**Plasmid DNA Prep (Large Scale $CsCl_2$ prep)**

**Growth and Amplification:**

1. Streak out strain of interest onto LB plate with the appropriate antibiotic selection. Incubate at 37°C overnight.

   (Meanwhile prepare reagents for the plasmid prep).

2. Inoculate 5ml LB containing the appropriate antibiotic with a single colony. Shake overnight at 37°C.

3. Inoculate 500 ml LB containing the appropriate antibiotic with the 0.5ml of the overnight. Shake at 37°C.

4. At $OD_{590}$ = 0.6 (mid-log phase; i.e., about 2-5 hrs later) add 100mg chloramphenicol powder. Continue to shake at 37°C for an additional 12-18 hrs.

**Plasmid Lysate:**

1. Pellet cells in SLA1500 rotor at 6000 rpm for 10min. Pour off the spent medium.

2. Quick spin cells down to pull down the last bit of medium; remove this with an L1000.

3. Resuspend cells in 8.0ml Tris-Sucrose.

4. Transfer the resuspended cells to an Oak Ridge tube. Place the tube on ice.

5. Prepare a 10mg lysozyme/ml Tris-sucrose solution (keep at RT and don't vortex because vortexing will denature lysozyme.

6. Add 2.0ml of the lysozyme solution to the cells. Blow the solution into the Oak Ridge Tube containing the resuspended cells. Do not mix, as this will cause overlysis; simply by blowing the solution into the tube sufficient mixing results.

7. Incubate on ice 5 min.

8. Add 4.0ml 0.5M EDTA in the same manner. Again, do not mix.

9. Incubate on ice 10 min.

10. Add 9.2ml 4% Triton X-100/Tris/EDTA sol'n in the same manner. No mixing.

11. Incubate on ice 20min.

12. Spin 16K rpm, 1h, 4°C. Chromosomal DNA and other cell stuff will precipitate.

13. Tare one Oak Ridge tube per prep (they're about 16g each).

14. Carefully decant the supernatant into the clean & tared tube. Try not to carry over any of the pellet (there is a ton of plasmid DNA in the supernatant so there is no need to get every last drop).

15. Add 1/10 volume of 5M NaCl and 1/10 weight of PEG 8000 (e.g., for 20g plasmid supernatant add 2.0ml 5M NaCl and 2.2 g PEG 8000).

16. Completely dissolve the PEG into the supernatant.

17. Incubate on ice 1-2h (overnight is okay too).

18. Spin 9K rpm, 30min, 4$^{o}$C to pellet the plasmid DNA.

19. Pour off the supernatant.

20. Dissolve the pellet in 20ml TE.

21. Add 24g CsCl. Dissolve completely.

22. Turn off room lights.

23. Add 1.5ml 10mg/ml Ethidium Bromide.

24. Check the refractive index of the sol'n. The refractive index should be 1.389. Adjust up with CsCl, down with TE.

25. Transfer the sol'n to a large quick seal tube. Add balance sol'n to bring the volume up to the neck of the tube.

    Balance solution: 22.2ml TE
    23.7g CsCl
    1.5ml 5mg/ml EtBr

    Refractive index = 1.389 (adjust like above, accordingly).

26. Balance tubes against each other.

27. Seal. Cleanse the outside of the tube of residual CsCl/EtBr (CsCl is extremely corrosive and can damage the titanium rotors). Place tubes in rotor slots, cap slots and torque to 220 psi.

28. Centrifuge 40K rpm, 16-18h (longer is okay), 25$^{o}$C.

29. Stop centrifuge.

30. Turn off room lights and remove tubes from the rotor. Visualize the DNA bands with long wave uv light source. The thick lower band is the supercoiled plasmid DNA. The thin upper band is nicked plasmid DNA and/or bacterial chromosomal DNA fragments.

31. Carefully puncture the top of the tube (to let air in when the bands are pulled) with a needle.

32. Just below the band to be pulled, insert into the tube, bevel side down, a 25g needle attached to a 10ml syringe. Be careful not to puncture your hand and/or the other side of the tube. Once inserted, turn the syringe so the bevel side is now facing up. Pull the band.

33. Reband the DNA. Transfer DNA to a small quick-seal tube. Bring the volume to the neck of the tube with balance solution.

34. Spin 40K rpm, ~18hrs, 25$^{o}$C.

35. Lights out. Remove the band as before.

36. Transfer the DNA solution to a sterile 15ml sealable tube.

37. Remove the EtBr from the plasmid DNA by repeated extractions with NaCl-saturated isopropanol:

(a) Add an approximately equal volume of NaCl-saturated isopropanol.
(b) Mix by a few quick flicks of the wrist.
(c) Let the two layers separate.
(d) Remove the upper pink isopropanol band.
(e) Repeat until no pink remains.
(f) Repeat twice more.

38. Dialyze the DNA into sterile $T_{10}N_5E_{0.1}$. A minimum of three 100:1 (dialysis buffer: DNA solution) changes should be performed.

39. Read $A_{260}$, $A_{280}$ and $A_{320}$.

Solutions and Stuff:

1. LB

2. 40mM $Tris_{(8.0)}$/25% Sucrose

| | |
|---|---|
| (a) Sucrose | 50g |
| (b) $ddH_2O$ | to 192ml |
| (c) Autoclave | |
| (d) 1M $Tris_{(8.0)}$ | 8.0ml |
| (e) Store 4°C | |

3. 40mM $Tris_{(8.0)}$/10mM EDTA/4% Triton X-100

| | |
|---|---|
| (a) 1M $Tris_{(8.0)}$ | 8.0ml |
| (b) 0.5M EDTA | 4.0ml |
| (c) Triton X-100 | 8.0ml |
| (d) $ddH_2O$ | 180ml |
| (e) A/C | |

4. 5M NaCl
5. 0.5M EDTA
6. $T_{10}E_{0.1}$
7. 10 mg/ml Ethidium Bromide
8. PEG 8000 (Polyethylene glycol 8000)
9. CsCl (technical grade)

10. NaCl-saturated isopropanol:

    (a) Heat 100ml $T_{10}N_5E_{0.1}$ to almost boiling and add as much NaCl as the solution will take.
    (b) Cool to RT.
    (c) Add 400ml Isopropanol.
    (d) Shake and allow to separate.
    (e) Use the upper, isopropanol phase to extract EtBr from the DNA.

11. Dialysis buffer ($T_{10}N_5E_{0.1}$):

| | |
|---|---|
| (a) 1M $Tris_{(8.0)}$ | 40ml |
| (b) 5M NaCl | 4ml |
| (c) 0.5M EDTA | 0.8ml |
| (d) $dH_2O$ | 3955ml |
| (e) A/C | |

Materials:

Notes:

The original protocol called for M9 minimal media. Sambrook claims this is unnecessary.
All recombinant bacteria must be A/C.
All solutions are stored at RT unless otherwise noted.