of the locus identifying code identifies the restriction enzyme combination used, the second letter identifies the strain that carries the variant locus and the third letter in parentheses is the strain identity of the second parental strain used in the cross. In this scheme, AD(B)#

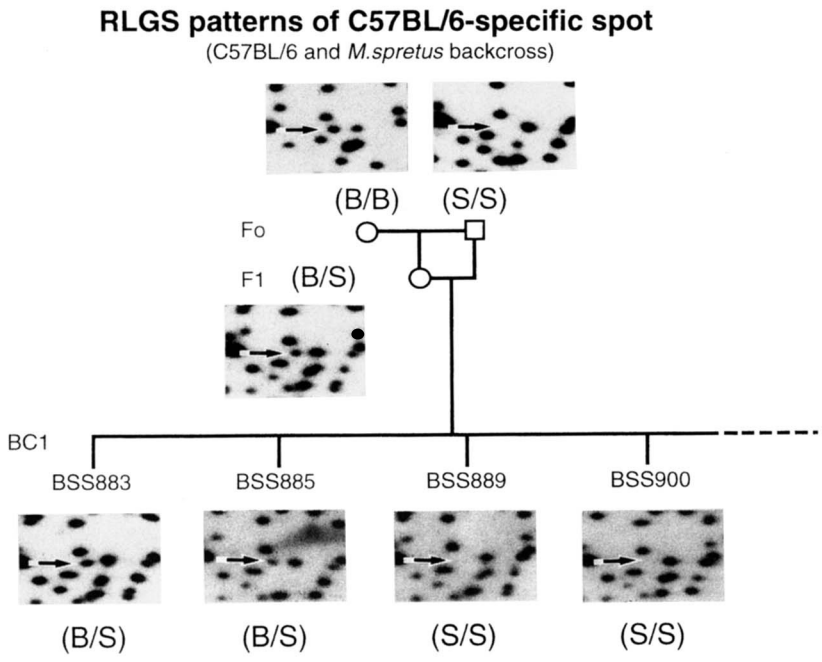


FIGURE 3.—Segregation of spot AB(S)323 (see Figure 2A, subsection IV) in the BSS backcross. The surrounding spot profiles are shown for C57BL/6 and *M. spretus* parent, the F_1 hybrid and four backcross progeny. Spot AB(S)323 is the locus D5Ncvs19 in the BSS genetic analysis. The (B/B), (S/S) and (B/S) identify the genotype for the arrowed spot in each of the subpanels shown for B6 and *M. spretus* alleles.

is an RLGS spot from the enzyme combination *Not*I, *Pvu*II, *Pst*I that is variant in D2 in the hybrid combination with B6.

Locus identification: Each spot was given an identification number as previously described (see Figure 2) and its location in the RLGS profile was defined by an area code (Table 2). Each of the variant spots that were analyzed in one of the genetic crosses was also given a locus name. The RLGS phenotypes of individual loci were identified as DNA or D numbers for specific chromosomes. For example, locus D1Ncvs1 identified spot AB(S) 393 in area code D3. Where D1 was a DNA locus on chromosome *1*, Ncvs1 represents National Cardiovascular Center-Shionogi locus 1. Initially, the loci were numbered sequentially from the proximal end of the chromosome within a cross. Loci linked to the same chromosome from different crosses are numbered sequentially. B6-specific RLGS spots that varied between both DBA/2 and *M. spretus* are given the same locus symbol in both crosses.

RESULTS

Variation in RLGS patterns: RLGS profiles of two laboratory mouse strains, C57BL/6 (B6) and DBA/2 (D2), the separate species, *M. spretus*, and their F_1 hybrids B6D2F_1 and (B6 × *M. spretus*) F_1 were examined. Variation was identified in the F_1 profile as a strain-specific spot that showed a 50% reduction in autoradiographic signal compared to the relative spot intensity in the parental profile. The identification of RLGS variation in the F_1 profile gave a more defined position of a spot compared to the RLGS spots of the opposite parent and it was independent of variation in the location of a specific spot between different gels. The extent of variation in RLGS spots between D2 and B6 and between

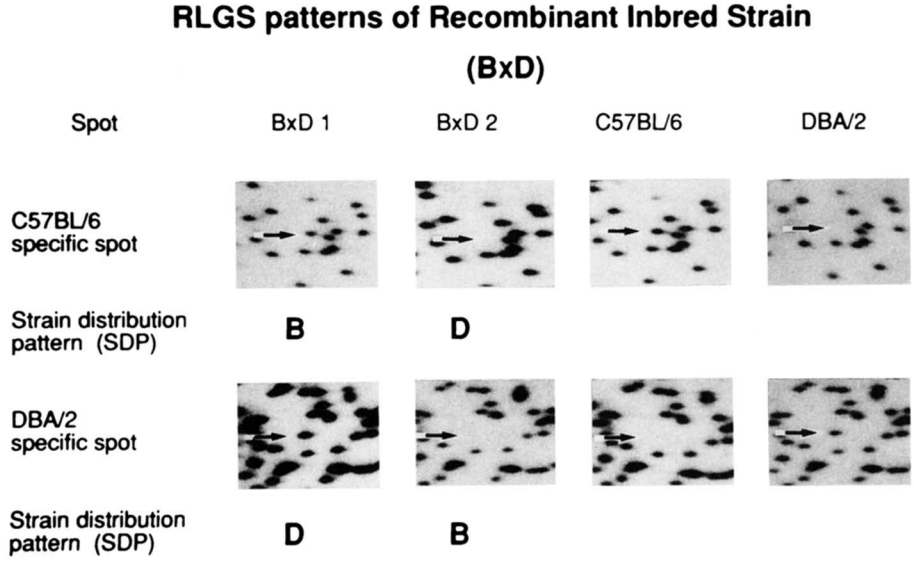


FIGURE 4.—Comparison of RLGS profile subsets for C57BL/6 and DBA/2 and two RI strains, BXD1 and BXD2. A D-specific spot AD(B)53 for D7Ncvs44 is shown.

TABLE 4

Distribution of RLGS loci in the BSS cross

Chr	RLGS loci	Total loci	Haplotypes: Nonrecombinant: Heterozygous	Haplotypes: Nonrecombinant: Homozygous	Haplotypes: Recombinant: Single	Haplotypes: Recombinant: Double	Haplotypes: Recombinant: Triple	Linked loci, no. haplotype	Length (cM)	Recombination events
1	17	21	9	4	38	19	2	2	114	82
2	19	22	7	15	33	16	1	3	94	68
3	9	11	10	14	42	6	0	5	75	54
4	24	25	15	9	34	14	0	4	86	62
5	16	18	19	14	28	10	1	7	71	51
6	10	14	14	14	30	14	0	2	81	58
7	22	23	14	9	40	8	1	3	82	59
8	19	21	16	9	37	10	0	2	79	57
9	19	20	12	14	36	10	0	1	78	56
10	11	12	11	13	42	6	0	2	75	54
11	21	26	16	10	34	12	0	2	81	58
12	12	14	17	21	29	5	0	4	54	39
13	14	17	20	15	36	1	0	0	53	38
14	16	17	21	21	24	6	0	2	50	36
15	8	12	21	11	37	3	0	2	60	43
16	6	9	25	17	29	1	0	7	43	31
17	9	10	20	17	35	0	0	2	49	35
18	6	8	21	19	32	0	0	2	44	32
19	8	9	25	18	29	0	0	4	40	29
X	4	7	4	24	37	6	1	0	72	52
Total	270	316	317	288	682	147	6	56	1381	994

Distribution of RLGS loci in the genome of the cross BSS showing the number of RLGS loci mapped by haplotype analysis to each chromosome (Chr), the details of the haplotypes for each chromosome, the total number of loci ordered on the chromosome and the number of RLGS loci that were linked but not ordered. The total length of the chromosome in map units is represented as centimorgans (cM) and the number of recombination events across each chromosome.

TABLE 5

Distribution of RLGS loci in the genome of the HSH cross

Chr	RLGS loci	Total loci	Haplotypes: Nonrecombinant: Heterozygous	Haplotypes: Nonrecombinant: Homozygous	Haplotypes: Recombinant: Single	Haplotypes: Recombinant: Double	Haplotypes: Recombinant: Triple	Linked loci, no. haplotype	Length cM	Recombination events
1	20	24	9	3	20	3	0	0	74	26
2	14	19	14	6	13	2	0	2	54	17
3	21	29	5	5	20	5	0	0	86	30
4	30	35	7	4	20	3	1	1	89	29
5	22	25	5	7	16	7	0	0	86	30
6	20	23	6	7	18	4	0	0	74	26
7	13	16	7	12	16	0	0	0	46	16
8	11	16	7	6	18	4	0	2	74	26
9	14	18	11	6	17	1	0	2	54	19
10	15	17	11	8	15	1	0	0	49	17
11	19	24	8	10	13	4	0	0	60	21
12	15	18	10	10	14	1	0	1	46	16
13	18	22	9	7	16	3	0	0	53	22
14	16	19	9	6	16	4	0	0	74	24
15	12	14	12	13	10	0	0	0	57	10
16	12	16	11	1	19	4	0	0	77	27
17	11	15	11	13	11	0	0	1	34	11
18	10	12	8	11	16	0	0	1	46	16
19	6	8	12	17	6	0	0	0	42	6
X	4	6	15	10	10	0	0	0	29	10
Total	303	376	187	162	304	46	1	10	1204	399

Distribution of RLGS loci in the genome of the cross HSH showing the number of RLGS loci mapped by haplotype analysis to each chromosome (Chr), the details of the haplotypes for each chromosome, the total number of loci ordered on the chromosome and the number of RLGS loci that were linked but not ordered. The total length of the chromosome in map units is represented as centimorgans (cM) and the number of recombination events across each chromosome.

TABLE 6

Distribution of RLGS loci in the BXD RI strain

Chr	Enzyme combination A			Enzyme combination C			Total RLGS loci	Paired loci
	Total	In B6	In D2	Total	In B6	In D2		
1	32	13	19	14	7	7	46	8
2	16	6	10	12	6	6	28	6
3	16	10	6	10	4	6	26	8
4	23	13	10	22	14	8	45	16
5	9	6	3	10	7	3	19	4
6	16	11	5	5	3	2	21	4
7	13	6	7	14	6	8	27	4
8	21	11	10	22	11	11	43	8
9	13	5	8	11	6	5	24	8
10	6	3	3	2	0	2	8	4
11	27	12	15	16	6	10	43	16
12	19	7	12	6	4	2	25	8
13	9	4	5	7	5	2	16	2
14	6	4	2	10	6	4	16	8
15	8	4	4	5	3	2	13	0
16	4	2	2	2	1	1	6	0
17	16	8	8	5	2	3	21	6
18	6	3	3	3	2	1	9	6
19	11	6	5	8	3	5	19	6
X	3	1	2	1	1	0	4	0
	274	135	139	185	97	88	459	122
Unknown	22	7	15	11	7	4	33	
Total	296	142	154	196	104	92	492	122

Distribution of RLGS loci in the BXD RI strain analyses for enzyme combinations A and C. The number of B6-specific and D2-specific loci is shown for each enzyme combination. The total number of loci mapped to each chromosome is shown and the number of loci pairs where a B6-specific RLGS locus SDP is coincident with the SDP of a D2-specific locus with adjacent spot numbers (paired loci).

B6 and *M. spretus* was established by selecting a sample of clearly identifiable spots in the F_1 profile of these strain combinations and determining the number of these spots that varied between strains. In $B6D2F_1$ hybrids, 51 of 378 spots (13.4%) examined varied between the parental B6 and D2 strains. In the $B6SF_1$, 188 of 369 spots (50.9%) varied between B6 and *M. spretus* parents. On average, we would expect half of the variant spots to be either B6-specific or S-specific spots and that the segregation of these spots could be followed as genetic loci in backcrosses to either parent strain.

The $B6SF_1$ RLGS spots produced by enzyme combination A were distributed uniformly to each of the five subsections (I–V) of the second dimensional gel (Figure 1A). The spots in the top portion of the gel at the lower molecular weights of the agarose dimension show some clustering that suggests that these were *Not*I-*Pvu*II fragments that were not cleaved by *Pst*I (see Figure 2A, section IV; Figure 2B). The distribution of variant RLGS spots in each of these profiles was determined by arbitrarily separating the overall profiles into subsections shown for each of the F_1 combinations shown in Figure 1 (Table 2). In general, the profiles observed with enzyme combination A appeared to have a distribution of RLGS loci that more uniformly covered the entire second dimensional gel than the profiles of enzyme combinations B and C. A comparison of the number of loci identified on the right (the high molecular weight agarose dimension) and left side (the low molecular size fragments) of the profile indicates that approximately half of the RLGS loci occurred on the left side of the profile in the enzyme combination A for both the BXD (subsections IV and V) and BSS (subsections IV and V) profiles (53% and 55%, respectively). By contrast, slightly less than half of the loci (44%) are found in the lower molecular size half of the profile (subsections IV and V) presented for the HSH comparison using enzyme combination B (Table 3). The differences in RLGS profiles reflect the larger fragment size produced by *Eco*RV compared with *Pvu*II as the second enzyme in the production of RLGS cleavages of genomic DNA. These results demonstrate that genetic variation for RLGS spots is detected across most of the molecular weight categories of both electrophoretic separations. The total RLGS profile was separated into subsections to facilitate the identification of strain-specific RLGS spots. The segregating RLGS spots in the overall profile were numbered sequentially and these numbers along with the other aspects of the identifying code for each spot are shown on the periphery of the profile subsection with a connecting line to its position in the gel (see Figure 2).

Area coding RLGS profiles: The use of the area codes provides information for each of the RLGS loci that will be included in locus identifying tables. The localization of RLGS loci into specific regions of the two-dimensional gels was accomplished by dividing each of the electrophoretic dimensions into 10 subdivisions (Table 2). The locations of these different areas within the overall dis-

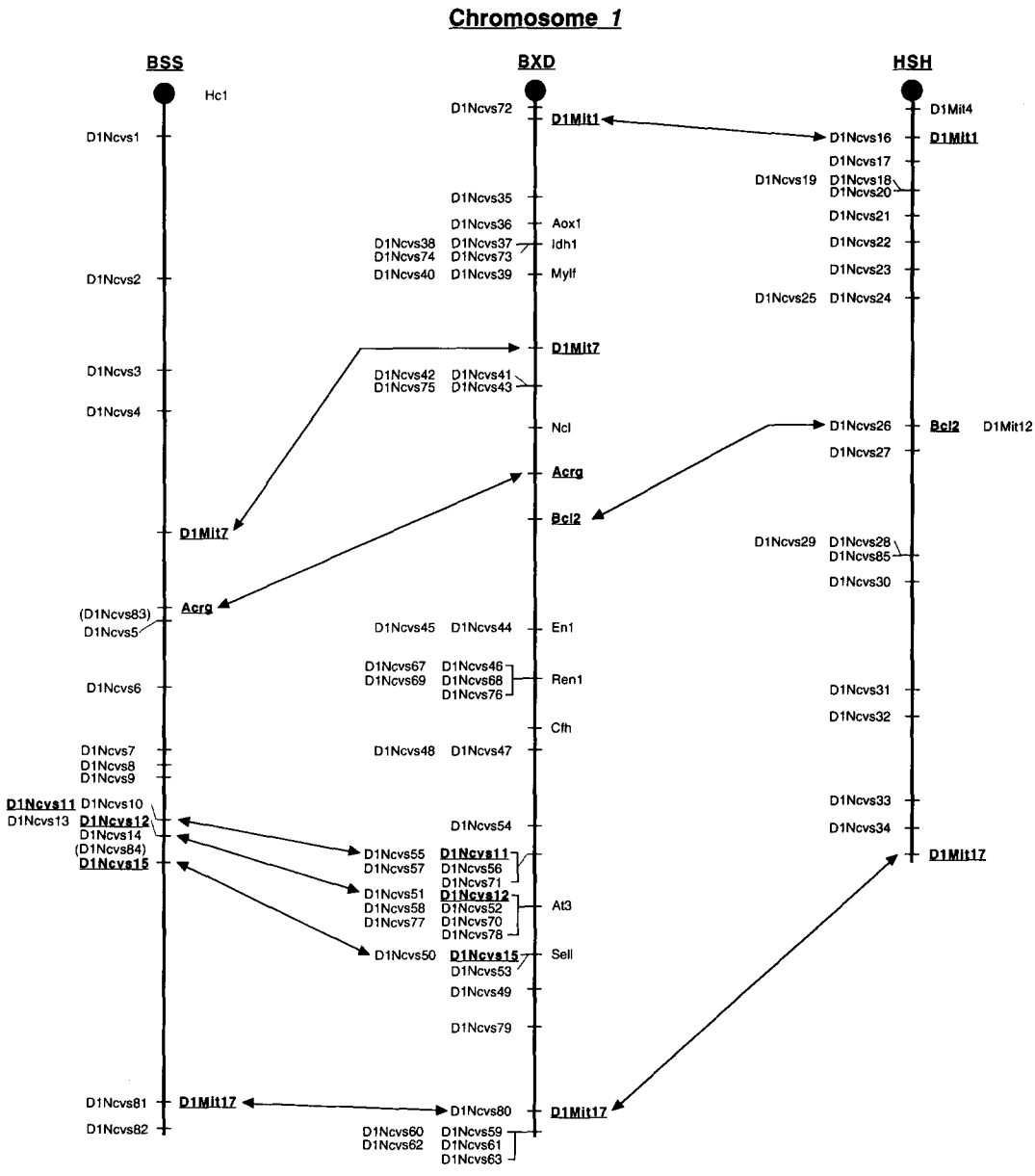


FIGURE 5.—Composite genetic maps for each mouse chromosome. The relative length of the BSS chromosomes represents the estimated cumulative recombination frequency from the haplotype analysis of Map Manager. The BXD chromosome length is normalized to the estimated size of the BSS chromosome. The length of HSH chromosome maps is also determined by recombination frequencies within the HSH cross. Loci that are common to each of the genetic maps are identified by arrows. Loci that have not recombined with neighboring loci are localized to the same position in the genetic map. In BXD, loci that show recombination, but cannot be ordered unambiguously are localized to the same position on the map. Loci that are provisionally linked to a chromosomal location by recombination and LOD score but not by haplotype analysis are identified by (D-Ncvs–). The location of provisionally linked loci is based upon the highest LOD scores for linkage with neighboring loci. Within each map, reference loci are listed on the right side of the genetic map and the RLGS loci identified as D-Ncvs are given on the left side.

tribution is shown for each of the profiles in Figure 1 A, B and C. For example, the B6-specific spot that is demonstrated in BSS backcross progeny in Figure 3 can be localized to the gel area coded as G4 from Figure 1A, subsection IV and Figure 2A. This indicates that there is a *Not*I-*Pvu*II cleavage fragment between 1882 and 2690 bp separated in the agarose dimension and a further cleavage by *Pst*I to a *Not*I-*Pst*I fragment size of 260–389 bp.

RLGS locus identification: The location of B6-specific spots from the B6SF$_1$ RLGS profile produced by enzyme combination A are shown in Figure 2A. The B6-specific spots, identified by a star in the figure, were given a spot reference number that was localized by code to an area in the profile as described in MATERIALS AND METHODS. An identical approach was used to identify and analyze RLGS spots in the BDF$_1$ hybrid with enzyme combination A (Figure 2B). In this case the B6-specific

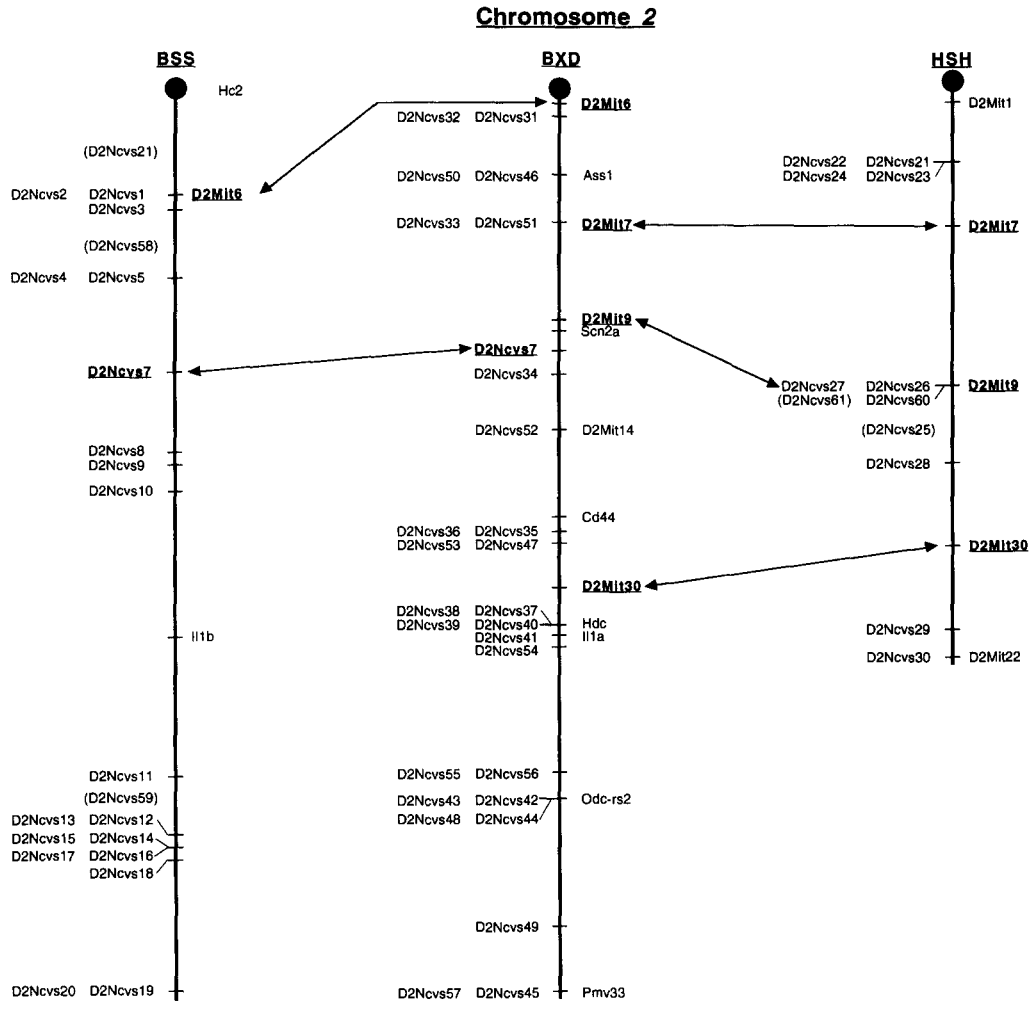


FIGURE 5.——Continued

spots are indicated by the second letter in the locus code with (D) as the second parent in the hybrid while the D2-specific spots were identified with D as the second letter in the code with (B) as the second parent in the cross. The identification of loci in the HSF_1 hybrid has B for the enzyme combination used, S for *spretus* as the segregating spots in the HSH cross and (C) for the C3H strain that is the non segregating parent in the cross (Figure 2C). The loci identified in the BDF_1 hybrid using enzyme combination C are shown in Figure 2D. The spot identifications for the $BXDF_1$ spots are given in Table 7. The complete list of loci that are identified by this coding system is available as an electronic data file both from Roswell Park Cancer Institute by anonymous FTP and also through MOSAIC to mcbio.med.buffalo.edu.

Segregational analysis of individual RLGS loci: A subset of RLGS spots from B6 ($n = 17$) and *M. spretus* ($n = 19$) and the F_1 hybrid ($n = 22$) are shown in detail (Figure 3). The spot, AB(S)323, that is identified by an arrow in each panel was present in B6 but absent in *M. spretus* and segregating in a sample of four backcross progeny. In addition, the relative intensity of the B6-specific spot in the F_1 and each of backcross progeny that was positive for the indicated spot was approximately 50% of that observed for the neighboring spots that are common to both parents. The F_1 profile shown also demonstrates the additivity of the parental profiles for additional spots. Three of the 17 B spots were B6-specific and 5/19 of the *M. spretus* spots were S-specific. The segregation of these spots and the effects of a single dosage were observed in the four backcross progeny shown.

The variation between inbred strains D2 and B6 is shown for two regions of an RLGS profile that demonstrates the segregation of B6-specific and D2-specific loci in BXD1 and BXD2 RI strains (Figure 4). These results also illustrate the lower frequency of variation between the inbred strain parents with 1 of 15 variant spots in the B6-specific group and 1 of 30 in the D2-specific panels.

Genetic analysis of RLGS loci

The method of genetic analysis of each backcross and RI strains was outlined in the MATERIALS AND METHODS. The complete description of the data as either PDPs for each backcross or as SDPs for RI strain analysis are avail-

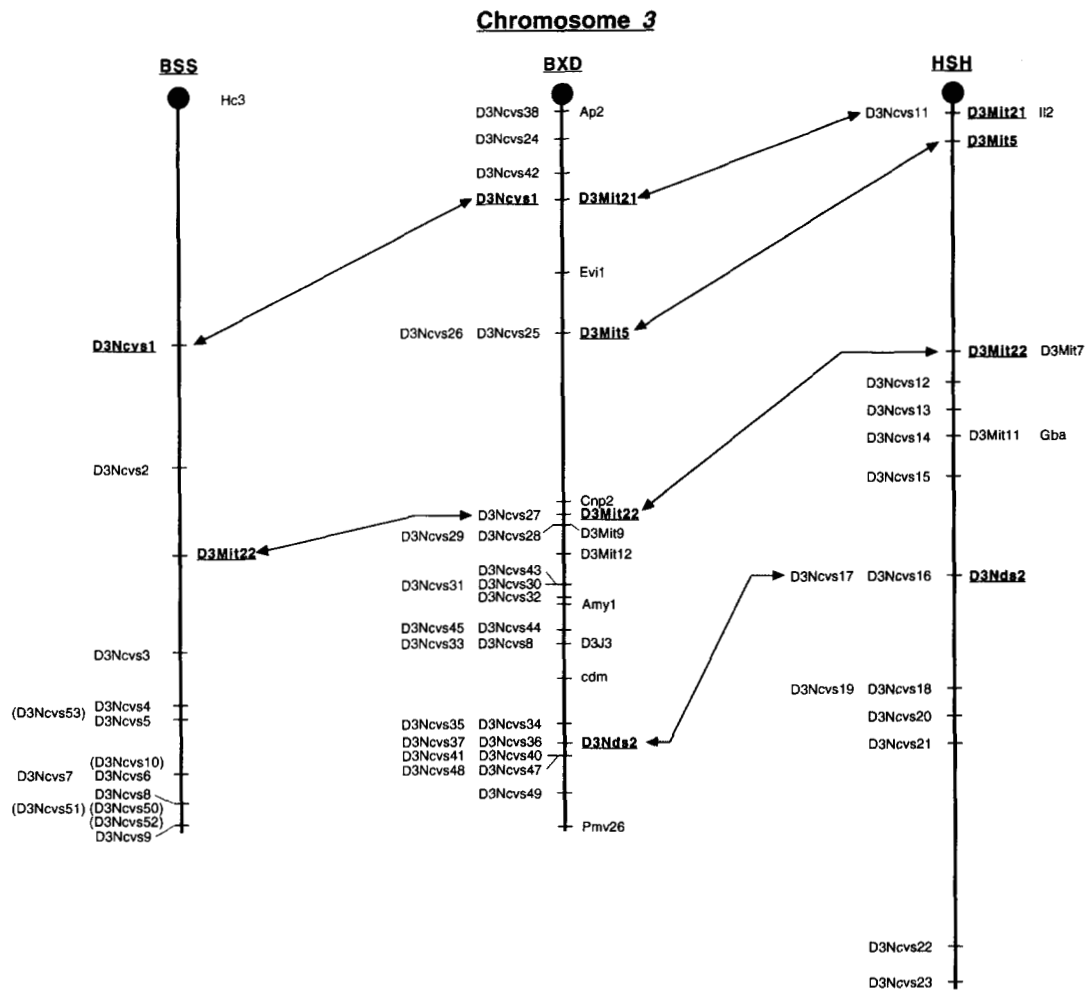


FIGURE 5.——Continued

able as Map Manager files that have been deposited with The Jackson Laboratory as indicated above. The Map Manager analysis format provides an opportunity for direct inspection of crossover events for each progeny and it allows a manipulation of locus orders that minimize multiple recombination events for each haplotype. A tabular description of the Map Manager analysis for each chromosome in the BSS cross and in the HSH backcross has been prepared that summarizes the number of haplotypes that were not recombinant, and the number of single, double, and triple recombination events that occurred for each chromosome (Tables 4 and 5). The total length of the mapped chromosome is given and the number of loci mapped is detailed. Genetic maps have been prepared for each chromosome from each cross (see Figure 5). The cumulative recombination frequencies in the two backcrosses were used to derive the overall lengths of the genetic maps and the relative distances between loci on each map. The lengths of the chromosomal maps for the BXD RI strains were normalized to the lengths of the BSS genetic maps.

Genetic analysis in the BSS backcross: The PDPs of 334 RLGS loci were analyzed for 72 BSS progeny. Of these, the PDPs of 270 loci identified linkages and defined haplotypes for each of the chromosomes. The distribution of the ordered RLGS loci in the BSS series is shown in Table 4. These loci have been placed on chromosomal genetic maps primarily by their association to the segregating, major satellite that determined the centromeric heterochromatin locus (*Hc*) for each chromosome using FISH and karyotype analysis. An additional 26 reference loci have been added to the maps of specific chromosomes to align and extend the genetic maps. These loci include SSR loci and some functional genes that can be identified on the chromosomal maps (Figure 5). Thus, there are a total of 316 loci in the BSS genetic map (270 RLGS + 20 *Hc* loci + 26 reference loci) and recombination among these loci resulted in an estimate of 1381 map units for the mouse genome. A total of 994 recombination events were identified in these analyses of which 682 or 68% were single recombinant events, 294 recombinants (147 × 2) or 30% were in double crossovers and 18 (6 × 3) or 2% were triple recombinations.

An additional 56 loci (linked but not ordered; Table 4) have been assigned to chromosomes by linkage analy-

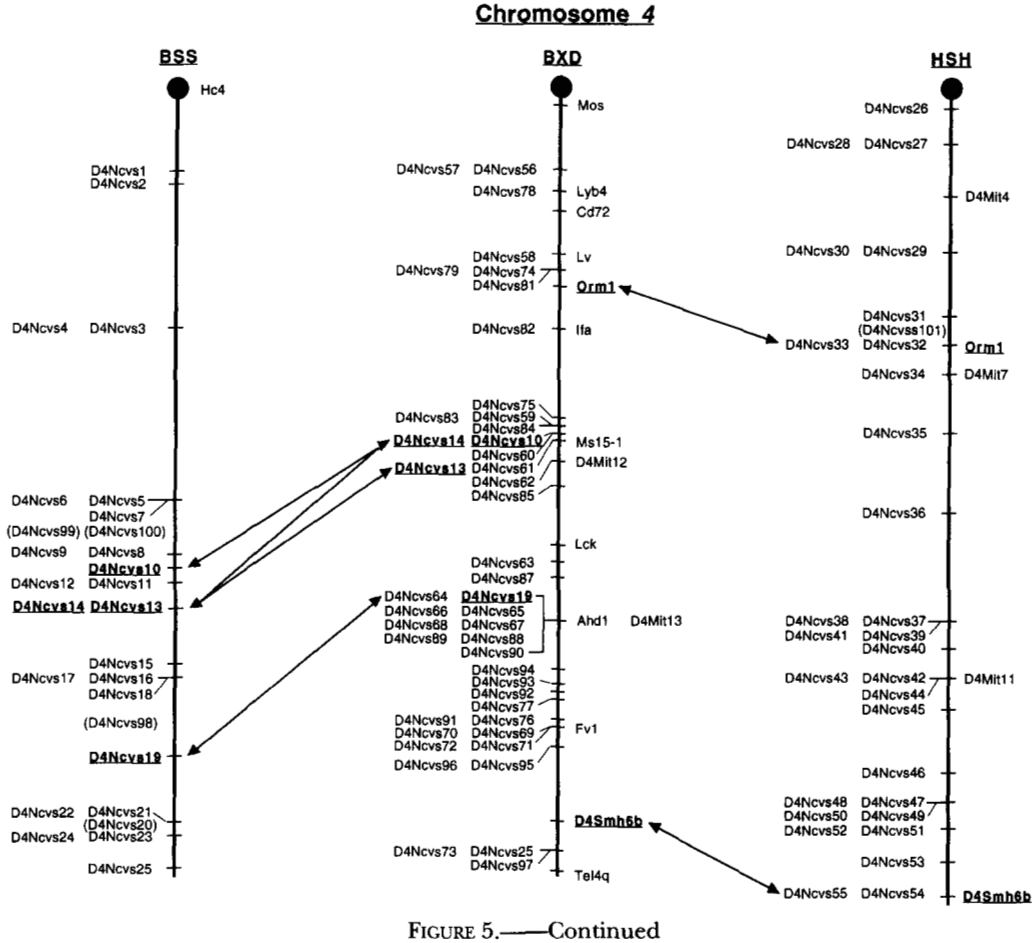


FIGURE 5.——Continued

sis and given a D-number for a specific chromosome. The location of these loci on the chromosome was determined by the highest LOD score (ranging from 4.0 to 10.0) but their order could not be established unambiguously by haplotype analysis and they are not included in the final estimation of chromosome size in the genetic map. These loci are bracketed to indicate the provisional nature of the localization on the genetic maps of chromosome (see Figure 5). Detailed analyses of these loci indicated that 38/56 (68%) have between 1 and 4 single locus double recombinations at the chromosomal location that has the lowest frequency of recombination events. Two of the loci are candidates for the most distal locus on separate chromosomes but the inclusion of these loci on the map extended the estimated length of the chromosome by more than 10%. The remaining loci have either multiple single locus double recombinants that are either all B6-specific allele types flanked by S alleles in the haplotypes, 10/56 (18%) or the converse 4/56 (7%). The segregation ratios were distorted for several of those loci that had multiple, single locus double recombinations of excess B6-specific alleles surrounded by S alleles. These results suggest that an RLGS spot with variable expression could have interfered with the analyses of some of these spots. An additional 12 RLGS loci were analyzed that did not link to any of the established chromosomes at the 99% confidence level and 26 RLGS spots have not been included in the final analyses because of insufficient data.

Genetic analysis in the HSH cross: The PDPs of 329 *M. spretus* (S-specific) RLGS loci were determined for 35 HSH progeny. Linkage of these loci compared to a series of 73 reference loci that were distributed on all of the chromosomes was determined using Map Manager at the 99.99% confidence level. The chromosomal distribution of 303 mapped RLGS loci across the mouse genome is described in Table 5 and illustrated in Figure 5. The genetic maps of the HSH cross cover 1204 cM of the mouse genome with the genetic maps for individual chromosomes ranging from 29 cM for the *X* chromosome to nearly 90 cM for chromosome *4*. The recombinational distance across the genome is based upon 399 recombination events of which 76% were single recombinants, 92/399 or 23% were double recombinations and 0.2% were triple recombinations. A frequency of 1 recombinant per 3.0 cM was observed in this cross which is consistent with the expected average recombination distance of 2.9 cM for 35 backcross progeny (Table 5).

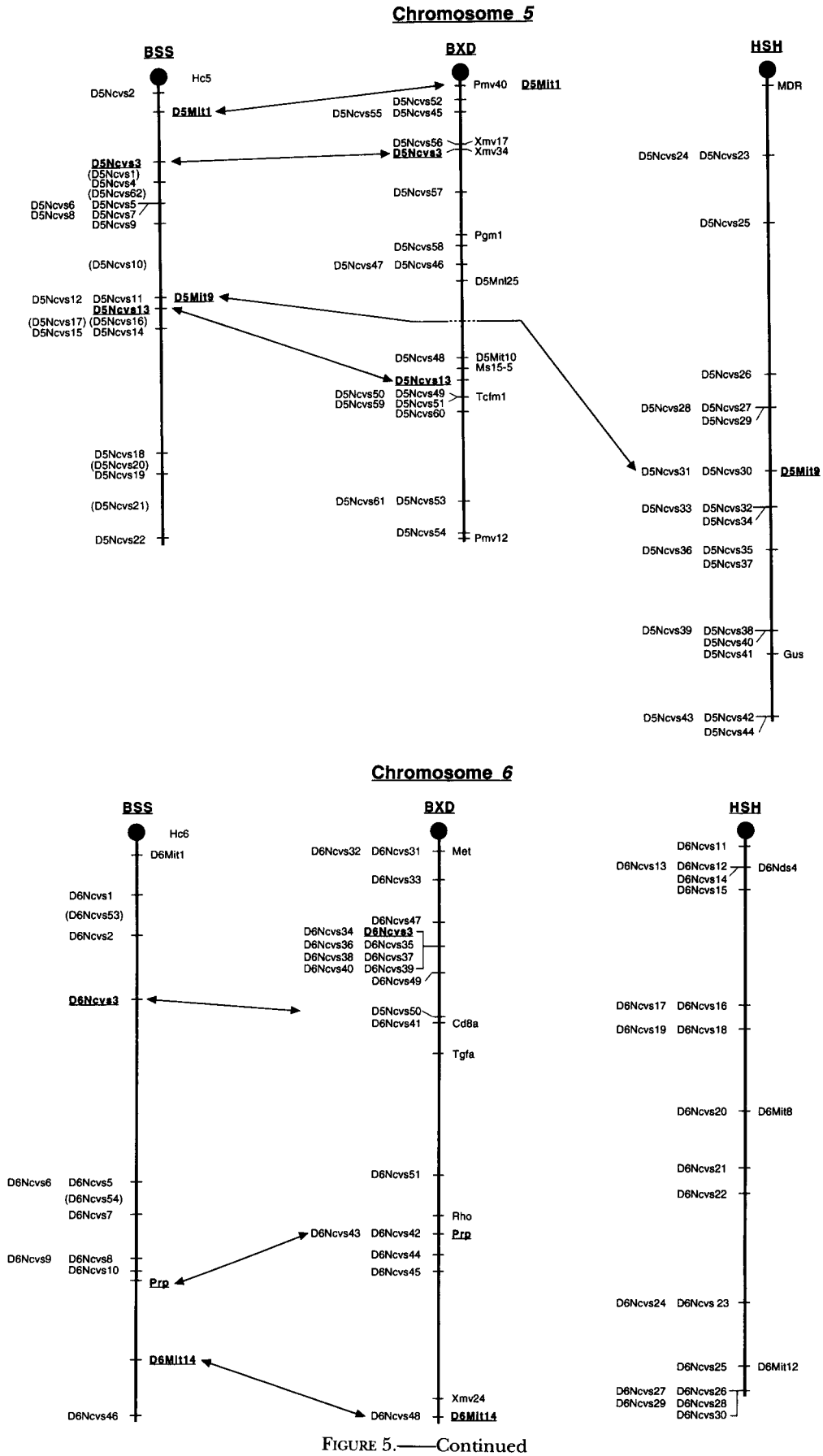
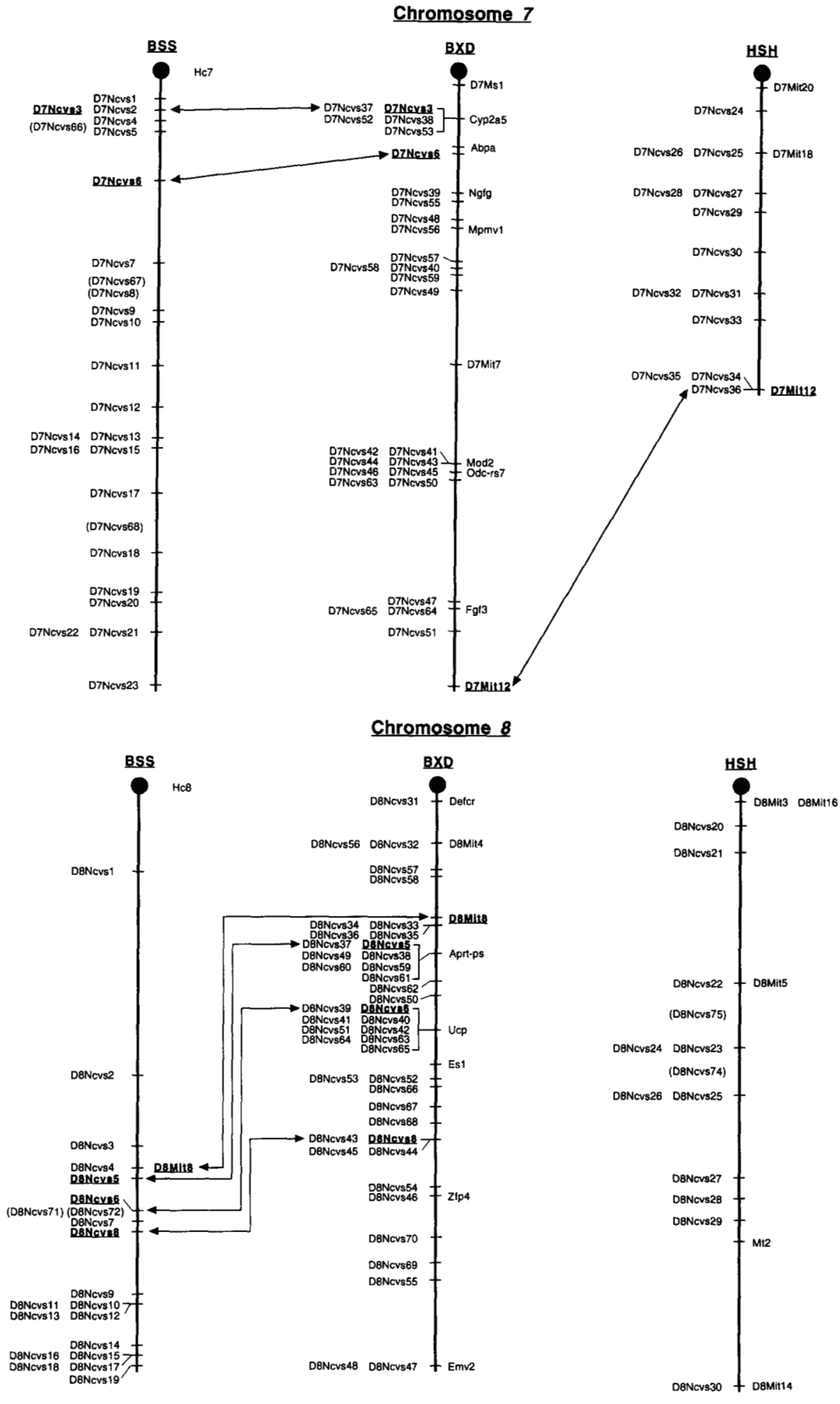


FIGURE 5.——Continued



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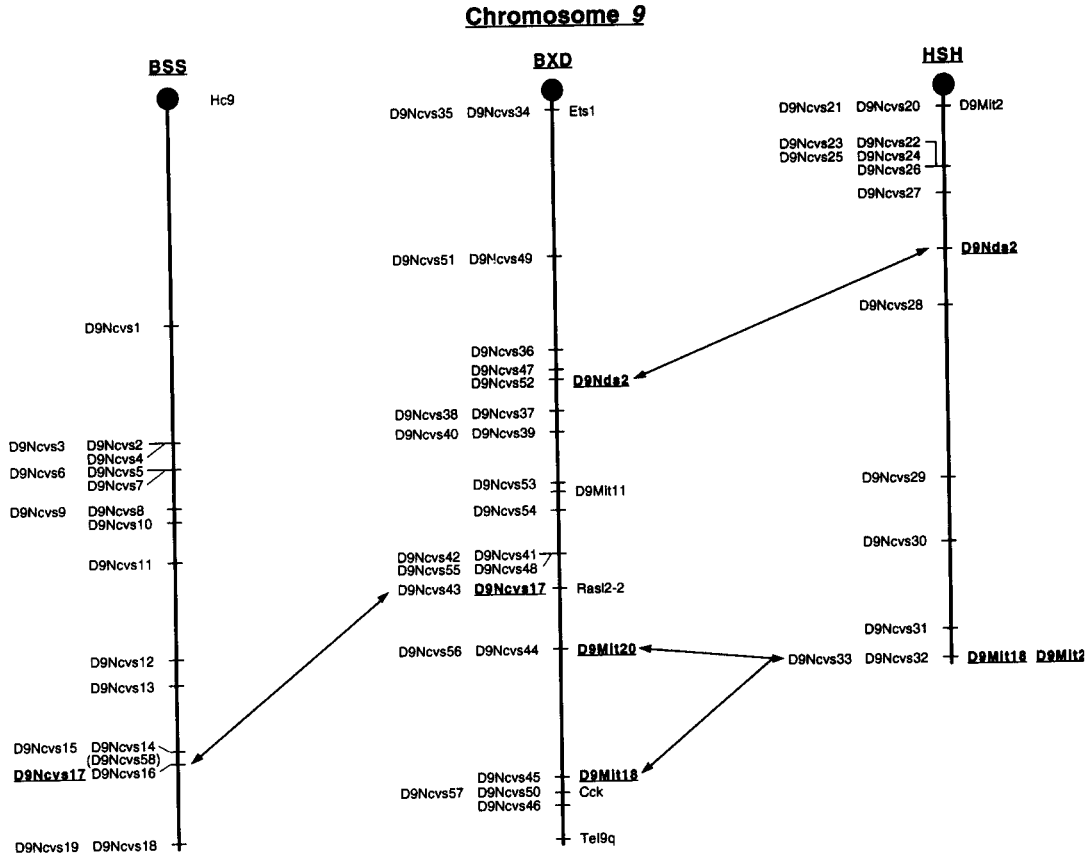

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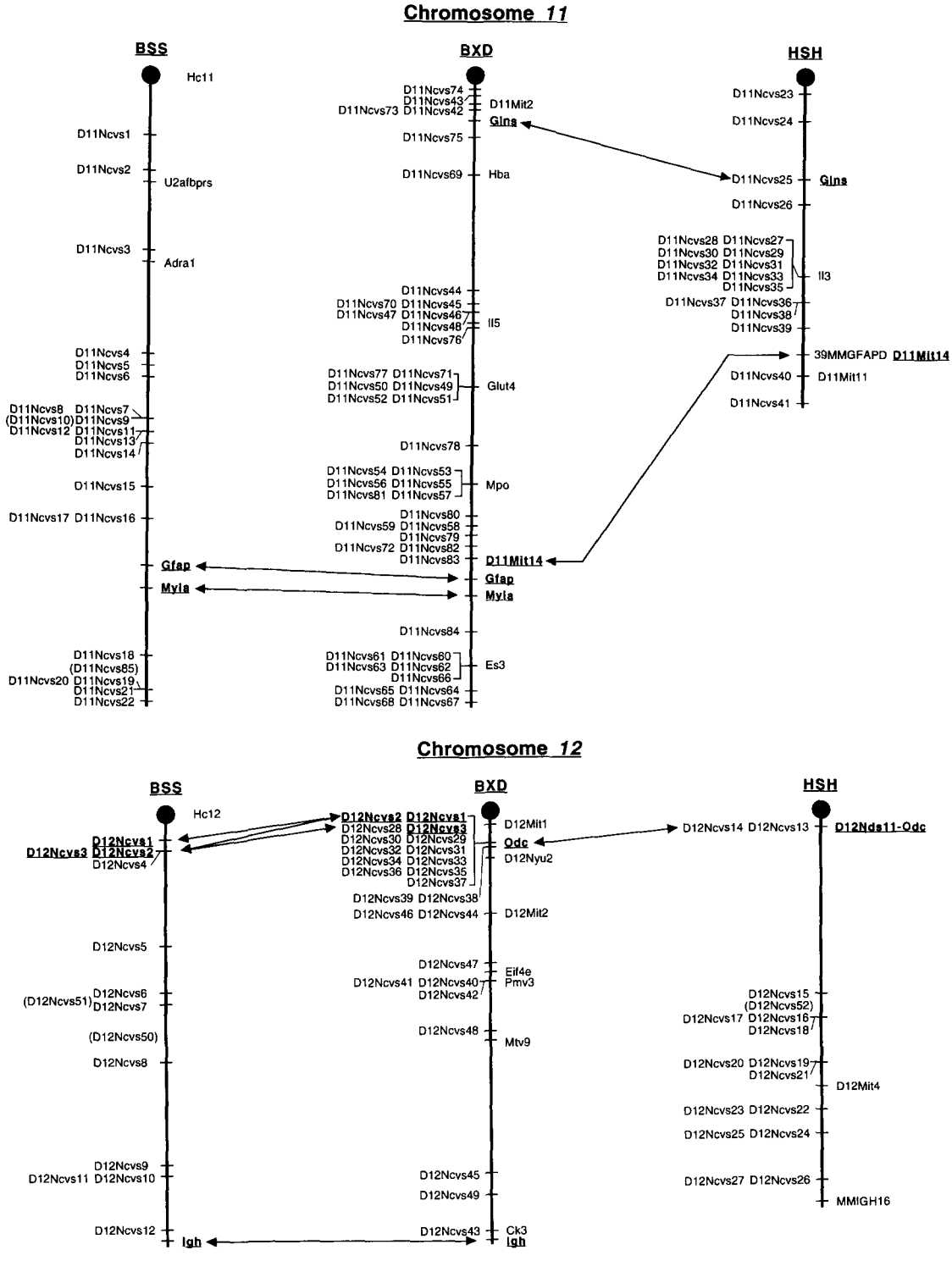


FIGURE 5.——Continued

Ten loci were identified in the HSH cross as linked but not ordered showing a LOD score for linkage of 2.9 or greater to a specific chromosomal location. However, these loci could not be unambiguously ordered using haplotype analyses. These loci have been provisionally placed on the genetic map relative to the position indicated by the highest LOD score (Figure 5). There were 31 additional loci identified in this cross that did not show linkage either to other RLGS loci or reference markers at the 99% confidence interval.

Genetic analysis in BXD RI strains: The DNA from BXD RI strains were analyzed with two enzyme combinations A and C (Table 6). Both B6- and D2-specific loci were identified in each analyses with 142 and 104 B6-specific loci in enzyme combinations A and C, respectively and 154 and 92 D2-specific loci in the same combinations. The SDPs for

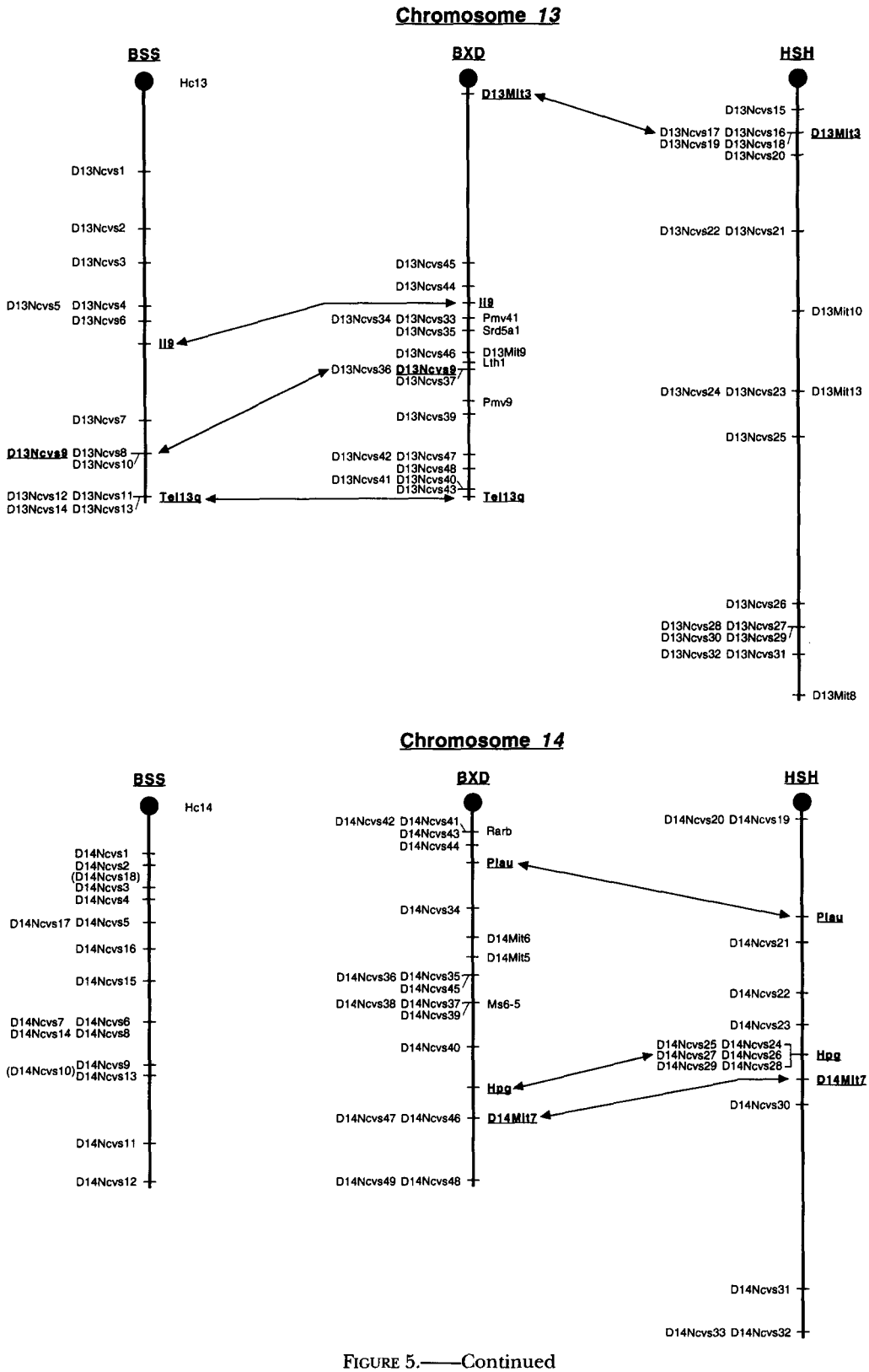


FIGURE 5.——Continued

these loci are presented in Table 7. The loci from these analyses were distributed to all of the chromosomes (Figure 5).

It is possible that some of the D2- and B6-specific loci with coincident SDPs are alleles of the same locus. In some instances these loci may be associated with RLGS spots that have similar physical properties as indicated by a similarity of area code locations in the second dimension of electrophoresis. For example the loci

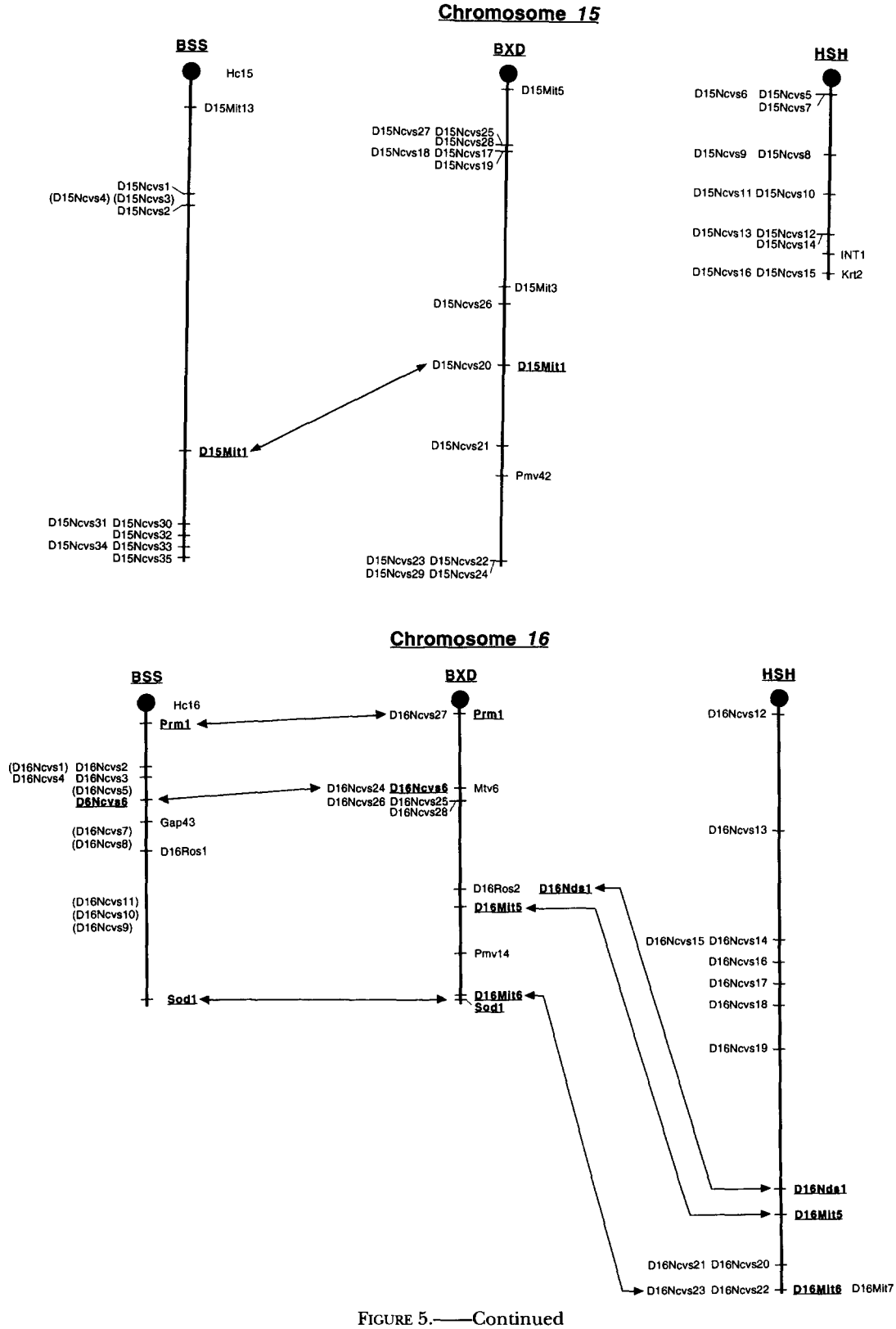
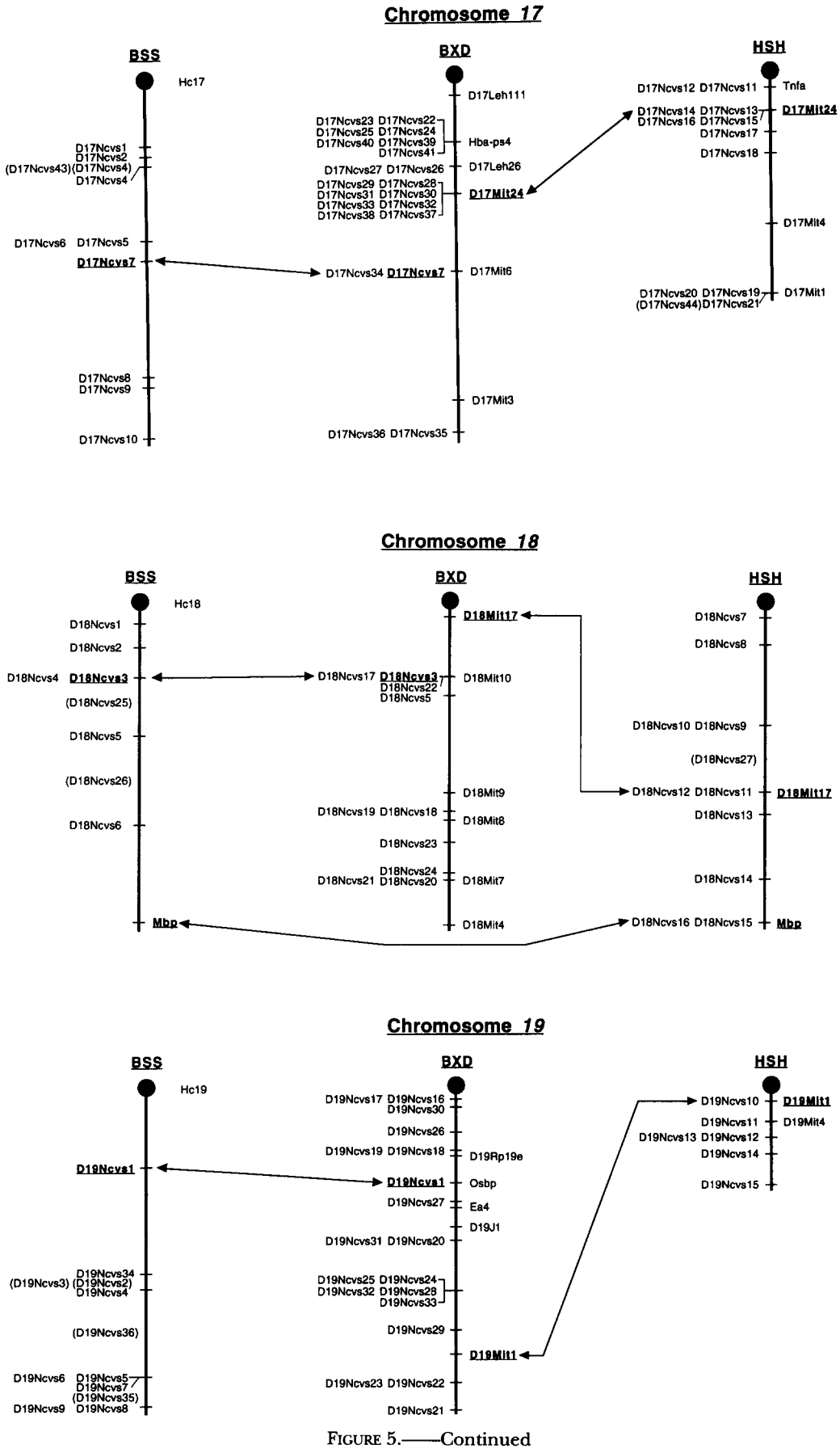


FIGURE 5.——Continued

D1Ncvs73 and -74 identify spots CB(D)564 and CD(B)563, respectively, and both of these spots localize to area code I4. Using this criterion we identified 61 pairs of loci or a total of 122 loci that showed the same properties across the genome. Thus, 25% or more of the loci identified in the BXD analyses could be allelic decreasing the total number of independent sites that are mapped to between 75 and 90% of the 473 loci that are



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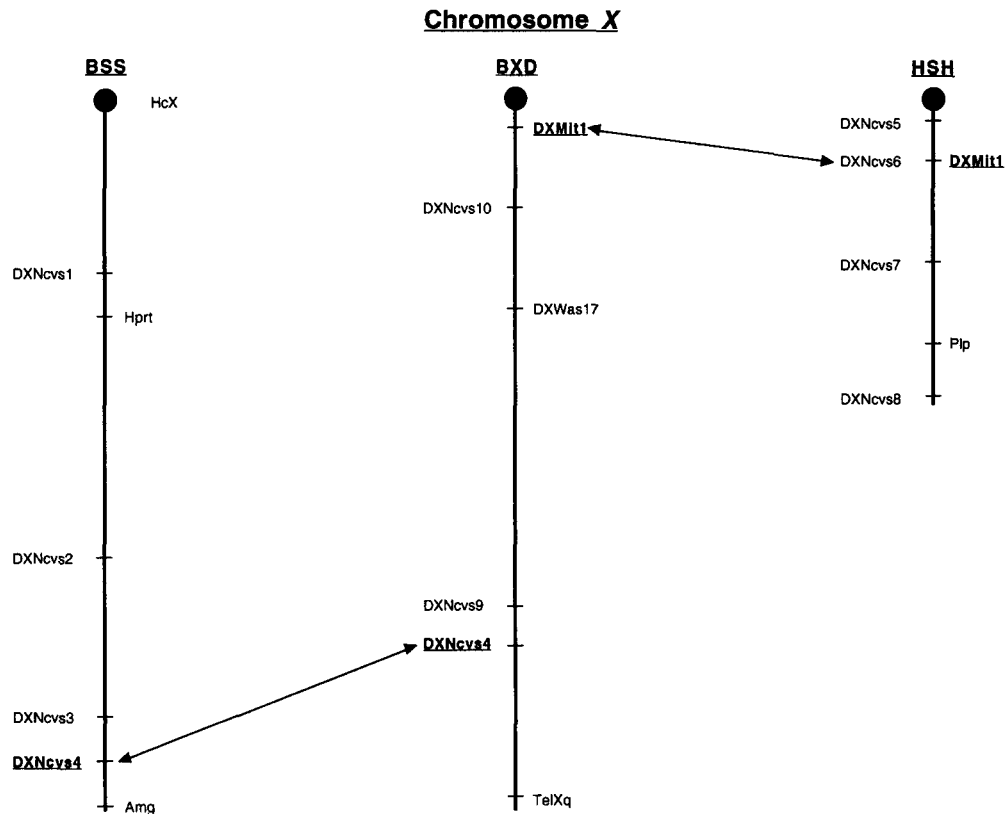

FIGURE 5.——Continued

distributed in the BXD maps (Figure 5). An excess of isolated "double crossovers" were found involving the BXD-31 strain. These data could be the result of either unusual patterns of genomic methylation in this strain or other technical problems with the analyses of data that were not readily detectable from the general quality of the RLGS analyses. Consequently, the SDPs for the BXD-31 should be considered provisional.

We examined the frequency of unknown alleles for each of the BXD strains used in these analyses. BXD28 had nearly 300 untyped loci which was probably due to partial degradation of the DNA sample. The data from the strain is retained in the analyses because the RLGS spots that were analyzed were consistent with the data derived from other strains. Overall, there were 211 of 460 loci (43%) that had no untyped strains while the frequency of unknown scores per locus showed ranged from 1 to 5 for an additional 45% of the strains if strain 28 was removed from consideration. In general, there was no evidence that there was any group or class of loci that was more difficult to type than others.

We identified 29 B6-specific RLGS loci that were variant in both BSF_1 and BDF_1 hybrids and they were mapped in the BSS cross and the BXD RI strains. Each of these loci mapped to chromosomal regions that were the same in both analyses. These results provided an independent verification of the genetic linkage of RLGS loci on 16 of the BSS and BXD chromosomes. An additional 13 loci that were not RLGS markers were analyzed in these same crosses that mapped to the same chromosomal region. The identification and location of the loci common to both the BSS and the BXD series is shown in the composite genetic maps (Figure 5). The validation of the chromosomal assignments in the HSH cross was based upon the coincident order of genes in this cross compared to the BXD RI strain maps. In this case, 33 of the 73 reference loci were typed in both analyses and they provide common references for the RLGS genetic maps in 16 of the autosomes of the HSH cross and the BXD series (Figure 5).

DISCUSSION

Our data demonstrate that RLGS profiles of different mouse genotypes are reproducible and that variation for specific spots in these profiles can be identified readily between inbred strains and between these strains and the separate species, *M. spretus*. Variants of individual landmark spots segregate as genetic loci in either backcrosses or RI strains. Thus, it is possible to analyze these loci in concert with other loci that serve as reference points for specific chromosomes to establish genetic linkage maps for individual mouse chromosomes. The composite genetic maps produced by these analyses cover all of the autosomes and the *X* chromosome with a combined map distances 1381 cM in the BSS backcross

TABLE 7

List of RLGS loci typed in the BXD analyses

Locus	Spot	Area code	RI strains																										
			1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	23	24	25	27	28	29	30	31	32
D1Ncvs11	AB294	D3	B	B	D	B	B	D	D	B	D	B	B	B	B	B	D	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs12	AB139	E7	B	B	D	B	B	D	U	B	D	B	B	B	B	B	B	B	D	D	U	D	U	U	U	D	B	B
D1Ncvs15	AB78	H5	B	U	D	B	B	D	D	B	D	B	D	B	B	B	B	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs35	AD40	I3	D	B	B	D	D	B	B	B	B	B	D	D	D	D	D	D	B	B	B	D	D	U	D	D	B	B
D1Ncvs36	AB260	D2	B	B	B	D	D	B	B	B	B	B	B	D	D	D	D	D	B	B	B	B	D	D	U	D	D	B	D
D1Ncvs37	AD165	G3	B	B	B	D	D	B	B	B	B	B	B	D	D	B	D	B	B	B	B	B	D	D	U	D	D	B	D
D1Ncvs38	AD360	D3	B	B	B	D	D	B	B	B	B	B	B	D	D	B	D	B	B	B	B	U	D	D	U	D	U	B	D
D1Ncvs39	AD100	H7	B	B	B	D	D	B	U	B	B	B	D	B	B	D	B	D	B	B	D	B	B	D	U	D	D	B	D
D1Ncvs40	AB101	H7	B	B	B	D	U	B	B	B	B	B	B	D	B	B	D	B	B	D	B	B	D	D	U	D	D	B	D
D1Ncvs41	AD67	J4	B	B	U	U	D	B	B	B	D	B	U	B	B	U	D	B	D	D	D	D	D	B	U	B	D	B	D
D1Ncvs42	AB110	17	B	B	B	D	D	B	B	B	D	B	D	B	B	B	D	B	D	D	D	D	D	B	U	B	D	B	D
D1Ncvs43	AD64	I4	U	U	B	D	D	B	B	B	D	B	D	U	B	B	D	B	D	D	D	D	D	B	U	B	D	U	D
D1Ncvs44	AD334	C4	B	B	D	D	B	B	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	U	D	D	B	D
D1Ncvs45	AD122	G9	B	B	D	D	B	B	D	B	D	U	D	D	D	B	D	B	B	D	D	B	U	U	D	D	B	U
D1Ncvs46	AD118	I8	B	B	D	D	B	B	D	B	D	B	D	B	U	U	D	U	B	D	D	B	D	U	D	U	B	D
D1Ncvs47	AB247	C1	B	B	D	U	B	B	U	B	D	B	U	B	B	B	D	B	B	D	B	B	D	U	U	D	B	D
D1Ncvs48	AB268	F2	B	B	U	B	B	B	D	B	U	B	B	B	B	B	U	B	B	D	U	B	D	U	D	D	U	B
D1Ncvs49	AD13	H1	U	B	D	U	U	D	D	B	D	B	D	U	B	B	B	B	D	D	D	D	D	U	D	D	D	D
D1Ncvs50	AB283	F2	B	B	D	B	B	D	D	B	D	B	D	B	B	B	B	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs51	AD103	G7	B	B	D	B	B	D	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	U	D	D	B	B
D1Ncvs52	AB265	E2	B	B	D	B	B	D	D	U	D	B	B	B	B	B	U	B	D	U	D	D	D	U	D	U	B	B
D1Ncvs53	AD284	F2	B	B	D	B	B	D	D	B	D	B	D	B	B	B	B	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs54	AB84	G5	B	B	D	B	B	D	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs55	AD236	C1	U	B	D	B	B	D	D	B	D	B	B	B	B	B	U	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs56	AD298	A3	B	B	D	U	B	D	D	B	D	B	B	U	U	U	D	B	D	D	D	D	U	U	D	D	B	D
D1Ncvs57	AD109	I7	B	B	D	B	B	D	D	B	D	B	B	B	B	B	D	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs58	AD85	H5	B	B	D	B	B	D	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	U	D	D	B	D
D1Ncvs59	AD113	J7	B	B	D	U	D	D	B	B	D	D	U	B	B	B	D	D	D	D	D	B	D	U	D	U	B	D
D1Ncvs60	AB34	I2	B	B	D	D	D	D	B	B	D	D	D	B	B	B	D	D	D	D	D	B	D	U	D	B	B	D
D1Ncvs61	AD33	I2	B	B	D	D	D	D	B	B	D	D	D	B	B	B	D	D	D	D	D	B	D	U	D	B	B	D
D1Ncvs62	AB27	H2	B	B	D	D	D	D	B	B	D	D	D	B	B	B	D	D	D	D	D	B	D	U	D	B	B	D
D1Ncvs63	AD25	H2	U	B	D	D	D	D	B	B	D	B	D	B	B	U	D	U	D	D	D	B	D	U	U	B	U	D
D1Ncvs67	CD559	I3	B	U	D	D	B	B	U	B	D	U	U	U	D	U	D	U	B	D	D	B	D	D	D	D	B	B
D1Ncvs68	CD658	H5	B	U	D	D	U	U	D	B	D	B	U	U	B	D	U	B	U	D	D	B	U	D	D	D	B	U
D1Ncvs69	CD493	E2	B	B	D	B	B	B	D	U	D	B	D	B	B	U	D	B	B	D	D	B	D	D	D	D	D	B
D1Ncvs70	CD446	G1	B	U	B	B	B	D	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	D	D	D	B	B
D1Ncvs71	CD665	I4	B	B	D	B	B	D	D	B	D	D	B	B	B	B	D	B	D	D	D	D	D	D	D	D	B	B
D1Ncvs72	CB457	H1	B	B	D	D	D	U	D	D	B	B	D	B	B	D	D	D	D	B	U	D	B	D	D	B	B	U
D1Ncvs73	CB564	I4	B	B	B	D	D	B	B	B	B	B	D	D	B	D	B	B	B	B	B	D	D	D	D	D	B	B
D1Ncvs74	CD563	I4	B	B	B	D	D	B	B	B	B	B	B	D	D	B	D	B	B	B	B	B	D	D	D	D	B	B
D1Ncvs75	CD688	H7	B	B	B	D	D	B	B	B	D	B	D	B	B	B	D	B	D	D	D	D	B	D	B	D	B	B
D1Ncvs76	CB492	F2	B	B	D	D	U	B	D	B	U	B	D	B	B	B	D	B	B	D	D	B	D	D	D	D	U	B
D1Ncvs77	CB447	G1	B	U	D	B	B	D	B	B	D	B	B	B	B	B	B	B	D	D	D	D	D	D	D	D	B	B
D1Ncvs78	CB507	F3	B	B	D	B	B	B	D	B	D	B	B	B	B	B	B	B	D	D	D	D	D	D	D	D	U	B
D1Ncvs79	CB449	G2	B	U	D	B	D	D	D	B	D	B	U	U	U	U	U	D	U	U	D	D	D	D	D	U	B	U
D1Ncvs80	CB613	E5	B	B	D	D	D	D	B	B	D	D	D	B	B	B	B	B	D	D	D	D	B	D	B	D	B	B	B
D2Ncvs7	AB145	G5	D	D	D	D	B	D	D	B	D	D	B	B	B	D	D	D	B	D	D	B	B	U	B	D	D	B
D2Ncvs31	AB250	C2	D	B	D	B	D	D	B	D	D	D	B	B	B	B	B	B	D	D	B	U	D	B	U	B	D	D	D
D2Ncvs32	AD303	C3	D	B	D	B	D	D	B	D	D	D	B	B	B	B	B	B	D	D	B	D	D	B	U	B	D	D	D
D2Ncvs33	AD82	H4	D	B	D	B	B	D	B	B	D	D	B	B	B	D	B	D	D	B	D	D	B	U	B	D	D	D
D2Ncvs34	AD117	I8	D	D	D	B	U	U	D	B	D	D	B	D	U	D	D	D	U	D	D	B	B	U	B	D	D	B
D2Ncvs35	AB133	C9	B	D	D	B	D	B	D	B	B	B	U	D	U	U	U	U	B	D	D	B	D	U	D	U	B	D
D2Ncvs36	AD132	C9	B	D	D	U	D	B	D	B	B	U	U	D	U	U	U	U	B	D	D	B	D	U	U	U	B	D
D2Ncvs37	AD243	A2	B	B	B	B	D	D	B	D	B	B	D	D	D	D	D	D	B	D	D	B	U	U	D	D	U	B
D2Ncvs38	AB337	A5	B	B	B	B	D	D	B	D	B	B	D	D	U	U	D	D	B	D	D	B	U	U	D	U	B	B
D2Ncvs39	AD339	C5	B	B	B	B	D	D	B	D	B	B	D	D	D	D	D	D	B	D	D	B	D	U	D	D	B	B
D2Ncvs40	AB244	B2	B	B	B	B	D	D	B	D	B	B	D	D	D	D	U	U	B	U	U	B	D	U	U	U	B	B
D2Ncvs41	AD41	J4	B	B	B	B	D	D	B	D	B	B	D	D	D	D	D	D	B	D	D	B	D	U	D	D	D	B
D2Ncvs42	AB59	I4	D	U	B	D	D	U	D	D	U	B	B	D	D	D	D	D	B	D	D	D	D	U	D	D	D	U
D2Ncvs43	AD60	I4	D	U	B	D	D	U	D	D	U	B	B	D	D	D	D	D	B	D	D	D	D	U	D	D	D	U
D2Ncvs44	AD137	D8	D	D	B	D	D	D	D	D	B	B	B	D	D	D	D	D	B	D	D	D	D	U	D	D	D	D
D2Ncvs45	AD177	H3	D	D	B	B	B	B	B	D	D	B	B	B	D	B	D	D	B	D	B	B	D	U	B	D	D	D
D2Ncvs46	CD670	I4	D	B	D	B	D	D	B	B	D	D	B	D	B	D	B	D	D	D	D	D	B	B	B	D	D	D
D2Ncvs47	CD693	G7	B	D	D	B	D	B	D	B	B	B	B	D	D	D	D	D	B	D	D	B	D	D	D	D	B	B
D2Ncvs48	CD479	H2	D	D	U	D	D	D	D	D	B	B	B	D	D	D	D	D	B	D	D	D	D	B	D	D	U	D
D2Ncvs49	CD608	F4	D	D	B	D	D	D	B	D	D	B	U	B	D	U	D	D	D	D	D	B	B	D	B	B	D	D	D

TABLE 7—Continued

Locus	Spot	Area code	RI strains																							
			1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	31	32
D2Ncvs50	CB671	I4	D	B	D	B	D	D	B	B	D	D	B	D	B	D	B	D	D	D	D	D	B	B	B	D	B	D
D2Ncvs51	CB506	F3	D	B	D	B	B	D	B	B	D	D	B	B	B	D	B	D	D	B	D	U	B	B	B	D	U	D
D2Ncvs52	CB633	F6	B	B	D	B	B	D	D	D	D	D	B	D	D	U	D	D	B	U	D	B	U	U	U	U	B	B
D2Ncvs53	CB485	G2	B	U	D	B	D	B	D	B	B	B	B	D	D	D	D	D	B	D	D	B	D	D	D	D	B	B
D2Ncvs54	CB490	E2	B	B	B	B	D	B	B	D	B	B	U	D	D	D	D	D	B	D	D	B	D	D	D	D	D	B
D2Ncvs55	CB540	G3	D	U	B	D	D	B	D	B	U	B	D	D	D	U	D	D	B	D	D	D	D	B	D	D	D	D
D2Ncvs56	CD541	G3	D	U	B	D	D	B	D	D	B	B	D	D	D	D	D	D	U	D	D	D	D	B	D	D	U	D
D2Ncvs57	CD624	D4	D	D	B	B	B	B	B	D	D	B	B	B	D	B	D	D	B	D	B	B	D	D	B	D	D	D
D3Ncvs1	AB74	I5	D	D	B	B	D	B	D	D	D	D	D	B	D	D	B	D	B	B	B	B	D	U	B	D	U	U
D3Ncvs8	AB126	G7	B	D	B	B	D	B	U	D	D	B	D	B	B	B	D	D	D	B	B	D	B	U	B	B	B	D
D3Ncvs24	AB68	J4	D	D	B	B	D	B	B	D	D	D	U	B	D	D	B	D	B	B	B	B	D	U	D	D	D	B
D3Ncvs25	AB245	C1	D	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	B	D	U	B	D	B	B
D3Ncvs26	AD246	C1	D	D	B	B	D	D	D	D	B	D	U	B	D	D	B	D	B	B	B	B	D	U	B	D	B	B
D3Ncvs27	AB75	I6	B	B	D	B	B	B	D	D	B	B	D	B	B	B	B	D	D	B	B	D	B	U	B	D	B	B
D3Ncvs28	AB266	F2	B	B	D	B	B	B	D	B	B	B	D	B	B	B	B	D	D	B	B	D	B	U	B	D	B	B
D3Ncvs29	AD263	E2	U	B	D	B	B	B	D	B	B	B	D	B	B	B	B	D	D	B	B	D	B	U	B	D	B	B
D3Ncvs30	AB328	C4	B	D	B	B	D	B	D	D	B	B	D	B	B	B	B	D	B	B	B	D	B	U	B	D	B	B
D3Ncvs31	AD329	C4	B	D	B	B	D	B	D	D	B	B	D	B	B	B	U	D	B	B	B	D	B	U	B	D	B	B
D3Ncvs32	AB143	G6	B	D	B	B	D	B	D	D	B	B	D	B	B	B	B	D	D	B	B	D	B	U	B	D	B	B
D3Ncvs33	AD125	G8	B	D	B	B	D	B	D	D	D	B	D	B	B	B	B	D	D	D	B	B	D	B	U	B	B	B
D3Ncvs34	AB206	G1	B	D	B	B	D	D	D	D	D	B	B	B	D	D	D	D	B	B	B	B	D	B	U	B	B	B
D3Ncvs35	AD207	G1	B	D	B	B	D	D	D	D	D	B	B	B	D	D	D	D	B	B	B	B	D	B	U	B	B	B
D3Ncvs36	AD22	H2	B	D	B	B	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	U	B	B	B	D
D3Ncvs37	AB21	H2	B	D	B	B	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	U	B	B	B	D
D3Ncvs38	CD662	H5	D	D	D	B	D	B	D	D	D	D	B	B	D	U	B	B	B	B	B	B	D	B	D	D	D	U
D3Ncvs40	CD590	H4	B	D	B	B	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	D	B	B	B	B
D3Ncvs41	CD647	G6	B	D	B	D	D	B	D	D	D	B	B	B	D	U	D	D	B	D	B	D	B	D	B	B	B	B
D3Ncvs42	CD547	H3	D	D	B	D	D	B	D	D	D	D	D	B	D	D	B	D	B	B	B	B	D	D	D	D	D	D
D3Ncvs43	CB495	E2	B	D	B	D	D	B	D	D	B	B	D	B	B	B	B	D	B	B	B	D	B	D	B	D	U	B
D3Ncvs44	CB527	F4	B	D	B	B	U	B	D	D	B	B	D	B	B	B	B	D	D	D	B	B	D	B	D	B	B	B
D3Ncvs45	CB508	F3	B	D	U	B	D	B	D	D	D	B	D	B	B	B	B	D	D	D	U	U	D	B	D	B	B	U
D3Ncvs47	CB589	H4	B	D	B	D	B	D	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	D	B	B	B	B
D3Ncvs48	CD686	H8	B	D	B	U	D	B	D	D	D	B	B	B	D	D	D	D	B	D	B	D	B	D	B	B	B	B
D3Ncvs49	CD521	E4	B	D	B	B	D	D	D	D	D	B	B	B	D	D	D	D	B	B	D	D	B	D	B	B	B	B
D4Ncvs10	AB340	D5	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	U	D	D	B	D
D4Ncvs13	AB102	G7	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	B	D	B	D	D	U	D	D	B	D
D4Ncvs14	AB152	F5	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	U	D	D	B	D
D4Ncvs19	AB287	F3	D	B	D	B	D	B	D	D	D	D	D	D	B	B	D	B	D	B	B	D	B	U	B	D	B	D
D4Ncvs25	AB18	H1	D	U	B	B	D	D	B	D	D	D	B	D	D	B	B	D	D	B	B	B	D	U	B	D	B	D
D4Ncvs56	AD317	F5	D	B	B	B	B	B	B	B	D	B	B	B	B	B	B	D	D	B	D	D	D	U	B	D	B	B
D4Ncvs57	AB318	F5	D	B	B	B	B	B	U	B	D	B	B	B	B	B	B	D	D	B	D	D	D	U	B	D	B	B
D4Ncvs58	AD223	E1	D	B	B	B	B	B	B	B	D	B	D	B	B	B	B	D	D	B	D	D	B	U	B	B	B	B
D4Ncvs59	AD319	E5	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	U	D	D	B	B
D4Ncvs60	AD341	D5	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	U	D	D	B	D
D4Ncvs61	AD105	H7	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	B	D	B	D	D	U	D	D	B	D
D4Ncvs62	AB65	I4	D	B	D	B	D	D	B	D	D	D	D	D	D	D	B	D	B	D	B	D	D	U	D	D	B	D
D4Ncvs63	AB205	H1	D	B	B	B	D	D	B	D	D	D	D	B	D	B	B	D	B	D	B	D	B	U	B	D	B	D
D4Ncvs64	AB153	G5	D	U	B	B	D	B	B	D	D	D	D	D	U	B	B	D	B	D	B	B	B	U	B	D	B	D
D4Ncvs65	AD154	G5	D	U	B	B	D	B	B	D	D	D	D	D	U	B	B	D	B	D	B	B	B	U	B	D	B	D
D4Ncvs66	AB66	J4	U	B	U	B	D	B	B	U	D	D	U	D	U	B	B	D	B	D	B	B	B	U	B	D	B	D
D4Ncvs67	AD99	H7	D	B	D	B	D	B	B	D	D	D	D	D	D	B	B	D	B	D	B	B	B	U	B	D	B	D
D4Ncvs68	AD171	F3	D	B	D	B	D	B	B	D	D	D	D	D	D	B	B	D	B	D	B	U	B	U	B	D	B	D
D4Ncvs69	AD234	C1	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	D	U	B	D	B	D
D4Ncvs70	AB48	I3	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	D	U	B	D	B	D
D4Ncvs71	AB357	H1	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	D	U	B	D	B	D
D4Ncvs72	AB241	B1	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	D	U	B	D	B	D
D4Ncvs73	AD17	H1	D	D	B	U	D	D	B	D	D	D	B	D	D	B	B	D	D	B	B	B	D	U	B	D	U	D
D4Ncvs74	CD459	H1	U	B	B	B	B	B	B	B	D	B	D	B	B	B	B	U	U	B	D	D	B	B	B	D	B	B
D4Ncvs75	CD470	I1	D	U	D	B	B	D	B	D	B	D	U	B	D	D	B	D	D	D	B	D	D	D	D	D	B	B
D4Ncvs76	CD668	I4	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	B	D	B	D	U	D
D4Ncvs77	CD421	E1	D	B	U	B	U	B	U	D	U	D	B	D	D	B	B	D	B	B	B	B	B	B	B	D	B	B
D4Ncvs78	CB478	H1	D	B	U	B	B	B	B	D	B	B	B	B	B	D	D	D	B	D	D	B	B	B	D	B	D	B
D4Ncvs79	CB460	H1	U	B	B	B	B	B	B	B	D	B	D	B	B	B	B	U	U	B	D	D	B	B	B	D	B	B
D4Ncvs81	CB410	G1	D	B	U	U	B	B	B	B	U	B	D	B	B	B	B	D	D	B	D	D	B	B	B	D	B	B
D4Ncvs82	CB513	F3	D	B	U	B	B	D	B	D	U	B	D	B	B	D	B	D	D	U	D	D	D	D	B	D	B	B
D4Ncvs83	CB480	H2	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	U	B
D4Ncvs84	CD481	H2	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	U	B	D	D	D	D	D	B	B
D4Ncvs85	CB641	G5	D	B	D	B	D	B	B	D	D	D	B	D	D	D	B	D	B	D	B	D	D	D	D	D	B	B

TABLE 7—Continued

Locus	Spot	Area code	RI strains																							
			1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	23	24	25	27	28	29	30	31	32
D4Ncvs87	CB586	H4	D	B	B	B	D	B	B	D	D	D	D	B	D	B	B	D	B	D	B	D	B	D	B	D	B	B
D4Ncvs88	CB543	G3	D	U	U	B	D	B	B	D	D	D	D	D	D	B	B	D	B	D	B	B	B	D	B	D	B	B
D4Ncvs89	CD627	D4	D	B	D	B	D	B	B	D	D	D	D	D	D	B	B	D	B	D	B	B	B	D	B	D	U	B
D4Ncvs90	CD529	F4	D	B	D	U	D	D	B	D	D	D	D	D	D	U	B	D	U	D	B	B	B	D	B	D	B	U
D4Ncvs91	CB491	E2	D	B	D	B	D	B	U	D	D	D	D	B	D	D	B	B	D	B	B	B	B	D	B	D	U	D
D4Ncvs92	CB669	I4	D	B	D	B	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	B	D	B	D	U	B
D4Ncvs93	CD524	D4	D	B	D	D	D	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	B	D	B	D	B	B
D4Ncvs94	CB525	D4	D	B	D	B	B	B	B	D	D	D	B	D	D	B	B	D	B	B	B	B	B	D	B	D	B	B
D4Ncvs95	CB651	G6	D	B	D	B	D	B	B	D	D	D	B	D	B	B	B	D	B	B	B	B	D	D	B	D	B	B
D4Ncvs96	CB652	G6	D	B	D	B	D	B	B	D	B	D	B	D	D	B	B	D	B	B	B	B	D	D	B	D	B	B
D4Ncvs97	CB618	E4	D	D	B	B	D	D	B	D	D	D	B	D	D	B	B	D	D	B	B	B	D	D	B	D	B	B
D5Ncvs3	AB187	G2	D	D	D	B	B	B	D	D	B	B	B	D	B	D	D	B	B	B	B	D	U	B	B	U	U	
D5Ncvs13	AB161	G4	B	D	D	B	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	U	B	D	B	D
D5Ncvs45	AB218	F1	D	D	D	D	B	B	D	D	B	B	B	D	B	D	D	B	B	U	B	B	D	U	B	B	D	D
D5Ncvs46	AB179	G3	B	B	D	B	B	D	B	D	B	D	B	D	B	B	D	D	D	D	D	B	B	U	B	D	D	D
D5Ncvs47	AD181	G3	B	B	D	B	B	D	B	D	B	D	B	D	B	B	D	D	D	D	D	B	B	U	B	D	D	D
D5Ncvs48	AB220	E1	B	D	D	B	B	B	D	D	B	U	B	D	D	B	B	D	D	B	D	B	B	U	U	D	B	D
D5Ncvs49	AD316	F4	D	D	D	D	B	D	D	B	B	U	D	D	B	B	D	D	B	D	U	B	U	B	D	B	D	D
D5Ncvs50	AB164	G3	B	D	D	D	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	U	U	D	U	D
D5Ncvs51	AD163	G3	B	D	D	D	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	U	U	D	U	D
D5Ncvs52	CD660	H5	D	D	D	U	U	D	D	D	B	U	U	D	U	D	D	B	U	U	B	B	D	D	B	B	D	D
D5Ncvs53	CD675	I6	D	U	D	D	D	D	D	D	D	B	B	D	B	D	D	D	B	B	D	D	B	B	B	D	B	B
D5Ncvs54	CD596	G4	B	D	D	D	D	D	D	D	D	D	B	D	D	B	B	D	D	B	B	B	B	D	B	B	B	B
D5Ncvs55	CB636	F5	D	D	D	D	B	B	D	U	B	B	B	D	B	D	D	B	B	B	B	B	B	D	B	B	B	B
D5Ncvs56	CB683	I7	D	D	D	B	B	B	D	D	B	B	B	U	B	D	D	B	U	B	B	B	D	D	B	B	D	D
D5Ncvs57	CB554	H3	B	D	D	B	B	B	D	B	B	B	D	D	B	D	D	D	D	B	D	B	D	D	B	D	U	D
D5Ncvs58	CB616	E4	B	D	U	B	B	D	D	D	U	D	B	D	B	D	D	D	D	D	D	D	B	B	D	B	D	D
D5Ncvs59	CB569	J3	U	D	B	U	D	B	D	U	B	B	U	U	D	B	B	D	U	U	D	U	B	B	B	D	B	U
D5Ncvs60	CB435	G2	B	U	D	B	D	B	D	D	B	B	B	D	D	B	B	D	D	B	D	B	B	B	B	D	B	B
D5Ncvs61	CB674	I6	D	U	D	D	D	D	D	D	D	B	B	D	B	D	D	D	B	B	D	D	B	B	B	D	B	B
D6Ncvs3	AB55	H4	B	B	B	D	D	B	D	B	D	B	B	B	B	D	D	D	B	B	D	D	U	B	D	B	B	B
D6Ncvs31	AD353	D7	U	B	B	D	D	D	D	D	D	D	U	D	D	B	B	B	D	D	D	B	D	U	U	D	B	D
D6Ncvs32	AB354	D7	B	B	B	D	D	D	D	D	D	D	B	D	D	B	B	B	D	D	D	B	D	U	B	D	B	D
D6Ncvs33	AB151	F5	B	B	B	D	D	D	D	D	D	D	B	B	B	D	D	B	B	D	D	B	B	D	U	B	D	B
D6Ncvs34	AD190	G2	B	B	B	D	D	B	B	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs35	AB274	F2	B	B	B	U	D	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs36	AB30	H2	B	B	B	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs37	AD169	F3	U	B	B	D	D	U	D	B	D	B	B	B	U	D	B	D	D	B	B	D	D	U	B	D	U	B
D6Ncvs38	AB170	F3	U	B	B	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs39	AB189	G2	B	B	B	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs40	AD56	H4	B	B	B	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	B	D	D	U	B	D	B	B
D6Ncvs41	AB77	I5	D	B	D	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	D	D	D	U	B	B	B	B
D6Ncvs42	AD237	C1	D	B	D	B	B	D	D	B	D	B	D	B	B	D	B	B	B	B	U	D	B	U	D	B	B	D
D6Ncvs43	AB210	G1	D	B	D	B	B	D	D	B	D	B	D	B	B	D	B	B	B	B	D	D	B	U	D	B	B	D
D6Ncvs44	AB138	D7	D	B	D	B	B	D	D	B	D	B	D	B	B	D	B	B	B	B	D	D	B	U	D	B	D	D
D6Ncvs45	AB148	F5	D	B	D	B	B	D	D	B	D	B	D	B	D	D	B	B	B	B	D	D	B	U	D	B	D	D
D6Ncvs47	CD661	H5	B	B	B	D	D	B	D	D	D	D	B	B	B	U	B	D	D	B	B	D	D	D	B	D	B	B
D6Ncvs48	CD605	F4	D	B	D	B	D	D	B	B	D	B	D	B	D	D	B	B	B	D	B	D	D	B	D	B	D	B
D6Ncvs49	CB405	H1	B	B	D	U	B	B	D	B	D	B	U	B	B	D	B	D	D	U	B	D	D	D	B	B	B	B
D6Ncvs50	CB614	E5	D	B	B	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	D	D	B	B	B	B	B	B
D6Ncvs51	CB486	G3	D	B	D	B	B	D	D	B	U	B	D	B	B	D	B	B	D	B	D	D	D	B	D	B	B	B
D7Ncvs3	AB312	E3	B	D	B	D	B	B	B	B	B	D	B	B	B	B	B	B	B	B	B	B	B	U	B	B	D	D
D7Ncvs6	AB54	H3	B	D	B	D	B	B	B	D	B	D	D	B	B	B	B	B	B	D	B	U	D	U	B	B	U	D
D7Ncvs37	AD310	E3	B	D	B	D	B	B	B	B	B	D	B	B	B	B	B	B	B	B	B	B	B	U	B	B	D	D
D7Ncvs38	AD204	H1	B	D	U	D	B	B	B	B	U	D	B	B	B	U	B	B	B	B	B	U	B	U	B	B	D	D
D7Ncvs39	AD134	C9	B	D	D	D	B	B	U	D	B	D	U	B	U	U	B	U	D	U	B	D	B	U	B	U	D	D
D7Ncvs40	AD160	G4	B	B	D	B	B	D	B	D	B	D	B	B	D	B	B	D	B	B	D	B	D	B	U	B	B	D
D7Ncvs41	AB61	I4	B	B	D	B	B	D	B	D	D	D	D	B	D	B	B	B	B	B	B	D	D	B	U	B	B	D
D7Ncvs42	AD62	I4	B	B	D	B	B	D	B	D	D	D	D	B	D	B	B	B	B	B	B	D	D	B	U	B	B	D
D7Ncvs43	AD28	H2	B	B	D	B	B	D	B	D	D	D	D	B	D	B	B	B	B	B	B	D	D	B	U	B	B	D
D7Ncvs44	AD53	H3	B	B	D	B	B	D	B	D	D	D	D	B	D	B	B	B	B	B	B	D	D	B	U	B	B	D
D7Ncvs45	AB326	D4	B	B	D	B	B	D	B	D	U	U	U	B	U	B	B	B	B	B	U	U	B	U	B	B	B	D
D7Ncvs46	AB229	D1	B	B	D	B	B	D	U	D	D	D	D	B	D	U	U	B	B	U	U	D	B	U	B	U	B	D
D7Ncvs47	AB16	H1	U	D	D	B	B	D	B	D	D	B	D	B	D	B	D	B	B	D	B	D	D	U	D	B	B	D
D7Ncvs48	CD593	H4	B	D	B	B	B	B	B	D	B	D	B	B	B	B	B	B	D	B	B	D	B	B	B	B	D	D
D7Ncvs49	CD667	I5	B	B	B	B	B	D	B	D	B	D	D	B	U	B	D	B	D	B	B	D	B	B	B	D	U	D
D7Ncvs50	CD424	F1	B	B	U	U	B	D	U	D	U	D	B	B	D	B	B	B	B	B	D	D	B	D	B	B	U	D
D7Ncvs51	CD466	H1	U	D	B	B	B	D	B	U	B	B	D	B	D	B	D	B	B	D	B	B	D	D	D	B	D	D

TABLE 7—Continued

Locus	Spot	Area code	RI strains																								
			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
D7Ncvs52	CD440	G2	B	U	D	D	B	B	B	B	B	D	U	B	B	B	B	B	B	B	U	B	B	D	U	B	D	D
D7Ncvs53	CD625	D4	B	D	B	D	B	B	B	B	B	D	B	B	B	B	B	B	B	U	B	B	B	D	B	B	D	D
D7Ncvs55	CB433	G2	B	D	U	D	B	U	U	D	U	D	B	B	U	B	B	B	D	B	B	D	B	U	B	D	U	D
D7Ncvs56	CB425	F1	B	D	U	U	U	B	B	D	U	D	D	B	B	B	B	B	D	U	B	D	B	B	B	B	D	U
D7Ncvs57	CB534	G4	B	B	D	B	B	D	B	U	B	D	B	B	B	B	B	B	D	B	B	D	B	B	B	B	D	U
D7Ncvs58	CD445	G1	B	U	U	B	B	D	B	D	B	D	B	B	D	B	B	B	D	B	B	D	B	B	B	B	D	D
D7Ncvs59	CB514	F3	B	B	U	B	B	D	B	D	U	D	D	B	D	B	B	B	D	B	B	D	B	B	B	B	D	D
D7Ncvs63	CB423	F1	B	B	D	U	B	D	U	D	U	D	B	B	D	B	B	B	B	U	D	D	B	D	B	B	U	D
D7Ncvs64	CD654	H6	D	D	D	B	B	D	B	D	D	B	D	B	D	U	D	B	B	D	B	D	D	D	D	B	D	D
D7Ncvs65	CB465	I4	U	U	D	B	B	D	B	U	D	B	D	B	D	B	D	B	B	D	B	D	D	D	D	B	U	D
D8Ncvs5	AB257	D2	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	D	B	D	B	B	U	B	B	D	D
D8Ncvs6	AB290	D2	D	B	B	D	D	D	D	B	D	B	B	D	D	D	D	B	D	B	D	B	B	U	B	B	D	D
D8Ncvs8	AB114	I8	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	D	B	D	U	B	D	B	D
D8Ncvs31	AB321	D4	B	B	D	B	B	B	D	B	D	D	B	D	D	D	B	D	D	B	D	B	D	U	D	D	D	D
D8Ncvs32	AD202	H1	B	B	B	B	B	B	D	B	D	B	B	D	D	B	B	B	D	B	D	B	B	U	D	D	D	D
D8Ncvs33	AD203	H1	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	B	B	D	U	B	U	B	B	D	D
D8Ncvs34	AD73	I5	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	B	B	D	B	B	U	B	B	D	D
D8Ncvs35	AB217	F1	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	B	B	D	B	B	U	B	B	D	D
D8Ncvs36	AB338	C5	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	B	B	D	B	B	U	B	B	D	D
D8Ncvs37	AD259	D2	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	D	B	D	B	B	U	B	B	D	D
D8Ncvs38	AD111	I7	D	B	D	B	D	D	D	B	D	B	B	D	D	D	B	B	D	B	D	B	B	U	B	B	D	D
D8Ncvs39	AD127	G7	D	B	B	D	D	D	D	B	D	B	B	D	D	D	B	D	B	D	B	B	U	B	B	D	D	
D8Ncvs40	AB342	D5	D	B	B	D	D	D	D	B	D	B	B	D	D	D	B	D	B	D	B	B	U	B	B	D	D	
D8Ncvs41	AD11	H1	D	B	B	D	D	D	D	B	D	B	B	D	D	D	B	D	B	D	B	B	U	B	B	D	D	
D8Ncvs42	AB12	H1	D	B	B	D	D	D	D	B	D	B	B	D	D	D	B	D	B	D	B	B	U	B	B	D	D	
D8Ncvs43	AD112	I7	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	U	B	D	B	D	U	B	D	B	D
D8Ncvs44	AB230	D1	D	B	B	U	D	U	D	B	D	B	B	U	D	B	D	B	B	B	D	B	D	U	B	D	B	D
D8Ncvs45	AD351	D6	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	D	B	D	U	B	D	B	D
D8Ncvs46	AB144	G5	D	B	D	D	D	D	D	B	D	B	B	D	D	B	D	D	B	B	D	D	D	U	D	D	B	D
D8Ncvs47	AB45	I3	D	B	D	D	D	D	D	B	B	D	D	B	B	D	D	D	B	D	D	D	U	D	B	D	D	
D8Ncvs48	AD46	I3	D	B	D	D	D	D	D	B	B	D	D	B	B	D	D	D	B	D	D	D	U	D	B	D	D	
D8Ncvs49	CD567	I3	D	B	D	B	D	D	D	B	B	B	U	D	U	D	B	B	D	B	D	B	B	B	B	B	D	U
D8Ncvs50	CD412	G1	D	U	B	U	D	D	D	B	D	B	B	D	D	B	B	B	D	B	D	B	B	B	B	B	U	D
D8Ncvs51	CD611	F4	D	B	B	D	D	U	D	B	D	B	B	D	D	D	B	D	B	B	B	B	B	B	B	B	D	D
D8Ncvs52	CD448	G1	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	D	B	D	B	B	D	U	B
D8Ncvs53	CD428	F2	D	B	B	D	D	D	D	B	D	B	B	D	D	B	D	B	B	B	D	B	D	B	B	D	U	B
D8Ncvs54	CD469	I1	D	B	D	D	D	D	D	B	D	B	U	D	D	D	U	D	D	B	U	D	D	D	D	D	B	B
D8Ncvs55	CD444	G1	D	U	U	D	D	D	D	B	D	B	B	D	D	B	D	D	B	U	D	D	D	B	D	B	B	B
D8Ncvs56	CB619	E4	B	B	B	U	B	B	D	B	U	B	B	D	D	B	B	B	D	B	D	B	B	B	D	D	U	D
D8Ncvs57	CD630	E6	B	B	B	D	B	D	D	B	D	B	B	D	D	B	B	B	B	B	D	B	B	B	D	D	D	D
D8Ncvs58	CB468	H1	B	B	B	B	B	D	D	B	D	B	B	D	D	B	B	B	B	B	D	B	B	B	D	D	D	D
D8Ncvs59	CB566	I2	D	B	D	B	D	D	D	B	D	B	B	D	U	D	B	B	D	B	D	B	B	B	B	B	D	U
D8Ncvs60	CD511	F3	D	B	D	B	U	D	D	B	D	B	B	D	D	D	B	B	D	U	D	B	B	B	B	B	U	D
D8Ncvs61	CB510	F3	D	B	D	B	U	D	D	B	D	B	B	D	B	D	B	B	D	B	D	B	B	B	B	B	U	D
D8Ncvs62	CB411	G1	D	B	D	U	D	D	D	B	D	B	D	D	D	B	B	B	D	B	D	B	B	B	B	B	D	D
D8Ncvs63	CB505	E3	D	B	U	U	D	D	D	B	D	B	B	D	D	D	D	B	D	B	B	B	B	B	B	B	U	D
D8Ncvs64	CB544	G3	D	B	D	D	D	D	D	B	D	B	B	D	D	D	D	B	D	B	D	B	B	B	B	B	D	D
D8Ncvs65	CD623	D4	D	B	U	D	D	D	D	B	D	B	B	D	D	D	D	B	D	B	D	B	B	B	B	B	U	D
D8Ncvs66	CB568	J3	U	B	B	U	D	D	D	B	B	B	U	D	D	B	B	B	D	B	D	B	B	D	B	D	U	U
D8Ncvs67	CD489	F2	D	B	B	D	U	D	D	D	D	B	B	D	D	B	D	B	B	B	D	B	D	B	B	U	U	B
D8Ncvs68	CB419	F1	D	B	U	U	D	D	D	B	D	B	B	D	D	B	D	B	B	D	D	B	D	B	B	D	B	B
D8Ncvs69	CB475	H2	D	B	B	D	B	D	B	B	U	B	B	U	D	B	D	D	B	B	D	D	D	B	D	B	B	B
D8Ncvs70	CB546	H3	D	B	D	U	U	D	B	B	D	U	U	U	U	D	D	D	U	D	D	U	B	D	B	B	U	
D9Ncvs17	AB308	D3	U	B	B	D	D	B	B	B	B	B	B	B	D	B	D	B	D	B	B	D	B	U	B	B	B	D
D9Ncvs34	AB323	D4	B	D	B	D	D	D	D	D	B	B	B	B	D	D	D	D	D	D	B	U	D	D	B	D		
D9Ncvs35	AD325	D4	U	D	B	D	D	D	D	D	B	B	U	D	D	D	B	D	D	D	D	D	B	U	D	D	B	D
D9Ncvs36	AD201	G1	D	B	D	D	D	D	B	D	B	D	D	D	D	D	B	D	D	B	B	D	B	U	D	D	B	B
D9Ncvs38	AD233	D1	D	B	B	D	D	D	B	D	B	B	D	D	D	D	D	D	B	B	B	D	B	U	D	D	B	B
D9Ncvs39	AB80	H4	D	B	B	D	D	B	B	D	B	B	B	D	D	D	D	D	B	B	B	D	B	U	U	D	B	B
D9Ncvs40	AD81	H4	D	B	B	D	D	B	B	D	B	B	B	D	D	D	D	D	B	B	B	D	B	U	U	D	U	B
D9Ncvs41	AD14	H1	D	B	B	D	D	B	B	D	B	B	B	B	D	B	D	D	D	B	B	D	B	U	B	D	B	D
D9Ncvs42	AB231	D1	D	B	B	D	D	B	B	D	B	B	U	B	D	B	D	D	D	B	B	D	B	U	B	B	B	D
D9Ncvs43	AD309	D3	D	B	B	D	D	B	B	B	B	B	B	B	D	B	D	B	D	B	B	D	B	U	B	B	B	D
D9Ncvs44	AD343	E5	B	D	B	B	D	B	B	B	B	B	B	B	D	B	D	D	D	B	B	D	B	U	B	B	D	D
D9Ncvs45	AB196	G2	B	U	B	U	B	B	B	B	B	B	B	U	B	B	B	U	D	D	U	B	B	U	B	D	B	D
D9Ncvs46	AD9	I1	D	D	B	D	B	B	B	B	B	B	U	D	B	B	B	D	D	D	D	B	B	U	B	D	B	D
D9Ncvs47	CD488	G3	D	U	D	U	D	D	B	D	B	B	B	D	D	D	B	D	D	B	B	D	B	D	D	B	B	
D9Ncvs48	CD594	H4	D	B	B	D	D	B	B	D	B	B	B	B	B	D	U	D	D	D	B	B	D	B	B	B	B	U

TABLE 7—Continued

Locus	Spot	Area code	RI strains																								
			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
D9Ncvs49	CD403	H1	D	D	D	U	D	D	B	D	B	B	U	D	D	B	B	D	B	D	B	D	B	B	D	B	B	B
D9Ncvs50	CD599	G5	B	D	B	D	B	B	B	B	B	B	B	D	B	B	B	D	D	D	D	B	B	B	B	D	B	B
D9Ncvs51	CB404	H1	D	D	D	B	D	D	B	D	B	B	U	D	D	D	B	D	B	D	B	D	B	B	D	B	B	B
D9Ncvs52	CB487	G3	D	U	B	D	D	D	B	D	B	B	U	D	D	D	B	D	D	B	B	D	B	B	D	D	B	B
D9Ncvs53	CB401	H1	D	B	B	D	D	B	B	D	B	B	D	D	D	B	B	D	D	B	B	D	B	B	B	D	B	B
D9Ncvs54	CB653	H6	D	B	B	D	D	B	B	D	B	B	B	D	D	B	D	D	D	B	B	D	B	B	B	B	B	B
D9Ncvs55	CB467	H1	U	U	U	D	D	B	B	U	B	B	B	B	D	B	D	D	D	B	B	U	B	B	B	D	B	B
D9Ncvs56	CD471	I1	B	D	B	B	D	B	B	B	B	B	B	B	D	B	D	D	D	B	B	D	B	B	B	B	D	D
D9Ncvs57	CB600	G4	B	D	B	D	B	B	B	B	B	B	B	D	B	B	B	D	D	D	D	B	B	B	B	D	B	B
D10Ncvs28	AD83	H5	D	D	D	B	D	B	B	B	B	B	B	B	D	D	B	B	B	D	B	D	B	U	B	D	B	D
D10Ncvs29	AB238	C1	B	D	B	U	B	B	B	B	D	B	B	B	D	D	D	D	D	B	D	D	D	B	U	B	B	D
D10Ncvs30	AB24	H2	B	D	B	D	B	B	D	B	B	B	B	D	D	D	D	D	D	B	D	D	D	B	U	B	B	D
D10Ncvs31	AD29	H2	B	D	B	D	B	B	D	B	B	B	B	D	D	D	D	D	D	B	D	D	D	B	U	B	B	D
D10Ncvs32	AB49	I3	B	D	B	D	B	B	D	B	B	B	B	D	D	D	D	D	D	B	D	D	D	B	U	B	B	D
D10Ncvs33	AD50	I3	B	D	B	D	B	B	D	B	B	B	B	D	D	D	U	D	B	U	D	D	D	B	U	B	B	D
D10Ncvs34	CD655	H6	D	D	D	B	D	B	U	D	D	B	B	B	D	D	B	B	B	D	B	D	B	D	B	D	D	B
D10Ncvs36	CB676	I6	D	D	D	B	B	B	B	B	B	B	D	B	D	B	B	D	B	D	B	D	B	D	B	D	B	B
D11Ncvs42	AD124	G8	B	B	B	D	B	D	B	B	D	D	D	B	D	B	D	B	D	D	B	D	U	D	B	D	D	D
D11Ncvs43	AD168	F3	B	B	B	U	B	B	B	B	D	D	U	B	D	B	U	B	B	U	D	B	D	U	D	B	D	D
D11Ncvs44	AD344	E6	D	D	B	D	B	B	B	D	D	D	D	D	B	D	B	D	B	B	B	B	B	U	D	B	B	D
D11Ncvs45	AD264	E2	D	D	B	D	B	B	B	D	D	D	D	D	B	D	B	D	B	B	B	B	B	U	D	B	D	D
D11Ncvs46	AD155	G4	D	D	B	D	B	B	B	D	D	D	B	D	B	D	B	D	U	B	B	U	U	U	D	B	D	D
D11Ncvs47	AB156	G4	D	D	B	D	B	B	B	D	D	D	B	D	B	D	B	D	D	B	B	U	U	U	D	B	D	D
D11Ncvs48	AB180	G3	D	D	B	D	B	B	B	D	D	D	B	D	B	D	B	D	B	B	B	B	B	U	D	B	D	D
D11Ncvs49	AB159	G4	B	D	B	D	B	B	B	B	D	B	D	D	B	D	B	D	B	B	B	B	B	U	D	B	B	D
D11Ncvs50	AB194	H2	B	D	B	D	B	B	B	B	D	B	D	D	B	D	B	D	B	B	B	B	B	U	D	B	B	D
D11Ncvs51	AB313	E3	B	D	B	D	B	B	B	B	D	B	D	D	U	D	B	D	B	B	B	B	B	U	D	B	B	D
D11Ncvs52	AD314	E3	B	D	B	D	B	B	B	B	D	B	D	D	B	D	B	D	B	B	B	B	B	U	D	B	B	D
D11Ncvs53	AD195	H2	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	B	U	D	B	B	D
D11Ncvs54	AB258	D2	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	B	U	D	B	B	D
D11Ncvs55	AD256	D2	U	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	U	B	U	D	B	B	D
D11Ncvs56	AB277	G2	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	B	U	D	B	B	D
D11Ncvs57	AD276	G2	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	B	U	D	B	B	D
D11Ncvs58	AD249	C2	B	D	D	B	D	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	D	U	D	B	U	D
D11Ncvs59	AB248	C2	B	D	D	B	D	D	B	D	B	D	D	D	B	D	D	D	D	B	B	B	D	U	D	B	B	D
D11Ncvs60	AD19	H1	D	D	B	D	D	D	B	U	B	B	U	D	B	B	D	D	D	B	D	U	D	U	B	D	B	D
D11Ncvs61	AD130	F9	D	D	B	D	D	D	B	D	D	U	D	D	B	B	D	D	D	B	D	D	B	U	B	D	B	D
D11Ncvs62	AD166	F3	D	D	B	D	D	D	B	D	D	B	D	D	B	B	D	D	D	B	D	D	D	U	B	D	B	D
D11Ncvs63	AB167	F3	U	D	B	D	D	D	B	D	D	B	D	D	B	B	D	D	D	B	D	D	D	U	B	D	B	D
D11Ncvs64	AD37	J3	D	D	B	U	D	D	B	D	D	B	U	D	B	U	D	D	D	B	D	D	D	U	B	D	U	D
D11Ncvs65	AB38	J3	D	D	B	D	D	D	B	D	D	B	U	D	B	B	D	D	D	B	D	D	D	U	B	D	B	D
D11Ncvs66	AB221	E1	U	D	B	D	D	D	B	D	D	B	D	D	U	D	D	D	D	D	D	D	D	U	B	D	B	D
D11Ncvs67	AB182	G3	D	D	D	D	D	D	B	D	D	B	D	D	B	B	U	D	D	B	D	D	D	U	B	D	D	D
D11Ncvs68	AD183	G3	D	D	D	D	D	D	U	D	D	B	D	D	B	B	U	D	D	B	D	D	D	U	B	D	D	D
D11Ncvs69	CD451	G1	B	U	B	D	B	B	B	B	B	D	D	B	D	D	D	B	B	B	D	B	D	D	D	D	D	D
D11Ncvs70	CD583	I4	D	D	B	D	B	D	B	D	D	D	D	D	B	D	B	D	B	B	B	B	B	D	D	B	D	D
D11Ncvs71	CD454	G1	B	U	B	U	U	B	B	B	U	B	U	D	B	U	B	D	B	B	B	U	B	D	D	B	B	B
D11Ncvs72	CD645	F6	B	D	D	D	D	D	B	U	U	D	D	D	B	D	D	D	D	B	D	B	D	B	U	D	U	D
D11Ncvs73	CD565	I2	B	B	B	D	B	D	B	B	D	D	D	B	D	B	D	B	B	D	D	B	D	D	D	B	D	U
D11Ncvs74	CB452	G1	B	U	B	D	B	B	B	B	D	D	B	B	D	B	D	B	B	B	D	B	D	D	D	D	D	D
D11Ncvs75	CD461	H2	B	D	B	D	B	D	B	D	D	D	D	D	D	D	D	B	B	D	D	B	D	D	D	D	D	D
D11Ncvs76	CB597	G5	D	B	B	D	B	B	B	D	U	D	B	D	B	B	B	D	B	B	B	B	B	D	D	B	D	D
D11Ncvs77	CB453	G1	B	U	B	U	B	B	B	B	U	B	U	D	U	U	B	D	B	B	B	U	B	D	D	B	B	B
D11Ncvs78	CB684	J8	B	D	D	B	B	D	B	D	D	U	U	D	B	D	D	D	U	B	B	B	B	U	U	B	B	B
D11Ncvs79	CD407	H1	B	U	U	B	U	D	B	D	B	D	U	D	U	D	D	U	D	U	B	B	U	B	D	D	U	U
D11Ncvs80	CD622	D4	B	D	D	B	D	D	D	D	U	D	D	D	D	B	D	D	D	B	B	B	B	D	D	B	B	B
D11Ncvs81	CB520	E4	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	D	B	B	B	D	B	B	B
D11Ncvs82	CB406	H1	B	U	D	U	U	D	B	D	U	D	U	D	U	D	D	U	U	U	D	B	U	B	D	U	B	U
D11Ncvs83	CD615	E4	B	D	D	D	D	D	B	B	B	D	D	D	B	D	D	D	D	B	D	B	B	B	D	D	B	B
D11Ncvs84	CD518	E3	D	D	U	D	D	D	B	B	D	B	D	D	D	U	D	D	D	B	D	D	D	B	D	D	B	D
D12Ncvs1	AB76	I5	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs2	AB87	H5	B	D	B	B	D	D	D	B	B	B	B	B	B	D	D	D	D	B	D	U	B	B	B	B	B	D
D12Ncvs3	AB289	D2	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs28	AD172	G3	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs29	AD96	I6	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs30	AD97	I7	B	D	B	B	D	D	U	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs31	AB94	H6	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	U	B	D
D12Ncvs32	AD93	H6	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D

TABLE 7—Continued

Locus	Spot	Area code	RI strains																								
			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
D12Ncvs33	AD88	H6	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs34	AB47	I3	B	D	B	B	D	U	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs35	AB356	E7	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs36	AD286	E2	B	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs37	AD352	D6	U	D	B	B	D	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs38	AD98	H7	B	D	B	B	B	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs39	AD311	E3	B	D	B	U	B	D	D	D	B	B	B	B	B	B	D	D	D	D	D	B	D	U	B	B	B	D
D12Ncvs40	AD267	F2	D	B	U	U	D	D	B	B	D	B	D	B	D	U	D	B	D	D	U	D	U	U	U	B	U	D
D12Ncvs41	AD269	F2	D	B	D	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	D	U	D	B	D	D
D12Ncvs42	AD5	I2	D	B	D	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	D	U	D	B	D	D
D12Ncvs43	AD355	D7	B	B	B	D	B	D	U	D	B	B	D	D	B	B	D	B	B	B	D	D	D	U	B	D	D	D
D12Ncvs44	CD602	G4	B	D	B	B	B	D	B	B	B	D	B	B	D	D	D	D	B	D	B	D	B	D	B	D	B	D
D12Ncvs45	CD581	I4	B	B	B	D	D	D	D	B	B	D	D	D	B	D	D	D	B	B	D	D	D	D	B	D	U	D
D12Ncvs46	CB603	G4	B	D	B	B	B	D	B	B	B	B	D	B	B	D	D	D	D	D	D	B	D	B	D	B	B	D
D12Ncvs47	CB635	F5	B	B	B	B	D	D	B	B	D	B	D	B	B	D	D	B	D	D	D	D	D	D	D	B	D	B
D12Ncvs48	CB523	E4	B	B	B	U	D	B	B	D	D	B	D	B	B	D	D	B	B	D	B	D	D	D	B	B	D	D
D12Ncvs49	CB664	I4	B	B	B	D	D	D	D	B	D	B	D	D	B	D	D	B	B	B	D	D	D	D	B	D	B	B
D13Ncvs9	AB348	D6	D	D	B	B	B	D	D	D	D	D	D	D	B	D	D	D	B	D	D	B	D	U	B	D	D	D
D13Ncvs33	AB8	I2	B	D	B	D	B	D	D	D	D	D	B	D	B	B	D	D	B	D	D	D	D	U	B	D	D	D
D13Ncvs34	AD23	H2	B	D	B	D	B	D	D	D	D	D	B	D	B	B	D	D	B	D	D	D	D	U	B	D	D	D
D13Ncvs35	AB211	F1	B	D	B	D	B	D	D	U	D	D	D	B	B	D	D	B	D	D	D	D	D	U	B	D	B	D
D13Ncvs36	AD350	D6	D	D	B	B	B	D	D	D	D	D	D	D	B	D	D	D	B	D	D	B	D	U	B	D	D	D
D13Ncvs37	AD188	G2	D	D	B	B	B	D	D	D	D	D	D	D	B	D	D	D	B	D	D	B	D	U	B	D	D	D
D13Ncvs39	AB213	F1	D	B	B	D	B	D	D	D	B	D	B	D	D	D	D	D	D	D	D	B	D	U	B	B	D	D
D13Ncvs40	AD296	C2	D	B	D	D	D	D	D	D	B	B	B	D	B	B	D	B	D	D	D	B	B	U	B	B	D	D
D13Ncvs41	AD92	H6	D	B	D	D	D	D	D	D	B	B	B	D	B	B	D	B	D	D	D	B	B	U	B	B	D	D
D13Ncvs42	CD591	H4	D	B	D	D	B	D	D	B	U	B	B	D	B	U	D	B	D	D	D	B	D	D	B	B	D	D
D12Ncvs43	CD638	F5	D	B	D	D	D	D	D	D	B	B	B	D	B	B	D	B	D	D	D	B	B	B	B	B	D	D
D13Ncvs44	CB417	F1	B	D	U	D	B	D	U	D	D	B	B	D	B	B	D	D	D	B	B	B	D	D	D	B	D	B	U
D13Ncvs45	CB515	E3	B	D	B	D	B	D	D	D	D	D	B	D	B	B	D	D	B	D	B	D	D	D	D	B	D	D	D
D13Ncvs46	CB530	G4	D	D	B	B	B	D	D	D	U	U	D	D	B	B	D	D	B	D	D	B	B	D	B	B	D	D	D
D13Ncvs47	CB592	H4	D	B	D	D	B	D	D	B	B	B	B	D	B	B	D	B	D	D	D	B	D	D	B	B	D	D
D13Ncvs48	CB522	E4	D	B	D	D	B	B	D	D	B	U	B	D	B	B	D	B	D	D	D	B	D	D	B	B	D	D
D14Ncvs34	AD176	G3	B	D	B	D	U	D	U	B	B	B	D	U	B	U	B	B	D	B	B	B	B	U	U	U	U	U
D14Ncvs35	AD192	G2	B	D	B	D	D	D	D	D	B	D	D	D	D	D	B	B	D	B	B	B	B	U	D	B	D	D
D14Ncvs36	AB191	G2	B	D	B	D	D	D	D	D	B	D	D	D	D	D	B	B	D	B	B	B	B	U	D	B	D	D
D14Ncvs37	AB198	G2	B	D	B	D	D	D	D	D	B	D	B	D	D	D	B	B	B	B	B	B	B	U	D	B	U	D
D14Ncvs38	AB193	H2	B	D	B	D	D	D	D	U	B	D	B	D	D	D	B	B	B	B	B	B	B	U	D	B	U	D
D14Ncvs39	AB325	C1	B	D	B	D	D	D	D	D	B	D	B	D	D	D	B	B	B	B	B	B	B	U	D	B	D	D
D14Ncvs40	CD438	G2	B	D	U	D	D	D	D	D	D	D	D	D	B	D	B	B	D	B	B	B	B	B	D	B	D	D
D14Ncvs41	CB576	J4	U	D	B	U	U	D	U	B	B	B	U	D	D	U	U	U	U	U	D	B	B	B	D	B	U	U
D14Ncvs42	CD575	J4	U	U	B	U	U	B	D	B	B	B	U	U	D	U	B	U	U	B	D	B	B	B	D	B	D	U
D14Ncvs43	CB538	G3	D	D	U	D	B	D	D	B	U	B	D	D	D	D	B	D	D	B	D	B	B	B	D	B	D	D
D14Ncvs44	CB648	G6	D	D	B	D	B	D	D	B	B	B	B	B	D	D	B	D	D	B	D	B	B	B	D	B	D	D
D14Ncvs45	CB436	G2	B	D	B	U	D	D	D	D	B	D	D	D	D	D	B	B	D	B	B	B	B	B	B	D	D	D	B
D14Ncvs46	CD474	I2	B	D	B	D	D	D	D	B	B	D	D	D	B	D	B	B	B	D	D	D	B	D	D	B	B	B
D14Ncvs47	CB473	I2	B	D	U	D	D	D	D	B	B	D	D	D	B	D	B	B	B	D	D	D	B	B	D	B	B	B
D14Ncvs48	CB610	F4	B	B	B	D	D	D	D	B	B	U	D	B	B	D	B	B	B	D	B	D	B	B	D	D	B	B
D14Ncvs49	CD609	F4	B	B	B	D	D	D	D	B	B	U	D	B	B	D	B	B	B	D	B	D	B	B	D	D	B	B
D15Ncvs17	AD214	F1	B	D	B	B	B	B	D	D	D	B	B	D	B	D	D	B	B	D	D	D	U	B	B	B	B	D
D15Ncvs18	AB79	H4	B	D	B	B	B	B	D	B	D	D	B	B	D	B	D	D	B	B	D	D	D	U	B	B	B	D
D15Ncvs19	AB359	F9	B	D	B	B	B	B	D	B	D	D	B	B	U	B	D	D	B	B	D	D	U	U	B	B	B	D
D15Ncvs20	AD293	D3	B	B	D	B	D	D	B	B	D	D	D	B	D	B	D	D	D	B	D	B	D	U	B	D	D	B
D15Ncvs21	AB335	B4	B	B	B	B	D	D	B	B	D	B	B	B	D	B	U	U	B	B	D	D	U	U	B	U	D	D
D15Ncvs22	AD185	G2	B	D	B	U	D	D	B	B	D	B	D	B	D	B	D	D	D	B	D	D	D	U	B	D	D	D
D15Ncvs23	AB107	I7	B	D	B	B	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	U	B	D	D	D	D
D15Ncvs24	AD147	G5	B	D	B	B	D	D	B	B	D	B	D	B	D	B	D	D	B	D	D	D	U	B	D	D	D	D
D15Ncvs25	CD595	H4	B	D	U	B	B	B	D	B	D	D	B	B	D	B	D	D	B	B	D	D	D	D	B	B	B	B
D15Ncvs26	CD531	G4	U	B	B	D	D	D	B	B	D	D	B	B	D	D	B	D	D	B	D	B	D	D	B	D	D	D
D15Ncvs27	CB598	G5	B	D	B	B	B	B	D	B	D	D	B	B	D	B	D	D	B	B	D	D	D	D	B	B	U	B
D15Ncvs28	CB582	I4	B	D	B	B	B	B	D	B	D	D	B	B	D	B	D	D	B	B	D	D	D	D	B	B	B	B
D15Ncvs29	CB631	E6	B	D	B	B	U	D	B	B	D	B	D	B	D	U	D	D	B	B	D	D	D	D	B	D	D	D
D16Ncvs6	AB136	D9	B	B	B	B	D	B	D	B	B	B	D	D	B	U	D	B	B	D	D	D	D	U	D	D	B	D
D16Ncvs24	AD123	G8	B	B	B	B	D	B	D	B	B	B	D	D	B	D	D	B	B	D	D	D	D	U	D	D	B	D
D16Ncvs25	AB349	D6	B	B	B	B	D	B	B	B	B	B	D	D	B	D	D	B	B	B	D	D	D	U	B	D	B	D
D16Ncvs26	AD347	E6	B	B	B	B	D	B	B	B	B	B	D	D	B	D	D	B	B	B	D	D	D	U	B	D	B	D
D16Ncvs27	CD504	D3	B	D	B	U	B	B	D	B	U	D	B	B	D	D	B	B	B	D	D	D	B	D	D	D	U	B
D16Ncvs28	CB463	H1	B	B	D	D	D	B	B	B	B	B	D	D	B	D	D	B	B	B	D	D	D	D	B	D	B	U

TABLE 7—Continued

Locus	Spot	Area code	RI strains																							
			1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	23	24	25	27	28	29	30	32
D17Ncvs7	AB89	H6	D	D	D	D	B	B	D	B	D	B	B	D	D	B	B	B	D	B	D	D	D	U	B	D	D	D
D17Ncvs22	AD128	G8	D	B	D	D	B	B	B	D	B	B	B	B	D	B	B	B	D	B	D	D	U	U	B	D	D	D
D17Ncvs23	AB129	G8	D	B	D	D	B	B	B	D	B	B	B	B	D	B	B	B	D	B	D	D	B	U	B	D	D	D
D17Ncvs24	AD69	I4	D	B	D	D	B	B	B	D	B	B	B	B	U	B	B	B	D	B	D	D	B	U	B	D	D	D
D17Ncvs25	AB72	I5	U	B	U	U	B	B	U	D	B	B	B	B	D	B	B	B	D	B	D	D	B	U	B	D	D	D
D17Ncvs26	AD31	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs27	AB32	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs28	AB4	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs29	AD3	I1	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs30	AD135	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs31	AB315	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs32	AB320	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs33	AB6	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs34	AD90	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs35	AD291	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs36	AB35	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs37	CD689	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs38	CD441	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs39	CD681	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs40	CB682	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D17Ncvs41	CB443	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs17	AD345	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs18	AB173	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs19	AD174	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs20	AD184	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs21	AB288	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs22	CB548	I2	D	B	D	D	B	D	D	U	B	B	B	D	D	B	B	D	D	B	D	D	B	U	B	D	D	D
D18Ncvs23	CD621	D4	B	D	B	U	U	D	U	D	B	B	D	B	D	U	D	B	D	B	D	B	B	B	B	B	D	D
D18Ncvs24	CB620	D4	B	B	B	D	U	D	U	D	B	B	D	D	D	B	D	B	D	D	D	B	B	B	B	B	U	D
D18Ncvs3	AB346	E6	B	D	D	D	D	D	D	D	B	D	D	D	D	B	B	B	D	D	B	B	B	U	B	D	B	D
D19Ncvs1	AB292	D3	B	B	B	D	D	B	D	B	D	B	B	B	B	B	B	D	U	B	D	U	B	D	U	B	D	D	B
D19Ncvs16	AB281	F2	B	B	B	D	D	B	B	B	D	B	B	B	B	B	B	D	B	B	D	D	B	D	U	B	B	B	D
D19Ncvs17	AD282	F2	B	B	B	D	D	B	B	B	D	B	B	B	B	B	B	D	B	B	D	D	B	D	U	B	B	B	D
D19Ncvs18	AB158	G4	U	B	U	D	D	B	B	B	D	B	U	B	B	B	D	B	B	D	D	U	U	U	B	U	D	B
D19Ncvs19	AD157	G4	U	B	U	D	D	B	B	B	D	B	U	B	B	B	D	B	B	D	D	U	U	U	B	U	D	B
D19Ncvs20	AB358	J4	D	B	B	U	D	B	D	B	D	B	B	B	B	B	D	B	B	B	B	B	D	U	D	D	B	B
D19Ncvs21	AD51	I3	D	B	B	D	D	B	D	B	D	B	B	B	B	D	B	B	B	D	B	B	D	U	B	U	B	D
D19Ncvs22	AB262	E1	U	B	B	D	D	B	D	B	D	B	B	B	B	D	B	B	B	D	B	B	B	U	B	B	B	D
D19Ncvs23	AD261	E1	D	B	B	D	D	B	D	B	D	B	B	B	B	D	B	B	B	D	B	B	B	U	B	B	B	D
D19Ncvs24	AD278	G2	D	B	B	D	D	B	D	B	B	B	B	B	B	B	D	D	B	D	B	B	D	U	B	B	B	D
D19Ncvs25	AB91	H6	B	B	B	B	D	B	D	B	B	B	B	B	B	B	D	D	B	D	B	B	B	U	B	B	B	D
D19Ncvs26	CD430	F2	B	U	B	D	D	B	U	U	D	B	B	B	B	B	D	B	B	D	D	B	D	B	B	B	B	B
D19Ncvs27	CD519	F3	B	B	B	D	D	B	D	B	D	B	B	D	B	B	D	B	B	B	B	B	D	B	B	D	U	B
D19Ncvs28	CD402	G1	D	B	B	D	D	B	D	B	B	B	B	B	B	D	D	D	B	D	B	B	B	B	B	B	B	B
D19Ncvs29	CD472	I1	D	B	B	D	D	B	D	B	D	B	B	B	B	D	D	D	B	D	B	B	B	B	B	B	U	U
D19Ncvs30	CB431	F2	B	B	D	B	D	B	B	B	D	B	B	B	B	B	D	B	B	D	D	B	D	D	B	B	U	B
D19Ncvs31	CB628	D5	D	B	B	D	D	B	D	B	U	B	B	B	B	B	D	B	B	B	B	B	B	D	B	D	D	B	B
D19Ncvs32	CD685	I8	D	U	D	D	D	B	D	D	B	B	D	B	B	B	D	D	D	B	D	B	B	B	B	B	B	B	B
D19Ncvs33	CB455	G1	D	B	B	U	B	B	D	B	B	B	B	D	B	B	D	D	D	B	D	B	B	B	B	B	B	B	B
DXNcvs4	AB285	F2	B	B	B	D	B	B	B	B	D	D	D	B	B	B	D	D	B	D	D	D	D	U	B	B	B	D
DXNcvs9	AD270	F2	B	B	B	D	B	B	B	B	D	D	D	B	B	B	D	B	U	D	B	D	D	U	B	B	B	D
DXNcvs10	CB555	H2	B	B	B	D	B	D	B	B	B	B	D	B	B	D	B	B	B	B	B	B	B	B	B	B	B	B
Unlinked Loci:																										
DONcvs	AB26	H2	B	D	D	B	B	B	B	D	B	B	D	B	B	B	B	U	B	D	B	D	D	U	U	D	U	B
DONcvs	AB104	H7	D	B	D	D	B	B	D	D	B	D	B	D	B	D	D	B	B	B	D	B	B	B	U	D	B	B	B
DONcvs	AB142	G7	B	B	B	D	B	D	D	D	B	D	D	D	D	D	D	B	D	D	D	B	B	U	B	D	D	D
DONcvs	AB186	G2	B	B	U	B	B	B	D	U	B	B	B	B	U	B	U	D	U	B	B	B	B	U	B	B	B	B
DONcvs	AB219	E1	U	B	B	B	B	B	B	B	B	B	B	U	U	D	D	B	B	B	U	B	B	U	B	D	B	B
DONcvs	AB275	F1	D	D	D	B	B	D	B	D	B	D	D	D	D	B	D	B	B	D	D	B	B	U	D	B	D	D
DONcvs	AD20	H1	D	D	D	D	D	D	D	D	U	D	B	D	D	D	U	D	D	U	D	D	D	U	D	D	D	D
DONcvs	AD42	I3	D	D	U	D	D	D	D	D	D	D	D	D	D	U	D	D	D	U	U	D	D	D	U	D	U	D	D
DONcvs	AD52	I3	B	D	B	B	B	D	B	D	B	D	D	D	D	B	B	D	D	D	B	D	D	B	U	D	U	D	B
DONcvs	AD162	G4	B	U	B	B	U	B	B	B	B	B	D	B	B	B	B	B	U	B	B	B	B	U	B	B	D	B
DONcvs	AD212	F1	D	D	B	D	B	D	D	D	D	D	D	D	B	B	D	D	D	D	D	D	D	D	U	B	D	D
DONcvs	AD222	E1	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	U	D	D	D
DONcvs	AD224	D1	D	D	D	D	D	D	B	D	D	D	D	D	D	D	D	D	D	D	D	B	D	U	D	D	B	D
DONcvs	AD225	D1	D	U	D	D	B	D	B	B	B	B	D	U	D	D	D	B	B	B	D	D	D	D	U	D	D	D	B

TABLE 7—Continued

Locus	Spot	Area code	RI strains 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
DONcvs	AD256	A1	U	D	D	B	B	D	B	D	B	D	D	D	B	D	D	D	D	B	B	U	B	U	D	B	B	D
DONcvs	AD327	D4	B	B	B	D	B	D	B	B	D	D	D	B	B	D	B	B	D	B	D	D	D	U	D	B	B	D
DONcvs	AD330	C4	B	D	B	D	B	B	B	B	D	B	B	U	U	B	U	B	B	B	D	U	B	U	U	B	D	B
DONcvs	AD333	C4	B	B	B	D	B	D	B	B	D	D	B	B	B	B	B	B	D	B	D	D	D	U	D	B	B	D
DONcvs	CB422	F1	D	B	D	D	U	B	U	D	U	B	U	D	D	B	B	B	D	U	D	U	B	B	B	U	D	B
DONcvs	CB434	G2	B	B	D	B	B	B	B	D	D	D	B	B	D	B	B	B	B	B	B	B	D	B	B	B	D	B
DONcvs	CB542	G3	B	U	B	B	D	D	B	D	U	B	D	B	D	B	B	D	B	D	B	D	B	B	B	D	U	B
DONcvs	CB560	I3	B	U	D	D	B	B	B	U	U	D	B	B	B	B	B	B	B	B	B	B	U	U	D	B	B	B
DONcvs	CB604	G4	B	B	D	B	B	B	B	U	D	D	B	B	B	B	B	B	B	B	D	D	B	D	D	B	D	B
DONcvs	CB639	F5	U	B	D	B	B	B	B	U	D	D	D	B	B	B	B	B	B	B	B	B	B	D	D	B	D	B
DONcvs	CB656	H5	B	U	B	U	U	B	U	B	B	B	U	D	D	B	B	B	B	B	D	D	B	D	B	B	B	U
DONcvs	CD456	H1	B	B	D	D	D	U	B	D	D	B	B	U	B	D	D	D	D	B	D	D	B	D	D	B	U	U
DONcvs	CD494	E2	B	D	D	D	D	B	D	D	D	B	D	B	D	B	B	D	B	B	B	D	B	D	B	D	U	B
DONcvs	CD509	F3	B	D	D	B	D	B	D	D	D	B	B	D	B	U	D	D	D	U	B	D	B	D	B	B	U	U
DONcvs	CD545	G1	D	B	D	U	U	B	B	B	D	U	U	U	U	U	B	D	D	U	D	D	U	B	D	D	B	B
DONcvs	CD549	G3	B	D	D	B	B	B	B	D	B	D	D	B	B	B	B	D	B	D	B	D	D	D	B	D	D	B

List of RLGS loci typed in the BXD analyses showing the locus identification number and area code in the RLGS profile. The SDP for each of these loci in the BXD series is given.

and 1204 cM in the HSH cross. These results indicate that the RLGS loci produce genetic maps that covered nearly 90% of the genome given an estimated size of the mouse genome of 1600 cM.

RLGS mapping has several experimental features that make it useful for genetic mapping. First, it offers the possibility of identifying several hundred variant spots simultaneously in the same electrophoretic gel. Second, RLGS spots represent robust genetic markers that identify sites/sequences that are common throughout the gene pool even though these spots are analyzed as dominant loci in genetic crosses. Variation for individual *Not*I landmark loci is dependent upon the total number of sequences that are recognized by each of the restriction enzymes used. In the case of *Not*I, *Pvu*II, *Pst*I there are a total of 20 bp that make up the sites that are essential for producing each two-dimensional spot. In addition to the probability of being variant for the enzyme sequence restriction sites there is the additional possibility of having insertions or deletions in the sequences separating these sites in the genome. In the initial analyses, the levels of RLGS variation among inbred strains and between inbred and *M. spretus* were similar to those reported for restriction fragment length polymorphism analysis using cDNA probes involving multiple restriction enzymes (V. M. CHAPMAN and R. W. ELLIOTT, unpublished data) and the levels of RLGS variation are 25–50% of that observed with PCR methods of molecular genetic analysis (LEROY *et al.* 1992; BEIER *et al.* 1992; DIETRICH *et al.* 1992). Third, it is possible to map these loci very rapidly. Each variant spot segregates as an independent genetic locus which means that several hundred loci can be identified simultaneously in the gel analysis of a single individual and crosses with up to 100 progeny can be analyzed in one month using existing laboratory protocols. Fourth, the number of loci can be extended by re-analyzing the same crosses using different restriction enzyme sets that identify additional landmarks. Restriction enzyme sets may differ either at the primary restriction site, which is labeled, or at the secondary sites, which determine the final size of the fragments. Fifth, RLGS methods can be applied directly to the genetic analysis of virtually any species without the previous development of either DNA probes or sequence information for PCR analyses. Finally, methods have been developed that allow the efficient recovery of *Not*I boundary clones corresponding to RLGS loci (HIROTSUNE *et al.* 1993). These can be used to characterize gene function for RLGS loci and establish sequence data for STS identification of the locus (HAYASHIZAKI *et al.* 1994).

The RLGS analyses of this study used horizontal electrophoretic methods. More recently, vertical electrophoretic approaches have been developed that will greatly reduce the costs of the RLGS analyses, particularly in the amounts of restriction enzyme required for the in-gel cleavage of the agarose-separated fragments. Moreover, the overall efficiency of the analysis has been improved by using a multiple gel approach for the acrylamide separation step in which up to 16 second dimensional gels are cast and run simultaneously. Thus, it is easily possible for one technician to produce 16 two-dimensional gels in one day (Y. OKAZAKI, H. OKUIZUMI, N. SASAKI, T. OHSUMI, S. HIROTSUNE, J. KUROMITSU, H. KATAOKA, M. MURAMATSU, A. IWADATE, N. HIROTA, C. PLASS, V. M. CHAPMAN and Y. HAYASHIZAKI, unpublished). Working at this level, a backcross such as the BSS used in this study could be analyzed in 1–2 weeks of laboratory time and an RI strain set such as BXD can be analyzed in less than a week. Moreover, the relative cost per locus with the newer methods has been greatly

reduced so that the RLGS data will cost the same or less than the same amount of genetic information derived from other methods.

The genetic maps that are produced in a backcross can be expanded very easily since the DNA of the same individuals can be reanalyzed for the same landmark enzyme by using additional second and third enzyme combinations. It is possible also to identify additional RLGS loci by using different landmark cleavage enzymes (E_L). In this study we used *Not*I but other enzymes such as *Asc*I (GGCGCG/CC) can be end-labeled landmarks. In this study, we were able to demonstrate the feasibility of this approach in the analysis of BXD by using the enzyme combinations A and C. In principle, the analysis of additional enzyme combinations within a cross or RI strain set will increase the number of RLGS loci in the genetic map that are ordered by haplotype analysis. It will be possible to integrate the RLGS loci into the composite maps of the mouse genome by adding reference loci from other analyses into the backcross resources used for RLGS mapping.

The advantage of mapping RLGS loci that have CpG landmarks is twofold. First, these sites are commonly associated with CpG islands so that the RLGS loci have a high probability of identifying functional genes in the genome (ANTEQUERA and BIRD 1993). Second, because these sites are associated with functional genes it is likely that there will be homology between the RLGS loci in mice and other mammals, including humans. Cloned probes containing RLGS sites such as *Not*I linking clones or *Not*I boundary clones will have a high probability of cross hybridizing between species. We have recently used RLGS methods to identify an imprinted gene, U2AF binding protein related sequence (*U2afbprs*), on mouse chromosome *11* (HAYASHIZAKI *et al.* 1994). We have used the *Not*I boundary clone for this locus to identify a homologous locus on human chromosome 5q31 employing a combination of somatic cell genetic analysis and FISH (I. KALCHEVA *et al.*, 1995). These results clearly demonstrate that RLGS loci in the mouse can be associated with functional genes and that cloned mouse probes can be used to identify homologous loci in the human genome.

The efficiency of using the RLGS method for genetic mapping depends upon the fraction of RLGS spots which vary between parental genotypes and the levels of heterozygosity for individuals in the population. In our analyses, we identified 6% B6-specific spots in the BXD series and 25% of the spots as B6-specific in the BSS cross. If the parental strain variation is independent in the two crosses we would have expected that 1.5% of the variant B6-specific spots would be common to both crosses. Thus, out of 1500–2000 spots in the B6 RLGS profile we would expect 25–30 B6 loci that are common to the BXD and BSS analyses. We observed 27 common loci.

There were 56 loci in the BSS cross and 10 loci in the HSH cross that showed a likelihood of linkage to specific chromosomal locations with LOD scores of 3.0 or greater. However, it was not possible to establish an unambiguous map order because of one or more single locus double recombinations. These results could have been the consequence of errors in the RLGS analysis or it could have been due to inherent limitations in the method itself. The reliability of the RLGS method depends upon the uniformity of the methylation status of landmark sites such as *Not*I. Two kinds of errors are possible. First, there may be some *Not*I sites that are variably methylated between different mice and the location of the RLGS spot for this *Not*I site is coincident in the RLGS profile with a spot that is variant in a cross being analyzed. Second, a variant spot itself may be subject to variable methylation so that the segregation pattern in backcross progeny is a function of both genetic segregation and variable expression. In the BSS cross there were 10 of 56 loci that were linked to chromosomes but showed excess double recombination events involving a single locus double crossover. The crossover events in these individual loci were B6-specific alleles flanked by S alleles. It is possible that some of these were due to either *M. spretus* polymorphisms or that they were an error of the first kind above, that is variable methylation of a coincident RLGS spot. There were four BSS loci which had single locus double recombinants of S-specific alleles flanked by B6 alleles. These would be consistent with the possibility of the second kind of error involving variable methylation of a variant spot. The basis of these errors will be better determined when cloned resources are available to examine individual loci in specific backcross progeny.

The multiplex nature of the RLGS method makes it an ideal technique for analyzing complex traits and quantitative trait loci. The RLGS loci are distributed to all of the mouse chromosomes in the BSS cross giving a global assessment of segregation for each chromosome region within a backcross. It is also possible to pool DNA from segregating backcross and even intercross progeny that share a common phenotypic characteristic and to analyze the pooled DNA as one sample. The resulting RLGS profile should be heterozygous for all of the unlinked loci but remain homozygous for those RLGS loci that are most closely linked to genes responsible for the selected trait. A similar approach can be used for positional cloning of mutant genes. The pooled sample can be analyzed with a large number of enzyme combinations to increase the number of RLGS loci that are linked. Experimental pools of up to 30 backcross progeny that are homozygous for the *Mdr* locus on chromosome *5* have been analyzed in this manner (Y. HAYASHIZAKI, unpublished data). The resulting RLGS profile did not differ from the BSF_1 profile for most of the RLGS pattern but the B6-specific spots were absent

for those loci that mapped most closely to the *Mdr* locus and there was an increasing intensity of the RLGS spots for B6-specific loci that mapped in increasing distances from *Mdr* on chromosome *5*. Thus, these approaches can identify a large number of candidate loci that map in the vicinity of a mutant gene. By coupling the RLGS identifications with the newly developed methods for recovering spot clones it should be possible to localize a mutant locus and recover genomic sequence clones for the physical region in a highly efficient manner.

Two approaches are available to identify the molecular sequences of the RLGS loci, direct spot cloning (HIROTSUNE *et al.* 1993) and mixing of *Not*I linking clones with genomic DNA (HAYASHIZAKI *et al.* 1992). The spot cloning method has been demonstrated for the recovery of a *Not*I-boundary clones that identify an imprinted locus on mouse chromosome *11* (HAYASHIZAKI *et al.* 1994). The direct spot cloning technique provides a highly efficient method of recovering specific RLGS-spot DNA that can be cloned and characterized with conventional methods such as Southern analysis. Once a series of RLGS related boundary clones have been recovered and characterized they can be made available through conventional clone repositories and they can be sequence analyzed and converted into STSs.

The RLGS methods described in this report have led to the identification of more than 1000 loci in the mouse genome. This number of loci could be doubled easily using the existing backcross resources and RI strains within two weeks. By coupling the RLGS information with the increasing number of SSR markers that are available for the mouse genome, it should be possible to integrate RLGS loci with the composite maps that relate SSR loci to functional genes (COPELAND *et al.* 1993).

We thank KEIKO MATSUI and YUKARI SHIGEMOTO for their technical assistance. This work was supported in part by a Research Grant for the Human Genome Project from the Science Technology Agency Japan, a Special Grant for Promotion of Research from The Institute of Physical and Chemical Research (RIKEN), the Comprehensive 10-Year Strategy for Cancer Control and a Research Grant for Aging from the Ministry of Health and Welfare, a Grant-in-Aid for the Human Genome Program, a Grant-in-Aid for Scientific Research on Priority Areas and other Grants-in-Aid from the Ministry of Education, Science and Culture Japan and a Research Grant from the Uehara Memorial Foundation to YH. This work was also supported by U.S. Public Health Service grants GM33160 and HG00170 to V.M.C., HD28623 to R.W.E., GM18684 to B.A.T.

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Communicating editor: R. E. GANSCHOW