Short PCR product yields improved by lower denaturation temperatures

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Polymerase chain reactions (PCR) typically involve template denaturation between 93° and 95°C for every cycle of amplification. Under these conditions *Taq* DNA polymerase is inactivated gradually, with a half life of 130 and 40 min at 92.5° and 95°C respectively (1) and its activity may be limiting in the later cycles. Since the amplified product acts as template in subsequent cycles, it may be unnecessary to use such high denaturation temperatures during the later cycles, when the original DNA template of longer length forms an insignificant fraction of the total template. The empirical melting point equations for DNA−DNA duplexes in solution predicts that hybrid length affects T_m by a factor of 650/L, where L is the length in base pairs (2). Hence, T_m of duplexes less than 350 base pairs in length would be expected to be 2°C or more lower than that for genomic DNA of similar GC content, though absolute T_m values have not been formally established for the conditions used in PCR. We discovered that lowering the denaturation temperature after the initial few cycles gives a much improved yield of product, and enables amplification to be performed for up to 70 cycles.

Primer pairs delimiting a 110 bp segment of the HPV 16 E6/7 genes (3), 110 bp (4) and 355 bp (5) segments of the β-globin gene, and a 500 bp fragment of λ-bacteriophage (Cetus) were used, the GC content of the sequences amplified were 31%, 53%, 53% and 57% respectively. *AmpliTaq* polymerase (Cetus) 2.5 U/100 μl was used to amplify plasmid and genomic DNA templates, after denaturation at 93° or 94° for 3 min.

Denaturation at 90° and 87°C after 10 initial cycles at 93°/94°C consistently yielded a greater amount of 110 bp products for HPV16 (4 to 6-fold in Fig. 1a) and β-globin (4-fold in Fig. 1b) compared to standard reaction conditions. No specific product was evident at 84°C. For the larger fragments, amplification occurred at 87°−93°C for the 355 bp β-globin (Fig. 1c), and at 92°C but not at 90°C for the 500 bp λ-phage (Fig. 1d). At least 5 initial cycles at 93°/94°C were required for maximum yield (data not shown). Increasing the number of late cycles to 60 (ie. total of 70) resulted in increasing product yield if lower temperatures were used (Fig. 2).

These results are consistent with predictions of T_m trends; short products are efficiently amplified at 90°C or below but higher temperatures are required for longer sequences. Furthermore *Taq* polymerase activity can be enhanced by the judicious use of lower denaturation temperatures for the later cycles, resulting in marked improvement in yield. However efficacy was not markedly increased when the yield was already high, presumably because of other limiting factors such as primer, nucleotide or pyrophosphate concentrations.

For the 110 bp sequences used, the optimum protocol is to cycle the reaction through the usual parameters for an initial 5−10 cycles, followed by cycles in which the denaturation temperature is lowered to 87°−90°C. Optimum temperatures should be determined empirically for the particular thermal cycler and primers used.

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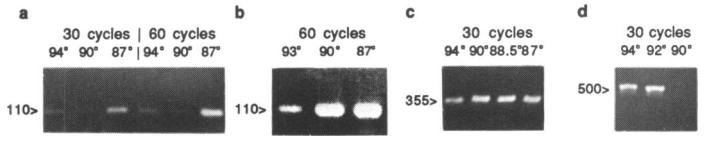

Figure 1. PCR products after 10 initial cycles with normal denaturation followed by 20−50 similar cycles at denaturation temperatures as indicated. **(a)** HPV16 amplified from genomic DNA: 94°-1 min, 50°-1 min, 72°-1 min **(b)** β-globin amplified from plasmid: 93°-1 min, 55°-1 min **(c)** β-globin amplified from plasmid: 94°-1 min, 55°-1 min, 72°-1 min **(d)** λ-phage amplified from whole DNA: 94°-1 min, 55°-1 min, 72°-1 min.

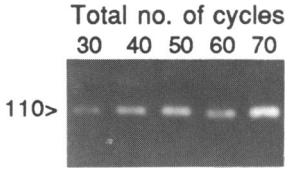

Figure 2. Products after 30−70 cycles of amplification of β-globin from plasmid including 10 initial cycles at: 93°-1 min, 55°-1 min, and subsequent cycles at: 90°-1 min, 55°-1 min.