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Genetics

Direct detection and isolation of restriction landmark genomic scanning (RLGS) spot DNA markers tightly linked to a specific trait by using the RLGS spot-bombing method

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ABSTRACT **We have developed a technique for isolating DNA markers tightly linked to a target region that is based on RLGS, named RLGS spot-bombing (RLGS-SB). RLGS-SB allows us to scan the genome of higher organisms quickly and efficiently to identify loci that are linked to either a target region or gene of interest. The method was initially tested by analyzing a C57BL/6–*Gus*^{S} mouse congenic strain. We identified 33 variant markers out of 10,565 total loci in a 4.2-centimorgan (cM) interval surrounding the *Gus* locus in 4 days of laboratory work. The validity of RLGS-SB to find DNA markers linked to a target locus was also tested on pooled DNA from segregating backcross progeny by analyzing the spot intensity of already mapped RLGS loci. Finally, we used RLGS-SB to identify DNA markers closely linked to the mouse reeler (*rl*) locus on chromosome 5 by phenotypic pooling. A total of 31 RLGS loci were identified and mapped to the target region after screening 8856 loci. These 31 loci were mapped within 11.7 cM surrounding *rl*. The average density of RLGS loci located in the *rl* region was 0.38 cM. Three loci were closely linked to *rl* showing a recombination frequency of 0/340, which is <1 cM from *rl*. Thus, RLGS-SB provides an efficient and rapid method for the detection and isolation of polymorphic DNA markers linked to a trait or gene of interest.**

To positionally clone genes of interest whose functions are unknown, efforts have been made to construct genetic maps whose density would be sufficient to link these genes to the physical map (1). Several methods have been used to increase the density of the genetic map of the mouse. (*i*) It has been possible to take advantage of widely divergent *Mus* subspecies and species to identify genetic variation and to analyze this variation in interspecific and intersubspecific crosses. (*ii*) It has been possible to use these crosses to analyze variation and to genetically map loci by a variety of molecular approaches for identifying genetic loci. These include (*i*) polymerase chain reaction (PCR) methods for detecting CA-repeat or simple sequence length polymorphisms (2); (*ii*) the identification of functional genes, gene families, and related sequences by established Southern blot analysis techniques (3); (*iii*) the PCR-mediated representational difference analysis methods (4); and (*iv*) the RLGS method (5). These approaches have been widely applied not only to mouse (2, 5) and human (6) but also other species such as rat (7), zebrafish (8), and cow (9). However, the construction of high-density genetic maps is time-consuming and laborious work. For most organisms, genetic maps are either nonexistent or too rudimentary to allow routine positional cloning. To make positional cloning broadly applicable, one would ideally want a method for directly generating markers that are tightly linked to a mutant of interest without the necessity of a preexisting high-density genetic map.

An approach has been recently proposed called genetically directed representational difference analysis (10). Several DNA markers in the vicinity of the genes of interests have been identified by this method. The method has proven to be effective but the efficiency of isolating DNA markers with genetically directed representational difference analysis that are closely linked to the target loci is not fully established. However, we have established (5) that RLGS methods can be used to produce moderate-density genetic maps of several hundred loci that span the entire mouse genome. In this paper we describe the expansion of this method for efficiently targeting a high density of loci in the vicinity of genes and chromosomal regions of interest. This method uses a targeted focus of analysis to a gene or chromosomal region in concert with several restriction enzyme combinations for RLGS analysis, RLGS spot bombing (RLGS-SB), to identify a high density of genetic markers within a defined genomic region. RLGS-SB does not require sequence information or cloned probes so that it is applicable to virtually any organism. We have evaluated RLGS-SB in three implementations. The first involves using a congenic C57BL/6 strain that carries a 4.2-centimorgan (cM) region of *Mus spretus* chromosome surrounding the *Gus* locus on chromosome 5. The second application uses pooled DNA derived from *M. spretus* and C57BL/6 interspecific backcross progeny. The validity of finding DNA markers linked to a target locus was tested by analyzing the spot intensity of already mapped RLGS loci (5). The last demonstration involves the use of a backcross that was segregating the reeler (*rl*) mutant. The experiment was specifically aimed at finding polymorphisms within an interval of 1 cM of *rl*. Thirty-one variant markers were detected within an interval of 11.7 cM surrounding *rl*. Three of them showed no recombination with *rl* in 340 meiotic events tested.

MATERIALS AND METHODS

Mouse Resources. Congenic strain C57BL/6–*Gus*^{S}, which has the *Gus* region from *M. spretus* (S), was produced by nine

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Abbreviations: RLGS, restriction landmark genomic scanning; RLGS-SB, RLGS spot bombing; cM, centimorgan(s); B, referring to C57BL/6 mouse or allele; S, referring to *M. spretus* mouse or allele; C, referring to BALB/cA mouse or allele.

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generations of backcrossing and selection. Backcross progeny used for the analysis of *Mdr1* locus were produced from C57BL/6 (B) and outbred *M. spretus* by using (C57BL/6 × *M. spretus*)F_1 females × *M. spretus* males (BSS). (BALB/cA-*rl*/*rl* × *M. spretus*)F_1 females (CS) were produced by *in vitro* fertilization, and backcross progeny were produced by breeding the F_1 females and BALB/cA-*rl*/*rl* males (CSC).

Pooled DNA and Fine Mapping. Pooled DNA used for the analysis of *Mdr1* locus was based upon genetic information of a previously analyzed BSS backcross (5). The concentration of DNA from each backcross progeny was carefully measured by spectrophotometer. Then 10 μg of DNA from each of the 20 backcross progeny that were either homozygous ($Mdr1^S/Mdr1^S$) or heterozygous ($Mdr1^B/Mdr1^S$) for *Mdr1* locus was sampled and mixed well by pipetting.

Fine mapping surrounding *rl* was performed with simple sequence length polymorphism, restriction fragment length polymorphism, or RLGS spot mapping (5). Specifically, the 5-hydroxytryptamine 5a receptor gene (*5ht5a*) was amplified (forward primer, 5′-CGTCTCCAGAAAGCAGGTATC-3′; reverse primer, 5′-AAGTCAGGACTAGCACTCGGA-3′), and genetic variation between parental strains was identified by *Eco*RI digestion of the amplified products followed by Southern blot analysis using the PCR product as a probe. The proximal flanking locus, *D5Mit47* (*Mdr1*), was analyzed by using the primer pairs identified for this locus. The pooled DNA samples used for the analysis of *rl* locus were selected from one of two groups; one consisted of 35 backcross progeny homozygous for *rl* phenotype (rl^C/rl^C) and the two flanking markers *D5Mit47* and *5ht5a* (C/C), and the other group consisted of 35 progeny that were heterozygous for these three loci (C/S). Here, 10 μg of DNA from each backcross progeny was sampled and pooled. The chromosomal location of the cloned DNA markers identified in this study was confirmed by genetic analysis of restriction variation by using the progeny that were recombinant between the distal and proximal flanking markers to the *rl* mutation. The linkage analysis was performed by using MAP MANAGER Version 2.6 (11).

RLGS Procedure. The protocol of RLGS Version 1.8 was described elsewhere (12). Eleven enzyme combinations for the first, second, and third digestions were used for the RLGS-SB (see Tables 1 and 2). A second digestion with a restriction enzyme was omitted when *Sse*8387I was used as the landmark because of the relatively frequent restriction sites compared with *Not* I or *Bss* HII.

Quantitative Analysis of RLGS Spot Intensity by PDQuest. The RLGS spot intensity was measured by PDQuest (PDI, Huntington Station, NY). The intensity was calibrated by comparing neighboring nonpolymorphic spots that show two copies of intensity as a control. In both congenic and pooled DNA analysis, we selected the candidate spots showing the greatest degree of differences in intensity between RLGS spots compared.

RESULTS

RLGS-SB with the C57BL/6-Gus^S Congenic Strain. We first applied RLGS-SB for the analysis of a congenic strain of mouse C57BL/6-Gus^S. The C57BL/6-Gus^S congenic strain is genetically identical to C57BL/6 except in a relatively small region surrounding the *Gus* gene. The RLGS profiles of C57BL/6-Gus^S, *M. spretus*, and C57BL/6 were compared to identify C57BL/6-specific spots that were missing from the congenic strain and *M. spretus*-specific spots that were present. Approximately 1000–2000 landmarks of RLGS spots were identified in each gel profile produced by one set of restriction enzymes. A total of 10,565 loci were examined by using seven enzyme combinations (Table 1), although this number includes redundancy to some extent. By using this approach, we detected 120 (66 C57BL/6 specific and 54 *M. spretus* specific) provisional candidate RLGS loci. Of these 120 RLGS loci, 33 (14 C57BL/6 specific and 19 *M. spretus* specific) were mapped to the *Gus* region by using the BSS backcrosses (5). An example of a C57BL/6-specific spot (*D5Ncvs19*) that was missing from the congenic strain and *M. spretus*-specific spots (*D5Ncvs63*, *D5Ncvs64*, and *D5Ncvs65*) that were present is demonstrated in Fig. 1. Combined with the genetic map information of these RLGS loci (*D5Ncvs18* and *D5Ncvs20*) (5), the *M. spretus* contribution to the congenic C57BL/6-Gus^S region was estimated to be <4.2 cM. These analyses identified 33 polymorphic DNA markers within a 4.2-cM region (0.13 cM per marker) on chromosome 5. A total of 21 RLGS gels were required to analyze the RLGS profiles of these seven sets of enzyme combinations. This work was accomplished in 4 days of laboratory effort by using the multiplex electrophoretic system RLGS Version 1.8 (12).

Table 1. RLGS loci that are mapped to the Gus^S region as either C57BL/6-specific spots lost or *M. spretus*-specific spots gained

Enzyme combination	Screened spots, no.	Candidate spots, no.			Mapped spots, no.		
		B	S	Total	B	S	Total
Not I/*Pvu* II/*Pst* I	1,531	8	6	14	1	3	4
Not I/*Eco*RV/*Hin*fI	1,600	14	5	19	4	2	6
Not I/*Bam*HI/*Hin*fI	1,786	12	13	25	3	2	5
Not I/*Pst* I/*Pvu* II	1,057	7	5	12	2	1	3
*Bss*HII/*Pvu* II/*Pst* I	2,545	6	12	18	2	5	7
*Bss*HII/*Bam*HI/*Eco*RV	777	14	8	22	2	4	6
*Bss*HII/*Bam*HI/*Eco*RI	1,269	5	5	10	0	2	2
Total	10,565	66	54	120	14	19	33

Validation of RLGS-SB by Using Pooled DNA. The analysis of congenic strains is an excellent application of the RLGS-SB, but producing congenic strains requires many generations of breeding, which can span several years or decades depending on the organism. An alternative approach to detecting and isolating variant markers that map in the vicinity of a gene of interest has been proposed that uses pooled DNA of backcross mice produced from two different progenitor strains (13). A collection of backcross siblings is produced so that their pooled DNA is homozygous in the region of a targeted gene but heterozygous for the rest of the genome. The design of this strategy indicates that each of the pooled samples should include at least 20 backcross progeny. By using this approach,

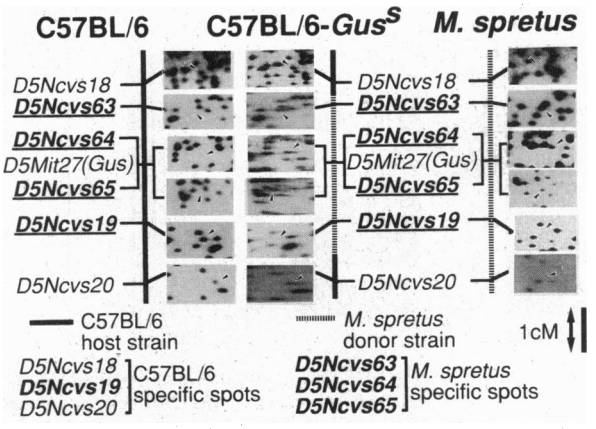

FIG. 1. Representative spots mapped within Gus^S congenic region after screening 10,565 RLGS loci. Each spot locus was magnified from an original RLGS profile and marked with an arrowhead. Only a part of the targeted RLGS loci detected by using the enzyme combination of *Not* I/*Pvu* II/*Pst* I is shown with the established genetic map using RLGS spot mapping (5). The vertical bold and dotted lines depict chromosome 5 derived from C57BL/6 and *M. spretus*, respectively. The underlined 4 RLGS loci are the members of targeted spot loci. The congenic region was estimated to be <4.2 cM (see text).

we evaluated the RLGS-SB for the analysis of pooled DNA. In this experiment, we used the *Mdr1* locus on proximal chromosome 5 as the target gene. Portions of the RLGS profile that have C57BL/6-specific spots are enlarged and shown in Fig. 2 in the sequential order of these spot loci on the genetic map (5). One of the parental alleles is always the S genotype in all BSS backcross progeny as shown in Fig. 2, since the father is *M. spretus.* The other allele is either the B or S genotype from the (C57BL/6 × *M. spretus*)F_1 mother. The intensity of the C57BL/6-specific spots (*D5Ncvs1*) are either null or nearly null intensity for the pooled DNA of $Mdr1^S/Mdr1^S$ mice in the vicinity of the *Mdr1* (0%, 0 of 40 alleles; Fig. 2), because all of the alleles present in the pooled DNA were S genotype. However, the corresponding spots in $Mdr1^B/Mdr1^S$-pooled DNA showed almost half the intensity (48%, 19 of 40 alleles), because the pooled DNA of heterozygotes at *Mdr1* locus was composed of S and B alleles at a 1:1 ratio. Meiotic recombination occurred between *Mdr1* and the spot loci that map distally on chromosome 5 so that an increasing proportion of the $Mdr1^S/Mdr1^S$ pools contain individuals that have C57BL/6-specific alleles for these distant loci. As a consequence, the intensity of C57BL/6-specific spots (*D5Ncvs4, 5, 9, 13,* and *22*) in the $Mdr1^B/Mdr1^S$ DNA decreased while the intensity of these spots increased in $Mdr1^S/Mdr1^S$ pooled DNA. In both pools of DNA, C57BL/6-specific spots converged to a quarter intensity at the most distal locus, *D5Ncvs22* (30%, 12 of 40 alleles in $Mdr1^S/Mdr1^S$ group; and 23%, 9 of 40 alleles in $Mdr1^B/Mdr1^S$ group), which is estimated to map >50 cM from *Mdr1.* This convergence at *D5Ncvs22* is due to the equalization of the genotypic frequency of B and S alleles by meiotic recombination. The population of B/S and S/S alleles in both pooled DNAs depends on the frequency of meiotic events so that the intensity of the spots reflected the distance from the target locus. The intensity of *D5Ncvs4, 5, 9, 13, 22* measured by two-dimensional densitometer (PDQuest) correlated well with the distance from *Mdr1* locus (Fig. 2 and data not shown).

The *M. spretus*-specific spots produced from pooled DNA homozygous ($Mdr1^S/Mdr1^S$) and heterozygous ($Mdr1^B/Mdr1^S$) for *Mdr1* locus showed full intensity and half intensity, respectively, nearest to the *Mdr1* locus (data not shown). As the distance of the RLGS loci from *Mdr1* locus increases, the intensity of *M. spretus*-specific spots on RLGS profiles produced from pooled DNA homozygous and heterozygous for *Mdr1* locus decreased and increased, respectively, to reach the same intensity (75% intensity, data not shown) for the RLGS spots produced by the loci that were most distal on chromosome 5.

These results clearly validated the RLGS-SB using pooled DNA. Moreover, the correlation of spot intensity with genetic distance from the target gene implies that we can estimate its approximate distance between a candidate RLGS locus and the target gene by its intensity. Thus, we can focus our primary attention on cloning those loci whose spots show the greatest differences between the RLGS profiles of the pooled DNA.

Application of RLGS-SB for the Isolation of Polymorphic Markers Linked to *rl*. We used RLGS-SB to detect and isolate polymorphic markers closely linked to the *rl* locus by using CSC backcross progeny. In the course of studies on *rl,* we produced 340 backcross progeny. These were typed for a series of genetic markers on chromosome 5 that identified the position of *rl* relative to these markers. By using this information, we selected two groups, one (group I) that was homozygous for *rl* (rl^C/rl^C) and also for the flanking markers (*D5Mit47* and *5ht5a*, C/C) and the other (group II) was heterozygous for *rl* (rl^C/wt) and for the flanking markers (*D5Mit47* and *5ht5a*, C/S). We screened 8856 RLGS loci by using four enzyme combinations. One hundred eleven provisional candidate spots were detected, and 31 (17 *M. spretus* specific and 14 BALB/cA specific) loci were finally mapped to the region near *rl* locus (Fig. 3). These were mapped with 48 backcross mice that had meiotic recombinations between *D5Mit47* and *5ht5a* by using RLGS spot mapping (Table 2 and Fig. 3) (5). These 31 RLGS loci were designated as *D5Rik87* through *D5Rik117* and their precise locations were determined (Fig. 3). The representatives of these loci are shown to the right in Fig. 3. Theoretically, all *M. spretus*-specific spots within the region flanked with

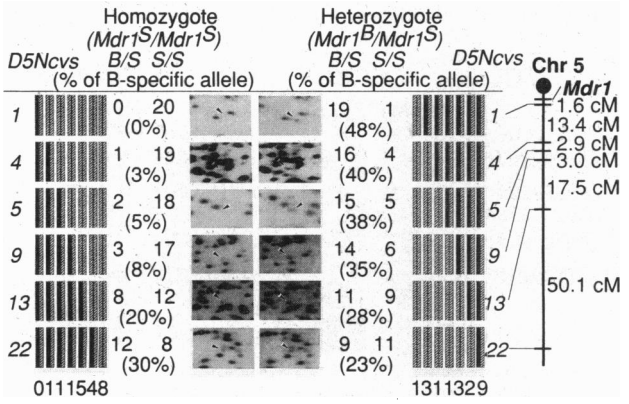

FIG. 2. Behavior of RLGS loci analyzed by using pooled DNA. An equal amount of DNA from two sets of the 20 backcross progeny carrying $Mdr1^S/Mdr1^S$ and $Mdr1^B/Mdr1^S$ genotype were pooled separately and subjected to RLGS. The number under the haplotype figure depicts the number of backcross progeny that showed the indicated haplotype pattern. The location of RLGS loci has been reported (5), and we compared the intensity of these spot loci sequentially in the order of their chromosomal location. The intensity of these RLGS loci correlated well with the genetic distance shown as haplotype figures. PDQuest (PDI) was used to measure the spot intensity. B-specific, C57BL/6-specific. Solid bars, B genotype; hatch bars, S genotype.

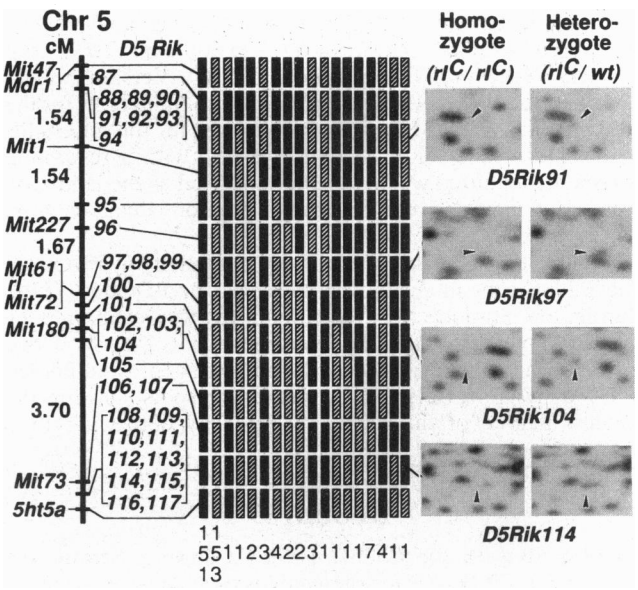

FIG. 3. RLGS spot markers (*D5Rik87–117*) detected by RLGS-SB between DNA markers *D5Mit47* and *5ht5a* are shown on the right of the map of chromosome 5. The genetic distance (in cM) between markers depicted in boldface type on the left of the map was calculated by using 340 backcross progeny. Only the maternal allele of the haplotype figures is shown in the center. Solid bars, C/C genotype; hatched bars, C/S genotype. The number of backcross progeny showing each haplotype pattern is described at the bottom. Four *M. spretus*-specific spots among the 31 RLGS loci mapped within the region are shown at the right as representatives. All the spots from the DNA of homozygotes showed null intensity, and those from the DNA of heterozygotes showed full intensity. The enzyme combinations for detecting these spot loci are shown in Table 2.

Table 2. Pooled analysis of BALB/cA- and *M. spretus*-specific loci linked to *rl* locus on chromosome 5 in (BALB/cA–*rl*/*rl* × *M. spretus*)F_1 × BALB/cA–*rl*/*rl* (CSC) backcross progeny grouped by rl^C/rl^C or rl^C/wild type

Enzyme combination	Screened spots, no.	Candidate spots, no.			Mapped spots, no.			Loci (*D5Rik*-)
		C	S	Total	C	S	Total	
*Bss*HII/*Hin*dIII/*Eco*RI	2108	19	24	43	8	8	16	*87–91, 94, 97, 98, 102–104, 106, 107, 109, 110, 114*
*Sse*8387I/*Hin*dIII	1820	4	16	20	0	5	5	*92, 93, 100, 108, 115*
Eag I/*Eco*RI/*Pvu* II	3191	14	6	20	4	1	5	*101, 105, 111, 116, 117*
Eag I/*Pst* I/*Hin*dIII	1737	7	21	28	2	3	5	*95, 96, 99, 112, 113*
Total	8856	44	67	111	14	17	31	*87–117*

n = 35 in each group.

D5Mit47 and *5ht5a* show null intensity for group I and almost half intensity for group II. By using this criterion, we observed 67 *M. spretus*-specific spots, of which 17 were mapped within this region. A part of these loci are shown in Fig. 3 (four *M. spretus*-specific loci, *D5Rik91, 97, 104*, and *114*) that coincide well with the expected results. The BALB/cA-specific spots should have full intensity for group I and half intensity for group II (data not shown). Altogether, we found 3 RLGS loci (*D5Rik97, 98, 99*) that showed no recombination with *rl* (Table 2). We cloned these three spots by using PCR-mediated (14) and restriction-trapper-mediated (15) spot cloning methods. Subsequent analysis using these clones in Southern blot analysis of restriction variation showed that the segregation of each of these was completely coincident with the segregation of *rl* in 340 meioses (Fig. 4). In summary, RLGS-SB detected 31 distinct polymorphic markers mapped within 11.7 cM surrounding the target locus (0.38 cM per marker) and 3 markers with no recombination for the *rl* locus were isolated.

DISCUSSION

We have demonstrated a technique, based on RLGS for isolating DNA markers that are tightly linked to a gene or trait of interest, named RLGS-SB. In this report, we demonstrated the implementation of RLGS-SB with three projects that targeted loci on mouse chromosome 5. Two projects were performed to evaluate the feasibility of this method and one was used to obtain DNA markers within a close interval surrounding the mutant locus *rl*. In the first instance, we applied RLGS-SB to the analysis of the congenic strain C57BL/6–Gus^S. We found 33 (14 C57BL/6 specific and 19 *M. spretus* specific) RLGS loci that correctly mapped within congenic region out of 120 (66 C57BL/6 specific and 54 *M. spretus* specific) provisional candidate RLGS loci. The congenic region was estimated to be <4.2 cM by using reported RLGS genetic map (5). Thus, the loci identified in this study provide an average density of 1 locus per 0.13 cM, which is well within the average physical size of most large fragment clones.

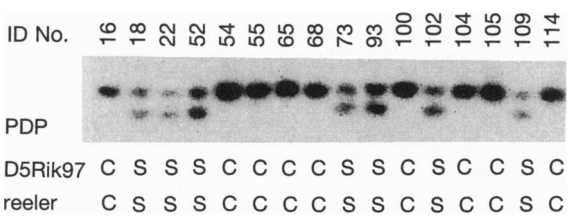

FIG. 4. Southern blot hybridization using the clone of RLGS spot locus *D5Rik97* as a probe, which showed no recombination with target *rl*. This panel shows only a part of the backcross progeny that have a recombination between the *rl* locus and flanking DNA marker. Three micrograms of genomic DNA digested with *Pst* I was loaded onto the 0.8% agarose gel and Southern blot analysis was performed. Genetic variation identified by Southern blot hybridization using the clones for *D5Rik98* and *99* also showed no recombination with the phenotype in 340 meioses with Southern blot hybridization (data not shown). PDP, progeny distribution pattern. ID no. refers to the backcross progeny.

The number of loci identified for this congenic region is plausible in the light of what would be expected from the total number of *Not* I sites (2380 ± 80 sites) (16) and *Bss*HII sites (≈9000 sites; unpublished data) that are estimated for the mouse genome. The identification of RLGS loci is based upon end-labeled restriction fragments that typically result from overlapping cleavages. Thus, both sides of the cleavage site are end-labeled and both parental chromosomes are assumed to carry the same cleavage site. As a consequence, there will be four possible cleavage products associated with each landmark site in the genome. However, if the rate of RLGS variation is ≈50% between B and S alleles, the probability that both sides of a landmark cleavage will be variant is 0.25. Moreover, each RLGS profile identifies only a portion of the total number of end-labeled restriction fragments for any particular enzyme set, and it is possible to derive an estimate for identifying allelic RLGS fragments. Thus, it is likely that some of the identified RLGS spots are either allelic or represent the same restriction landmark, but the total number of independent loci still represents a density of at least 0.18 cM.

The rest of the spots that did not map within the target region were false-positive. A major reason for this apparently large number is that the congenic strain used still had a significant level of *M. spretus* genome that was independent of the *Gus* locus on chromosome 5. We can estimate that the amount of remaining (R) donor genome (expressed in cM) is $R_n = (0.5)^{n-1} \times (LE_{UL} - LE_L)$, where n is the number of generations backcrossed, LE is the length of the total genetic map (1600 cM), UL is unlinked donor genome, and L is the linked donor genetic material. For nine backcross generations, there will be an estimated 6.25 cM of unlinked genome remaining or about double the interval mapped in the chromosome 5 congenic region. Thus, the expected number of unlinked loci, 66 loci, does not differ greatly from the 87 loci that were observed.

RLGS-SB provides an efficient method of identifying the donor strain genes that are present in a congenic strain and provides a direct method of determining the occurrence of both linked and unlinked donor genotypes that remain in the final congenic strain. This method of assessment will become increasingly valuable in characterizing congenic strains that have been constructed in accelerated breeding schemes that use expanded breeding populations in early generations to identify parents that have reduced levels of donor genotype across the genome (17). The RLGS method will be particularly useful for analyzing selected congenic strains to check for unlinked donor genotype that may have been missed by other marker methods.

The validity of using pooled samples of DNA from segregating backcross progeny was demonstrated with the analysis of the chromosome 5 region surrounding *Mdr1*. Chromosome 5 loci that had been identified and mapped previously were detected in these analyses. Moreover, it was possible to identify candidate *M. spretus*-specific allelic spots and C57BL/6-specific spots that were linked to the *Mdr1* locus on the basis

of quantitative levels of spot intensity within the RLGS profile. In addition, the spot intensity measured by PDQuest correlated well with the genetic map distance of the RLGS locus from the target *Mdr1* locus. Finally, we applied RLGS-SB to detect and isolate DNA markers in the vicinity of *rl*. The detection of three RLGS loci that completely cosegregated with *rl* in 340 meioses paves the way for applying RLGS-SB to positional cloning not only in the mouse but also in other organisms whose genetic maps are either nonexistent or too immature to allow linkage analysis.

In the latter case, the boundary clones that are recovered in the spot cloning procedure are from rare-site cleavages in the genome that are typically associated with CpG islands that in turn are indicative of functional gene promoter regions (18). Thus, the RLGS method of analysis is biased toward the identification of functional genes, and the boundary clones for these sequences will frequently crosshybridize with homologous genes in different species (19). By using this feature, it should be possible to recover spot clones that are closely linked to mutations in species that have limited genetic and physical maps and to analyze the comparative map region of the mouse or even human genomes where more complete genetic and physical definition and resources are available. In this case, it may be possible to identify useful candidate genes for a mutational defect that maps to the same region. Alternatively, it should be possible to initiate a search for all of the genes that share the comparative map location and to narrow the positional cloning to a more defined region.

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