Comparative Medicine
Copyright 2004
by the American Association for Laboratory Animal Science

Vol 54, No 5
October 2004
Pages 524-527

Microsatellite Analysis in FVB/N Mice

Patricia V. Basta, PhD, Sarah P. Whitmore, Karen B. Basham, and Carol C. Whisnant, PhD*

The purpose of the study reported here was to identify, by size, a set of microsatellite markers for use in diagnostic genetic monitoring of FVB/N mouse colonies. A large panel of microsatellite markers were chosen on the basis of their high degree of allelic variability. These markers were then tested for their ability to amplify well under a standard set of polymerase chain reaction analysis conditions and to present an easily identifiable band on an agarose gel. From this panel, we chose at least one marker on each chromosome that amplified well under our standard high-throughput conditions. Using this approach, we identified the allele sizes for 27 microsatellite markers in the FVB/N strain of mice. Each autosomal chromosome and the X chromosome were analyzed using at least one locus marker. We have determined a precise size for FVB/N microsatellite alleles, as opposed to a description of size in relation to that of a known allele.

The FVB/N strain of mice was originally derived in 1935 from an outbred colony of Swiss mice at the National Institutes of Health (NIH). From this original outbred colony, two lines were derived that were selected either for resistance (HSFR/N) or sensitivity (HSFS/N) to challenge with histamine after exposure to a pertussis vaccine. Later, at the eighth inbred generation in the early 1970s, the HSFS/N strain was determined to carry the *Fv1*^{b} gene for sensitivity to the B strain of Friend leukemia virus. Mice homozygous for *Fv1*^{b} sensitivity were then inbred as FVB mice without regard to histamine sensitivity.

Since the description by Taketo and co-workers (14) of the superiority of the inbred FVB/N strain to other alternatives for the production of transgenic mice, this strain has been used more and more for the production of transgenic strains by the government, commercial breeders, and academic researchers. Therefore, because this strain has become so important for the biomedical research community, it is important that genetic quality control be maintained in the various FVB breeding colonies. The objective of the study reported here was to determine and describe the simple sequence length polymorphism (SSLP) or microsatellite analysis for 27 microsatellite markers in the FVB/N colonies maintained by the National Toxicology Program of the National Institutes of Environmental Health Sciences, by the Biological Testing Branch of the Frederick Cancer Research and Developmental Center (NCI), at the National Institutes of Health Genetics Unit (Bethesda, Md.), and by a commercial breeder. These 27 markers are distributed so that at least one marker on each autosomal chromosome and the X chromosome is used. Although 441 SSLP markers in the FVB strain of mice have been analyzed (7, 9, 10), to the authors' knowledge, the sizes (in basepairs) of the amplified microsatellite markers have not been defined. Previously the sizes of the FVB alleles were described as either within a range or as smaller or larger than the size of a known allele. Fourteen of the 27 markers that we analyzed for FVB mice in this relational manner have been described in literature, whereas the other 13 microsatellite markers have not previously been described to our knowledge.

Simple sequence length polymorphisms (SSLP), also called microsatellites, are simple sequence repeats of variable length that are randomly distributed throughout mammalian genomes (3, 5, 13, 15, 17). The abundance of these repeats and their varied polymorphism make these microsatellite markers highly useful for genetic monitoring. Primer sets for the flanking regions of these repeat sequences have been developed for mouse and rat genome-mapping studies, allowing these markers to be easily amplified by use of polymerase chain reaction (PCR) analysis (1, 8). The advantages of using microsatellite markers for genetic monitoring of rodents strains are as follows. This type of analysis is straightforward and easy to perform. Large panels of primer pairs have been identified, allowing analysis of markers on all chromosomes. In addition, this method has, in some instances, been documented to be more sensitive than alloenzyme genetic monitoring analyses (19). Finally, one of the biggest advantages is that necropsy tissue is not needed.

The microsatellite markers for this investigation were chosen on the basis of their high degree of allelic variability, and the ease to which amplification occurs under a standard set of high-throughput PCR conditions. Attempt was not made to optimize PCR conditions for primer pairs that did not perform adequately on first screening. Basically we chose primer pairs that produce a scorable marker under a standard set of laboratory (PCR) conditions, rather than attempting to optimize conditions for any particular primer pair.

We were successful in amplifying markers with high allelic variability; of the 27 microsatellite markers we analyzed, 10 of 27 displayed an FVB allele with a different-sized marker from C57BL/6 and C3H/N mice, and the other 17 markers were different from those of at least one of these two strains. Finally, we analyzed, on average, 93 animals with each marker (range, 4 to 131); these animals were from five breeding colonies, and nearly half were transgenics. The 27 markers that were analyzed did not have size differences on the basis of our criteria, being within 5% of the mean for each marker in our base FVB/N strain among these five groups of FVB/N mice.

Received: 2/24/04. Revision requested: 3/30/04. Accepted: 6/14/04.
Proteomics and Genomics Applications, Science and Engineering Group, RTI International, P.O. Box 12194, Research Triangle Park, North Carolina 27709-2194.
**Corresponding author.*

Materials and Methods

Materials. Agarose (Cambrex, Rockland, Maine), ethidium bromide (Promega, Madison, Wis.), MapPair primers (Invitrogen, Carlsbad, Calif.), molecular mass markers (Promega), nucleotide mix (Promega), platinum *Taq* polymerase, 10X PCR buffer (Invitrogen), sterile water (Sigma, St. Louis, Mo.), and Tris acetate EDTA (TAE) buffer 40X (Promega) were obtained.

Animals. All procedures involving animals were approved by the appropriate IACUC. Tail tips obtained from mice of FVB/N colonies maintained by the National Toxicology Program (NTP) of the National Institutes of Environmental Health Sciences, by the Biological Testing Branch of the Frederick Cancer Research Cancer Research Development Center (National Cancer Institute [NCI]), at the NIH Genetics Unit (Bethesda, Md.), and by a commercial breeder were used to isolate DNA from which microsatellite analyses were performed. All colonies tested free of Sendai virus, pneumonia virus of mice, mouse hepatitis virus, mouse polio virus, reovirus type 3, epizootic diarrhea of infant mice, *Mycoplasma pulmonis*, polyoma virus, mouse pneumonitis virus (K), mouse cytomegalovirus, lymphocytic choriomeningitis virus, ectromelia virus, and mouse parvovirus. All colonies tested free of Hantaan virus, mouse adenovirus, mouse thymic virus, and *Encephalitozoon cuniculi*, except those from the NIH, which did not list test results for these agents. All colonies tested free of cilia-associated respiratory bacillus, except those from Taconic Farms (Germantown, N.Y.), which did not list test results for this agent. All colonies tested free of lactate dehydrogenase-elevating virus, except those from the NCI and NIH, which did not list testing for this agent. All colonies tested free of minute virus of mice, except those from the Charles River (Raleigh, N.C. and Kingston, N.Y.), which did not list test results for this agent.

The Charles River, NCI, and Taconic colonies tested free of *Bordetella bronchiseptica*, *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Salmonella* spp., *Streptobacillus moniliformis*, *Helicobacter hepaticus*, *H. bilis, Helicobacter* spp., *Klebsiella pneumoniae*, *K. oxytoca*, *Pasteurella multocida*, *P. pneumotropica*, *Pasteurella spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and β-hemolytic *Streptococcus* spp. Taconic colonies also tested free of *Pneumocystis carinii*, *Haemobartonella muris*, *Eperythrozoon coccoides*, *Clostridium piliforme*, and *Corynebacterim kutscheri*. The NIH colonies tested positive for *H. bilis* and *H. hepaticus* and negative for *Salmonella* spp.

Mice of all colonies were tested for some of the following endo- and exoparasites, with no positive results reported: *Giardia* sp., *Spironucleus* sp., *Syphacia obveolata*, *S. muris*, *Aspicularis tetraptera*, *Hymenolepis* spp., *Heterakis spumosa*, *Trichuris muris*, *Radfordia ensifera*, *R. affinis*, *Myobia musculi*, *Mycoptes musculinus*, *Polyplax* spp., *Trichoecius romboutsi*, *Gyropus ovalis*, *Gliricola porcelli*, *Ornithonyssus* spp., *Demodex* spp., coccidia, trichomonads, *Hexamastix* spp., *Entamoeba* spp., *Psoregates* sp., *Notoedres* sp., *Liponyssus* sp., *Klosiella muris*, and *Eimeria* sp.

Isolation of DNA. The DNA from the tail tip of adult mice was extracted according to the method of Truett and co-workers (16). Tail tips tested were obtained from most of the animals of the FVB/N colonies maintained by the NTP and were collected at the NTP site according to approved IACUC protocols from each institute. Tail tips were then sent on ice to our laboratory at RTI International. Additional tail tips from FVB/N animals from additional government sources (NCI) and commercial breeders were collected after sacrifice of the animals with permission from the appropriate institutes. Briefly, approximately 2 mm of tail tissue was removed from the tail tip and placed in a 0.65-ml tube containing 75 µl of alkaline lysis buffer (25 m*M* NaOH, 0.2 m*M* disodium EDTA [pH 12]). The sample was incubated at 95°C for 35 min, then was cooled to 4°C and placed on ice. Seventy-five microliters of neutralizing reagent buffer (40m*M* Tris-HCl in water without adjusting the pH) was then added to the tube and was mixed well. The DNA was aliquoted and stored at –20°C.

Polymerase chain reaction (PCR) amplification. Two micoliters of DNA was pipetted into the appropriate well(s) of a 96-well polycarbonate V-bottom plate. The DNA was allowed to dry in the wells while reaction master mixes were prepared. The amplification protocol consisted of a 2-min, 95°C denaturation step followed by 30 cycles at 94°C for 45 sec, 57°C for 45 sec and 72°C for 1 min, with a final extension step at 72°C for 7 min. Master mixes consisted of 1X PCR buffer, 0.2 m*M* nucleotide mix, 1.5 m*M* $MgCl_2$, 0.2 µ*M* MapPair primers, 0.24 U of platinum *Taq* polymerase (10 µl of master mix per reaction was used).

Gel analysis. After PCR amplification, 2 µl of a 6X loading buffer (0.25% bromophenol blue [wt/vol], 0.4% Orange G [wt/vol], and 15% Ficoll in water) was added to each reaction well. Eleven microliters of the loading dye/reaction product mix was analyzed by use of electrophoresis on a 3% agarose gel (MetaPhor agarose) in 1X TAE buffer (diluted from a 40X concentration of TAE buffer) containing 27 µl of ethidium bromide (10 mg/ml). Running conditions were 90 V for 3.5 to 4 h. Molecular mass markers, consisting of a 25-base pair (bp) and 100-bp ladder, were analyzed along with the test PCR reaction mixes.

Microsatellite size analyses. Digital images of the agarose gel(s) were obtained directly, using the Bio-Rad Gel Doc 2000 (Hercules, Calif.) with an 8-bit charge-coupled device (CCD) camera to capture images in real time and allow accurate imaging and focusing of the image using an 848 zoom lens and a manual aperture.

Microsatellite size determination. Bio-Rad's *Quantity One* image analysis software was used to determine the size (bp) of each band representing a PCR microsatellite reaction. Molecular mass markers on the gel were used as reference standards for determining size.

Results and Discussion

The study reported here involved use of a standard semi-high throughput agarose gel electrophoresis method to evaluate the size of 27 FVB/N microsatellite marker alleles and to compare these sizes to those determined for C57BL/6N and C3H/HeNMTV mice, using the same amplification and electrophoretic techniques. This method has been shown by us and others (10) to have the analytical power to distinguish alleles that have a minimum of a 6-bp size difference. Using the aforedescribed procedures, we have been able to maintain an intragel variability (coefficient of variation) of < 2% and an intergel variability of < 3%, depending on the particular microsatellite marker being evaluated. In our hands as in those of others (2, 11), the larger alleles have higher variabilities.

The size determinations for 27 FVB/N microsatellite markers are detailed in Table 1 and are compared with the sizes that we routinely obtain for C57BL/6N and C3H/HeNMTV mice. Fourteen of these 27 markers have been analyzed and reported in lit-

Table 1. Microsatellite marker sizes of FVB compared with C57BL/6N and C3H/HeN mice

Locus Strain	FVB	C57BL/6N	C3H/HeN
D1MIT17	177 (< C3H; > BL/6)*	173 (170)	187 (183)
D2MIT17	243 (NP)	208 (205)	226 (220)
D2MIT458	99 (NP)	125 (122)	99 (98)
D3MIT203	149 (> C3H)	155 (154)	140 (138)
D4MIT15	295 (NP)	294 (279)	361 (329)
D5MIT79	145 (NP)	107 (106)	106 (106)
D5MIT240	177 (< C58)	155 (156)	175 (176)
D6MIT102	184 (NP)	148 (146)	126 (126)
D7MIT323	108 (NP)	119 (116)	144 (142)
D8MIT45	136 (NP)	125 (122)	132 (132)
D8MIT215	164 (> C3H**; < BL/6)	185 (182)	164 (164)
D9MIT18	218 (> BL/6)	185 (180)	218 (210)
D10MIT233	112 (< BL/6)	135 (130)	112 (108)
D11MIT20	132 (< C3H; > BL/6)	114 (116)	147 (150)
D11MIT236	83 (NP)	107 (106)	109 (108)
D12MIT12	158 (< C3H; > BL/6)	149 (146)	167 (164)
D12MIT124	150 (NP)	149 (144)	128 (124)
D13MIT13	154 (> C3H)	154 (148)	137 (138)
D14MIT228	144 (NP)	182 (174)	142 (138)
D15MIT245	105 (NP)	121 (119)	105 (101)
D15MIT7	112 (NP)	112 (109)	102 (100)
D16MIT212	210 (NP)	207 (200)	178 (178)
D16MIT4	152 (>> C3H; > BL/6)	136 (132)	125 (123)
D17MIT21	113 (NP)	144 (137)	128 (122)
D18MIT12	121 (NP)	121 (120)	134 (130)
D19MIT68	119 (< BL/6)	136 (136)	120 (122)
DXMIT64	120 (NP)	135 (134)	116 (114)

*Information in parentheses is the size found in the Mouse Genome Database maintained at the Jackson Laboratory.
**Published relative size different from that described herein.
NP = Not published.

erature for FVB mice (7, 9, 10, 18), but only relative to the size of another strain. For 13 of 14 of these markers, the allele size that we elucidated for the FVB/N strain of mice was consistent with these relative sizes reported in literature, with one exception: the microsatellite marker D8MIT215. Neuhaus and co-workers (10) reported that the size of the allele in FVB mice should be greater than the size of the allele in C3H mice, but smaller than the size of the allele in C57BL/6 mice. We found the size of the FVB strain's allele for this marker to be 164 bp. This size for the FVB/N strain's allele was consistent with what Neuhaus and co-workers (10) reported relative to the size of the C57BL/6 strain's allele; however, our results indicated that the FVB/N strain's allele was equivalent in size (164 bp) to that of the C3H strain's allele, rather than greater, as reported by the Nehaus group (10). Since our protocols were similar and the primers were derived from the same source, the reason for this discrepancy is unclear. Although the fact that normal laboratory-to-laboratory variability exists due to technical differences such as gel conditions, instrumentation, and molecular mass standards (6, 11, 12), relative sizes of markers between laboratories are usually consistent.

In fact, although the sizes we report for C57BL/6N and C3H/HeN mice are slightly different (in most cases, < 6 bp, which is the resolution limit of our electrophoresis system) from the published standards, their relative sizes are highly consistent. It has been suggested that laboratory-to-laboratory variability could be eliminated, if the experimental need exists, by including an internal standard with each sample, and by placing an allelic ladder (which had been determined by sequencing) in adjacent wells (11). A commercial breeder that performs in-house microsatellite analyses has reported that the size of the FVB/N strain's allele for microsatellite marker D6MIT102 was significantly lower than the size that we reported for this same marker (4). In that instance, insufficient information was given to determine whether the same primers were used and what were the electrophoresis conditions for the size determination analysis. These are the only discrepancies between our data and those of any other published studies.

In addition to describing the actual size of previously reported FVB/N strain alleles, we also document the size of 13 additional microsatellite markers for the FVB/N strain. With the 27 markers that we used, we were able to determine that the FVB/N strain varied from the C3H/HeN strain by > 6 bp for 18 of 27 (67%) of the markers and from the C57BL/6N strain for 21 of 27 (78%) markers (Table 1). We did not observe variation in the size of any of these 27 markers among the four inbred or inbred transgenic FVB/N mice from the five sources that were analyzed. In addition, we did not find relative differences in the sizes we obtained for these 27 markers between the C3H/HeNMTV and C3H/HeJ lines, the microsatellite allele sizes of which were determined initially by Dietrich and co-workers (1) and were catalogued in the Mouse Genome Database maintained by the Jackson Laboratory (9).

In conclusion, using a standard set of PCR and gel conditions, we have identified a panel of highly variable (on average, at least five alleles) microsatellite markers that can be used to monitor the genetic integrity of the FVB/N strain of mice.

Acknowledgments

This work was supported in part by NIEHS contract N01-ES-15469 "Genetic Monitoring of Inbred Rodents."

References

1. **Deitrich, W., J. Miller, R. Steen, M. A. Merchant, D. Damron-Boles, Z. Husain, R. Dredge, M. J. Daly, K. A. Ingalls, T. J. O'Connor, C. A. Evans, M. M. DeAngelis, D. M. Levinson, L. Kruglyak, N. Goodman, N. G. Copeland, N. A. Jenkins, T. L. Hawkins, L. Stein, D. C. Page, and E. Lander.** 1996. A comprehensive genetic map of the mouse genome. Nature **380:**149-152.
2. **Delmotte, F., N. Leterme, and J. Simon.** 2001. Microsatellite sizing: difference between automated capillary electrophoresis and manual technique. Biotechniques **31:**810-818.
3. **Hamada, H., M. G. Petrino, and T. Kakunaga.** 1982. A novel repeat element with Z-DNA-Forming potential is widely found in evolutionary diverse eukaryotic genomes. Proc. Natl. Acad. Sci. USA **79:**6465-6469.
4. **Harlan Genetic Monitoring Reports.** 2004. Harlan. [Online]. Available at http://www.harlan.com. Accessed January 2004.
5. **Hilbert, P., K. Lindpaintner, J. S. Beckman, T. Serikawa, F. Soubrier, C. Dubay, P. Cartwright, B. De Gouyon, C. Julier, S. Takahashi, M. Vincent, D. Ganren, M. Georges, and G. Lathrop.** 1991. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. Nature **353:**521-529.
6. **Iakoubova, O., C. L. Olsson, K. M. Daines, J. Choi, I. Kalcheva, L. G. Bentley, M. Cunanan, D. Hillman, J. Louie, M. Machrus, and D. West.** 2000. Microsatellite marker panels for use in high-throughput genotyping of mouse crosses. Physiol. Genomics **3:**145-148.
7. **Le Voyer, T., and K. Hunter.** 1999. Microsatellite DNA variants among the FVB/NJ, C58/J, and I/LnJ mouse strains. Mamm. Genome **10:**542-543.
8. **Love, J., A. M. Knight, M. A. McAleer, and J. Todd.** 1990. Towards construction of a high resolution map of the mouse genome using PCR-analysed microsatellites. Nucleic Acids Res. **18:**4123-4130.
9. **Mouse Genome Database (MGD).** 2003. Mouse genome informatics web. The Jackson Laboratory. [Online]. Available at http://www.informatics.jax.org. Accessed October 2003.

10. **Neuhaus, I., C. S. Sommardahl, D. K. Johnson, and D. Beier.** 1997. Microsatellite DNA variants between the FVB/N and C3HeB/FeJLe and C57BL/6J mouse strains. Mamm. Genome **8:**506-509.
11. **Rossouw, M., R. Warren, and E. Hoal.** 2002. Accurate microsatellite typing and inter-study comparison: pitfalls and solutions using interferon-γ (IFNG) and natural resistance-associated macrophage protein 2 (NRAMP2) genes as examples. Clin. Chem. Lab. Med. **40:**926-929.
12. **Schalkwyk, L., M. Jung, M. Daser, M. Weiher, J. Walter, H. Himmelbauer, and H. Lehrach.** 1999. Panel of microsatellite markers for whole-genome scans and radiation hybrid mapping and a mouse family tree. Genome Res. **9:**878-887.
13. **Stalling, R., A. F. Ford, D. Nelson, D. C. Torney, C. E. Hildebrand, and R. Moyzis.** 1991. Evolution and distribution of (GT)n repetitive sequences in mammalian genomes. Genomics **10:**807-815.
14. **Taketo, M., A. C. Schroeder, L. E. Morbateb, K. B. Gunning, G. Hanten, R. R. Fox, T. H. Roderick, C. L. Stewart, F. Lilly, C. T. Hansen, and P. Overbeek.** 1991. FVB/N: an inbred mouse strain preferable for transgenic analyses. Proc. Natl. Acad. Sci. USA **88:**2065-2069.
15. **Tautz, D.** 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. **17:**6463-6471.
16. **Truett, G. E., P. Heeger, R. L. Mynatt, A. A. Truett, J. A. Walker, and M. L. Warman.** 2000. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques **29:**52-54.
17. **Weber, J.** 1990. Informativeness of human (dC-dA)n·(dG-dT)n polymorphisms. Genomics **7:**524-530.
18. **Whitehead Institute/MIT Center for Genome Research.** 1999. Genetic and physical maps of the mouse. Broad Institute. [Online]. Available at http://www.broad.mit.edu. Accessed October 2003.
19. **Wood, P., D. A. Hamm, S. C. Cartner, and J. Lindsey.** 1986. Simple sequence length polymorphism analysis of mice for genetic monitoring. Contemp. Top. Lab. Anim. Sci. **35:**60-62.