# Hank's Maxi Prep

1. Grow 200 ml TB c plasmid of choice O/N.
2. Pellet bugs at 5000 RPM/15 min./4ºC (can freeze @ –20ºC).
3. Resuspend in 20 ml GTE + 2mg/ml lysozyme (180 ml GTE + 0.36 g lysozyme). Vortex vigorously to get all pieces into a homogenous solution.
4. Keep at R.T. 5 min.
5. Add 40 ml of 0.2 M NaOH/ 1% SDS (prepared fresh: 14 ml 2 M NaOH + 14 ml 10% SDS). Mix thoroughly but do not vortex (can shear DNA at this point).
6. Keep at R.T. 5 min.
7. Add 30 ml of ice-cold 3M KCl/5 M acetate (same as solution III of minipreps) and mix.
8. Place on ice for 10 minutes.
9. Spin 9000 RPM/ 20º/4ºC.
10. Transfer supernatant through two Kimwipes to a new bottle. Add 0.6 vol (54 ml) isopropanol, mix thoroughly. Allow to stand for 10' at RT. Spin 9000 RPM/RT/15.
11. Drain, vacuum dry pellet, allow to air dry or dry in warm room.
12. Dissolve pellet in 10X TE to 9 ml. Add 1.06 g/ml of CsCl (e.g. 9.54 gm for 9 ml) and 50-100 ul of 10 mg/ml EtBr soln.
13. Spin at 8000 RPM at R.T. for 5' to remove protein.
14. Transfer to an ultracentrifuge tube, top off with 1.06 g/ml CsCl/10X TE solution.
15. Ultracentrifuge @ 60K/20C/overnight.
16. Transfer bottom band to new ultratube, add 25-50 ul of EtBr, top off with CsCl/TE as before and repeat step 13.
17. Pull band and extract with water saturated n-Butanol until the aqueaous phase is colorless.
18. Ppt. DNA for 1 vol of DNA:
    - vol. dW
    - vol EtOH (100%)
    - vol 3M NaOAc
19. Cool @ -20 > 1 hr.
20. Spin 10K RPM/20'/4C
21. Wash pellet 1X 70% EtOH
22. Resuspend pellet in TE pH 8.0