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Cesium Chloride Prep:

1. Spin down the bacteria at 8,000 rpm for 10 minutes at 4 C (starting culture about 500 ml).
2. Invert tube with paper towel stuck into the neck of it to absorb excess liquid. Make sure the paper towel does not disturb the pellet.
3. Prepare 50 mls of soln 2 (1 mL 10M NaOH to 0.2 M, 5 mLs 10% SDS to 1%, 44 mLs ddH2O) and chill soln 3.
4. Resuspend bacteria in 10 mLs of soln 1 by vortexing and using a pipet

   Soln 1: 50 mM Glucose 0.91 g glucose
   25 mM Tris-HCl (pH 8.0) 2.5 ml of 1M
   10 mM EDTA (pH 8.0) 2 ml of 0.5 M
   total volume 100 ml
5. Pipet in 10 mLs of soln 2 and shake to lyse bacteria. Let sit ~5-10 minutes at room temp.
6. Pipet in 15 mLs chilled soln 3 and shake vigorously for 5 seconds

   Soln 3: 5 M Potassium acetate 60 ml
   Glacial acetic acid 11.5 ml
   ddwater 28.5 ml
   total volume 100 ml
7. Keep on ice for 10 minutes.
8. Spin at 4,000 rpm (2600 x g) for 15 minutes at 4 C.
9. Set aside and label 2 Oakridge tubes for each sample.
10. Filter through lab gauze to remove the white flaky stuff from the liquid part.
11. Add 10.5 mLs (0.6 volume) of Isopropanol to each tube, mix well and incubate for 10 mins at room temp.
12. Spin at 9,000 rpm for 15 minutes.
13. Decant supernatant, and pipet in 3 mLs of 70% Ethanol to each tube to rinse the pellet.
14. Vacuum off any excess liquid, wait 10-15 minutes until pellet is dry.
15. Add 3.8 mLs of TE containing 100 ug/ml RNase-A to resuspend the pellet (carried out in 15 ml polypropylene tubes). Use this volume to dissolve both tubes and combine into one.
16. Put samples in 37 C water bath for 20-30 minutes.
17. Measure 4. 078g of Cesium Chloride into one 15 mL opaque tube for each pair of Oakridge tubes.
18. Pipet DNA into tube with Cesium Chloride.
19. Add 185 uL of 10ml/ml Ethidium Bromide to each tube and vortex carefully (~setting of 4). Also prepare $CsCl_2$ balancer: 32.8 ml 1 X TE, 35.2 g $CsCl_2$, 1.6 ml 10 mg/ml EtBr.
20. Spin DNA samples for 7 minutes at 8,000 rpm to remove impurities from the $CsCl_2$.
21. Pipet supernatant into optiseal tube, filling up the rest with $CsCl_2$ balancer solution.

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22. Put into ultra centrifuge at 80,000 rpm, 18 C, maximum acceleration, no brake for at least 3 hrs. and 30 minutes; otherwise you should do overnight for this first step.
23. When first spin has come down, remove bottom band and put into another optiseal tube and as before, top with Ethidium Bromide with Cesium Chloride. Spin in ultra centrifuge at same settings (3 hrs and 30 minutes minimum is sufficient).
24. Transfer DNA to 15 ml polypropylene tubes (from Sarstedt) and carry out cleaning up of DNA. Add 1 ml of salt-saturated isopropanol (top layer), vortex and allow the phases to separate. Aspirate off the top layer and repeat 5 times or more until the bottom layer is clear (at this time all the the EtBr should have been removed.

    TO prepare salt-saturated isopropanol: make 200 mls soln to just beyond 6 M NaCl and mix overnight. Next day add to 1L and mix overnight again.
25. Top solution to 3 mls with 1 X TE, add 6.5 ml of 100% EtOH, and place at -20 ^{o}C for 30 mins. If precipitate forms after the 30 mins, add 3 mls of TE to dissolve the salts. Add 6mls of 100% EtOH and all to sit for 30 mins at -20 ^{o}C.
26. Centrifuge at 9000 rpm fro 10 mins at 4 ^{o}C.
27. Pour off supernatant and all the pellet to air dry.
28. Resuspend in 300 ul of TE and transfer to an eppendorf tube. Add 150 ul of 7.5 M (or 112.5 ul of 10 M) Ammonium acetate, 1 ml of 100% EtOH and incubate at room temp for 5 mins.
29. Spin at max speed in a microcentrifuge for 15 mins. Wash the pellet with 70% EtOH and resuspend to desired concentration.

THAT's ALL FOLKS!!!