# Large Scale Plasmid DNA Purification (CsCl Gradient Banding)

**by TCH 1/25/02**

1. Grow 100-150ml *coli* culture overnight.
2. Collect bacterial culture to 250ml bottles (for Eppendorf 5810), and centrifuge at **3,800**rpm x 25 min (in Eppendorf 5810 at cold room).
3. Decant supernatant, and resuspend pellets in 10ml **BDI**. Vortex well.
4. Add 10ml **BDII**. Invert tubes gently.
5. Transfer resuspended pellets/lysates to 50ml polypropylene conical tubes.
6. Add 10ml **BDIII**. Invert sharply several times.
7. Centrifuge @ **3,500**rpm x 15min (Eppendorf 5810 at 4°C).
8. Collect cleared supernatant by passing through funnel/Kimwipes in beakers or 50ml conical tubes.
9. Transfer about 20ml of cleared supernatant to two clean 50ml conical tubes, and add 15ml isopropanol (2-propanol, or 2/3 volume of the DNA solution). Mix well.
10. Spin @ **3,500** rpm x 20min at 4°C.
11. Decant supernatants, wash pellets with 20ml of 70% ethanol (vortex and spin down for 5-10min).
12. Remove the ethanol carefully, and dry the pellets by inverting the tubes on paper towels for 5-10min.
13. Dissolve pellets in **8.2ml dd$H_2O$** (combine two tubes to one for each sample), and add **8.8g CsCl**. Vortex well.
14. Add 100ul ethidium bromide (10mg/ml), mix well.
15. Transfer DNA solution to **Ti50** ultracentrifuge tubes (SETON 6041) w/ transfer pipettes. Screw on collars, and put tops on. Tighten with nut screwdriver.
16. Place in **Ti 50** rotor and spin ~20 hrs @ **45,000**rpm, @ 20°C, following instruction on **Beckman L7** ultracentrifuge.
17. Prepare two syringes (3 cc) with one 22 gauge needles for each prep. Label syringes so you won't mix them up.
18. Carefully remove tubes from rotor with hemostat. Loose or remove inner nut from centrifuge tubes. Put on face shield. Insert needle just beneath supercoiled band and bevel up to aspirate the band.
19. Pour remaining CsCl in chemical waste and wash centrifuge tube cones and nuts. The plastic centrifuge tubes are disposable but **the nuts and cones are not disposable**.

20. Extract with an equal volume of $H_2O$ saturated 1-Butanol in 15 ml conical tubes (**Saturated 1-butanol** = 40 ml 1-butanol + 10 ml H2O; the butanol stays on top).
21. Vortex and sit 10-60 sec @ RT. Pipette off/discard top (red/pink) layer.
22. Repeat three times (total of 4 extractions) or until the red/pink color completely disappear. Add equal volume of $H_2O$-saturated 1-butanol each time. (If begin with 1.5 ml, end with ~ 2.5 ml of solution).
23. Add ddH2O to 10ml mark (or 5 volumes ddH2O to final volume of DNA) in a 50 ml tube. Mix well and add 20-30ml (or 2 volumes) of 100% ethanol (e.g., if 2.5 ml DNA, add 12.5 ml ddH2O, mix, then add 30 ml ethanol).
24. Centrifuge 30 min @ **3,500** rpm in Eppendorf 5810.
25. [***Optional:*** *Wash pellets with 70 % ethanol twice, Re-spin and remove last drop of 70% ethanol.* ***You may be at risk losing a lot of DNA using this method!***].
26. Dissolve pellets in 200ul ddH2O (it may take up to 30min, or place the tube in 37°C water bath). Briefly spin down, and transfer the DNA solution to a 1.7ml microfuge tube. Add 100ul 7.5M ammonium acetate and 600ul 100% ethanol.
27. Mix well and spin in microcentrifuge at top speed for 5 min at room temperature.
28. Wash pellets with 70% ethanol twice.
29. Dissolve in 300-500ul of dd$H_2O$ (depending on the thickness of supercoiled band; it may take up to 30min, or place the tube in 37°C water bath). Read **OD260 *vs.* OD280** to determine the quality and quantity (concentration) of plasmid DNA. Stock DNA should be kept at –20°C.

**STOCK SOLUTIONS**:

**BDI (Lab Stock):**
50 mM glucose, 25 mM Tris pH 8.0, 10 mM EDTA stored @ 4°C
(made as follows: 4.5 gm glucose, 12.5 ml 1 M Tris-HCl pH 8.5, 25 ml 0.2M EDTA, 460 ml H2O, pH 8.05).

**BDII (Made by yourself):**

0.125N NaOH/1% SDS (made by yourself: 1 ml 50%NaOH, 10 ml 10%SDS, q.s. with $H_2O$ to100 ml).

**BDIII (Lab Stock):**

3M K+, 5M Acetate pH 4.8

(made as follows: 147.3 g K.Acetate, 57.5 ml Acetic Acid, $H_2O$ to 500 ml, pH ~4.8).