Chlamy Chloroplast DNA Protocol
(8/1/92)

Follow these steps exactly with NO variations:

1. Grow 750mL of a cell wall deficient strain of C. reinhardtii at 25° C in high light to 1 x 10^7 cells in TAP medium.

2. Harvest the cells by centrifugation at 3000g for 5 minutes at 4° C (use Oakridge tubes in JA10).

3. Pour off supernatemt.

4. Wash cells by resuspending the pellet in 20mL TAP (gently use a paintbrush).

5. Transfer the suspension to a 30mL Sarstedt tube and centrifuge at 3000g for 5 minutes at 4° C (use JA17 or JA20).

6. Pour off supernatent.

7. Gently resuspend the cell pellet with a paintbrush in 8mL of "A" buffer at 4° C.("A" buffer is: 0.1 M NaCl, 50mM EDTA, 20mM Tris-HCl pH 8.0)

8. Transfer the suspension to a 50mL Fisher tube.

9. Add 0.5mL Pronase (10mg/mL in 0.01M NaCitrate, pH 5.0 predigested at 37° C for 2 hours made the same day) and mix by swirling.

10. Add 2.0mL 20% SDS and mix.

11. Incubate tubes in a 50° water bath for 45 minutes.

12. Add 0.5mL more Pronase.

13. Incubate tubes in 50° C water bath for 45 more minutes.

14. Again add 0.5mL more Pronase.

15. Incubate tubes in 50° water bathe for 2.5 hours. The cells should appear browninsh at this point.

16. Cool on ice. Add 20mL phenol. Mix by inversion and leave on ice for 20 minutes.

17. Transfer suspension to a 30mL Sarstedt tube.

18. Centrifuge the phenol-cell mixture at 7000rpm for 15 minutes at 4° C.

19. The upper aqueous phase should appear a light pink in color. Transfer the upper phase to a new 30mL Sarstedt tube using a glass Pasteur pipet.

20. Add 2 volumes 100% EtOH. Leave at -20° C overnight.

21. Precipitate the DNA by centrifugation at 8000K for 10 minutes at 4° C.

22. Drain by inverting the tubes on a kimwipe and letting the tubes sit about 5 minutes. Wipe off the outer edge of the tube with a kimwipe.

23. Dissolve the pellet in 13mL 10mM Tris, pH 7.5. Let tubes rock for 2 hours.

24. Place tubes in a 65° water bath for 1 hour in order to completely redissolve the DNA.

25. Centrifuge at 5000rpm for 5 minutes at 4° C in order to pellet particulate matter that has not resuspended.

26. Transfer the upper aqueous to a Fisher tube.

27. Add 1.6 mL 1xTEN.
    1xTEN: 10mM Tris-Hcl, pH 8.0
    10mM EDTA
    150mM NaCl

28. Add 2.4mL EtBr (0.4mg/mL in 10mM Tris, pH 7.5).

29. Add 23mL NaI saturated in 1xTEN (about 2g NaI/mL 1xTEN).

30. Mix by inversion.

31. Take the refractive index of each tube. Adjust to 1.4350. Do not stray over 1.4385. Otherwise he gradient will not separate the DNA properly.

32. Transfer to quick-seal tubes.

33. Weigh and balance to 0.05g

34. Seal caps.

35. Spin in Vti50 for 44 hours at 46 krpm, 20° C. Set brake to OFF.

36. Pull topmost chloroplast DNA band with an 18g needle.

Chloroplast DNA Prep (Cont.)

37. Extract EtBr with iso-amyl alcohol (equilibrated with saturated NaI in 1xTEN).

38. Dialyze DNA overnight against 4L TE (50/10), pH 8.0 with one change.

39. Dialyze DNA overnight against 4L TE (10/1).

40. Precipitate DNA with 2 volumes 100% EtOH, 1/10 volume 3M NaOAc, pH 5.2, overnight at -20° C.

41. Spin at 8000rpm for 10 minutes at 4° C. Drain pellet.

42. Rinse with 1mL 70% EtOH.

43. Spin at 8000rpm for 10 minutes at 4° C. Drain pellet.

44. Dry in speed-vac.

45. Resuspend DNA in H_2O to 1mg/mL.

*Use quartz cuvetted. Measure at OD_{260} and OD_{280}.
OD(50)(dilution factor)=ug/mL