Microsatellite marker panels for use in high-throughput genotyping of mouse crosses

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Several microsatellite genotyping panel sets have been developed that are polymorphic between C57BL/6J and CAST/Ei mice, or C57BL/6J and DBA/2J. One set of markers for each strain pair has an intermarker distance of ~20 cm, and a second set has an intermarker distance of 5 cm. The 20-cM set contains 105 markers for C57BL/6J × DBA/2J and 108 for C57BL/6J × CAST/Ei, divided into 13 panels. Each 5-cM set includes 350 markers arranged into 45 panels. A panel contains a number of primer pairs whose fluorescently labeled PCR products can be pooled together and separated on one lane of a polyacrylamide gel. The sets are arranged by the size of the PCR product and by the type of fluorescent dye; 5-cM sets are also arranged by chromosomal region. The 20-cM sets are most useful for full-genome scans, the 5-cM sets are useful for full-genome and/or for region-specific chromosome screens. Both sets were proven as useful tools for speed congenic production of congenic strains, (speed congenics) (6, 11, 18), quantitative trait loci (QTL) analyses (7, 12, 17, 19), and the genetic dissection of polygenic traits (2, 8). We have constructed two sets of marker panels whose PCR products are polymorphic between either C57BL/6J and CAST/Ei, or between C57BL/6J and DBA/2J, for use in full-genome or chromosomal region-specific genotyping screens.

MATERIALS AND METHODS

The panel sets were initially developed using a 96-well format with the Perkin-Elmer 9600 thermocycler and tested on the ABI 377. PCR conditions were not optimized for each individual marker, but instead were chosen based on their performance under the conditions indicated below. PCR products for each individual mouse are pooled together by panel prior to electrophoresis. Each 96-well tray contained progenitor DNA from C57BL/6J, DBA/2J, CAST/Ei, a negative control, (C57BL/6J × DBA/2J) F1, and (C57BL/6J × CAST/Ei) F1 to establish that alleles were not subject to preferential amplification.

The following conditions were used to achieve optimal performance for all panels. Mouse tails are harvested in 1,536–2,304 mouse genotypes daily per one gel-based system. Whole genome scans of one animal require 13 or 48 gel lanes, with 20 cm or 5 cm density, respectively.

C57BL/6J; CAST/Ei; DBA/2J; quantitative trait loci analyses; congenic mice

MICROSATELLITE MARKER PANELS, that allow concurrent detection of multiple PCR products, are readily available and widely used for linkage studies in human molecular genetics and in diagnostic molecular biology (9, 10, and 15). However, in mouse genome research the absence of this type of highly reliable tool has been limiting. The ability to perform high-throughput genotyping can dramatically facilitate the marker-assisted production of congenic strains, (speed congenics) (6, 11, 18), quantitative trait loci (QTL) analyses (7, 12, 17, 19), and the genetic dissection of polygenic traits (2, 8). We have constructed two sets of marker panels whose PCR products are polymorphic between either C57BL/6J and CAST/Ei, or between C57BL/6J and DBA/2J, for use in full-genome or chromosomal region-specific genotyping screens.

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10-min incubation at 95°C, a thermocycling profile is performed: 10 cycles of 95°C for 30 s; 53°C for 60 s; 72°C for 120 s, then 31 cycles at 89°C for 30 s; 53°C for 60 s; 72°C for 90 s and a final extension for 20 min at 72°C. The PCR products are pooled by panels and run on a 5% polyacrylamide 36-cm well-to-read (WTR) gel using an ABI 377 DNA Sequencer (Applied Biosystems) 96-well upgrade. The markers are typed using ABI GeneScan 3.1 and Genotyper 2.1 Software.

RESULTS

Supplementary Table 1 presents the 20-cM marker panel set for the C57BL/6J × DBA/2J cross. (Tables 1–4 have been published online as Supplementary Material and can be viewed at the Physiological Genomics web site.) Each panel contains the name of the marker, the chromosomal map position, the confirmed allele size, and dye label. The C57BL/6J × DBA/2J panel set contains a total of 105 primer pairs arranged into 13 panels with up to 8 markers per panel. Table 2 of the Supplementary Material shows the 20-cM marker panel sets for the C57BL/6J × CAST/Ei cross containing 108 primer pairs. The microsatellites indicated by “GC tag” contain 6–12 GC repeats added to the 5′ end sequence of the primers to adjust product size. The allele size indicated for these markers includes the size of the GC tag. Each of the 13 panels contains 8–10 primer pairs whose fluorescently labeled PCR products can be pooled together and separated on one lane of a polyacrylamide gel. The average distance between adjacent markers in both sets is ~16 cM with the range between 2.2 and 30.6 cM.

Supplementary Table 3 presents the 5-cM panels for the C57BL/6J × DBA/2J cross. The number of primer pairs used is 355, spaced at an average distance of 4.19 cM with the largest gap of 16.4 cM. Thirty-four percent of the markers are spaced at a distance greater than 5 cM. Each of these 48 panels is arranged by size of the PCR product and by the chromosomal region. For example, panel for chromosome 1 proximal (1.1) in Table 3 shows the name of the marker, the chromosomal map position (based on the MIT map), the confirmed allele size, and fluorescent label. Table 4 of the Supplementary Material lists the 5-cM panels for the C57BL/6J × CAST/Ei cross, which contains 307 markers organized into 48 panels with markers spaced at an average distance of 4.65 cM with the largest gap of 11.7 cM. Forty-four percent of these markers have an intermarker distance greater than 5 cM. The panels are arranged by chromosomal location, facilitating region-specific genome scans. Figure 1 illustrates the relative chromosomal location of the markers used in the 20-cM and 5-cM density mouse marker panels.

DISCUSSION

Although though single nucleotide polymorphic markers (SNPs) will eventually replace microsatellites as a major source of polymorphisms for high-resolution mapping, microsatellites are currently the most reliable source of genetic markers in mouse molecular genetics/genomics. Undoubtedly, microsatellites will continue to be widely used at least for the next few years, because of the unavailability of high-throughput, automated genotyping assays for SNPs. Although the development of technology for genotyping by SNPs is in progress, at the present time the assays are complicated, cost inefficient, and are not sufficiently reliable to use for whole genome scans in the mouse.

Microsatellites have been successfully used for linkage studies in both human and mouse, and these have identified numerous QTLs, regions of the genome associated with polygenic disorders (1, 12, 13, 17). The use of human microsatellite markers became much more efficient with the development of microsatellite panel sets. Fluorescent-based, semi-automated genome mapping with marker panel sets permits simultaneous runs of 6–12 PCR products on the same gel.
lane and therefore makes genome-wide scans 6–12 times more efficient (15). The lack of similar sets of markers in mouse genomics can be explained by the following: 1) the smaller range of sizes in PCR products generated makes binning difficult (size range is only between 100 and 160 base pairs); 2) many mouse markers produce a much stronger PCR stutter artifact; 3) each mouse cross requires independent marker panel development due to significant differences in allelic sizes between various inbred strains. These features of mouse microsatellites limit the number of PCR products that can be reliably resolved on a gel lane.

To facilitate the genotyping that is critical for efficient dissection of complex genetic traits, we have developed two panel sets of markers that are polymorphic between C57BL/6J and DBA/2J strains and C57BL/6J and CAST/Ei strains. The choice of these specific strains has special importance due to their genetic and phenotypic divergence. The C57BL/6J and DBA/2J strains are among the most thoroughly characterized strains for metabolic, neurological, behavioral, autoimmune disorders and cancer-related traits, as well as asthma, glaucoma, and osteoporosis. Therefore, numerous QTLs associated with these traits have been mapped using C57BL/6J × DBA/2J crosses as well as BXD recombinant inbred (RI) strains (7, 13), and many more QTLs will likely be identified in the future. In addition, C57BL/6J is the strain that is currently being sequenced in the mouse genome project and has also been used as the source for the most widely used bacterial artificial chromosomes (BAC) libraries (14).

The markers have been individually tested prior to the assembly of panels. Special care was taken regarding preferential amplification of the strain-specific alleles. For example, we found that 50% of the markers tested for C57BL/6J × CAST/Ei crosses were subject to preferential amplification, and we were not able to reliably identify the heterozygotes. Additional testing was done on the progenitors and F1 DNAs using assembled panels to confirm that markers of the same dye perform well together and that genotypes can be reliably called despite the existence of stutter bands.

In a number of instances, the allele size of a marker was discordant with the published MIT size. For example, the D3Mit328 published size is 127 and 122 for C57BL/6J and DBA/2J, respectively, and our data show this marker at 128 and 124 base pairs. Some major discrepancies exist; for example, the published size of D1Mit221 is 132 and 125, but our data indicate a 117 and 123 call. Our size data were reproduced in multiple genotyping experiments. The differences in the published calls are most likely due to technical differences such as gel conditions and instrumentation. Information regarding these discrepancies is available by request from the authors.

We have successfully utilized both sets of these panels for the genotyping of 12,000 N2-N6 progeny during the development of multiple congenic lines between C57BL/6J and DBA/2J and between C57BL/6J and CAST/Ei. Marker failure rates in these experiments ranged between 1–7%. Another worthwhile application for the C57BL/6J × DBA/2J panel set is the genotyping of BXD RI strains. In recently published work, 319 genetic markers were used for genotyping to aid in the construction of 9 novel BXD RI strains (16). Interestingly, only a small portion of these markers overlap the C57BL/6J × DBA/2J panel set (<15%) reported here. Therefore, this panel set can be used to quickly generate more data to characterize the newly developed BXD RI strains. An additional application is the use of the high-density marker panel sets for genetic mapping in different mouse crosses. Since ~40% of microsatellites are polymorphic between any two inbred laboratory mouse strains, any one of the 5-cM panels is likely to provide polymorphic markers across the genome at 10- to 12-cM intervals. Use of both the C57BL/6J × DBA/2J and the C57BL/6J × CAST/Ei panels in a different mouse cross would provide polymorphic markers at an average interval of 8 cM, due to sharing of some markers between the two panel sets.

The successful use of these marker panels described herein has allowed us to increase our mouse genotyping throughout six- to eightfold using commonly available laboratory equipment without the addition of extra personnel. There are now significant efforts to identify SNPs useful for genetic mapping in human, mouse, and other species. The genotyping of SNPs will require a different technology than that used for microsatellite genotyping. However, no standardized robust technology is now widely available for the genotyping of SNPs. During the transition period when SNP genotyping technology is still under development, the microsatellite marker panels described in this report will be of significant utility.

REFERENCES


