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Specific amplification with PCR of a refractory segment of genomic DNA

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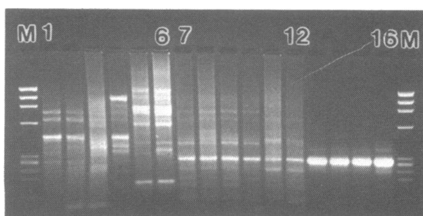
We have amplified and sequenced more than 30 regions of mammalian DNA using our previously published protocols (1,2). In brief, thirty cycles of PCR are currently performed on an automated thermal cycler with Taq polymerase in the presence of 10 ng/λ genomic DNA and 1 μM of each PCR primer. Optimal magnesium (Mg) concentrations for the PCRs have ranged from 1.2-2.5 mM. The amplified product (which contains a phage promoter sequence) is then transcribed and sequenced by reverse transcriptase using an internal sequencing primer.

Despite multiple attempts under different conditions, it was not possible to obtain specific amplification for a region of the coding sequence of the mouse Vβ6 T-cell receptor gene. Two different pairs of PCR primers failed to give the specific amplification required, presumably because the primers cross-hybridized to repetitive sequences (Fig. 1, lanes 1-12). This problem was overcome by diluting the products of the first amplification 100,000-fold and reamplifying with the second set of primers (Fig. 1, lanes 13-16). A large dilution seems to be important because a 1,000-fold dilution of the first PCR produced multiple spurious bands after the second amplification (data not shown). When sequencing was performed as described above, the data confirmed that the product of nested amplification was the Vβ6 segment (3).

Nested PCR with an appropriate dilution between the two rounds of PCR can allow the specific amplification of refractory regions of the genome. For such segments, the method can provide a more rapid and possibly a more specific alternative to PCR after size fractionation of a restriction enzyme digestion of genomic DNA (4).



LEGEND TO FIGURE 1: Amplification of the Vβ6 gene of mouse strain B10 with oligonucleotides that should amplify a 333 bp segment (lanes 1-6) or a 274 bp internal sequence (lanes 7-12). In lanes 1-6, the stringency of annealing was altered by varying the Mg concentration from 0.9 mM to 3.0 mM (0.9, 1.1, 1.3, 1.5, 2.0, 3.0 mM, respectively). In lanes 7-12, the identical Mg titration was performed with the second set of primers. Additional amplifications with Mg concentrations varying between 0.3 and 0.9 mM were also nonspecific (data not shown). In lanes 13-16, nested PCR was performed with a Mg concentration in both PCRs of 0.9, 1.3, 2.0, and 3.0 mM, respectively. Lane M: Size markers produced by HaeIII digestion of OX174.

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