An improved method for sequencing double stranded plasmid DNA from minipreps using DMSO and modified template preparation

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DNA sequencing by the dideoxy chain termination method can be performed on double stranded DNA templates from quantities and purities achieved with miniprep procedures (1). Depending on the procedure used to isolate the DNA, sequencing reactions can be subject to high backgrounds and artifacts in band patterns, particularly presence of bands across two or more lanes on the sequencing gel. Dimethyl sulphoxide (DMSO) has been used in sequencing polymerase chain reaction (PCR) products (2). Described herein is a procedure coupling a modified method for isolating and treating plasmid DNA and a protocol for using a DMSO-modified dideoxy chain termination sequencing reaction to overcome such artifacts to give unambiguous DNA sequence determination.

E. coli DH5 α bearing the yeast heat shock factor gene yHSF (3' Δ 54 and 3' Δ 63) in plasmid pOTSNco12 (3) was freshly grown to late log phase: overnight cultures were diluted, re-grown, and harvested in a single day. 1.5 ml of this 5 ml culture was treated using a modified procedure of Holmes and Quigley (4). This miniprep protocol consists of the following steps: 1) resuspending the bacterial pellet in 300 μ l STET (8% sucrose, 5% Triton X-100, 50 mM EDTA, 50 mM Tris-Cl, pH 8.0) and 20 μ l 10 mg/ml lysozyme; 2) incubating at room temperature for 5 minutes; 3) boiling this suspension for 2 minutes; 4) centrifuging and discarding the pellet; and 5) precipitating with 500 μ l 2.5 M ammonium acetate–75% isopropanol at room temperature for 20 minutes. This final pellet was resuspended in 50 μ l of H₂O. To ensure complete double stranded template separation, two denaturation steps were utilized. Per 50 μ l miniprep DNA, 4 μ l 2 N NaOH and 4 μ l 2 mM EDTA was added, and the sample incubated at 90°C for 5 minutes. To this was added 6 μ l 3 M NaAcetate (pH 5), 14 μ l H₂O and 160 μ l ethanol. The DNA was precipitated at room temperature for at least 10 minutes. Samples were centrifuged for 20 minutes at room temperature and the DNA pellet was washed twice with 70% ethanol and vacuum dried. The pellet was dissolved in 25 μ l H₂O; of this, 7 μ l was heated along with 2 μ l 5 $\times$ sequencing buffer (50 mM Tris-Cl, pH 7.5, 60 mM NaCl, 35 mM MgCl₂, 5 mM DTT), 1 μ l DMSO and 1 μ l (2.9 pmole) primer, at 65°C for 5 minutes and quickly frozen in dry ice. After quick thawing and aliquoting the annealed sample into individual G, A, C and T reaction tubes, subsequent DNA sequencing reactions similar to protocols described by Koop *et al.* (5) were performed with the modification of adding 10% v/v DMSO (final concentration) to both the labeling and terminating steps. The US Biochemical Corp. (Cleveland, OH) Sequenase kit may also be used with miniprep DNA template and DMSO inclusion. Figure 1 shows

Figure 1. Comparison of sequencing reactions with and without DMSO. Standard dideoxy chain termination DNA sequencing reactions using miniprep plasmid DNA as template were performed without (A) and with (B) DMSO (10% v/v final concentration), and run on a denaturing 6% acrylamide gel. The arrows note the spurious band problems resolved by the inclusion of DMSO into the sequencing reaction mixture.

that DMSO has alleviated the artifact problems of spurious bands across two or more lanes.

Alternatively, denaturation of CsCl-banded double stranded

DNA template is less harsh and was performed as follows: 2 μ l 4 N NaOH and 20 μ l template were incubated for 5 minutes at room temperature and neutralized with 4 μ l 3 M NaAcetate (pH 5). To this was added 2.9 pmole of primer and the entire sample was ethanol precipitated as described. Following recovery, the pellet was dissolved in 8 μ l H₂O, to which was added 2 μ l 5 $\times$ sequencing buffer and 1 μ l DMSO. This was additionally heat denatured at 65°C for 5 minutes, placed immediately on dry ice and treated further as described above.

Although these reactions were performed in microcentrifuge tubes, there should be no problems incorporating DMSO into large scale radioactive, and possibly fluorescent, DNA sequencing protocols such as described by Koop *et al.* (5). The enhancement of the sequencing reaction with two template denaturation steps and with the inclusion of DMSO, along with the slight modification of the miniprep procedure, can produce cleaner sequences. Together these modifications allow unambiguous DNA sequence determination.

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