DNA PREPARATION FROM *CHLAMYDOMONAS REINHARDTII*

1. 1000 ml of cells grown to stationary (thicker cultures work better).

2. Pellet 5K 2-5 mins.

3. Drain well and resuspend in 8.3 ml of H_2O.

4. 11 ml of resuspended cells are pipetted into 50 ml Corning orange cap tube.
   Add 20 ml of SDS-EB.

   SDS-EB = 2% SDS
   400 mM NaCl
   40 mM EDTA
   100 mM tris-Cl, 8.0

   Note: This solution precipitates on storage.

   Mix gently by hand.

5. 15 min. 50°C.

6. Add 32g solid CsCl.

7. Dissolve by shaking on rocker.

   Note: Can take a long time.

8. Add 2 ml of 10 mg/ml Ethidium bromide. Mix gently.

9. Load into 38.5 ml quick seal tube. Use funnel provided by Beckman. The solution is very viscous and will not go through a needle.

10. Seal according to Beckman instructions. If tubes aren't full, use CsCl solution (1 g dissolved in 1 ml TE--**NOTE**: Final volume is greater than 1 ml).

11. 45K, VTi 50, 24 h, 15°-20°

12. Remove band with 5 ml syringe and 18 g needle.

13. Extract with CsCl solution saturated i-Butanol.

14. Dialyze against 0.4M NaCl in TE 24 h.
    Change dialysis buffer.
    Dialyze against TE 24 h.

15. Phenol extract, chloroform extract.

16. Ethanol ppt, using NH_4OAc, and resuspend in 1.5 mls TE buffer.

17. Add 1.5 g CsCl and 100λ 10 mg/ml EtBr.

18. Spin 90 Krpm 15°C 5 hours in TL 100.3 rotor in TL 100 ultracentrifuge (J. Erickson's lab) using polycarbonate tubes.

19. Pipet out genomic DNA band. Extract EtBr with 1-butanol saturated with CsCl solution.

20. Add to final aqueous phase:

    2 volumes TE buffer
    6 volumes 100% Ethanol

    Let precipitate on ice.

21. Centrifuge and wash pellet with 70% EtOH.

22. Drain and dry final pellet and resuspend in SH_2O (sterile H_2O).