Derived from Gardiner, et al. 2006

Non-commercial Plasmid Mini Preparations

Ilsa León, Llinás Lab 2007

Reagents

- **Alkaline Lysis Buffer**: 0.1 M Tris-HCl pH 8.0; 0.1 M NaOH; 0.5% SDS; 1 mM EDTA; add RNase A to 50 µg/ml prior to use from a 10 mg/ml stock stored in -20ºC
  For 10 ml: 1 ml 1 M Tris-HCl, pH 8.0; 1 ml 1 M NaOH; 20 µl 0.5 M EDTA; 500 µl 10% SDS. Add 5 µl of a 10 mg/ml RNase A stock for every 1 ml of Alkaline Lysis Buffer needed in the below protocol.
- 3 M Na-Acetate pH 5.3
- Isopropanol (-20ºC)
- Tris-EDTA (TE) buffer
- Phenol:Chloroform (1:1 v/v, pH of phenol must be between 7.8-8.0)
- 100% ethanol
- 70% ethanol
- LB

Materials

- 50 ml conical tubes
- 1.5 ml tubes
- Allegra® 25R and TA10.250 rotor plus yellow adapters for 15 ml conical tubes
- Microcentrifuge in 4ºC
- Microcentrifuge at room temperature
- Vortex
- Disposable pipette tip attached to vacuum line and side armed flask
- Laminar air flow cabinet

A. Preparation of Cells

1. Start a 25 ml O/N culture with the proper antibiotic.
2. Take 1.5 ml of culture and pellet the cells by centrifuging for 60 seconds in a microcentrifuge. Remove remaining media by aspiration, leaving the bacterial pellet as dry as possible. Repeat 2 more times.

B. Lysis of Cells

3. Add 400 µl alkaline lysis buffer and loosen the pellet with vigorous vortexing to lyse the cells. (Run your Eppendorf tubes along a microfuge rack several times to assist you. It is very important that you resuspend your pellet completely). Place on ice for 5 min.
4. Add 200 µl 3 M Na-Actetate pH 5.3 and mix well by briefly vortexing.
5. Centrifuge sample at maximum speed in a tabletop centrifuge for 5-10 min.

C. Recovery of Plasmid DNA

6. Transfer supernatant to a fresh tube and precipitate by adding 550 µl cold isopropanol. Mix well by vortexing. Incubate for 10 min on ice and centrifuge for 30 min at 4ºC, max speed (or incubate in -20 ºC indefinitely).
7. Dry the pellet briefly and resuspend in 500 µl TE or water.

D. Phenol:Chloroform Extraction

8. Add an equal volume of phenol:chloroform.
9. Mix by vortexing until an emulsion forms.
10. Centrifuge the emulsion at maximum speed for 2 minutes.
11. Transfer the aqueous upper layer to a fresh tube without pipetting the interface or organic phase.
12. Repeat steps 8-11 with the extracted aqueous layer.

E. Ethanol Precipitation

13. Add 3 M Na-Acetate pH 5.3 at 1/10 the volume of the aqueous phase.
14. Mix the solution well.
15. Add 3 volumes of (ice-cold) 100% ethanol and mix the solution well.
16. Incubate the ethanol solution on ice for 15-30 minutes to allow the DNA to precipitate (this solution may be left indefinitely at 0ºC or at -20ºC).
17. Pellet DNA by centrifugation at max speed in 4°C for 30 min. Carefully remove the supernatant by aspiration.
18. Wash the pellet with 500 µl of 70% ethanol. Centrifuge the sample at max speed for 2 minutes at 4ºC. Carefully remove the supernatant as above.
19. Perform the following steps in a laminar air flow cabinet. Dry the pellet at room temperature until the last traces of fluid have been evaporated. The pellet represents approximately 40-60 µg DNA.
20. Resuspend the pellet in appropriate amount of cytomix and store in 4ºC until ready to use.
21. Spec 1:100 dilution checking OD_{260} and $OD_{260/280}$.