Current protocols in Molecular Biology

Bacteria from a saturated liquid culture are lysed and proteins removed by digestion with proteinase K. Cell wall debris, polysaccharides, and remaining proteins are removed by selective precipitation with CTAB, and high-molecular-weight DNA is recovered from the resulting supernatant by isopropanol precipitation.

Materials

- TE buffer (see below)
- 10% sodium dodecyl sulphate (SDS)
- 20 mg/ml proteinase K (stored in small single-use aliquots at -20°C)
- 5 M NaCl
- CTAB/NaCl solution (see below)
- 24:1 chloroform/isoamyl alcohol
- 25:24:1 phenol/chloroform/isoamyl alcohol
- Isopropanol
- 70% ethanol

1. Inoculate a 5-ml liquid culture with the bacterial strain of interest. Grow in conditions appropriate for that strain (i.e., appropriate medium, drug selection, temperature) until the culture is saturated. This may take several hours to several days, depending on the growth rate.

2. Spin 1.5 ml of the culture in a micro centrifuge for 2 min, or until a compact pellet forms. Discard the supernatant.

3. Resuspend pellet in 567 µl TE buffer by repeated pipetting. Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K to give a final concentration of 100 µg/ml proteinase K in 0.5% SDS. Mix thoroughly and incubate 1 hr at 37°C. The solution should become viscous as the detergent lyses the bacterial cell walls. There should be no need to predigest the bacterial cell wall with lysozyme.

4. Add 100 µl of 5 M NaCl and mix thoroughly. This step is very important since a CTAB-nucleic acid precipitate will form if salt concentration drops below about 0.5 M at room temperature (Murray and Thompson, 1980). The aim here is to remove cell wall debris, denatured protein, and polysaccharides complexed to CTAB, while retaining the nucleic acids in solution.

5. Add 80 µl of CTAB/NaCl solution. Mix thoroughly and incubate 10 min at 65°C.

6. Add an approximately equal volume (0.7 to 0.8 ml) of Phenol/Chloroform/Isoamyl-alcohol, mix thoroughly, and spin 4 to 5 min in a microcentrifuge. This extraction removes CTAB-protein/polysaccharide complexes. A white interface should be visible after centrifugation.
7. Remove aqueous, viscous supematant to a fresh microcentrifuge tube, leaving the interface behind. Add an equal volume of chloroform/isoamyl alcohol, extract thoroughly, and spin in a microcentrifuge for 5 min.

With some bacterial strains the interface formed after chloroform extraction is not compact enough to allow easy removal of the supernatant. In such cases, most of the interface can be fished out with a sterile toothpick before removal of any supernatant. Remaining CTAB precipitate is then removed in the phenol/chloroform extraction.

8. Transfer the supernatant to a fresh tube. Add 0.6 vol. isopropanol to precipitate the nucleic acids (there is no need to add salt since the NaCl concentration is already high). Shake the tube back and forth until a stringy white DNA precipitate become: clearly visible. At this point it is possible to transfer the pellet to afresh tube containing 70% ethanol by hooking it onto the end of a micropipet that has been heat-sealed and bent in a Bunsen flame. Alternatively, the precipitate can be pelleted by spinning briefly at room temperature.

If no stringy DNA precipitate forms in the above step, this implies that the DNA has sheared into relatively low-molecular-weight pieces. If this is acceptable, i.e., if DNA is to be digested to completion with restriction endonucleases for Southern blot analysis, chromosomal DNA can often still be recovered simply by pelleting the, precipitate in a microcentrifuge.

9. Wash the DNA with 70% ethanol to remove residual CTAB and respin 5 min at room temperature to repellet it. Carefully remove the supernatant and briefly dry the pellet in a lyophilizer.

10. Redissolve the pellet in 100 µl TE buffer.

This may take some time (up to 1 hr) since the DNA is of high molecular weight. 1.5h of this DNA will typically digest to completion with 10 U EcoRI in 1 hr which is sufficient to be clearly visible on an agarose gel, or to give a good signal during Southern hybridization.

REMOVAL OF POLYSACCHARIDES FROM EXISTING GENOMIC DNA PREPS

Steps 4 through 10 of the basic protocol can be adapted for removing polysaccharides an other contaminating macromolecules from existing bacterial chromosomal DNA prepa rations. Simply adjust the NaCl concentration of the DNA solution to 0.7 M and add 0. vol. CTAB/NaCl solution. A white interface after the chloroform/isoamyl extraction. indicates that contaminating macromolecules have been removed. The CTAB extraction step (steps 5 and 6) can be repeated several times until no interface is visible.
MINIPREP OF BACTERIAL GENOMIC DNA

1. Grow bacterial strain to saturation.

2. Spin 1.5 ml for 2 min in microcentrifuge.

3. Resuspend in 567 μl TE buffer, 3 μl of 20 mg/ml proteinase K. Mix and incubate 1 hr at 37°C.

4. Add 100 ~1 of 5 M NaCl. Mix thoroughly.

5. Add 80 μl of CTAB/NaCl solution. Mix. Incubate 10 min at 65°C.


7. Transfer aqueous phase to a fresh tube. Extract DNA with phenol/chloroform/isoamyl alcohol. Spin 5 min in microcentrifuge.

8. Transfer aqueous phase to a fresh tube. Extract DNA with 0.6 vol. isopropanol. Wash precipitate with 70% ethanol. Remove supernatant and briefly dry pellet in lyophilizer.

9. Resuspend pellet in 100 μl TE buffer.

REAGENTS AND SOLUTIONS

CTAB/NaCl solution (10% CTAB in 0.7 M NaCl)
Dissolve 4.1 g NaCl in 80 ml water and slowly add 10 g CTAB (hexadecyltri-methyl ammonium bromide) while heating and stirring. If necessary, heat to 65°C to dissolve. Adjust final volume to 100 ml.

Tris-Cl 1 M (pH 8.0)
solve 121 g Tris base (mw = 121.14) in 800 ml aqua adjust the pH to 8.0 with conc. HCl (appr. 45 ml), add bidest to make the volume 1 liter.

EDTA 0.5 M (pH 8.0)
solve 186.1 g Na₂EDTA-2H₂O (mw = 372.24) in 700 ml aqua by adjusting the pH to 8.0 with 10 M NaOH (appr. 45 ml), add bidest to make the volume 1 liter.

TE (pH 8.0)
10 mM Tris-Cl 10ml/l 1M Tris-Cl pH 8.0
1 mM EDTA 2ml/l 0.5M EDTA pH 8.0
COMMENTARY

Background Information
Most commonly used protocols for the preparation of bacterial genomic DNA consist of lysozyme/detergent lysis, followed by incubation with a non-specific protease, and a series of phenol/chloroform/isoamyl alcohol extraction's prior to alcohol precipitation of the nucleic acids (Meade et al., 1984; Silhavy et al., 1982). Such procedures effectively remove contaminating proteins, but are not effective in removing the copious amounts of exopolysaccharides that are produced by many bacterial genera, and which can interfere with the activity of molecular biological enzymes such as restriction endonucleases and ligases. In this procedure, however, the protease incubation is followed by a CTAB extraction whereby CTAB complexes both with polysaccharides and with residual protein; both groups of contaminating molecules are effectively removed in the subsequent emulsification and extraction with chloroform/isoamyl alcohol. This procedure is effective in producing digestible chromosomal DNA from a variety of Gram-negative bacteria, including those of the genera Pseudomonas, Agobacterium, Rhizobium, and Bradyrhizobium all of which normally produce large amounts of polysaccharides. If large amounts of exceptionally clean DNA are required, the procedure can be scaled up and the DNA purified on a cesium chloride gradient, as described in the alternate protocol. The method can also be used to extract high-molecular-weight DNA from plant tissue (Murray and Thompson, 1980).

Critical Parameters
Contributed by Kate Wilson
The most critical parameter is the salt (NaCl) Massachusetts General Hospital concentration of the solution containing the Boston, Massachusetts lysed bacteria prior to adding CTAB. If the NaCl concentration is <.5 M then the nucleic acid may also precipitate; indeed, CTAB is frequently used for just this process (Murray and Thompson). It is also important to maintain all solutions above 15°C, as the CTAB will precipitate below this temperature.

Anticipated Results
The typical yield from both the MINIPREP and the large-scale prep is 0.5 to 2 mg DNA per 100 ml starting culture (10^8 to 10^9 cells/ml).

Time Considerations
The miniprep takes ~2 hr, including the 1-hr incubation. The large-scale prep takes slightly longer to reach the point where the CsCl gradient is loaded. Subsequent steps will spread over 1 to 2 days, depending on the time of the CsCl gradient spin,