Improvement of PCR amplified DNA sequencing with the aid of detergents

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The problem of sequencing short double stranded DNA like products of the polymerase chain reaction (PCR) is the tendency of the templates to reanneal. The use of dimethyl sulphoxide (DMSO) can hep to overcome this problem[1]. Using the T7 sequencing96 kit (Pharmacia), we have found that addition of 0.5% of nonidet P-40 (NP-40) or the combination of 0.5% NP-40 with 0.5% Tween 20 to the sequencing reaction mix[2] enhances the intensity of signals obtained and also reduces the frequency of unspecific bands appearing in certain positions.

Double stranded DNA spanning 115bp of the gag region of HIV-2ben[3] (1875–1989) was amplified by the polymerase chain reaction[4] (PCR). Amplified DNA was purified by electrophoresis on 2% agarose gels. 0.25 pmole of the electroeluted DNA and 20 pmoles of one PCR primer were denaturated by boiling for 3 min in the presence of detergent and immediately snap-cooled on dry ice to minimize reannealing. For sequencing of the DNA 10 μCi of ^{35}SdATP, T7 DNA polymerase, and labeling mix were added to the annealing mixture. The resulting mixture was divided into four equal parts in tubes each containing another 'short'-termination mix. The sequencing reaction was kept at 37°C for five minutes. Then 2μ of solution containing 0.25 mM dNTP, 50 mM NaCl and detergent (see below) were added, and the reaction was left for five minutes at 37°C. The detergent concentration was maintained throughout all additions to the reaction mixtures. Several detergents were tested alone and in combination; DMSO 10%, NP-40 0.5%, Tween 20 0.5% each individually, as well as NP-40 0.5% and Tween 20 0.5% together. The 0.5% solution of Tween 20 and NP-40 was described elsewhere as yielding better *Thermus aquaticus* DNA polymerase activity in high temperature[5] reactions.

Figure 1 shows a comparison of sequences obtained following the above protocol with different detergents and, for comparison, with no detergent and DMSO. The sequence obtained without detergent was not readable, whereas the sequence obtained with DMSO gave signals of low density. The highest intensity of the signals and the lowest background at specific positions were found in the sequences obtained with NP-40 or with the combination of NP-40 and Tween 20. This effect is presumably due to the prevention of secondary structure formation and the suppression of DNA-annealing by detergents.

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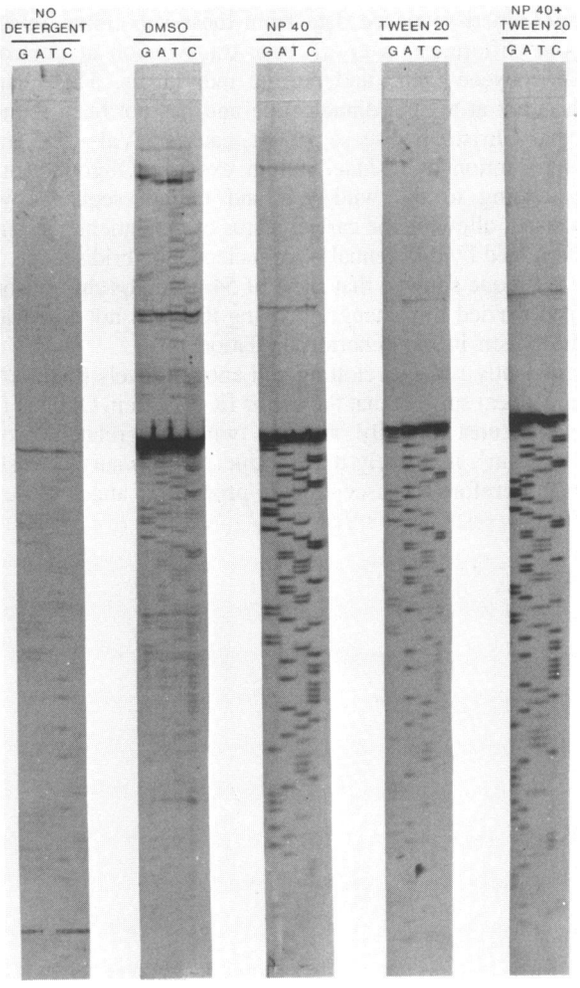

Figure 1. Sequencing gel from a HIV-2ben gag region. 115bp of double stranded DNA, amplified by PCR, were sequenced without detergent, with 10% DMSO, or 0.5% NP-40, or 0.5% Tween 20, or 0.5% NP-40 and 0.5% Tween 20. The DNA fragments were separated in a 6% denaturating sequencing gel.