**LYSOZYME-TRITON PLASMID PREP**

1) **Grow up 5ml culture during the day; inoculate O/N culture – 500 LB Broth in a 2L flask. Add appropriate antibiotic.**

2) **Harvest cells, 5K, 15 minutes. Beckman JA-10 rotor.**

3) **Resuspend into 12.0 ml, 25% sucrose, 0.05M tris pH 8.**

4) **Make fresh - add 2.0 ml, 10mg lysozyme/ml.**
   - 0.05M tris. pH 8
   - Incubate on ice for 15 minutes.

5) **Add 4.0 ml 0.25M EDTA, pH 8. Incubate on ice for 15 minutes.**

6) **Add 16.0 ml 2% triton.**
   - 0.05M tris, pH 8
   - 0.0625M EDTA, pH 8
   - Incubate on ice for 15 minutes

7) **Pour into screw-top bottle.**
   - Boil 10 minutes
   - Tighten lids
   - Spin 18K (30 minutes) in Beckman JA-10 rotor
   - 30 minutes

8) **Recover supernatant into 50ml plastic tube. (Should produce 28-30 ml, if not, re-boil and re-spin).**

9) **Add 0.96 g CsCl/ml of lysate recovered.**

10) **Add 0.036 ml of 10 mg EtBr/ml per ml of lysate recovered.**

11) **Pour into screw-top bottle, spin 18K, 30 minutes (to clear).**

12) **Filter through glass wool into Beckman Quick Seal (30 ml size) tubes. (12 cc syringe & 18 g needle, a bit of glass wool).**

13) **Balance tubes to within 0.01g, add oil (light mineral oil) and seal.**

14) **Spin in VTi 50 rotor, 48K, 16 hours.**

15) **Recover the bottom band and place into Beckman Quick Seal (5ml) tube, spin again in VTi 65, 55K, 5 hr or 48K O/N.**

16) **Recover bottom band and extract 4 times with Butanol/water saturated.**

17) **Add 2 volumes of TE to the bottom layer (contains DNA).**

18) **Add 2 volumes of 100% EtOH and precipitate O/N. (Don’t add salt, you want to get rid of CsCl).**

19) **Spin 7.5K, 15 minutes in JS-13 rotor.**

20) **Wash 2 times with 70% EtOH and then dry.**

21) **Re-suspend in 200-400 µl of TE.**

22) **Add 3M NaOAc to make a 1:10 dilution of the NaOAc.**

23) **Transfer to Eppendorf tubes and do 2 PCI extractions (25:24:1) and 2 CIA (24:1) extractions.**

24) **Precipitate DNA with 2 volumes of 100% EtOH O/N.**

25) **Wash 2 times with 70% EtOH and then dry.**

26) **Resuspend in 200-400µl of TE and take ODs.**