Biology 324 **Leaf/Moyer**
Winter 2000 **Lab 3 - DNA minipreps**

This is a protocol modified from Brian Seed, a legendary Cal Tech graduate student who pioneered many of the techniques in molecular cloning. You will use this protocol to characterize the insert in the pTA.cad3 plasmid which was transformed into *E.coli* XL-1 Blue cells last week.

Preparing bacterial cells
1.Grow cells to near saturation in 5 ml liquid culture.

2. Pour cultures into 15 ml centrifuge tubes.

3. Centrifuge in table top centrifuge 5 minutes (4000 rpm). Pour off liquid. Remove all of the supernatant. Samples can be frozen or used immediately.

Lysing bacterial cells
4. Add 200 µl of Soln I and resuspend cells. Avoid bubbles.

5. Transfer to 1.5 ml eppendorf tube. Make sure cells are completely resuspended.

6. Add 400 µl of fresh Soln II, Mix gently but thoroughly. The solution should clear as the cells lyse. Do not leave the sample in solution II for more than 5 minutes.

Precipitating chromsomal DNA, proteins, and lipids
7. After the cells are lysed, Add 300 µl of Soln III . Shake the tubes by hand and mix well.

8. Spin in eppendorf for 10 min.

9. Draw the clear supernatant into a fresh tube. Phase gel will be provided. Phase gel has an intermediate density between the phenol and aqueous layers and physically separates them.

Extracting protein from nucleic acids
10. Add 400 µl of phenol, pH 6.

PHENOL IS VERY HAZARDOUS. IT CAUSES PAINFUL BURNS. ALWAYS WEAR SAFETY GLASSES AND LAB COATS AROUND PHENOL.

11. Vortex. Spin in eppendorf for 3-4 min.

12. Transfer the aqueous (top) phase into a new tube.

AFTERWARDS PLACE ALL PHENOL TUBES INTO THE PHENOL WASTE CONTAINER IN THE HOOD.

Precipitating nucleic acids
13. Add 600 µl of isopropanol (or until full), shake, spin in eppendorf for 1-2 min.

14.Rinse with 70% EtOH.

15.Dry the pellets. This can be done by air drying or by speed-vac'ing.
(Speed vac'ing is drying under a vacuum and is a little faster).

Precipitating large RNA
16. Resuspend in 100 µl TE. Add 1.5 volumes of 8M LiCl. Set in -20 for 30 minutes to overnight. This should precipitate the large RNA molecules.

17. Spin in eppendorf for 15 minutes. Transfer the supernatant into a new eppendorf tube.

Precipitating plasmid DNA
18. Add 1/2 volume 7M NH_4OAC and 2 volumes EtOH to supernatant. Put into -20 freezer for 30 minutes to overnight.

19. Spin sample for 15 minutes to pellet.

20. Resuspend in 20 µl TE. Use 10 µl for restriction digestion.

Diagnosing plasmid for insert
21. Set up appropriate restriction digestion to determine if the plasmid has an insert. Check the map of the plasmid first and verify with your instructor.

22. Run out the restriction digest and the undigested DNA on a 1.0% agarose gel with 0.5X TBE and the appropriate amounts of EtBr.

Solution I
50 mM glucose (MW 180)
25 mM Tris-HCl pH 8
10mM EDTA pH 8
filter sterilize or autoclave

Solution II: Must be FRESH.
0.2 N NaOH, 0.1% SDS

Solution III
For 100 ml
60 ml of 5 M KOAc, 11.5 ml Acetic Acid and 28.5 ml water.