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Microsatellite-Directed Selection of Breeders for the Next Backcross Generation By Using a Minimal Number of Loci

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To establish a minimal number of markers for direct selection of candidate mice used for the next mating to produce congenic mice, recombination frequencies of 53 microsatellite loci on chromosomes (chr.) 1 and 19 were examined using 41 N2 mice: the donor strain was BALB/c, and recipient strain was C57BL/6J (B6J) or C57BL/6N (B6N). These markers were spaced at 0.1 to 24.2 centimorgans (cM). Among the 41 mice, B6/B6 homozygosity ranged from 18 to 24 animals (mean, 20; 2 standard deviations, 1.36) for a given locus. There was no difference in recombination frequency between chr. 1 and 19. The recombination frequency of B6J was higher than that of B6N ($P < 0.05$). Various densities of markers, 10 (5 markers/chr.), 8 (4 markers/chr.), and 6 (3 markers/chr.) spaced at 12.0 to 29.3, 9.0 to 45.0, and 24.5 to 53.0 cM, respectively, were selected from the 53 markers, and homozygosity was compared in each mouse. In mice with decreased homozygosity when tested using 53 markers, homozygosity differed depending on the density of the markers. The results suggested that 3 markers/chr. are sufficient for selection of the highest percentages of homozygosity but are not suitable to define mice with lower percentages of homozygosity.

Congenic strains have been used for analysis of the effect of genetic background of mice on established mutations. The backcrossing system between the donor animal and recipient inbred partners has been used to create these congenic strains (4). The traditional protocol calls for 12 backcross generations to obtain congenic mice with < 0.1% donor genome. The "speed congenic technique" is a powerful tool to obtain congenic mice rapidly (8). Because mice with the lowest level of donor genome are used as breeding stock for the next generation, congenic strain founders with < 1% residual contaminating donor genome are produced by the fourth breeding generation. Microsatellite markers have been used to screen the residual donor genome in congenic mice and breeding stocks for the next generation. The higher the density of the markers, the more the status of the background can be analyzed in detail. However, it is not practical to use hundreds of markers for this purpose. Wakeland and colleagues reported computer simulation showing that the contaminating donor genome reached 0.5% at N5 when mice with the lowest level of donor genome were selected for the next mating from 16 males screened by low-density (25-cM spacing) markers (8). In the present study, to prove the simulation, we clarified the recombination frequency of each locus on chromosomes (chr.) 1 (the longest) and 19 (the shortest), compared the recombination frequency between C57BL/6J (B6J) and C57BL/6N (B6N), and determined how many microsatellite loci per chromosome should be monitored.

Materials and Methods

Animals. Four BALB/cA, two B6J, and two B6N mice (age, 7 weeks) were obtained from a commercial supplier (CLEA Japan, Inc., Tokyo, Japan). BALB/cA females were mated with B6J males or B6N males to obtain F1 mice, and F1 progeny were backcrossed with B6J or B6N to obtain N2 mice. Finally 19 (13 male and 6 female) and 22 (10 male and 12 female) N2 mice were obtained from the F1 mice derived from B6J and B6N, respectively. These mice were housed in a conventionally conditioned room at 23 ± 2°C, 55% ± 5% humidity and a 12:12-h light:dark cycle (lights on, 8 a.m. to 8 p.m.), and were given ad libitum access to commercial diet (CA-1, CLEA Japan Inc.). All animal handling procedures were approved by the Central Institute for Experimental Animals, Care and Use Committee.

Health status. Vendor and in-house monitoring reports indicated that the mice were free of the following bacterial, viral and parasitic pathogens. On the basis of serologic test results, mice were free of *Clostridium piliforme*, *Mycoplasma pulmonis*, ectromelia virus, lymphocytic choriomeningitis virus, mouse hepatitis virus, and Sendai virus. *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, and *Salmonella* spp. were not detected by the culture method. Parasite screening indicated that the mice were free of ectoparasites, intestinal protozoa, and pinworms. *Helicobacter hepaticus* was not detected by polymerase chain reaction (PCR) assay.

Genotyping. Genomic DNA was isolated from tails of the mice by using GENEXTRACTOR TA-100 (Takara Shuzo Co., Ltd., Shiga, Japan). The DNA samples were subjected to microsatellite analysis by PCR with 53 microsatellite markers (34 markers on chr. 1 and 19 markers on chr. 19; Fig. 1). PCR was performed as described previously (2). Briefly, each 25-µl reaction volume contained 10 to 100 ng DNA, 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 to 4 mM $MgCl_2$, 2.5 mM of each dNTP, 0.5 µM PCR primers, and 0.5 U *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif.). The tubes were placed in a ther-

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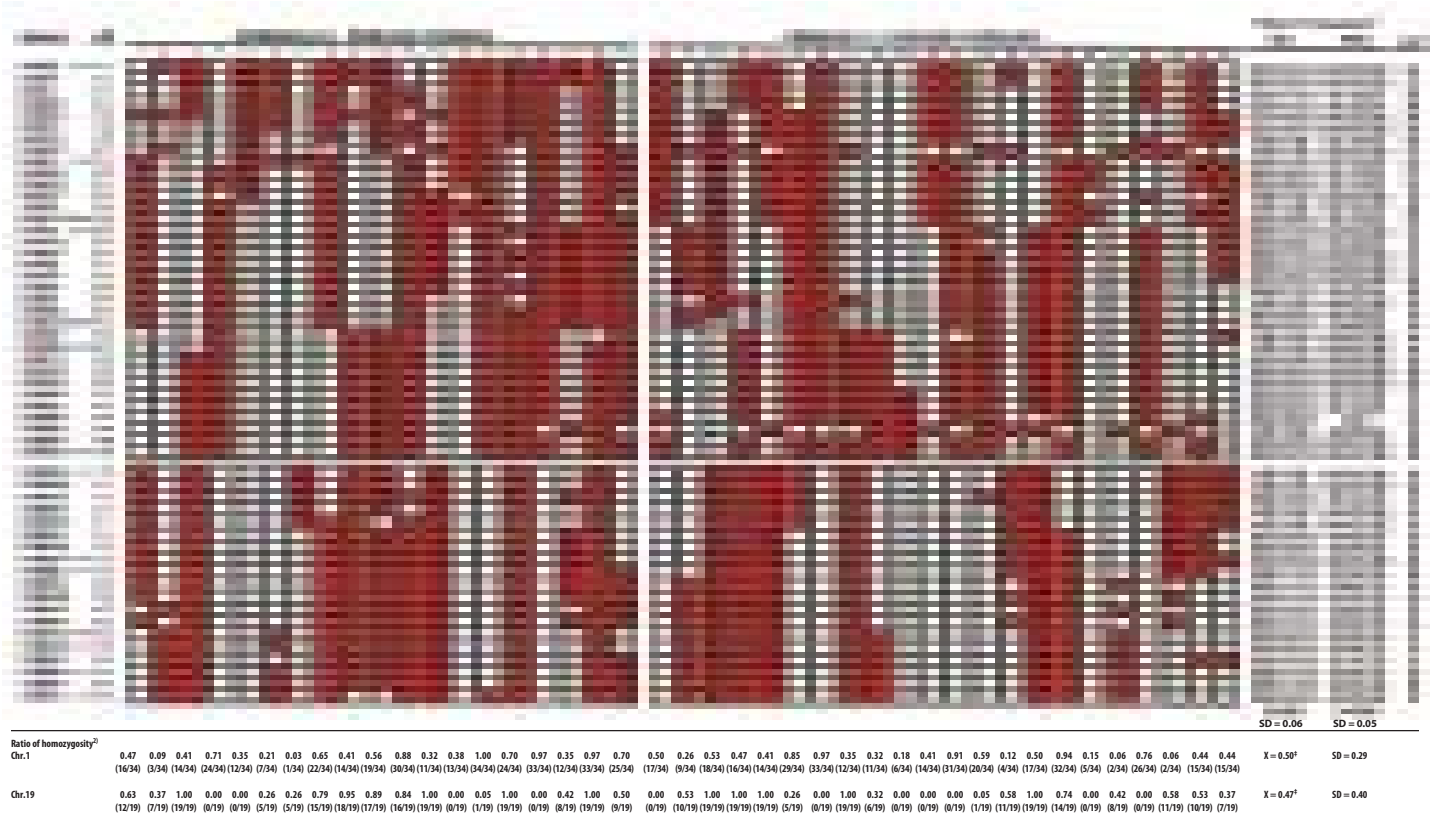

Figure 1. Recombination in tested loci on chromosomes 1 and 19 from 41 N2 (C57BL/6×BALB/c)×C57BL/6 mice.
1) No. of animals showing B6/B6 homozygosity/no. of animals tested. 2) No. of animals showing B6/B6 heterozygosity at a locus (mean, 20; 2 standard deviations, 1.36). 3) No. of loci showing B6/B6 homozygosity/ no. of loci tested. †, $P < 0.05$; ‡, $P > 0.05$.
Five markers (**), 4 markers (***, D19Mit93 and D19Mit71), and 3 markers (** exept D19Mit23 and D19Mit89) were selected for N2 mouse checking.
■B6/B6 homologous type □B6/BALB heterozygous type.

mal cycler (Model TP600, Takara Bio Inc., Shiga, Japan). The thermal profile involved 32 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 30 sec.

Selection of microsatellite markers. The 34 markers from chr. 1 and 19 markers from chr. 19 that showed visible differences between BALB/c and C57BL/6 on 4% agarose gel electrophoresis were selected. These markers were selected to cover the entire chromosome. For evaluation of suitable density of the markers, three loci from chr. 1 (D1mit65, located near the centromere; D1mit155, located near the telomere on the long arm; and D1Nds2, located between these two markers) and three loci from chr. 19 (D19mit93, located near the centromere; D19mit71, located near the telomere on the long arm; and D19mit119, located between these two markers); four loci per chromosome (D1mit65, D1mit178, D1mit218, and D1mit155 and D19mit93, D19mit28, D19mit19 and D19mit71); and five loci per chromosome (D1mit65, D1mit155, D1Nds2, D1mit18, and D1mit399 and D19mit93, D19mit71, D19mit119, D19mit23, and D19mit89) were selected. The percentage of homozygosity of each mouse was compared using these combinations of markers. The locations (cM) of the markers are shown in Fig. 1.

Statistical analysis. Data were compared using Student's *t* test, and $P < 0.05$ was considered statistically significant. Unless otherwise stated, the values in the text are expressed as mean ± 2 standard deviations (SD).

Results

Loci that showed homozygosity in each mouse are depicted in Fig. 1. Among the 41 mice, B6/B6 homozygosity ranged from 18 to 24 animals (mean, 20; SD, 1.36) for a given locus. There was no difference in recombination frequency between chr. 1 and 19. In a comparison of B6J and B6N, a significant difference in recombination frequency was observed ($P < 0.05$).

As shown in Tables 1 and 2, the mouse that revealed the highest percentage of the recipient genome as determined by using 53 microsatellite markers was the same as the mouse that revealed the highest percentage as determined by using selected markers, i.e., 6, 8, or 10 markers selected from chr. 1 and 19 and spaced at 24.5 to 53.0, 9.0 to 45.0, or 12.0 to 29.3 cM, respectively.

Discussion

In this study, we proved that it is possible to select congenic mice showing the highest percentage of the recipient genome by using three microsatellite markers per chromosome. Congenic strains are used widely for analysis of established mutations, but the traditional protocol requires more than 3 years to produce a congenic mouse strain. A "speed congenic technique" by which congenic mice can be obtained in five backcrossings by selecting mice with the lowest level of donor genome and using them for the next mating has been reported (5, 7, 8). To clarify the background of these mice, microsatellite markers commonly are used, and it is necessary to take the number of markers, marker density, and recombination frequency of these animals

Table 1. Percentage of microsatellite loci on chromosomes 1 and 19 that showed B6J/B6J type

No. of markers used	Spacing (cM)	Animal no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
53	0.1-24.2	52.8	18.9	62.3	45.3	22.6	22.6	11.3	69.8	60.4	67.9	86.8	56.6	24.5	66.0	81.1	62.3	37.7	98.1	64.2
10	12.0-29.3	40.0	40.0	70.0	30.0	20.0	50.0	20.0	70.0	70.0	70.0	80.0	60.0	20.0	60.0	90.0	50.0	30.0	100.0	70.0
8	9.0-45.0	50.0	37.5	75.0	37.5	12.5	37.5	12.5	75.0	62.5	62.5	75.0	62.5	25.0	62.5	87.5	50.0	37.5	100.0	50.0
6	24.5-53.0	33.3	50.0	83.3	33.3	16.7	50.0	16.7	66.7	66.7	66.7	66.7	66.7	16.7	66.7	100.0	50.0	33.3	100.0	50.0

Table 2. Percentage of microsatellite loci on chromosomes 1 and 19 that showed B6N/B6N type

No. of markers used	Spacing (cM)	Animal no. 20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
53	0.1-15.9	32.1	35.8	69.8	66.0	62.3	64.2	62.3	58.5	32.1	11.3	26.4	58.5	39.6	28.3	67.9	86.8	9.4	18.9	49.1	24.5	47.2	41.5
10	12.0-29.3	30.0	40.0	70.0	70.0	70.0	50.0	50.0	70.0	50.0	10.0	20.0	50.0	30.0	30.0	70.0	90.0	10.0	30.0	40.0	20.0	50.0	30.0
8	9.0-45.0	37.5	37.5	62.5	75.0	75.0	50.0	50.0	62.5	37.5	12.5	25.0	50.0	25.0	37.5	62.5	87.5	12.5	25.0	37.5	25.0	62.5	37.5
6	24.5-53.0	33.3	50.0	66.7	83.3	66.7	50.0	50.0	66.7	50.0	16.7	16.7	50.0	33.3	33.3	66.7	83.3	0	33.3	50.0	16.7	50.0	33.3

into account when the markers are selected.

In our study, the number of animals that showed B6/B6 type at a given locus ranged from 18 to 24 (mean, 20; SD, 1.36). Jensen-Seaman and coworkers suggested that recombination levels vary among animal species, chromosomes, and regions within chromosomes (3). In the present study, we observed a difference in recombination frequency between B6J and B6N, differences in phenotype between B6J and B6N as were previously reported (1, 6), and differences in the length of PCR products from B6J and B6N by using D1mit356. Differences also could be detected using other markers such as D2mit1 and D19mit100 (data not shown). The results suggest that it is not necessary to consider such variations, as long as microsatellite markers are used for the purpose of selection of candidate mice for production of the "speed congenic mouse."

In this study we used BALB/c mice as a donor strain and C57BL/6 as a recipient strain and proved that three markers per chromosome are sufficient for selection of the highest rate of recipient genome. The background of the animal was not always reflected when low-density markers were used; therefore, to confirm the background strain in the final congenic line, a larger number of markers may be needed.

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