Arbitrarily primed PCR fingerprints resolved on SSCP gels

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Arbitrarily primed PCR methods (1), including RAPD (2), generate a reproducible fingerprint of DNA products from complex nucleic acids. Differences detected between fingerprints derived from different genomes have been used extensively as polymorphisms in genetic mapping (e.g., 3).

Various strategies can be used to maximize the number of polymorphisms that are scored in each fingerprinting experiment. First, parents can be selected for the mapping population that have highly divergent genomes. Second, a large, but manageable, number of fragments in each fingerprinting experiment can be generated by using Stoffel fragment rather than Taq polymerase holoenzyme (4). Third, primers can be directed against sequences such as purine–pyrimidine microsatellite repeats that are intrinsically more polymorphic (5). Fourth, gel systems that detect more polymorphisms can be used, e.g., denaturing gradient gel electrophoresis (6).

Another extremely simple method to increase the number of scorable polymorphisms would be to include single stranded conformation polymorphisms (SSCP) (7). This method could increase the number of scorable polymorphisms for two reasons. First, the two DNA strands from the same PCR product often run in different places on SSCP gels. This gives two opportunities to score a polymorphism whereas other gel systems afford only one such possibility for each polymorphism. Second, some PCR products from identical places in the two parental genomes may have internal sequence polymorphisms that will resolve as mobility differences on an SSCP gel.

To test the utility of this approach we mapped polymorphisms in the mouse genome using the C57BL/6J×DBA and the A/J×C57BL/6J recombinant inbred mapping populations. Figure 1 shows a fingerprint for two parental strains separated on a denaturing polyacrylamide gel (lanes 1 and 2) and on an SSCP gel (lanes 3 and 4). Two conspicuous examples of resolved strands are labeled 'A' and 'B'.

Figure 2 shows a fingerprint generated by a pair of primers used on genomic DNAs from a set of recombinant inbred lines. Strands derived from the same polymorphic PCR product could be assigned because of their completely concordant segregation patterns. The polymorphism indicated by an 'A' is known to be an SSCP polymorphism because it could not be scored on a denaturing gel (not shown). Length polymorphisms, labeled B, C, and D, could be scored at up to four places on the SSCP gel (Figure 2).

In a series of five arbitrarily primed PCR experiments on recombinant inbred mouse genomes, the rate of detection of

Figure 1. 60 ng and 15 ng of genomic DNA from mouse strains C57BL/6J (lanes 1 and 3) and DBA (lanes 3 and 4) were fingerprinted using the primer S2 (5'-CC-TCTGACTG). Experiments were performed using 0.4 μ M primers (Operon Tech. Inc., Alameda, CA), 100 μ M dNTPs, 1 μ Ci of 3000 mCi/mmol α - 32 P dCTP, 2 units Taq polymerase Stoffel fragment (Perkin Elmer, Branchburg, NJ) in 20 μ l of 10 mM KCl, 10 mM Tris pH 8.3, 5 mM MgCl₂. The amplification profile was 40 cycles at 94°C, 1 min; 35°C, 1 min; 72°C, 1 min. 4 μ l of each reaction was diluted in 18 μ l of 95% formamide, 10 μ M NaOH and heated to 94°C for 2 mins. 1.5 μ l were loaded on a 5% polyacrylamide–50% urea sequencing gel (lane 1 and 2), and on 5% MDE (lanes 3 and 4) and electrophoresed in 0.5×TBE at 7 watts for 18 hrs at room temperature. Hydrolink-MDE was purchased from Baker, Phillipsburg, NJ. Gels were dried and autoradiography performed with X-Omat film (Kodak) for 12 hrs. Fragments visualized range from about 150 bases to 700 bases. Polymorphisms 'A' and 'B' show dramatic shifts in mobility of the two strands of the PCR product.

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Figure 2. 15 ng of genomic DNA from mouse strains in the A/J×C57BL/6J recombinant inbred mapping population were fingerprinted using the pair of primers S2 and U18 (5'-GAGGTCCACA) and the conditions described in Figure 1. The fingerprints were resolved on 5% MDE + 5% glycerol. Only a portion of the gel is shown. Fragments visualized range from about 150 bases to 700 bases. Polymorphisms are labeled 'A' through 'D'.

polymorphisms was about 20% greater using SSCP gels than using denaturing gels. About 30 easily visualized PCR products were detected on denaturing gels, of which an average of five were polymorphic. Almost twice as many bands were visible on SSCP gels and an average of six different polymorphisms were scorable. Most of these polymorphisms were scorable in at least two different parts of the gel (data reviewed but not shown).

Primers used in pairwise combinations (8), such as in Figure 2, generally yield high quality fingerprints in SSCP gels. Fingerprints generated by most but not all individual 10-mer primers could be resolved well on SSCP gels. Individual 18-mers sometimes failed to give good patterns on SSCP gels perhaps because the ends form stable panhandle structures (8). It is recommended that primers be used in pairwise combinations. Pairs of primers can be tested on the two parental DNAs and resolved on an SSCP gel. Those pairs that give good fingerprints and many scorable polymorphisms can then be chosen for mapping.

In previous work 43% of specific PCR products derived from C57BL/6J and DBA genomes could be distinguished by SSCPs (9). However, we observed fewer than expected SSCPs using mouse strains that have a similar level of genomic sequence

divergence even when we used identical electrophoresis conditions. The number of SSCPs observed was not increased by using 5% glycerol (7). We have no explanation for the lower than expected number of SSCPs we observed. Nevertheless, the number of resolved SSCPs is expected to be significantly greater in mapping populations derived from parents that are more divergent than the ones we used, for example, an interspecific cross between C57BL/6J and *M. spretus*. It should also be possible to extend the method to mapping using arbitrarily primed PCR of RNA (10).

SSCP gels allowed increased confidence in scoring the usual presence/absence polymorphisms that result from arbitrarily primed PCR and yielded SSCPs that could not be scored by other gel systems. It is worthwhile to use SSCP gels to increase throughput and reliability of scoring when mapping by PCR fingerprinting.

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