Plasmid DNA extraction

Miniprep Alkaline Lysis Method

Protocol

Reagents and solutions

- Phenol chloroform isoamyl alcohol (25:24:1)
- Sodium acetate 3.0 M.
- TE (10 mM tris pH 7.9, 1 mM EDTA pH 7.9)
- Nuclease-free water
- Solution 1 - STE (15% saccharose, 50 mM tris pH 7.9, 50 mM EDTA pH 7.9)

Saccharose	15 g
Tris	0.6057 g
EDTA	1.8612 g

Dissolve the reagents in 80 mL of distilled water, adjust pH to 7.9 and complete to 100 mL. Sterilize by autoclaving, store at room temperature.

- Solution 2 - Lysis solution (0.2 M NaOH, 1% SDS)

NaOH	8 g
SDS	1g

Dissolve the reagents in 80mL of distilled water, adjust volume to 100 mL.
Store at room temperature.

- Solution 3 (3.0M Potassium acetate pH 5.5)

KCH_3COO	29.442 g
CH_3COOH	11.5 ml

Dissolve the potassium acetate in 60 mL of distilled water, add the glacial acetic acid and complete the volume to 100 mL. It is not necessary to adjust pH.

Protocol

1. Bacterial clones are growth overnight at 37 ºC in shaker incubator (250 rpm).
2. Centrifuge 1.5 mL at room temperature for 1 minute 3 times (to reach 4.5 mL). Remove media.
3. Resuspend in 100 µl of STE, vortex at room temperature.
4. Add 200 µL of lysis solution, mix by inverting.
5. Add 100 µL of potassium acetate solution, shake softly.
6. Chill on ice for 10 min.
7. Centrifuge for 15 min at 12000 rpm at 4 ºC. remove supernatant and transfer to eppendorf tube.
8. Add 400 µL of phenol chloroform isoamyl alcohol, shake manually for 5 minutes until the mixture acquires a milky look.
9. Centrifuge at room temperature for 5 minutes at 12000 rpm. Three layers must be formed: An upper aqueous layer, a white part in the middle, and the phenol in the bottom.

10. Remove aqueous phase (350 µL approximately), transfer to another tube and add 20 µL of sodium acetate.

11. Add 800 µL of 95% ethanol, mix by inverting the tube twice. Store at -20ºC overnight.

12. Centrifuge for 15 min at 4ºC and 12000 rpm. Remove alcohol by decantation.

13. Wash with 1mL of 70% ethanol, centrifuge for 5 min at 12000 rpm.

14. Remove alcohol by decantation, being careful not to detach the pellet. Leave the tube open to allow the remaining alcohol to evaporate.

15. Resuspend the pellet in 25 µL of TE, divide into 5-10 µL aliquots.

16. DNA Quantification: Quantify DNA concentration by measuring at 260 and 280 nm to obtain 260/280 absorbance relation:

D.O. = 50 µg/µl DNA

Use the following relations to asses the DNA quality:
$D.O._{260}/D.O._{280}$ = 1.8 (Pure DNA)
$D.O._{260}/D.O._{280}$ = 2.0 (Pure RNA)
$D.O._{260}/D.O._{280}$ = <1.8 (Contamination by phenol or proteins)