A specificity enhancer for polymerase chain reaction

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Submitted June 1, 1990

The polymerase chain reaction (PCR) is one of the most rapid and versatile methods of producing large quantities of DNA for molecular analysis (1). Because of the powerful amplification potential of the PCR technique, undesirable multiple satellite bands, in addition to the single expected band, are often present on the gel when complex cDNA or genomic DNA is used as the PCR template (Lanes 2 and 4, Figure 1). These multiple bands, frequently attributed to non-specific priming, often cannot be eliminated even when all experimental parameters are optimized. The use of DMSO (2) and non-ionic detergents (3) has been reported to improve the DNA sequencing reaction, perhaps due to decreased inter- and intra-strand reannealing. Recently, the inclusion of 10% DMSO in the PCR mixture was found to be essential for amplification of the retinoblastoma gene; little or no PCR fragment was produced without DMSO (4). However, DMSO was also found to inhibit DNA synthesis by Taq polymerase by 50% in the PCR assay (5).

Tetramethylammonium chloride (TMAC, M.W. 109.6) has been used successfully to reduce potential DNA/RNA mismatch and to improve the stringency in hybridization reactions (6, 7). We have found that the inclusion of TMAC (Fisher Scientific) in the PCR mixture can dramatically reduce and even eliminate non-specific priming events, thereby enhancing the specificity of the reaction. We have selected two well-characterized mouse cDNAs, Tumor Necrosis Factor-β (TNF-β) and Interleukin-1α (IL-1α) to demonstrate the effects of TMAC on the reduction of extraneous bands (Figure 1). Titration studies showed that TMAC used at concentrations of 1×10^{-4} M-1×10^{-5} M can effectively eliminate non-specific amplification without any inhibitory effects on Taq polymerase. Further studies are now in progress to explore the feasibility of using TMAC as a general specificity enhancer for PCR.

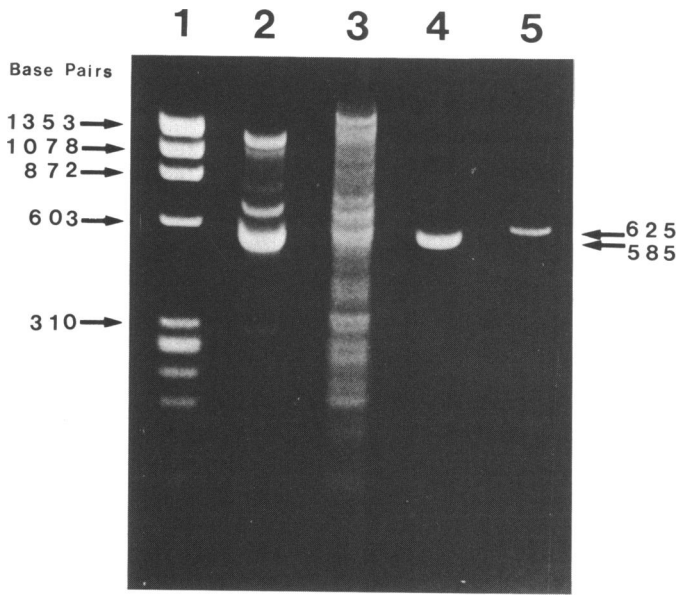

Figure 1. Mouse liver cDNAs were amplifed with a set of two primers for either TNF-β or IL-1α. One ng of mouse liver cDNA was amplified in 50 μl PCR buffer (10 mM Tris-Cl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% gelatin), 50 pmols of primers, 200 μM dNTPs and 1.25 units AmpliTaq® DNA polymerase. PCR was carried out at 94°C for 1 min, 62°C for 1 min and 72°C for 1 min for 30 cycles in an automated DNA thermal cycler (Perkin-Elmer Cetus). Lane 1: ϕX174 DNA/*Hae*III size markers; Lanes 2 and 3; TNF-β and IL-1α; Lanes 4 and 5: TNF-β and IL-1α, each with 5×10^{-5} M TMAC added to the PCR mixture.

ACKNOWLEDGEMENT

We thank Mingjie Cai for his valuable suggestions.

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