Benchmarks

Plasmid Mini-Preparations from Culture Streaks


The rapid alkaline lysis protocol for the preparation of plasmid DNA from *E. coli* (1) has been extensively modified in recent years. For example, better quality plasmid preparations have been achieved through the use of extra purification steps (4) or by using solid phases that reversibly bind DNA (2). In these cases, however, the modifications call for more elaborate processing of a standard alkaline lysate. Other recent methods have included direct cell growth in the extraction tube (6) or even the use of a microwave oven for cell lysis (3). We have found that high-quality plasmid DNA can be efficiently isolated from a cleared alkaline lysate simply by direct precipitation using polyethylene glycol (PEG) (5). The only requirement is that the cleared lysate be prepared by substituting sodium acetate for potassium acetate, which results in cleaner lysate recovery and provides the sodium needed for efficient precipitation. The concentration of PEG used (ca. 6%) is at a threshold level that results in the preferential precipitation of large-sized nucleic acids over contaminating oligodeoxynucleotides and ribonucleotides (5). Under the conditions described, predominantly covalently closed circular plasmid DNA is isolated (Figure 1). In addition, we have simplified the steps needed to produce the cell lysate (Table 1). Rather than grow individual liquid cultures for each preparation, we collect cells directly from heavily grown streaks on agar plates. Up to eight culture streaks can be grown per plate and the cells are harvested by scraping the agar surface with a toothpick (Figure 1). After the cells have been harvested, the agar plates can be stored as temporary stocks, and the toothpick is used in subsequent steps as a mixing stick. The cells are easily collected and suspended if the toothpick is partially broken at its thick end before use.

The modifications described reduce the time and materials required for the mini-preparation of plasmid DNA. In addition, the simplified protocol is easily reproduced for consistent results. DNA recovery is typically 5–10 µg (as determined by absorbancy at 260 nm). The plasmid quality is suitable for all major applications including restriction analysis and DNA sequencing (see Figure 1).

Table 1. Protocol for Preparation of Plasmid DNA from Culture Streaks

1) Using a clean pair of forceps, partially break a toothpick near its thick end so that it resembles a hockey stick. Use the toothpick to recover 10–15 medium-sized colonies (or equivalent) from a culture streak (Figure 1). Suspend cells in 200 µL of solution 1 [25 mM Tris-HCl, pH 7.5, 10 mM EDTA, 1% (wt/vol) glucose and 50 µg/mL RNase A (2)] in a 1.5-mL microcentrifuge tube by gently spinning the toothpick between thumb and forefinger and let stand at room temperature for 10 min.

2) Add 300 µL of freshly prepared solution 2 [0.2 N NaOH, 1% (wt/vol) sodium dodecyl sulfate], mix thoroughly by again spinning the toothpick (not too violently) and let stand for 10 min at room temperature.

3) Add 230 µL of solution 3 (3 M sodium acetate adjusted to pH 5.2 with glacial acetic acid), mix thoroughly and place on ice for 15 min. Discard the toothpick, pellet the debris by centrifugation for 5 min (12,000×g) and pour the cleared lysate into a new tube.

4) Add 200 µL of solution 4 [30% (wt/vol) PEG 8000 (Sigma Chemical, St. Louis, MO, USA)], mix thoroughly by repeatedly inverting the tube and incubate on ice for at least 30 min. Solution 4 should be stored in small aliquots at -20°C to prevent microbial growth. Recover the DNA by centrifugation for 5 min (12,000×g) and wash the pellet with 70% ethanol and then 95% ethanol.

Resuspend the dried DNA in 200 µL TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7.5).

Figure 1. Preparation of plasmid DNA from cells grown on agar plates. Cells were directly harvested from agar plates using a partially broken toothpick (Panel A). Panel B shows a high-quality preparation that was isolated from a cleared alkaline lysate by direct PEG precipitation. The plasmid DNA (pTZ19R; Amersham, Arlington Heights, IL, USA) with a 2.5-kbp ribosomal DNA insert was fractionated in a standard 1.0% agarose gel. Lane 1 shows the plasmid as predominantly a covalently closed circle, and lane 2 shows a complete HindIII/BamHI restriction endonuclease digestion. Panel C shows an example autoradiograph of a standard dideoxy DNA sequencing reaction.
Method for Preparation of RNA from Ruminococcus albus

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Ruminococcus albus is one of the bacteria of the rumen microflora that is difficult to lyse. Rapid lysis is crucial for the isolation of RNA since the average half-life of bacterial mRNA is approximately 3 min (1). There are many procedures for the isolation of good-quality RNA from various sources (3–8,10). Some procedures use physical disintegration methods that require special equipment such as a French press (9) or a nitrogen decompression chamber (5). We tried many chemical and enzymatic lysis methods on R. albus, but these lysis methods gave very low RNA yields. Here we report a rapid and simple procedure for the extraction of total RNA from R. albus (Table 1).

RNA yield, determined spectrophotometrically, was 6.3 μg from 1 mL of culture. Upon analysis of RNA by agarose gel electrophoresis, discrete 23S and 16S rRNA bands were visible in a background smear of high and low molecular weight RNA (Figure 1, line 2). Reverse transcription polymerase chain reaction (RT-PCR) was also used to assess the structural integrity of the RNA preparation. The RT-PCR primers were designed for amplification of the 229–1793 region of the xynA gene (GenBank® Accession No. U43089) of

Table 1. Protocol for the Extraction of Total RNA from R. albus

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tr>
<td>1.</td>
<td>Grow R. albus 7T (ATCC 27210, Rockville, MD, USA) to an optical density (OD&lt;sub&gt;600&lt;/sub&gt;) of 0.7–1.0 in RGCA medium (2) at 37°C.</td>
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<td>2.</td>
<td>Harvest the cells by centrifugation at 10,000×g for 3 min at 4°C, and store the pellet at -80°C until use.</td>
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<td>3.</td>
<td>Melt the cells harvested from 1-mL cultures and add 50 μL TESKD lysis buffer (200 mM Tris-HCl, pH 8.0, 20 mM EDTA, 2% sodium dodecyl sulfate, 2 mg/mL proteinase k, 25 mM dithiothreitol).</td>
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<td>4.</td>
<td>Mix the solution well and incubate for 3 min at room temperature.</td>
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<td>5.</td>
<td>Add 300 μL solution I, 300 μL solution II and 600 μL solution III of SepaGene®-RV (SancoJunyaku, Ltd., Tokyo, Japan), which is the total RNA isolation kit based on the agglutination partition method (9), and mix for 10 s at room temperature.</td>
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<td>6.</td>
<td>Put the tube on ice for 15 min and centrifuge at 15,000×g for 3 min at 4°C.</td>
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<td>7.</td>
<td>Collect the upper phase and precipitate with an equal volume of isopropanol.</td>
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<td>8.</td>
<td>Centrifuge at 10,000×g for 3 min at 4°C, and treat the RNA pellet with RNase-free DNase (Stratagene, La Jolla, CA, USA).</td>
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REFERENCES


Figure 1. Agarose gel electrophoresis of R. albus RNA. Samples from a 1-mL culture were run on a 0.7% agarose gel and stained with ethidium bromide. Lane 1, SepaGene-RV extract without TESKD lysis treatment; lane 2, SepaGene-RV extract with TESKD lysis treatment; and lane M, 1-kb DNA ladder (Life Technologies, Gaithersburg, MD, USA).

Figure 2. Purity check of RNA by RT-PCR. RT-PCR amplification was performed using R. albus RNA template, specific primers for the xynA (U43089) gene of R. albus and RT-PCR high (To-yobo, Ltd., Tokyo, Japan). Samples were run on a 2.0% agarose gel and stained with ethidium bromide. Lane 1, reverse transcription reaction with primers and PCR with primers; lane 2, reverse transcription reaction without primers and PCR with primers; and lane M, 1-kb DNA ladder.