Utility of the Interval Mapping Technique Using DNA Pools of Inbred Mice

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Abstract

We report an application of the interval mapping technique using DNA pools of inbred mice. The latest challenge in molecular genetics is to use microsatellite markers for gene mapping and cloning. Little is known about the interval mapping technique for detecting candidate linkages in inbred mice. We investigated the optimum interval length of microsatellite markers for gene mapping using DNA pools on mouse chromosomes, and found that between 25 and 35 centi Morgans (cM) was sufficient. We estimated that at least two to four microsatellite markers required for interval mapping should be present on each chromosome. The number required would depend entirely on the linkage map.

Introduction

Inbred mice are very useful for the study of mammalian genetic systems. Historically, the mouse has been the mammal of choice for genetic analysis primarily because of its short gestation period and large litter size, the availability of inbred strains, and its suitability for controlled matings. It has also served as an important model of human genetic diseases such as anemia, cancer, diabetes, epilepsy, immunological disease and various reproductive anomalies[1-6].

The explosive progress made in mouse gene mapping in recent years was sparked by the advent of new types of genetic markers such as simple sequence repeats (SSRs) and simple sequence length polymorphisms (SSLPs)[7,8]. The next challenging frontier is considered to be the use of some types of microsatellite markers for gene mapping and cloning, and molecular genetics studies have already made advances in this area. Recombinant DNA techniques allow the identification and mapping of DNA polymorphisms[9], and have provided an abundant source of biologically interesting loci on the mouse gene map. DNA markers were initially scored as restriction fragment length polymorphisms (RFLPs) on Southern blots. More recently, many of them have been developed and can be assayed by the polymerase chain reaction (PCR). One powerful system involves dinucleotide (tri- and tetranucleotide) repeat polymorphisms that can be typed using PCR assays. This approach has been used in both human and mouse gene mapping.

Linkage analysis is one of the most important strategies for cloning mutant genes because a genetic map allows initial localization of the genes and then provides starting points for cloning them. In addition, the first step of gene mapping requires a candidate linkage. As a quick test for candidate linkages, pools of genomic DNA rather than DNA samples from individual mice would be useful. When using DNA pools, the Ter mutation gene has already been mapped on chromosome 18[10].

Little is known about interval mapping based on DNA pools for detecting has been candidate linkages in inbred mice. However, the need for interval mapping widely recognized in recent studies[10,11].

To evaluate interval mapping technique with DNA pools, we investigated how many microsatellite markers and the interval length between them were needed for linkage mapping on mouse chromosomes.

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Materials and Methods

Mice

All mouse stocks used in this study were obtained from the Mouse Mutant Resource Colony of The Jackson Laboratory (Bar Harbor, ME). All mice were maintained under standard Jackson Laboratory conditions

Genomic DNA preparation

DNA was isolated from the spleen and brain of all the mouse stocks. Standard methods were used to prepare high-molecular-weight genomic DNA

DNA pool preparation

Two sets, (C3H/HeJ vs CAST/Ei) and (C57BL/6J vs CAST/Ei), were used to prepare DNA pools. Two sets of somatic DNA samples were originally pooled together. Subsequently, these DNA samples were pooled separately as two distinct phenotypes. A total of 21 samples of each set were prepared using stock DNAs previously standardized to 12.5 ng/µl in dH2O (Table 1). After thorough mixing, 2.0 µl of each DNA to be included in a given pool was placed in a microfuge tube and used to prime the PCR (Fig.1).

PCR conditions

A Perkin-Elmer Cetus 4800 DNA thermocycler was used for PCR. Four microsatellite markers, D3Mit11, D7Mit76, D11Mit71 and D19Mit19, were used to prime the PCR (Table 2). The reagents were 1.0 µl (0.25 U) of 1:20-diluted Taq polymerase (Takara), 1.0 µl 10 x reaction buffer (magnesium-free), 1.6 µl 10 mM dNTPs, 0.9 µl 25 mM magnesium chloride, 2.0 µl DNA (total 25 ng) template genomic DNA, 3.0 µl primers (final concentration 0.52 µM), and 0.5 µl dH2O in a final volume of 10 µl. Amplification conditions were as follows: DNA denaturation at 94°C for 3 min, followed by 25 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 2 min, extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were size-fractionated in 10 % polyacrylamide gels and electrophoresed at 50 V (running buffer: 1 x TBE) for 5 min followed by 150 V for 2 h. PCR products were visualized with ethidium bromide fluorescence, examined on a UV light box and photographed.

Results

PCR conditions of DNA pools

The PCR conditions tested allowed efficient amplification of the DNA segments we expected. The polymorphism among the tested mice was clearly detectable (Figs. 2-5).

DNA pooling method

In this study, we tested a protocol involving DNA pooling. Genomic DNA (group A) from both the spleen and brain of C3H/HeJ mice was pooled with that of CAST/Ei mice in different ratios (Table1). In the same way, genomic DNA (group B) from both the spleen and brain of C57BL/6J mice was pooled with that of CAST/Ei mice in different ratios. The recombination frequencies for each of the samples are listed in Table 1. A total of 21 samples of pooled DNA were amplified by PCR and then separated by 10 % gel electrophoresis.

To screen the genome for candidate linkages, DNA pools from three inbred strains, C3H/HeJ, C57BL/6J and CAST/Ei were used. The intensity of the bands varied with the percentage of each type of DNA in the recombinant pools, as shown in Figs. 2-5.

Good quantitative data were obtained with all the MIT primers tested. As shown in Fig.2, we were able to identify the intensity of the bands of the recombinant pool (sample 1) with those of C3H/HeJ and of the other recombinant pool (sample 21) with those of CAST/Ei. We obtained the same results for D7Mit76, D11Mit71 and D19Mit19 using PCR (Figs. 3-5).
Interval mapping using DNA pools

To estimate the number of microsatellite markers required for interval mapping, we adopted the following criteria: If there was a difference in intensity between the tested band and the adjacent one on the right side upon visual inspection, we entered an open square in Fig. 6. On the other hand, if the difference in the intensity of the bands could not be identified as a ratio, we entered a solid square in Fig. 6. Based on these results, we tested different recombinant frequencies of DNA pools with several MIT markers. As shown in Table 3, we evaluated a standard scale for effective interval length using the DNA pools (Table 3). It was found that the effective interval length on the chromosome necessary for detecting linkage was between 25 and 35 cM. We also estimated that at least two to four microsatellite markers were needed on each mouse chromosome.

Discussion

Linkage maps for the mouse suitable for typing intraspecific crosses by PCR, which may be useful for finding the chromosomal locations of many recessive and dominant mutant genes, have been described by Dietrich et al.\[16\]. However, for only a few genetic studies, an enormous number of PCRs are needed. We have reported here one of the most efficient mapping strategies for linkage testing. The pooling of DNA from several mice at a time is an excellent protocol, since it reduces markedly the number of PCR runs and gels required\[13\].

In this study, we tested a protocol with DNA pools from C3H/HeJ, C57BL/6J and CAST/Ei mice. The intensity of the bands varied with the recombinant frequencies of each DNA sample in both group A and group B, as shown in Figs. 2-5. However, it was noted that preferential amplification of the allele of one strain sometimes occurred. In some cases, the DNAs of the two strains were not equally amplified for the protocol, since the control 1:1 ratio of the two DNA types can be used as a scale (Figs. 2-5). On the other hand, if a marker locus was linked to a mutant allele, the linked loci in the affected mice would display excess homozygosity. By contrast, unlinked marker loci would show independent assortment and Mendelian levels of homozygosity.

As shown in Figs. 2-5, we were able to identify the intensity of the bands of the recombinant pool (sample 1) with those of C3H/HeJ or C57BL/6J and of the other recombinant pool (sample 21) with those of CAST/Ei. If a SSLP was near and linked to a target gene, one would expect the two to segregate together almost 100 % of the time. The results we obtained from all the primers were skewed towards 100 % frequency of segregation with the two pooling samples (samples 1 and 21).

If the results has been reciprocally skewed toward an alternative template in the pools, the individual samples would then have to be tested independently to verify the linkage. Since interval mapping methods provide a powerful system for finding linkages in DNA pools, the number of microsatellite markers needed for preliminary linkage analysis is very important. To investigate the number of microsatellite markers required for interval mapping, we stipulated the criteria shown in Fig.6. On this basis, we were able to estimate the standard scale for effective interval length (Table 3).

Our findings suggest that the chromosomal positional interval of MIT primers required is between 25 and 35 cM. The utility of microsatellite markers on mouse linkage maps has advanced in recent years. The longest linkage distance is chromosome 1, approximately 114.6 cM, and the shortest is chromosome 18, approximately 44.5 cM\[8\]. Thus the interval length of MIT primers needed for linkage analysis with DNA pools is very important. We concluded that at least two to four microsatellite markers are required for interval mapping on mouse chromosomes. The exact number would depend entirely on the length of each chromosome based on the linkage map.

Conclusion

We studied the interval length of microsatellite markers required for gene mapping on mouse chromosomes. By pooling DNA from three inbred mouse strains, we obtained similar results with
several MIT primers. The present findings have several important implications. We were able to identify the intensity of the bands of the recombinant pools with MIT primers, and we also found that the length of microsatellite markers needed was between 25 and 35 cM on each mouse chromosome. Therefore, the interval length determined in this study should provide useful genetic information for linkage mapping with DNA pools. The required number of microsatellite markers will be entirely dependent on the mouse linkage map. Our interval mapping technique should facilitate identification and characterization of candidate genes.

Acknowledgments

We thank Joseph H. Nadeau for the CAST/Ei, C57BL/6J and C3H/HeJ mice. This work was funded by Nihon University School of Dentistry at Matsudo.

References


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**Fig. 1** Procedure for making DNA pools of each group.

- **C3H/HeJ**
  - Stock DNA (conc. 12.5 ng/μl)
  - Mixing
  - 20.0 μl in each tube
  - 1 - 21
  - 2.0 μl of DNA mix is placed in the microfuge tube
  - 1 - 21

- **CAST/Ei**
  - Stock DNA (conc. 12.5 ng/μl)
  - Mixing
  - 20.0 μl in each tube
  - 1 - 21

- **C57BL/6J**
  - Stock DNA (conc. 12.5 ng/μl)
  - Mixing
  - 20.0 μl in each tube
  - 1 - 21

**Group A**

**Group B**
Fig. 2 D3 Mit 11 PCR products for the tested samples and for the C3H/HeJ (C3H), CAST/Ei (CAST) and 1:1 mix controls. The 1:1 mix showed the relative amplification of alternative alleles in a simulated F1 hybrid.

Fig. 3 D7 Mit 76 PCR products for the tested samples and for the C3H/HeJ (C3H), CAST/Ei (CAST) and 1:1 mix controls. The 1:1 mix showed the relative amplification of alternative alleles in a simulated F1 hybrid.

Fig. 4 D11 Mit 71 PCR products for the tested samples and for the C57BL/6J (B6), CAST/Ei (CAST) and 1:1 mix controls. The 1:1 mix showed the relative amplification of alternative alleles in a simulated F1 hybrid.
Fig. 5  D19 Mit 19 PCR products for the tested samples and for the C57BL/6J (B6), CAST/Ei (CAST) and 1:1 mix controls. The 1:1 mix showed the relative amplification of alternative alleles in a simulated F1 hybrid.

Fig. 6  Estimation of interval mapping using several primers in the mouse genome. Arrow indicates the longest interval for use in gene mapping with DNA pools. a: Based on the different ratio of the mixed DNA, all the samples were divided into two regions which were upstream (from sample 1 to sample 10) and downstream (from sample 12 to sample 21).
### Table 1 DNA pool preparation using high-molecular-weight genomic DNA.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C3H&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CAST&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
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<td>20</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup> : Volume of dilution DNA (concentration : 12.5 ng/µl); 21 samples for each group were prepared for the PCR reaction. The concentration of each sample varied with the conditions of mixing.

<sup>b</sup> : Recombinant percentage (%) = Volume of dilution DNA of CAST/20 x 100

<sup>c</sup> : Linkage map distance (centiMorgan)

### Table 2 Assay locus, chromosome location, primer sequence, and product size among the inbred mice.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Locus</th>
<th>Chromosome location</th>
<th>Primer sequence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Product size (bp)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>L38</td>
<td>D 3 Mit 11</td>
<td>3</td>
<td>CCAACCACAGTAACACATGT TGGAGCCAATGCGAACAAC</td>
<td>163 (C3H) 201 (CAST)</td>
</tr>
<tr>
<td>MPC2109</td>
<td>D 7 Mit 76</td>
<td>7</td>
<td>CATGAGCACTGGAGAAGAAGA CGTGGGAAACCTGAATAAAGTA</td>
<td>250 (C3H) 192 (CAST)</td>
</tr>
<tr>
<td>MPC1313</td>
<td>D11Mit 71</td>
<td>11</td>
<td>GCCATACCTGGTAGCGTT AATTTCTAGATGATGGCCATAAGCC</td>
<td>214 (C57BL) 280 (CAST)</td>
</tr>
<tr>
<td>A658</td>
<td>D19Mit 19</td>
<td>19</td>
<td>CCTGTGTCCATACAGGCTCA ACCATACAGGGAAGCACCCTG</td>
<td>142 (C57BL) 98 (CAST)</td>
</tr>
</tbody>
</table>

<sup>a</sup> : The chromosome location is published in Copeland, N.G. et al. (1995) <sup>14</sup>.

<sup>b</sup> : PCR primer sequences were taken from the Massachusetts Institute of Technology (MIT).

Primer sequences for forward (upper) and reverse (lower) are both shown in the 5’ to 3’ orientation.

<sup>c</sup> : The product size given is the observed size of the C3H/HeJ and C57BL/6J allele (upper), and the CAST/Ei (lower) allele on agarose gels.
Table 3 The best interval for linking analysis with DNA pools.

<table>
<thead>
<tr>
<th>Group</th>
<th>Primer</th>
<th>upstream (cM)</th>
<th>downstream (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>D3 Mit 11</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>A</td>
<td>D7 Mit 76</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>B</td>
<td>D11Mit 71</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>B</td>
<td>D19Mit 19</td>
<td>25</td>
<td>35</td>
</tr>
</tbody>
</table>

Note: The left column lists the type of group. The base sequences for each primer are shown in Table 2. The criteria for both upstream and downstream are shown in Fig. 6. The best interval for linkage analysis is listed in the right-hand column.