

Optimization of the PCR program for RAPD analysis

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Random amplified polymorphic DNA (RAPD) analysis has been used for genome mapping, gene tagging, and population studies (1–3). The Polymerase Chain Reaction (PCR) program commonly used for RAPD analysis with random 10-mers includes a 1 min template denaturing step at 94°C, a 1 min primer annealing step at 36°C and a 2 min primer extension step at 72°C (1). Usually, 45 cycles of the three steps are run to obtain a RAPD pattern. This program can last as long as 5 hours depending on the thermal cycler that is used. A lengthy PCR program can be a limitation when large numbers of samples are analysed. In this report we show that by a systematic examination of the effects of the length of each step and the number of cycles on RAPD patterns the time for the PCR program can be reduced to 2.5 hours without changing the RAPD patterns obtained from alfalfa genomic DNA.

Genomic DNA was isolated from single alfalfa leaflets using a rapid procedure (4) with some modifications. Specifically, the leaf tissue was homogenized in the extraction buffer (rather than dry) and an additional centrifugation step was included to remove debris from the DNA solution used for initiating the PCR. A 25 μl PCR mixture containing 1 μl of the genomic DNA solution (containing 20 ng DNA), 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.1 mM each of dATP, dCTP, dGTP and dTTP (Pharmacia), 0.36 μM random primer (Biotechnology Laboratory, University of British Columbia), and 2 units of Taq DNA polymerase (Promega) was incubated in a MJ Research Programmable Thermal Controller (PTC-100). In order to test whether the PCR program could be shortened, the effects of different denaturing, annealing and extension times on the RAPD patterns from several primers were determined. A PCR program with a denaturing time of 5 sec gave better PCR product yields than programs with 30 sec or 60 sec denaturation times (Fig. 1a). This result can probably be attributed to the fact that Taq DNA polymerase has a limited lifespan at high temperatures (5). Therefore, it is better to use a denaturing time that is as short as possible. Figure 1b shows that there is an interaction between the time required for primer annealing and the GC content of the primer. For all the primers used in this study (which had GC contents from 50–80%) 30 sec of annealing time appeared to be sufficient to obtain a complete RAPD pattern (Fig. 1b). However, for the primers containing 50% or 60% GC the amounts of PCR products were reduced considerably when a 5 sec annealing time was used. In contrast, the amounts of PCR products from primers with 70% or 80% GC were no different with 5 or 60 sec of annealing time (Fig. 1b). Figure 1c shows that between 5 and 60 sec extension time there is a direct correspondence between the extension time and the maximum size of fragment that is amplified. For amplification of PCR products shorter than 1.5 kb 30 sec of extension times was sufficient but longer PCR products required a longer extension time. Fragments as large as 3 kb were amplified with 1 min extension time (data not shown). No large differences in band intensity were found among PCR products obtained from programs run for 35, 55 or 75 cycles (Fig. 1d). This result may be attributable to Taq DNA polymerase inactivation over time or be indicative that some other components in the reaction mixture become limiting at high cycle numbers.

The 35 cycle program that we are currently using to produce RAPD patterns with alfalfa genomic DNA and random 10-mers has a 5 sec denaturation step at 94°C, a 30 sec annealing step at 36°C and a 60 sec extension step at 72°C. It requires only 2.5 hours to complete and results in sharper banding patterns for most of the primers than the original program (data not shown). The time for each step may need to be optimized for different sized random primers and/or DNA templates from different species but our results suggest that there is considerable opportunity for increasing the efficiency of the PCR program used for RAPD analysis.

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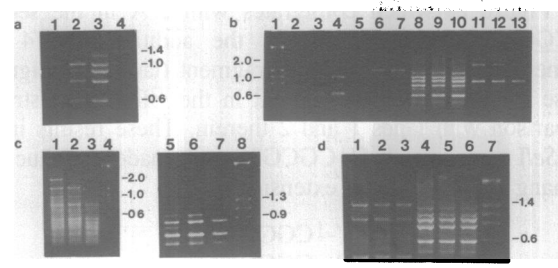

Figure 1. (a) Effects of denaturing time on RAPD patterns obtained from alfalfa genomic DNA and primer AGCAGCGTGG. PCR program: 35 cycles of denaturing at 94°C for 5 sec (lane 3), 30 sec (lane 2) or 60 sec (lane 1), annealing at 36°C for 30 sec and extension at 72°C for 60 sec. (b) Effects of primer/template annealing time and primer GC content on RAPD patterns obtained from alfalfa genomic DNA. PCR program: 35 cycles of denaturing at 94°C for 5 sec, annealing at 36°C for 5 sec (lanes 2, 5, 8 and 11), 30 sec (lanes 3, 6, 9 and 12) or 60 sec (lanes 4, 7, 10 and 13) and extension at 72°C for 60 sec. Patterns in lanes 2–4, 5–7, 8–10, and 11–13 were obtained with primers TTAGCGGTCT (50% GC), CAAGGGAGGT (60% GC), AGCAGCGTGG (70% GC), and C-TGGCGGCTG (80% GC), respectively. (c) Effects of primer extension time on RAPD patterns obtained from alfalfa genomic DNA. PCR program: 35 cycles of denaturing at 94°C for 5 sec, annealing at 36°C for 30 sec and extension at 72°C for 5 sec (lanes 3 and 7), 30 sec (lanes 2 and 6) or 60 sec (lanes 1 and 5). Patterns in lanes 1–3 and 5–7 were obtained with primers GCTTGTGAAC and AGCAGCGTGG, respectively. (d) Effects of the number of amplification cycles on yield of PCR products. The RAPD patterns were obtained from alfalfa genomic DNA. PCR program: 35 cycles (lanes 3 and 6), 55 cycles (lanes 2 and 5) or 75 cycles (lanes 1 and 4) of denaturing at 94°C for 5 sec, annealing at 36°C for 30 sec and extension at 72°C for 60 sec. Patterns in lanes 1–3 and 4–6 were obtained with primers CAAGGGAGGT and AGCAGCGTGG, respectively.