Preparation of Miniprep-DNA for Automated Non-radioactive Sequencing


Numerous rapid miniprep procedures for isolation of plasmid DNA have been described during the last years, mostly based on the standard alkaline lysis protocol (1,3,4,6). However, in our hands none of these techniques that we have tested so far resulted in DNA pure enough to be used for automated sequencing. Most of the preparations did contain contaminations like bacterial DNA, RNA or proteins, which inhibited automated sequencing of the DNA using an ALF DNA Sequencer™ (Pharmacia Biotech, Freiburg, Germany). Furthermore, commercially available kits were too expensive to be used in our project of differential display of myocardial RNA (2), where large numbers of cloned PCR products had to be screened by restriction digest and automated sequencing. Therefore, we had to modify the standard alkaline lysis protocol in such a way that the technique was accurate and reliable for the isolation of plasmid DNA suitable for automated sequencing. The procedure is described in Table 1.

In this procedure, RNAs are removed by treatment with RNases like RNase A or RNase-It™ (Stratagene, Heidelberg, Germany). The subsequent phenol-chloroform-isoamyl alcohol extraction removes all residual proteins including the RNases, whereas all other inhibiting contaminations seem to be excluded from the preparation by isopropanol precipitation. The average plasmid yield of this procedure is 15 μg for high-copy number plasmids like pGEM® (Promega, Madison, WI, USA) or pBluescript® (Stratagene) at a concentration of about 0.5 μg/μL and less for low-copy-number plasmids like the pBR series. The plasmid DNA is mostly supercoiled and can be used for further applications like restriction analysis (Figure 1), isolation of inserts and labeling for Northern or Southern blot analysis. Most important, the DNA isolated with this procedure is suitable for automated nonradioactive sequenc-

**REFERENCES**


*We thank Ali T. van Loo-Bhattacharya for skillful technical assistance. This work was supported by National Science Foundation Grant MCB-9118757. Address correspondence to Timothy J. Larson, Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0308, USA. Internet: tilarson@vt.edu*

Received 9 January 1996; accepted 12 April 1996.

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Table 1. Protocol

1. Grow an overnight culture (15 h maximum) of the bacterial clone of interest in 3 mL LB medium supplemented with the appropriate antibiotics at 37°C with shaking at 250 rpm in a tube of 10 mL or more.

2. Pour about 1.5 mL in a microcentrifuge tube (store the rest at 4°C for further use) and centrifuge bacteria for 2 min at top speed in a microcentrifuge at room temperature.

3. Remove the supernatant completely using pipet tips and dry it by inverting for 5 min.

4. Resuspend the pellet in 150 µL of standard ice-cold GTE-buffer (50 mM glucose, 25 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0, optional; lysozyme at 2 mg/mL) by pipetting up and down. Do not vortex mix!

5. Add 200 µL of freshly prepared lysis buffer (0.2 N NaOH, 1% sodium dodecyl sulfate) and mix by inverting tubes carefully. Do not vortex mix! Leave on ice for 5 min.

6. Add 150 µL of 3 M potassium acetate solution, prepared as described previously (3), mix by inverting several times and centrifuge at top speed in a microcentrifuge for 10 min at room temperature.

7. Transfer supernatant in a new tube, add 0.5 volume of isopropanol (room temperature) and spin down for 10 min at top speed in a microcentrifuge at room temperature. Be careful not to disturb the pellet.

8. Discard supernatant, invert tube and dry pellet for 10–15 min. Do not let it dry down completely, otherwise the pellet will be difficult to resuspend.

9. Redissolve the pellet in 150 µL of RNase-A (10 mg/mL) or 6 µL of RNase-It (Stratagene) and incubate at 37°C for 30 min.

10. Extract twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), then add 1/10 vol 3 M sodium acetate, pH 5.2, and 0.5 vol isopropanol at room temperature, invert tube several times and centrifuge the plasmid DNA at top speed in a microcentrifuge for 5 min at room temperature.

11. Finally, wash the DNA twice with 1 mL of 70% ethanol, dry under a vacuum, redissolve in 30 µL water (TE buffer seems to inhibit the sequencing reactions) and store at 4°C until further use.

REFERENCES


Received 26 December 1995; accepted 9 April 1996.

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