Alkaline Lysis Large Scale Plasmid Preparation

1. Set up 10 ml overnight culture.

2. Add overnight to 500 mls of sterile LB with appropriate selective agent (e.g. amp, tet...)

3. Incubate at 37°C with shaking (12-16 hr at 225 rpm) until the culture reaches log phase (O.D. = 0.4 at 600 nm).

4. Aliquot 2 mls and miniprep if desired.

5. Spin culture into a pellet in large bacterial centrifuge containers at 5,000 rpm x 10 minutes.

6. Pour off supernatant and resuspend pellet in 20 mls of 50mM Tris, 5mM EDTA solution.

7. Separate the resuspension into two 50 ml centrifuge tubes.

8. Spin the tubes at 5,000 rpm x 10 minutes (or at 5,000 rpm x 15 minutes in the tabletop centrifuge.)

9. Freeze pellet at -70°C for 15-30 minutes or at -20°C for 2-3 days.

10. Resuspend the pellets in 5 mls cold filter sterilized solution I.

11. Add 50 mgs of lysozyme to each tube, vortexing if necessary so that the solution becomes viscous.

12. Allow tubes to sit at room temperature for 5 minutes.

13. Add 10 mls of fresh solution II per tube.

14. Invert tubes to an upside down position and vortex gently. Invert tubes to a right side up position and vortex gently again.

15. Place tubes on ice for 15 minutes.

16. Add 7.5 mls of cold autoclaved solution III per tube.

17. Invert tubes vigorously until the solution becomes cloudy.
18. Place tubes on ice for 10 minutes.

19. Spin tubes at 12,000 rpm for 40 minutes.

20. Transfer supernatants to two 50 ml centrifuge tubes, discard pellets.

21. Add 0.6 volumes of isopropanol (0.6 volumes in comparison to the amount of supernatant) and 1 ml of 5 M NaCl. Allow DNA to precipitate at room temperature for 15-30 minutes.

22. Spin in a centrifuge at 10-12,000 rpm for 15 minutes.

23. Remove supernatant and dry pellet.

24. Add 5 mls TE pH8.0 to each tube, resuspend pellet, and combine into one tube.

25. Add 10 µl of 10 mg/ml RNase A under the fume hood with disposable tips. Use 1µl of 10 mg/ml stock for every ml of solution in the tubes. Incubate at 37°C for 1 hour.

26. Add an equal volume of phenol to the solution in the tubes, vortex to form an emulsion, and leave on ice for 5 minutes. Spin at 8,000 rpm x 15 minutes to separate the phases.

27. Remove supernatant and extract it with an equal volume of phenol:chloroform (1:1). Spin in a centrifuge at 6,000 rpm x 15 minutes. Repeat extraction and separation with chloroform alone.

28. Remove supernatant and add it to an equal volume of isopropanol and 0.1 volume of NaCl.

29. Place at -20°C for 1-2 days or at -70°C for 2-3 hours to precipitate DNA.

30. Spin at 12,000 rpm for 45 minutes.

31. Discard supernatant and wash pellet with 70% ethanol. Remove as much ethanol as possible.

32. Cover the tubes with parafilm. Poke holes in the film so that the pellets can be vacuum dried for about 10 minutes.
33. Resuspend the pellets in 1 ml of TE pH 8.0. Aliquot 5 µl and save to run on a 0.8% gel to check for plasmid DNA.

34. Bring volume up to 7.6 mls with room temperature TE.

35. Add 8.2 grams of cesium chloride per tube. Make certain that it goes into solution. Heat tubes at 55°C if necessary.

36. Cover tubes with aluminum foil and add 0.42 mls of 10mg/ml EtBr.

37. Put solution into an ultracentrifuge tube with pastuer pipettes.

38. Add immersion oil to the top of the solution in the ultracentrifuge tube. Use a syringe (best) or an pastuer pipette and avoid getting oil into the neck of the tubes.

39. Balance tubes (with caps if using that type) to within 0.01g.

40. Note tube positions in rotor as labels may wear off while spinning. Ultracentrifuge at 45,000 rpm for 46-48 hours at running temperature of 15°C, maximum temp of 30 C.

41. Check tubes with a hand held UV light for band of plasmid DNA (top band is genomic DNA, towards bottom of the tube is RNA and junk).

42. Puncture tubes at the bottom center with a heated needle, or from the side with a heated needle or syringe to isolate the band.

43. Remove EtBr with a butanol/water saturated solution. Dilute DNA first with 2 volumes of TE pH 7.6. Extract with butanol until solution becomes clear.

44. Place DNA into dialysis tubing and dialyse in 2 liters of dialysis buffer. Cover buffer with aluminum foil and allow DNA in tubing to spin at 4°C for two hours, then change buffer solution and allow DNA to spin overnight.

45. Collect DNA from tubing and determine concentration with a spectrophotometer. Run gels to determine purity.

**Solutions:**

- 50mM Tris/ 5mM EDTA: per 100 mls
1M Tris pH 8.0..... 5mls
0.5 M EDTA..... 1 ml
ddH2O..... 94 ml

Solution I: per 100 mls:
50mM sucrose..... 5ml of 1M sucrose
10mM EDTA..... 2.0 ml of 0.5M EDTA
25mM Tris 8.0..... 2.5 mls of 1M Tris pH8.0
ddH2O..... 90.5 mls

Solution II: per 100mls:
0.2N NaOH..... 4 mls of 0.5N NaOH
1% SDS.....10 mls of 10% SDS
ddH2O..... 86 mls

Solution III: per 100mls:
11.5 mls of glacial acetic acid
28.5 mls of ddH2O
60.0 mls of 5M potassium acetate

Dialysis Buffer: per 2 liters:
4 mls of 0.5M EDTA
20 mls of 1M Tris pH 8.0
1986 ml of ddH2O

LB Media

5 g of yeast extract
10 g of NaCl
10 g of tryptone
Add 800 ml of dH2O; bring the pH to 7.0 with NaOH. Bring the volume up to 1 liter with dH2O. For plates add 15 g of agar per liter.

Addition of antibiotics:
<table>
<thead>
<tr>
<th>Stock Solution(-200C)</th>
<th>Working concentration</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>concentration</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>25-50 mg/ml</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>50 mg/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>34 mg/ml in EtOH</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>10mg/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10mg/ml</td>
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<tr>
<td>Tetracycline</td>
<td>5mg/ml in EtOH</td>
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Stock solutions of antibiotics dissolved in dH₂O should be filter sterilized through a 0.22 micron filter. Antibiotics in EtOH need not be sterilized. Store solutions in light-tight containers. Magnesium ions are antagonists of tetracycline. Use media without magnesium salts (e.g. LB media) for selection of bacteria resistant to tetracycline.

**NOTE:** Add filter-sterilized antibiotics to LB liquid before use. Add sterile antibiotics to cooled LB agar after autoclaving before plates are poured. These concentrations are those suggested by Sambrook et.al. Different laboratories/experiments may call for concentrations differing from those listed above.

Notes: Holly and Ken use this prep. Entered by TAA 1/25/91 Updated by D.P. 8/17/95