碱裂解提取质粒 DNA*

Materials

Glucose/Tris/EDTA (GTE) solution
TE buffer
NaOH/SDS solution
Potassium acetate solution
95% and 70% ethanol
10 mg/ml DNase-free RNase (optional)
1.5-ml disposable microcentrifuge tubes

1. Inoculate 5 ml sterile LB medium with a single bacterial colony. Grow to saturation (overnight).

2. Spin 1.5 ml of cells 20 sec in a microcentrifuge at maximum speed to pellet. Remove the supernatant with a Pasteur pipet.

The spins in steps 2 and 6 can be performed at 4°C or at room temperature. Longer spins make it difficult to resuspend cells.

3. Resuspend pellet in 100 µl GTE solution and let sit 5 min at room temperature.

Be sure cells are completely resuspended.

4. Add 200 µl NaOH/SDS solution, mix by tapping tube with finger, and place on ice for 5 min.

5. Add 150 µl potassium acetate solution and vortex at maximum speed for 2 sec to mix. Place on ice for 5 min.

Be sure mixing is complete.

6. Spin 3 min as in step 2 to pellet cell debris and chromosomal DNA.

7. Transfer supernatant to a fresh tube, mix it with 0.8 ml of 95% ethanol, and let sit 2 min at room temperature to precipitate nucleic acids

8. Spin 1 min at room temperature to pellet plasmid DNA and RNA.

9. Remove supernatant, wash the pellet with 1 ml of 70% ethanol, and dry pellet under vacuum.

10. Resuspend the pellet in 30 µl TE buffer and store as in support protocol. Use 2.5 to 5 µl of the resuspended DNA for a restriction digest.

Contaminating RNA may interfere with detection of DNA fragments on the agarose gel; it can be destroyed by adding 1 l of a 10 mg/ml RNase solution (DNase-free) to the digestion mixture.

Glucose/Tris/EDTA (GTE) solution
50 mM glucose
25 mM Tris~Cl, pH 8.0
10 mM EDTA
Autoclave and store at 4°C

NaOH/SDS solution
0.2 N NaOH
1% (wt/vol) sodium dodecyl sulfate (SDS)
Prepare immediately before use

5 M potassium acetate solution, pH 4.8
29.5 ml glacial acetic acid
KOH pellets to pH 4.8 (several)
H_2O to 100 ml
Store at room temperature (do not autoclave)

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