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.