Plasmid Maxi-Prep On CsCl Gradients

1. Grow 1 liter of E. coli + plasmid overnight at 37°C in LB + antibiotic. Transfer culture volume to four 250 ml centrifuge bottles. Chill cells on ice for 10 minutes.

2. Centrifuge at 4°C for 10 minutes at 5500 rpm (~5000 g) in Sorvall GSA rotor.

3. Remove as much supernatant as possible. Add 40 mls of GTE and resuspend pellet completely by repipetting. Add 50 mg Lysozyme and swirl to dissolve. Hold at room temperature for 10 minutes.

4. Add 80 mls of freshly made 0.2 N NaOH + 1% SDS (dilute 2 M NaOH and 10% SDS). Mix by swirling until all clumps are dispersed. Place on ice 15 minutes.

5. Add 60 ml of 3M KOAc, pH 5. Swirl liquid in centrifuge bottle while adding KOAc slowly. Mix completely. Stir gently with glass rod if clumps don’t disperse. Place on ice 15 minutes.

6. Centrifuge at 4°C for 10 minutes at 5500 rpm in Sorvall GSA rotor.

7. Pour supernatant into 250 ml bottle through 4 layers of cheesecloth to remove small pieces of precipitate. Total volume should be about 150 mls (estimate total volume by eye).

8. Add 90 mls (0.6 volumes) of isopropanol. Mix. Hold at room temperature for 5 minutes.

9. Centrifuge at 4°C for 10 minutes at 5500 rpm in Sorvall GSA rotor.

10. Remove as much supernatant as possible. Disperse pellet in 50 ml of 70% EtOH.

11. Centrifuge at room temperature for 5 minutes at 5500 rpm in Sorvall GSA rotor.

12. Remove as much supernatant as possible. Invert bottle on Kimwipe for 5 minutes to drain off 70% EtOH. Dry pellet for about 5 minutes using gentle stream of filtered air. Do not dislodge pellet.

13. Add 10 mls of TE. Cap top of bottle. Dissolve pellet at room temperature for 1-2 hours or at 4°C overnight. Place bottle at angle to submerge pellet. Pellet must be completely dissolved.

14. Transfer DNA/TE to 50 ml tube. Rinse bottle twice with 5 mls TE and pool in tube. Carefully adjust volume to 20.0 ml with TE buffer. Use a pipette to measure volume.

15. Add exactly 22.0 g CsCl to tube. Mix. Place at 37°C for 5 minutes to bring to room temperature. Mix until completely dissolved.

16. Add 2.0 ml of 10 mg/ml EtBr (5 mg/ml OK). Mix completely. A flocculant precipitate is normal. Make up extra CsCl solution (10.0 mls TE + 11.0 g CsCl + 1.0 ml EtBr).

17. Load quick-seal polyallomer tube for VTi50 rotor. Wear gloves: EtBr is toxic. Do not use ultraclear tubes. Use a Pasteur pipette. Fill to bottom of neck with extra CsCl solution. Seal tube with heat. Test for leaks by squeezing tube over beaker. The precipitate will pellet during spin. Tubes should be balanced to within 0.1 g. Do not forget spacers above tubes in VTi50 rotor.

18. Spin in VTi50 at 50,000 rpm at 20°C for 24-48 hours. No brake. After spin, handle tubes carefully to avoid turbulence and mixing of gradient. Examine gradients with long wavelength UV lamp. Use eye protection. Two bands should be visible near the middle of the tube. If bands are not near the middle of the tube, repeat the ultracentrifugation in a new tube after adjusting the density.
of the CsCl solution. If the bands are not tight and well-separated, continue ultracentrifugation for
24 hours. Red bands will be visible in room light if >100 µg of DNA is present per band.

- Top band = chromosomal DNA.
- Bottom band = plasmid DNA.

19. Harvest bottom band. Note position of protein “pellet” along side of tube. Handle tube carefully,
and avoid dislodging protein pellet from side wall of tube. RNA is at bottom of tube.

A. Place tube in stable rack and turn down room lights. Place a piece of Scotch tape on side of the
tube away from pellet. Middle of tape about 1-1.5 cm below bottom band.

B. Insert small (e.g., 25) gauge needle in top shoulder of tube to allow air to enter.

C. Insert 18-20 gauge needle with 5-10 ml syringe through tape about 1-1.5 cm below bottom
band. Beveled side of needle up. Position tip of needle just below bottom band. Before
inserting needle, loosen plunger by moving back and forth in syringe. This avoids the jerk that
accompanies movement of the plunger when starting to remove DNA.

D. Slowly remove bottom band keeping needle tip below band as it sinks and disappears. Volume
collected will be about 4-5 mls. Do not collect any of the top DNA band. Measure volume in
syringe.

20. Transfer to 30 ml Corex tube. Extract with 5 ml ddH$_2$O-saturated 1-butanol. Cap tube with
rubber stopper (#4). Vortex well and allow phases to separate. Remove upper butanol layer
(pink). Discard in proper EtBr waste container. Repeat extractions (3-5 times) until no pink
color appears in the butanol layer.

21. Add 2 volumes of ddH$_2$O. Mix thoroughly. Divide equally between 2 X 30 ml Corex tubes. Measure
volume of solution with pipette.

22. Add 2 volumes of 100% EtOH at room temperature to each tube. Total volume in both tubes = 6 X
volume at step 20. Mix thoroughly. Hold on ice for 15 minutes. DNA precipitate should be
visible.

23. Spin in HB-4 rotor at 8000 rpm (10,000 g) for 15 minutes at 0-4°C. Use adaptors for tubes.

24. Remove supernatant. Invert tubes on Kimwipe to drain off excess liquid. Leave tubes open to air
on bench to dry.

25. Dissolve each pellet in 200 µl TE. Transfer to microfuge tube. Rinse Corex tube twice with 100
µl TE and pool in microfuge tube. Measure total volume in microfuge tube (400 - 450 µl).

26. Ethanol precipitate: Add 1/10 volume of 3 M NaOAc, pH 5. Mix thoroughly. Add 2 volumes of
100% EtOH. Mix thoroughly (rotate 10 minutes at room temperature). Hold at room temperature
for 10 minutes.

27. Spin in microfuge 10 minutes at top speed at room temperature.

28. Add 1 ml 70% EtOH to each pellet. Disperse pellets. Hold at room temperature for 5 minutes.

29. Spin in microfuge 5 minutes at top speed at room temperature. Dry in speed-vac using tubes
covered with parafilm + holes.

30. R/S each pellet in 0.5 ml TE. Place at 37°C to dissolve DNA. Pool DNA into one tube. Dilute
1/1000 and measure DNA concentration.