Typed 30 June 2007
Original 2/27/91

PLASMID MINI-PREP PROTOCOL
(from Promega for high quality DNA)

1. Inoculate 5 ml of LB broth containing 100 µg/ml ampicillin with a single bacterial colony. Incubate at 37ºC overnight with vigorous shaking.

2. Place 1.5 ml of culture into a microcentrifuge tube and centrifuge for 1 minute. The remainder of the overnight culture can be stored at 4ºC.

3. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.

4. Resuspend the pellet by vortexing in 100 µl of an ice-cold solution containing:

	Stock	10ml
50 mM glucose	1 M	0.5 ml
10 mM EDTA	0.5 M	0.2 ml
25 mM Tris-HCl, pH 8.0	1 M	0.25 ml

5. Store 5 minutes at room temperature.

6. Add 200 µl of a freshly prepared solution containing 0.2 N NaOH, 1% SDS. Mix by inversion. **DO NOT VORTEX.** Incubate 5 minutes on ice.

7. Add 150 µl of ice-cold potassium acetate (pH 4.8). (The solution is 3 M with respect to potassium and 5 M with respect to acetate.) (See Maniatis p. 447). Mix by inversion for 10 seconds. **DO NOT VORTEX.** Incubate on ice for 5 minutes.

8. Centrifuge for 5 minutes.

9. Transfer supernatant with a pipetman to a fresh tube avoiding the white precipitate.

10. Centrifuge the supernatant for an additional 5 minutes. Transfer the supernatant to a fresh tube, avoiding the white precipitate.

11. Add RNase A to a final concentration of approx. 20 µg/ml. Mix well by gentle vortexing.

12. Incubate at 37ºC for 20 minutes.

13. Add an equal volume of phenol/chloroform. Mix. Centrifuge 5’ and transfer 0.4 ml of aqueous phase to a fresh tube.

14. Add 2.5 volumes of absolute ethanol. Mix well by vortexing and precipitate 5 minutes on dry ice.

15. Centrifuge 4 minutes. Rinse the pellet with 1 ml of 70% ethanol (prechilled) and dry pellet in speedvac.

16. Dissolve the pellet in 16 µl deionized water. Add 4 µl 4M NaCl, mix, and then add 20 µl 13% PEG (polyethylene glycol; MW 8,000). Mix well, centrifuge 2 seconds and incubate on ice for 20 minutes.

17. Centrifuge 10 minutes. Orient tubes in rotor when spinning so as to keep track of location of invisible pellet. Remove supernatant with pipetman set at 40 µl and wash the pellet with 1 ml 70% ethanol (prechilled). Dry pellet. The pellet is almost invisible.

18. Dissolve the dried pellet in 20 µl of sterile deionized water. A yield of 1-3 µg of DNA should be expected.

19. Digest 2 µl with 3-4 units of restriction enzyme – 15-20 µl reaction volume.

NOTE: All solvents (phenol, $CHCl_3$, EtOH) should be pipetted out of a secondary container (not out of stocks). All "ice" incubations should be in <u>contact</u> with ice.

This DNA is of very high quality.