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Novel Mouse Microsatellites: Primer Sequences and Chromosomal Location

Takeshi SUDA,[1,2†] Mitsuru OYANAGI,[1*] Shigeharu WAKANA,[3] Yoshiaki TAKAHASHI,[1] Hideki KANADA,[4] Hiromichi YONEKAWA,[4] Nobumoto MIYASHITA,[5] Toshihiko SHIROISHI,[5] Kazuo MORIWAKI,[5] and Ryo KOMINAMI[1*]

First Department of Biochemistry,[1] and Third Department of Internal Medicine,[2] Niigata University School of Medicine, Asahimachi-doori 751, Niigata 950, Japan, Central Institute for Experimental Animals, Nogawa, Miyame-ku, Kawasaki, Kanagawa 211, Japan,[3] Department of Laboratory Animal Science, The Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyou-ku, Tokyo 113, Japan,[4] and Department of Cell Genetics, National Institute of Genetics, Mishima, Shizuoka 411, Japan[5]

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Abstract

Sixty-nine sequences containing microsatellites were determined by analysis of clones from a pUC118 library of total genomic mouse DNA. These sequences were examined for size variation using polymerase chain reaction and gel electrophoresis. Fifty-one of them showed allelic variations between C57BL/6 and MSM, the two strains used for genetic mapping. Hence, their chromosomal location was determined using a panel consisting of 131 backcross mice that had been typed with 85 anchor loci. The microsatellites were distributed to most chromosomes except for chromosomes 16 and 19. These novel markers with defined locations are useful in linkage and genome mapping studies.

Key words: microsatellite; polymorphism; mouse gene mapping

1. Introduction

Microsatellites are tandem repeats of simple sequences with repeat lengths of six base-pairs or less.[1,2] They occur frequently and are randomly distributed in most eukaryotic genomes.[1] Interest was increased by the discovery that these arrays exhibit considerable polymorphism due to variation in the number of repeat units. Since microsatellites are usually less than 100 bp long, their polymorphisms can be efficiently analyzed by polymerase chain reaction (PCR) followed by agarose gel electrophoresis.[3] Therefore, microsatellites are ideal markers on chromosomes that serve as anchors for genetic analyses.[4,5]

The majority of published mouse microsatellite sequences were first derived from mouse database sequences,[4,6] but recently were identified by analyzing randomly cloned fragments of total genomic DNA.[5,7] To date, most known microsatellite polymorphisms are based on dinucleotide repeats, particularly $(CA)_n$ repeats.[5] Therefore, we aimed at the isolation of novel microsatellite sequences through hybridization to a $(CA)_{10}$ oligonucleotide probe. The present paper describes the primer sequences and chromosomal locations of 51 newly isolated microsatellites.

2. Materials and Methods

2.1. Isolation of clones containing $(CA)_n$ repeats

A random genomic library was constructed in a pUC118 vector from female C57BL/6 DNA. DNA was digested with *Mbo*I and fragments ranging from 400 to 800 bp were recovered from a gel after agarose gel electrophoresis. The DNA fragments were ligated with pUC118 vector DNA that was digested with *Bam*HI. After transformation of *Escherichia coli* DH-5α strain[8] with the recombinant plasmids, approximately 10^3 colonies were plated on agarose gels and transferred to nylon filters. Clones carrying microsatellites were identified by hybridization to end-labelled $(CA)_{10}$ oligonucleotide probes. Hybridization condition was in 5X SSC (1X SSC=0.15 M NaCl, 0.015 M Na-citrate), pH 7.6, 1% SDS, at 42°C for 18 h, and washing was carried out in 1X SSC, 0.5% SDS at 40°C for 30 min. Strongly hybridized colonies were picked up and cultured in L broth.[8] Plasmid DNA was purified from the cells according to a standard method.[8]

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* To whom correspondence should be addressed. Tel. +81-25-223-6161 (Ext, 2250), Fax. +81-25-223-0237

† The first two authors contributed equally to this work.

2.2. *DNA sequencing*

To sequence regions flanking a run of $(CA)_n$ repeats in recombinant plasmid DNA, we used a set of degenerate sequencing primers which annealed directly to $(CA)_n$ or $(GT)_n$ microsatellites.[9] DNA sequencing was carried out using the sequenase sequencing kit (Toyobo Co., Japan) according to the manufacturer's specifications. From the sequence information on one strand obtained by this analysis, a complementary oligonucleotide (forward primer) was synthesized, and this primer was used to sequence the other strand. The second primer (reverse primer) was then synthesized according to the sequence obtained. These primer pairs were designed to prevent self-annealing and achieve equivalence of Tm.

2.3. *DNA samples and PCR experiments*

Oligonucleotides for PCR primers were synthesized on an Applied Biosystems 380B synthesizer (Mountain View, CA, USA). Sequences for genes, and the *Nds* and *Mit* markers used for anchor loci were synthesized according to previous reports.[2,4,6,7]

DNA samples were prepared by a standard method[8] from kidney of four inbred strains, a mouse of *Muridae spretus* and the offspring of an intersubspecific backcross between C57BL/6 and MSM. This MSM strain is derived from Japanese wild mice, *M. m. molossinus*.[10] PCR was carried out in a volume of 10–20 μl under the conditions described by Saiki et al.[11] Samples were processed through 30–35 cycles of amplification consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with the last elongation step lengthened to 10 min. In some cases, Mg^{2+} in the buffer was changed to 1 or 3 mM. Most of the products were analyzed by 4% NuSieve agarose gel or 8% polyacrylamide gel electrophoresis.[3,12] Some were subjected to PCR-SSCP analysis for better resolution of alleles; i.e., the products were heat-denatured and electrophoresed in an 8% polyacrylamide gel containing 5% glycerol.[13]

2.4. *Genotyping and linkage analysis*

Size variations on the amplified region were examined for DNA samples prepared from the C57BL/6(B6) strain and a wild-derived inbred strain, MSM. When a length variation was detected between them, its segregation pattern was examined for the backcross mice that were obtained by mating (B6 X MSM)F_1 females to MSM males. The mapping panel consisted of 131 mice which were already typed with 85 anchor microsatellite loci spanning the entire genome. Some microsatellites were typed for all the mice, but most of the microsatellites were typed for only 61 mice.

Genetic distances are calculated and given in centiMorgans(cM). Lod scores were estimated as $\log_{10}[r^x(1-r)^y/(0.5)^{x+y}]$, where r is the recombinant fraction, x is the number of recombinants, and y is the number of nonrecombinants. A lod score of 3.0 or greater is taken as evidence for linkage.

3. Results

3.1. *Isolation and characterization of microsatellites*

Bacterial colonies harboring recombinant plasmid DNA were screened for the presence of $(CA)_n$ repeats, and 146 colonies were isolated. Plasmid DNA was purified from 97 of the clones and subjected to sequence analysis. According to the sequence information obtained, a PCR primer pair flanking the $(CA)_n$ repeats was designed and synthesized to 72 microsatellites and each pair was examined for optimizing the PCR reaction.

The primer pairs were tested to determine whether they revealed variation between C57BL/6(B6) and MSM, the two strains used for genetic mapping. These strains were chosen because they belong to different subspecies and are likely to show a high degree of variation, but they are so closely related that F_1 progeny of both sexes are fertile. Sixty three primer pairs produced discrete products of the expected size, 49 of which showed variation. In the course of this study, we obtain two microsatellites consisting of trinucleotide repeats which also showed variation. These microsatellite markers are designated as a *Jpk* series and are listed in Table 1. The other 14 markers did not exibit variation between the two strains. The primer pairs were further examined for size variations for DBA/2, C3H/He and a mouse of *M. spretus* (Table 1).

3.2. *Mapping novel* Jpk *markers*

Fifty-one *Jpk* markers were typed in 61 or 131 backcross mice that were obtained by mating (B6 X MSM)F_1 females to MSM males. In parallel, a well-spaced collection of 85 authentic genes and markers (*Nds* and *Mit* markers)[4–7] were typed to locate *Jpk* markers relative to the existing genetic map.[5,14] Comparison of their strain distribution patterns allowed us to define the map position of *Jpk* markers (Fig. 1). The positioning of the markers suggests that they may be useful for linkage analysis.

4. Discussion

We have isolated and mapped 51 novel microsatellite markers of the mouse. The majority of the size variations of these markers were detectable with agarose or acrylamide minigel electrophoresis, but some showed a difference only by SSCP analysis.[13] In all those cases, however, analysis of the PCR products did not require the use of radioactive labeling. When we started this study, a few hundred markers were available. Very recently, however, four thousand microsatellites have been identified by an MIT group,[15] which provide a map with

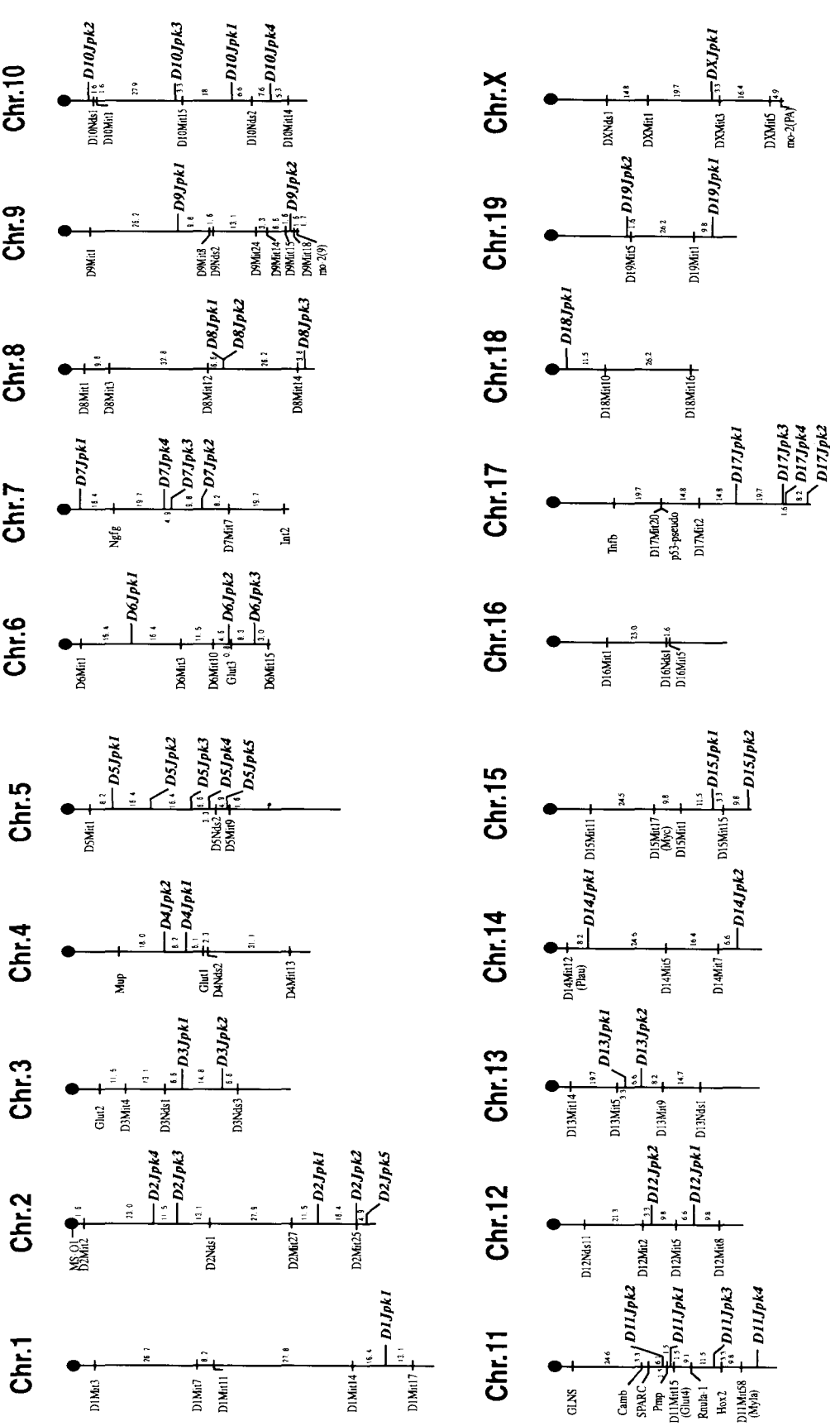

Figure 1. A genetic linkage map of the mouse. Chromosomes are illustrated by vertical lines and centromeres are indicated by filled circles. Anchor loci used in this study are indicated to the left side of each chromosome and new *Jpk* markers to the right. Genetic distances between markers are indicated in centiMorgans (cM). The order of *Jpk* microsatellite markers relative to the map[14] is supported by a LOD score of at least 5.2.

Table 1. Novel *Jpk* microsatellites of the mouse.

No.	Locus name[a]	Primer Sequences	PCR Product[b] Size(bp)	PCR Condition Temp.	Cycle	Mg^{2+} [e]	Size Variation[c] D2.	MSM.	B6.	SPR.	C3H	4%NuSieve[d] 8%PAGE or SSCP
1	D1Jpk1	AATCAGACACTACACCACAG TGTGGGAAGATGTGAACTTAC	155	53	35	1.5	2	3	2	1	1	PAGE
2	D2Jpk1	CCAACATCCTTCTTCAGCATCT CCCATAGGTATTTGTCTGTAATG	165	58	35	1.5	B	B	1	1	1	NuS
3	D2Jpk2	AACAAAAGAGAGAGCAGA GTCGTCAGGAAGCCTGTCTAT	150	52	35	1.5	1	1 1	1 2	1	1	PAGE SSCP
4	D2Jpk3	TGTATTTAGATGACCTCATTTGAG TGTTACTGACCAGCTAAAATGA	135	45	35	1.5	2	3	1	3	2	PAGE
5	D2Jpk4	AAATGGACACCACAAATAGGCCAG ACAAATACACAGACACAGAGATGC	180	55	35	1	2	1	2	1	2	PAGE
6	D2Jpk5	TGCTAGGTAGATGACTCTGTCAGT ACAAGGTTAATAATCATGGTA	210	55	35	1.5	2	1	2	2	2	PAGE
7	D3Jpk1	GCTCCAGGGAATTTGATGCCT CGCCTCATTGACAAGTGCTAA	245	58	35	1.5	4	1	2	4	3	PAGE
8	D3Jpk2	TTGAAATATCTGCCCACGGC TGGAAGGAAAGAAGTGACTTG	150	58	35	1.5	1	2	1	1	1	NuS
9	D4Jpk1	CAAGTCCTGCCTGGTCTATAA ACCAGGGTTATCCACTCCAT	220	58	35	1.5	2	4	1	3	1	PAGE
10	D4Jpk2	TTACTCTTTATGGTCAAAAGTAAG CACACATTCATTCAAATCTGTCTT	150	49	35	1.5	2	1	3	4	2	PAGE
11	D5Jpk1	TCTTCCAAAGAATATCTGTCA CTGTTCCCATTCAGAGCTGTA	140	55	35	1.5	1	2	1	1	1	NuS
12	D5Jpk2	CAGGTCCCAGCACTCAGCAT CCTGGGTGGGGGCCAGAAGA	140	58	35	1.5	3	5	1	2	4	NuS
13	D5Jpk3	GCACCCACACAGCTAGTGAG CTCACATGTGTACTGTTGTCT	100	55	35	1.5	1	B	1	2	1	PAGE
14	D5Jpk4	CACATGACCAGGATTCTTAC GATATCAGATGTTGTCAACTTGAC	150	55	35	1.5	4	3	2	1	4	PAGE
15	D5Jpk5	GATCTGCTTATTACTTTCAGT CTCCACATGTGTGCTGTGTGGTAC	130	55	35	1.5	1	2	1	2	1	PAGE
16	D6Jpk1	ATCTGATTTGTTATTCCCTCA GGATTCTATCACCCAGT	180	55	35	1.5	1	2	1	3	2	PAGE
17	D6Jpk2	ACCCAGGGACACACATATAA CACTGTGAATATAGAAAGCAC	145	58	30	1.5	2	1	3	1	4	NuS
18	D6Jpk3	CAGACTGAAGCAGACTGGATT CAGAATCATGGCCTGTATTTCT	200	55	35	1.5	2	1	2	1	2	PAGE
19	D7Jpk1	ATATATGTGAACCTAAGGGAATCT AGCATATATGGTATGTGTATGCAG	110	55	35	1.5	3	2	1	1	2	PAGE
20	D7Jpk2	GATCTGATATTCTTTGCTGGCC CGTTTGAAGGAGGATTATAGTGA	200	58	35	1.5	1	1	2	1	1	PAGE
21	D7Jpk3	CATTCTGACAGTAATGGACTCC CTGCTGTCTTGCATTCAGCAT	150	55	35	1.5	1	1 2	1 1	1	1	PAGE SSCP
22	D7Jpk4	CATGTTTTCAAGAAAGGACAGTGA TTTCTGAAATCTTCATGGGTATAC	180	58	35	1.5	3	5	1	4	2	NuS
23	D8Jpk1	CAATTCAAAACGGAGTAGTTCCT AATGTTTTCCTCCCTGCCTCT	160	55	35	1.5	3	1	2	3	2	NuS
24	D8Jpk2	ATGGAACCTGAGGACGACACA CTATGCAGTAATACTGTGTCTCA	150	55	35	1.5	2	1	2	3	2	PAGE
25	D8Jpk3	GAATTCAAGCACTCACATAAACAC ACTGTGGAGTTACTGAGGCA	130	58	35	1.5	4	2	3	1	5	PAGE
26	D9Jpk1	AGGATTTCAGTAACTCCGCT CACATACTCATACATATGTGC	100	48	35	1.5	3	1	2	4	3	PAGE
27	D9Jpk2	GATCTTGTCTTCAACCTTTAATTC CCAATACCACCCCATGATTTTGTA	150	55	35	1.5	1	3	1	4	2	PAGE
28	D10Jpk1	TCCTACTACATTTAGACTGCCT CTAGAGAACTGCATAGGAATATATG	100	58	35	1.5	1	2	1	3	1	PAGE
29	D10Jpk2	TTCTGATAGCTGGAGGCTAGA TATATCACCTGCTGCTCATG	140	58	35	1.5	2	1	2	2	2	PAGE
30	D10Jpk3	TCAAAGCTCATTTATGTCTTCCC	135	55	35	1.5	3	2	3	1	3	PAGE

Table 1. Continued

No.	Locus name[a]	Primer Sequences	PCR Product[b] Size(bp)	PCR Condition Temp.	Cycle	Mg^{2+} [e]	Size Variation[c] D2.	MSM.	B6.	SPR.	C3H	4%NuSieve[d] 8%PAGE or SSCP
		TACACACAAGCACATGTACCC										
31	D10Jpk4	CTGAATCAAAATGGCCTCCTT TGAGAGGCATCCACGAGCAT	160	57	35	1.5	3	2	3	1	3	PAGE
32	D11Jpk1	TCTTTCATTGGGATGAGCCGTTTTAG CATCCCTAATCCTCCAGTGTTTTG	120	53	35	1.5	1	2	1	3	1	PAGE
33	D11Jpk2	CAGCTGTGACTGGGGCAGTT GAACACATGGGGTCTTCGTAT	110	61	35	1.5	2	3	2	1	2	PAGE
34	D11Jpk3	GTGCTGTAGCACATGCGTCCCCAT TACTATGTTTAGGCATAGTACACG	300	58	35	1.5	3	2	3	1	3	NuS
35	D11Jpk4	GGTAGGCCATATCACAGTGA CACCTGGCACCTGAGTTTGC	220	60	35	1.5	4	3	4	1	2	SSCP
36	D12Jpk1	TGTAGARGTGTATATGTGTGGGT ATAAACGAGAACATCTACCTGG	155	58	35	1.5	1	3	2	3	1	PAGE
37	D12Jpk2	GGACTCCACAAAAAGCTGTGA TAGCAATTACATATAGTGGCTTG	160	58	35	1.5	2	1	2	1	2	NuS
38	D13Jpk1	GGTCTATACTGCTTACCACCAGCT CAGCTGTCAGAATCATCTCAAGGT	145	55	35	1.5	1	2	1	3	1	PAGE
39	D13Jpk2	AATCTGCCTGATGCTTACCCAGAT TTCCGTCAGAGCCACACCCTACAG	200	55	35	1.5	3	2	3	1	2	PAGE
40	D14Jpk1	CCTTGTGGCAAAGCTGACAGCT CACTTAAATAGTATTCTAGAACTGA	250	45	35	1.5	1	2	1	1	1	PAGE
41	D14Jpk2	GATCCTACCCCAAATTGTCC GATCTGGAATATACATCTTTTAAA	120	58	35	1.5	1	2	1	3	1	PAGE
42	D15Jpk1	CCAGTGGAGAGGACCTTCATT GTGTTGAGAAGGCTCAGGTGA	200	58	35	1.5	2	1	2	3	3	PAGE
43	D15Jpk2	CACCCTGCACATAAACATGTG CTAGTAGAGCACCTATGTGTG	200	58	35	1.5	1	B	1	1	1	NuS
44	D17Jpk1	CACAGGGAAGAAGAATAATCAG GAAGAATTATGTGATACCTAAATATG	150	58	35	1.5	1	1 1	1 2	1	1	PAGE SSCP
45	D17Jpk2	TCTCGAGTGCCAGGGCTGCT CCTTGCTTCTCAGAAGCTAAAC	160	45	35	1.5	3	5	4	1	2	PAGE
46	D17Jpk3	GATCTGCCTGACTTTTTCTCC GAGAACTGACTTCCACAGCTT	160	55	35	1.5	2	3	2	1	1	NuS
47	D17Jpk4	TGGACCACTGACTTCCATGTATAG TCTTAGATGTTAAATATCTTTGAC	120	49	35	1	2	1	2	2	2	PAGE
48	D18Jpk1	CATGCTTACGGTCTGGACAA AGGAAAGTCACAAACCAGTTC	200	45	35	1.5	3	1	4	1	2	NuS
49	D19Jpk1	CTAAGTACATCTTTGACACTCC TGGTCCTTCTGTGCTCTTTGG	160	58	35	1.5	3	4	2	3	1	PAGE
50	D19Jpk2	GATCTTTGAGGCTCCTCACTC CAGGCGACTGAGAGACCTGT	155	55	35	1.5	1	3	2	3	1	PAGE
51	DXJpk1	AAGAGTTAACATGGTCTATGTGTCT GATGACCATATCCCACAAATGG	155	58	35	1.5	2	1	3	B	2	NuS

[a] Chromosomal location was determined by linkage analysis. The map position of *Jpk* markers is shown in Fig. 1. Two microsatellites, D2*Jpk*3 and D6*Jpk*2, consist of $(CCT)_n$ and $(CTT)_n$ repeats, respectively. [b] The PCR product size was estimated by comparison of the mobility of products with those of standard size markers. The number shown is that observed for the C57BL/6 allele. [c] The strain(s) giving the smallest product size is numbered 1; strains showing product sizes larger than the smallest size are given numbers from 2 to 5 in order of size. B indicates no PCR products generated. [d] Recommended methods to detect size variations are abbreviated: PAGE, 8% polyacrylamide gel; NuS, 4% NuSieve agarose gel; SSCP, single-strand conformation polymorphism.[13] [e] The concentration is expressed in mM.

an average spacing of 0.35 cM between markers. Since more markers are still required for dense linkage maps that facilitate positional cloning of genes of interest, this set of *Jpk* microsatellites will contribute to mouse genome analysis.

We made a mapping panel consisting of 131 backcross progeny, which had been typed with 85 anchor loci.[14] This panel provided a rapid means of mapping the *Jpk* microsatellites. The accuracy of information on their map position was estimated by statistical analysis; two-point linkage analysis of *Jpk* markers and flanking anchor loci gave a lod score of 5.2 or greater for all *Jpk* markers, indicating that the relative position of the novel markers are reliable although distances among the genes and the

markers should be taken as a very approximate guide.

As a result of this study, we had a new mapping panel that was eventually typed with 136 markers. The usefulness of this panel has been demonstrated by several mapping experiments of repetitive sequences and genes. The studies demonstrated that a hypervariable minisatellite, Pc-2, is mapped to chromosome 6,[16] and a minor satellite variant, MS-O1, is located at the centromeric region of chromosome 2.[17] A putative tumor suppressor gene, *mgl-1*, and a gene encoding tyrosine kinase, *srm*, are mapped to chromosomes 11[18] and 2,[19] respectively.

In addition, microsatellites are valuable for studies of loss of heterozygosity (LOH) in murine tumors, because DNA polymorphisms detected with these markers are useful in recognizing LOH. *Jpk* and other microsatellites were applied to a LOH analysis of 505 cells derived from an F_1 mouse, a mouse model used for metastasis studies.[20] Clear data on LOH were obtained, suggesting that mouse chromosome 16 may harbor a gene(s) involved in metastasis.[12] Thus, the utility of microsatellites in LOH analysis as well as genetic mapping is well established.

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