Alkaline Lysis Procedure for Minipreparation of Plasmid DNA

by TCH 08/18/01

1) Transfer 1.5 to 2.0 ml overnight *E. coli* cultures into 2.0 ml microfuge Eppendorf tubes.

2) Spin down the cells at top speed for 1.0 min at room temperature, and aspirate the supernatant.

3) Add 200ul of BD-I, and vortex vigorously (***Note: it's very important to resuspend the cell pellet completely***).

4) Add 200ul of BD-II and mix gently by inverting the tubes several times.

5) Add 200ul of BD-III and mix well by inverting the tubes.

6) Spin the tubes at top speed for 2 minutes at room temperature.

7) Pour supernatants to a new set of 1.5ml microfuge tubes; add 500ul 2-propanol; mix well.

8) Centrifuge at top speed for 4-5 minutes at room temperature.

9) Aspirate supernatant; Add 500μl of 70% ethanol, vortex well, and spin down for 1 min. **Repeat the washing step once**.

10) Aspirate supernatant; Spin down for 10 seconds and aspirate the residual liquid completely.

11) Dissolve DNA in 70 ul of ddH_2O (or LoTE).

NOTE: 1) In most cases, 5 ul of miniprep DNA are sufficient for restriction reaction.

2) To reduce RNA contamination in the miniprep DNA, one can add DNase-free RNase to BD-I.

BD-1: 50mM glucose, 25mM Tris-HCl pH8.0, 10mM EDTA pH8.0
BD-2: 93ml ddH_2O, 2ml 10N NaOH, 5ml 20%SDS

BD-3: 5M potassium acetate 60ml, glacial acetic acid 11.5ml, ddH_2O 28.5ml