PLASMID EXTRACTION

Plasmid DNA preparation

Method

Many different methods are available for isolation of plasmid DNA. In this practical you will be using a commercially available kit, which utilizes an affinity column to purify the plasmid DNA. Bacteria (*E. coli* in this case) containing plasmid is grown from a single colony in a suitable medium such as Luria Broth (LB). An aliquot of the culture is used for preparing the plasmid DNA.

Principle

Reagent	Principle
Centrifugation Bacterial culture	• Centrifugation results in pelleting of bacterial cells
Solution I (Tris pH 8.0 EDTA- Ethylene diamine tetra acetic acid and Glucose) RNase Lysozyme- optional	• Glucose gives osmotic shock that leads to the rupture of cell wall and membrane • EDTA, inhibits nucleases • Rnase: Dergrades RNA • Lysozyme is used for rupturing cell wall of bacteria
Solution II Sodium hydroxide SDS- Sodium dodecyl sulphate (Alkaline pH `12.0)	• Lyses the cell completely • Alkaline pH denatures chromosomal DNA but not the covalently closed circular plasmid DNA
Solution III Sodium or potassium acetate (Acidic pH 5.4)	• Neutralizes the alkaline pH • Precipitates protein and forms SDS-protein complex • Chromosomal DNA renatures and aggregates with protein
Centrifugation	• Pellets the Protein –DNA aggregates • Plasmids will be present in the supernatant (These plasmid can be precipitated with ethanol- This used to be the conventional method)
Spin column Silica membrane	• Silica membrane binds to plasmid DNA at high salt buffer condition
Elution	• Under low salt condition (Distilled water), plasmids can be eluted from the silica membrane

Protocol

Each group is provided with 10ml of an *E.coli* (containing plasmid) culture that has been grown overnight at 37^{o}C.

1. Transfer 1.5ml of bacterial culture to a micro centrifuge tube.
2. Pellet cells by centrifuging at 12,000 rpm for 2 minutes
3. CAREFULLY remove the supernatant.
4. Add another 1.5ml of culture to the same tube and centrifuge and repeat step 2 &3
5. Add 250 µl of Solution I /RNAase.
6. Resuspend the pellet by vortexing briefly or by pipetting up and down.
7. Add 250 µl of Solution II
8. Mix GENTLY by inverting and rotating the tube several times. DO NOT vortex!!!
9. Leave at room temperature for 5 minutes but NOT MORE than 5 minutes.
10. Add 350 µl of Solution III
11. Mix by inverting the tube 6-8 times
12. Centrifuge at 12,000 rpm for 12 min
13. While your tubes are spinning place a Spin column in a 2-ml collection tube.
14. After centrifugation, CAREFULLY transfer the supernatant to the column. DO NOT disturb the pellet.
15. Centrifuge the column and collection tube at 12,000 rpm for 1 min
16. Discard the flow through collected in the collection tube.
17. Replace the column in the collection tube
18. Add 250 µl of HB Buffer into the column and centrifuge for 1 minute at 12,000 rpm and discard the flow through.
19. Add 700 µl of wash buffer into the column and centrifuge for 1 minute at 12,000 rpm and discard the flow through.
20. Centrifuge again to remove residual buffer for 90 seconds
21. Place the column in a sterile 1.5ml tube.
22. Add 50µl of deionised water or TE buffer into the column
23. Centrifuge at 12,000 rpm for 1 min.
24. Discard the column. Plasmid DNA is in the deionised water or TE buffer will be collected in the 1.5ml tube.
25. Place the tube containing plasmid DNA on ice for further use or store at –20^{o}C