Biology 324 **Moyer**
Winter 2002 **Lab 3 - Plasmid DNA minipreps**

This is a protocol modified from the classical "Molecular Cloning Lab Manual" protocol to add in dealing with HMW RNA. You will use this protocol to characterize a cloned insert in a pTA plasmid which was transformed into *E.coli* XL-2-Blue or TOP10F' cells.

Preparing bacterial cells

1. Grow cells to near saturation in 50 to 500 ml liquid culture in LB, YT, or TB with required antibiotic.

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 ice-cold Soln I and resuspend cells. Avoid bubbles.

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

6. Add 400 µl of fresh Soln II, Mix gently but thoroughly by inverting and/or finger-flicking. The solution should clear as the cells lyse. Store on ice.

Precipitating chromsomal DNA, proteins, and lipids

7. After the cells are lysed, Add 300 µl of ice-cold Soln III. Shake the tubes by hand and mix well. Ice for 3 - 5 min.

8. Spin in microfuge for 10 - 15 min in cold room.

9. Draw the clear supernatant into a fresh MF 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:chloroform mixture (~equal volumes between phases).

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

11. Vortex. Spin in microfuge 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 microfuge 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 freezer for 30 minutes to overnight. This should precipitate the large RNA molecules.

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

Precipitating plasmid DNA

18. Add 1/2 volume of 7.5 M NH_4OAC (~125 µl) and 2 volumes (~750 µl) EtOH to supernatant. Put into -20 freezer for 30 minutes to overnight.

19. Spin sample for 15 minutes at top speed in MF 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 against the Hi-Lo DNA marker with 1X TBE and the appropriate amounts of EtBr.

Alkaline Lysis Solutions:

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

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

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