Experiment (1)
Plasmid Isolation and Purification

Materials:

Chemical

LB medium, Ethylene diamine tetra acetate (EDTA), NaOH, Tris-HCl, glucose, potassium acetate, acetic acid, Sodium dodecyl sulphate (SDS), NaCl, Tryptone, Yeast extract, Tris-Cl, Ethanol.

Preparation of solutions

1- LB medium

To 950 ml of deionize H_2O add 10g Tryptone, 5g yeast extract, and 10g NaCl. Shake until the solution dissolve. Adjust pH to 7.0 with 5N NaCl. Adjust the volume to 1L with deionize H_2O. Sterilize by autoclave for 20 minutes at 15 psi on liquid cycle.

2- Alkaline lysis solution I

50 mM glucose, 25 mM Tris-Cl (pH 8.0), 10 mM EDTA (pH 8.0), deionized water.

3- Alkaline lysis solution II

0.2 N NaOH, 1% (w/v) SDS, deionized water.

4- Alkaline lysis solution III

5 M potassium acetate, acetic acid, deionized water.

Equipment and Glassware

Microfuge centrifuge, electronic balance, microcentrifuge tube, centrifuge tube, Pasteur pipette, micropipette, tips.

Protocol:

1. Centrifuge the bacterial samples at 4 °C, maximum speed for 5 minutes, using microcentrifuge device.
2. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.
3. Resuspend the bacterial pellet in 100 µl of ice-cold alkaline lysis solution I then vortex vigorously.
4. Add 200 µl of freshly prepared alkaline lysis solution II to the bacterial suspension. Invert the tube rapidly 5 times. Store the tube on ice for 1 min.
5. Add 150 µl ice cold alkaline lysis solution III to the microcentrifuge tube. Invert the tube 3-5 times, then incubate the tube on ice for 3-5 minutes.
6. Centrifuge the bacterial lysate at maximum speed for 3 minutes.
7. Transfer the supernatant to a new labelled microcentrifuge tube.
8. To the tube, add 2 volumes of 95% ethanol.
9. Vortex and allow the tube to stand at room temperature for 2 minutes.
10. Centrifuge at maximum speed for 5 minutes.
11. Remove the supernatant by gentle aspiration.
12. Stand the tube in an inverted position over a paper towel to allow all fluid to drain away.
13. Add 20 µl of 70% ethanol, then invert the closed tube several times.
14. Centrifuge at maximum speed for 5 minutes.
15. Remove the supernatant by gentle aspiration.
16. Remove any beads of ethanol from the sides of the tube. Leave tube open at room temperature until residual ethanol has evaporated.
17. Dissolve the pellet in 25-50 µl sterile water or TE buffer and vortex the solution gently for few seconds.
18. The plasmid DNA can be stored at -20 °C.

Results:

- Concentration of plasmid DNA (ng/µl) = _____
- Plasmid purity: A_{260}/A_{280} = _____