RAPIDPREP PLASMID DNA PREPARATION PROTOCOL

Adapted from Pelham, H. (1985) Trends in Genetics, ***1****:6 by Vinh Pham.*
Last modified: May 22, 2002.

MATERIALS:

TEG Buffer (25mM Tris, 50mM Glucose, 10mM EDTA, adjust to pH 8.0 with HCl)
Alkaline Lysis Buffer/ALB (0.2N NaOH, 1% SDS)
3M Potassium Acetate, pH 5.5 (or **3M Sodium Acetate**, pH 4.8)
24:1 Chloroform:Isoamyl Alcohol ($CHCl_3$:IAA)
10mM Tris, pH 8.5 (or **TE**, or **dH_2O**)

PROCEDURE:

1. Inoculate desired culture (1.5ml to 3ml) and grow overnight.

   Note: The incubation time varies with culture medium used and the size of the inoculum. Cultures inoculated into LB can be extracted by about 4-5 hours.

2. Pellet cells at 14Krpm for 30 sec and discard s/n.
3. Add 100µl TEG, resuspend cells.

   Note: Clumping of cells reduces plasmid DNA yield.

4. Add 200µl ALB, mix by inverting 5-6 times.

   Note: Atmospheric CO_2 reacts with the OH^- and acidifies the ALB. For optimal plasmid DNA extraction, only use ALB that is less than one week old.

5. Add 150µl 3M Potassium Acetate (pH 5.5) or 3M Sodium Acetate (pH 4.8).
6. Pellet at 14Krpm for 10 min.
7. Transfer 400µl of s/n to new tube containing 1ml $CHCl_3$:IAA, mix by inverting.
8. Spin at 14Krpm for 10 min.
8. Transfer aqueous phase to 800µl of ice-cold 95% EtOH.
9. Precipitate at -20ºC for at least 30 min.
10. Pellet at 14Krpm for 10 min.
11. Discard s/n, wash pellet with 1ml 70% EtOH, then 1ml 95% EtOH.

    Note: The 70% EtOH wash removes salts due to the relatively high [water].

12. Discard EtOH and dry by Speed-Vac (or air-dry).

13. Dissolve in 30µl-50µl distilled water, 10mM Tris-HCl (pH 8.5), or TE.

14. Store at -20ºC.