细菌 Genomic DNA 提取*

Materials

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

Method

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 microcentrifuge 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 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 supernatant to a fresh microcentrifuge tube, leaving the interface behind. Add an equal volume of phenol/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 becomes clearly visible. At this point it is possible to transfer the pellet to a fresh 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. 15 l 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

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 (hexadecyltrimethyl ammonium bromide) while heating and stirring. If necessary, heat to 65°C to dissolve. Adjust final volume to 100 ml

***:**出自 Current Protocols in Molecular Biology [B], *Wiley Online Library,*2007