Improved Alkaline Lysis Method for Rapid Isolation of Plasmid DNA

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Isolation of highly purified plasmid DNA from *E. coli* is an essential step in many techniques of current molecular biology. Traditional techniques for plasmid isolation are the alkaline lysis technique of Birnboim and Doly (2) and the boiling method of Holmes and Quigley (4), which are normally followed by a further purification by cesium chloride gradient centrifugation (9). Though the purity of the plasmids purified by these methods is sufficient for most routine uses, these procedures are both expensive and time-consuming. Many investigators have proposed alternative methods for isolating plasmid DNA on a small or large scale while eliminating the above drawbacks (1,6,10–12), but sometimes these methods are not very time- or cost-efficient. In addition, the partially purified plasmid DNA can only be used for less demanding applications.

Recently, we have developed an improved alkaline lysis method for plasmid purification that simultaneously maximizes time efficiency, yield and quality. The procedure uses alkaline lysis, high-concentration potassium acetate precipitation of cellular debris that can eliminate bacterial protein in supernatant efficiently and improve plasmid yield (3), enzymatic digestion of RNA (5) and recovery DNA with PEG-$MgCl_2$ (13% polyethylene glycol in 10 mM $MgCl_2$ at room temperature), which gives better recovery and takes less time than the PEG-NaCl method (7). Our protocol consistently provides high-quality plasmid DNA suitable for most routine uses in molecular cloning, and the whole process for a minipreparation can be easily completed in less than one hour with plasmid yields up to 8–9 µg/mL culture for high-copy-number plasmids such as pUC19 and pBluescript® (both from Stratagene, La Jolla, CA, USA). The procedure is outlined in Table 1.

Our modified protocol does not require toxic reagents such as phenol/chloroform, and the whole procedure can be carried out at room temperature. In the conventional method, it is always difficult to resuspend the bacterial cells completely with GTE by vortex mixing, but in this procedure, using a toothpick can make it easy. It is important to centrifuge the tubes at room temperature in step 6 because at high salt concentration, low temperature will lead to protein contamination and salt precipitation. Both low- and high-copy-number plasmids isolated with this method are largely supercoiled (Figure 1), and the optical density (OD) ratio at 260/280 nm is 1.92–2.0. These plasmids are suitable for all routine use such as restriction enzyme digestion analyses (Figure 1), double-stranded DNA sequencing (Figure 2), polymerase chain reaction (PCR) etc. Also, this labor- and time-saving method has proven to be very helpful in screening hundreds of recombinant clones at one time. It can be easily scaled up for a large-scale preparation of plasmid DNA with its yield up to 6–7 mg DNA/L culture for high-copy-number plasmids. Since the main reagent cost in this method is associated with the use of RNase A, the method represents great reduction in cost compared with the commercial kits (8) and is thus especially attractive for some laboratories where the reagents cost, rather than the labor cost, is the main concern.

In conclusion, our improved alkaline lysis method has the best features for a purification method: high yield and purity of the final products, low

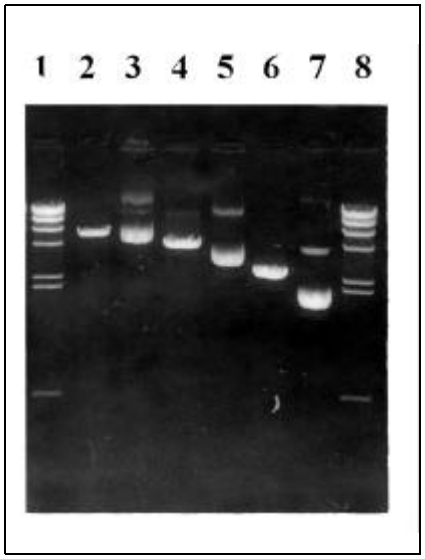

Figure 1. Agarose gel electrophoresis of plasmids purified by this method. Lanes 1 and 8: *Hin*dIII-digested λDNA marker. Lane 2: *Nco*I digestion of pET32a(+) (Stratagene). Lane 3: isolated pET32a(+) plasmid (5.9 kb, medium copy number). Lane 4: *Eco*RI digestion of pBR322 (Stratagene). Lane 5: isolated pBR322 plasmid (4.3 kb, low copy number). Lane 6: *Bam*HI digestion of pUC18. Lane 7: isolated pUC18 plasmid (2.7 kb, high copy number). Digestions of these plasmids with restriction enzymes were performed according to the instructions of Promega (Madison, WI, USA). Gel electrophoresis was done on a 1% agarose gel in 1× TAE buffer (20 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 5 V/cm.

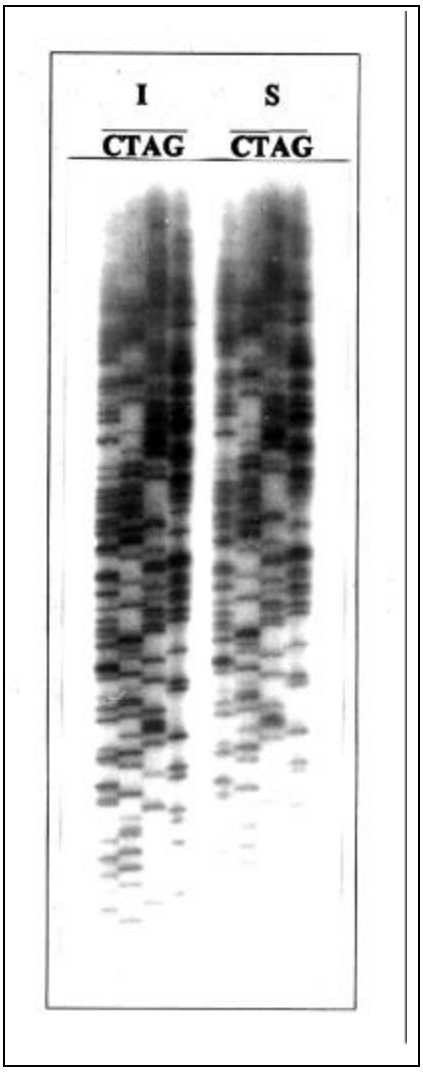

Figure 2. Double-stranded DNA sequencing. Plasmids purified by standard alkaline lysis (S) and the improved method (I) were used for comparison. Each sequencing reaction was carried out using 3 µg template DNA (plasmid pET-TPO), 1 pmol T7 promoter primer and T7 DNA Polymerase Sequencing Kit (Promega).

Table 1. Protocol for Alkaline Lysis Method

1. Grow *E. coli* (DH5αF′™ [Life Technologies, Gaithersburg, MD, USA] and TG1 [a gift from Dr. B. Cen, Shanghai Biochemistry Institute] were tested) transformed with the plasmid of interest in 3 mL LB medium containing appropriate antibiotics in a tube (1.5 × 15 cm). After overnight (15–16 h) growth at 37°C, harvest the bacterial cells by centrifugation at top speed for 20 s in a microcentrifuge.
2. Resuspend the bacterial pellets completely in 120 µL ice-cold GTE (50 mM glucose, 25 mM Tris-HCl, 10 mM EDTA, pH 8.0) by putting a toothpick in the tube and then vortex mixing for several seconds.
3. Remove the toothpick and add 240 µL freshly prepared lysis buffer (0.2 M NaOH, 1% sodium dodecyl sulfate [SDS]); mix quickly by inverting and swirling the tube several times until the contents become clear. Do not vortex mix.
4. Precipitate cellular debris by adding 360 µL ice-cold 4 M potassium acetate solution (prepared by mixing 80 mL 5 M potassium acetate, 11.5 µL glacial acetic acid and 8.5 mL H_2O). Mix the contents thoroughly by immediately swirling the tube. Do not vortex mix.
5. Centrifuge at top speed for 3 min at room temperature and transfer about 660 µL of the supernatant into a clean tube with a disposable pipet. Avoid collecting floating film of denatured material by submerging the pipet tip well into the solution before suction.
6. Add 330 µL isopropanol and invert tubes to mix well. Centrifuge at top speed for 3 min. Pour out the supernatant and place each tube back in the rotor in the original orientation. Quickly centrifuge the tubes at maximum speed for several seconds. Aspirate all residual supernatant from the tubes carefully.
7. Resuspend the pellet in 200 µL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing 20 µg/mL DNase-free RNase A. Keep warm at 37°C for 30 min.
8. Add 100 µL 40% PEG-8000 in 30 mM $MgCl_2$, mix well and then centrifuge at top speed for 5 min at room temperature.
9. Rinse the pellet with 1 mL ice-cold 75% ethanol, resuspending and centrifuging 1 min to remove PEG. Discard all the residual supernatant as described in step 6.
10. Air-dry and dissolve the plasmid DNA in 100 µL TE.

cost, rapidity and no use of toxic reagents.

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