An improved method for directly sequencing PCR amplified material using dimethyl sulphoxide

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The use of the dideoxy chain termination procedure[1] to sequence double stranded DNA templates is subject to problems related to the template strands reannealing. I have found that when using Sequenase™ (USB),the inclusion of dimethyl sulphoxide (DMSO) during the sequencing reactions helps to overcome these problems.

Double stranded DNA fragments spanning 332bp of the 5'region of the factor IX gene (residues -275 to 75[2]) produced after DNA amplification by the polymerase chain reaction procedure[3] were purified by elution from agarose gels onto Gene-Clean™(Bio 101) according to manufacturers instructions. 140ng (20 pMol) of one of the 20 base pair amplification primers (5'GATGAGGCCTGGTGATTCT3') was mixed with 60ng (0.25pMol) of the purified template in 6µl of 40mM Tris-chloride pH7.5, 25mM $MgCl_2$, 50mM NaCl, 10% DMSO. After boiling for three minutes to denature the template and immediately snap-cooling on dry ice to minimize renaturation, 4µl of labelling mix (0.025M DTT, 10 µCi ^{35}S dATP(1200 Ci/mmol. Amersham,plc) containing 2 Units of Sequenase™ (USB) was added. The resulting 10µl mixture was divided equally into four tubes each containing 2µl of 80µM dCTP, dGTP, dTTP, 50mM NaCl, 10% DMSO and 0.08µM ddATP (Tube A), 8µM ddCTP (Tube C), 8µM ddGTP (Tube G) or 8µM ddTTP (Tube T). The tubes were incubated at 37°C for five minutes, 2µl of 0.25mM dATP, dCTP, dGTP, dTTP, 50mM NaCl, 10% DMSO was then added and the reaction was left for a further five minutes at 37°C.

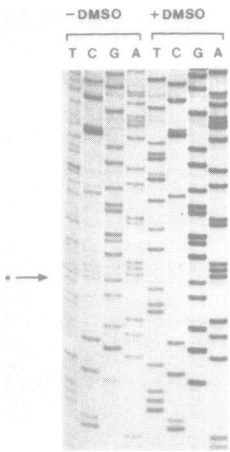

Fig 1 shows the sequence obtained following the above protocol with DMSO in the sequencing reagents. Sequence obtained without DMSO in the sequencing reagents[4] is shown for comparison. It is clear that DMSO not only enhances the intensity of signal obtained but also reduces background at specific positions in the sequence (see * Fig 1). This latter effect is presumably due to the prevention of secondary structure formation. Therefore, the inclusion of DMSO should also prove beneficial when sequencing from single stranded templates.

Fig1 8% denaturing poly-acrylamide gel showing the effect of using DMSO.

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