A fast and simple procedure for sequencing double stranded DNA with Sequenase

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Double stranded DNA sequencing of plasmids isolated from minipreps is now a routine practice. Here I provide a procedure for sequencing double stranded DNA using Sequenase (USB) where the sample maneuvering time can be reduced tremendously.

Conventionally, the DNA isolated from miniprep cultures is treated to remove RNA, followed by alkaline denaturation, neutralization and precipitation steps before being dissolved and hybridized to primers. The routine is rather time consuming especially when a large number of samples is involved.

The protocol described here modifies the steps after pelleting the miniprep DNA. It combines alkaline denaturation of the DNA, alkaline hydrolysis of RNA and annealing of the template and primers into a single step; thus RNAse digestion and phenol/chloroform extraction are totally eliminated. Furthermore, it does not require the DNA to be precipitated after alkaline denaturation. The result of these modifications not only saves time and improves the yield, but also reduces the exposure to hazardous chemicals.

The quality of the sequence information produced by this method is excellent (Figure 1). This method is presented in a step by step protocol as follows:

1. Dissolve the miniprep DNA pellet in 25 to 50 μl of TE buffer depending on the DNA yield (In my hands, the amount of DNA yield from the rapid-boiled method (1) is about twice as much as that of the alkaline-lysis method (2), i.e., 50 μl for the rapid-boiled method and 25 μl for the alkaline-lysis method). Use 5 μl per sequencing reaction.
2. Add 1 μl of sequencing primers (10 ng/μl).
3. Add 1 μl of 1N NaOH and mix by pipetting.
4. Incubate at 37°C for 10 min.
5 Add 1 μl of 1N HCl to neutralize and mix by pipetting.
6. Add 2 μl of 5 × Sequenase reaction buffer.
7. Continue to incubate at 37°C for 5 min to ensure primer/template annealing (optional).
8. Follow the remaining steps as described in the Sequenase Handbook (USB), i.e., add 0.1 M DTT (1 μl), reaction mix (2 μl), labeled dATP (0.1 to 1 μl) and diluted Sequenase (2 μl) ... etc.

I often prepare 24 or 36 minipreps (rapid-boiled method) and sequence 12 samples at a time. From the start of the miniprep (removal of the overnight cultures from the shaker) to the end of loading 12 sequencing samples (48 lanes), the procedure takes about 3 to 3.5 hrs.

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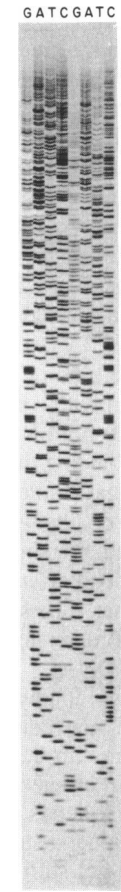

Figure 1. DNA sequence ladders determined by the method described.