

Steve's CsCl Maxi Prep

1. Grow 400 ml LB c plasmid of choice O/N.
2. Pellet bugs at 8000 RPM (JLA 10.5) /10 min./4°C (can freeze @ -20°C).
3. Resuspend in 20 ml of Solution I (Maniatis). My favorite combination is vortexing vigorously followed by violent shaking (by hand). Using a pipet to stir the pellet can help. Get it all into solution (no bits of pellet).
4. Add 40 ml of 0.2 M NaOH/ 1% SDS (prepared fresh: 14 ml 2 M NaOH + 14 ml 10% SDS). Mix thoroughly (swirl gently) but do not vortex (can shear DNA at this point).
5. Keep at R.T. 5 min.
6. Add 30 ml of 3M KCl/ 5 M acetate (same as solution III of minipreps) and mix. Solution should be at room temperature.
7. Spin 10000 RPM (JLA 10.5)/ 10 minutes/ RT.
8. Transfer supernatant through a Kimwipe to a new bottle. Add 0.6 vol (54 ml) isopropanol, mix thoroughly. Spin 10000 RPM/ 10 minutes / RT.
9. Drain, vacuum dry pellet, allow to air dry or dry in warm room. You know its dry when the pellet goes from white and opaque to clear and translucent.
10. Dissolve pellet in 10X TE to 9 ml. Pipet up and down and vortex. It should all go into solution. Add 1.06 g/ml of CsCl (e.g. 9.54 gm for 9 ml).
11. Transfer to an ultracentrifuge tube, add 100 ul of EtBr (10 mg/ml) and top off with 1.06 g/ml CsCl/10X TE solution.
12. Ultracentrifuge @ 60K/20°C/overnight.
13. Transfer bottom band to new ultratube, add 50 ul of EtBr, top off with CsCl/TE as before and repeat step 13.
14. Pull band and extract with water saturated n-butanol until the aqueous phase is colorless. Transfer to Beckman tube (for J17 rotor).
15. Ppt. DNA. For 1 vol of DNA add the following in order (mix after each addition):
 - 2 vol dW
 - 0.3 vol 3M NaOAc
 - 6 vol EtOH (100%)
16. Cool @ -20 > 1 hr.
17. Spin 10K RPM/20 minutes/4°C. Decant supernatant and allow DNA to air dry to a clear colorless pellet. Circle the pellet with a marker so you know where to resuspend.
18. Resuspend pellet in TE pH 8.0