Protocol for Plasmid DNA Extraction using Alkaline Lysis Method
Protocol code: Alk_Lys

Materials

- RNAase
- Isopropanol
- Absolute Ethanol
- Glucose
- EDTA
- NaOH
- SDS
- Potassium acetate
- Glacial acetic acid
- Tris
- Concentrated HCl
- 2mL Centrifuge tubes

Solutions

1. **Resuspension Solution** [50 mM glucose, 10 mM EDTA(pH 8.0), 25 mM Tris (pH 8.0)]
    a. Prepare the solution as described in the following table.

Reagent	Volume (mL)
1M Glucose (filter sterilized)	5
1M Tris (pH 8.0)	2.5
0.5M EDTA (pH 8.0)	1
De-ion water	91.5

    b. Autoclave or filter sterilize using membrane milipore (0.20µm).
    c. Store at 4°C.

2. **Lysis Solution** (0.2 M NaOH 1% SDS)
    a. Prepare the solution as described in the following table.

Reagent	Volume (mL)
0.2M NaOH	0.2
1% SDS	1
De-ion water	8.8

3. **Neutralizing Solution** [3 M Potassium acetate (pH 6.0), 1.2 M acetic acid]
    a. Prepare the solution as described in the following table.

Reagent	Volume (mL)
5M Potassium acetate (pH 6.0)	60
Glacial acetic acid	11.5
De-ion water	28.5

Procedure

1. Grow 4 mL bacterial culture in LB medium with appropriate antibiotics overnight with shaking.
2. Transfer culture to a 2 mL tube and spin down cell culture at highest speed for 1 min at table-top centrifuge.
3. Discard the supernatant. To remove the liquid completely, place the tube upside down onto a piece of paper towel for a few seconds.
4. Add 250 µL of Resuspension Solution into each tube and vortex to completely resuspend cell pellet.
5. Add 250 µL of Lysis Solution and mix by gently inverting the tube 5-6 times. The solution should quickly turn colorless and become more viscous which indicates bacterial lysis has taken place.
6. Add 350 µL of Neutralizing Solution and mix by inverting the tubes several times. At this point bacterial chromosomal DNA is usually seen as a white precipitate.
7. Centrifuge the tubes at highest speed for 10 minutes.
8. Carefully transfer the supernatant (try to not disturb the white precipitate) to a new labeled 2mL tube.
9. Add 1 volume of cold isopropanol (stored at -20 °C) to each tube and mix by inverting the tubes a few times. Spin down plasmid DNA precipitate (transparency pellet) at highest speed for 5 min.
10. Discard the supernatant and remove the remaining liquid as much as possible by leaving the tube upside-down on a piece of paper towel
11. Add 500 µL of cold ethanol 70% (stored at -20 °C) to each tube and mix by inverting the tubes a few times. Spin down plasmid DNA precipitate (transparency pellet) at highest speed for 2 min.
12. Discard the supernatant and remove the remaining liquid as much as possible by leaving the tube upside-down on a piece of paper towel. To dry faster, keep tubes at 42°C heat block for 20 minutes.
13. Resuspend the DNA pellet with 50 µL TE buffer + 1 µl ARNase. Completely dissolve the pellet by pipetting solution several times.
14. Incubate at 37 °C for 30 mins.
15. Store at 4°C.

References

Birnboim, H. C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res,* 7(6): 1513-1523.

Birnboim, H. C. (1983). A rapid alkaline extraction method for the isolation of plasmid DNA. *Methods Enzymol,* 100: 243-255.