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Increased efficiency of the Taq polymerase catalyzed polymerase chain reaction

Stephen A.Krawetz, Richard T.Pon and Gordon H.Dixon

Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada
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The Polymerase Chain Reaction (PCR; 1,2) is rapidly becoming the preferred means to quickly clone genes or gene products of interest when sequence information is available (3,4). In our studies on the human protamine genes and cDNAs, we observed that substituting RTB (Reverse Transcriptase Buffer; 5) for the PCR buffer markedly increased the efficiency of the reaction.

A 100 μl PCR reaction mixture contained 200 μM of each dNTP, 1 μM of each primer (4), 2.5 units of Taq Polymerase, 5 μl of previously amplified gel-purified product buffered with either RTB (50 mM Tris-HCl, pH 8.5 @ 37°C, 50 mM NaCl, 5 mM $MgCl_2$, 2 mM DTT; Fig. 1, lanes RTB), or PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3 @ 37°C, 1.5 mM $MgCl_2$; Fig. 1, lanes PCRB). The reaction mixture was then overlaid with 100 μl of paraffin oil and initially incubated for 90 sec at 94°C. The reactions were then carried out for a total of 60 cycles, with the use of a Perkin Elmer Cetus thermal cycler. The cycle times were as follows: annealing, 2 min @ 50°C; primer extension, 3 min @ 72°C; denaturation 1 min @ 94°C. The primer extension reaction was increased by 3 sec for each subsequent cycle.

With a simple modification to the buffer system the efficiency of the PCR can be enhanced. As shown in Fig. 1, this is independent of the template. For example, an increase of approximately 2 fold (compare Fig 1 of PCR products from lane R RTB to lane R PCRB) in the synthesis of the human P1 protamine cDNA (lane R) was observed when RTB was substituted for PCRB. The effect was even more pronounced when human P1 genomic DNA (lane D) was amplified. As shown in lane D, upon buffer substitution an increase of approximately 5 fold in the synthesis of human genomic P1 protamine (compare Fig 1. of PCR products from lane D RTB to lane D PCRB) DNA was observed. A similar effect was also noted utilizing four additional primer-template sets (data not shown). This observation, may, in part, reflect the altered Mg^{+2} concentration, which can effect PCR efficieny (6).

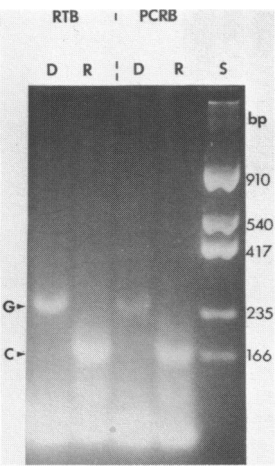

Fig. 1 Legend: Ethidium bromide stained 4 % agarose gel of 30 μl of the reaction mixture. The cDNA copy of human P1 protamine (lane R) and the genomic DNA copy of human P1 protamine were amplified with the use of the PCRB buffer (PCRB) or the RTB buffer (RTB). Lane S, shows the relative migration of the molecular weight (bp) standards. The arrows indicate the positions of the genomic (G) and cDNA (C) copies of the human P1 protamine.

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