8/1/92

I.) Chloroplast DNA prep protocol:

A.) 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 ~1x10⁷ cells/ml (in TAP medium).
2.) Harvest the cells by centrifugation at 3000g for 5' at 4°C (in Oakridge tubes in JA1
3.) Pour off supernatant.
4.) Resuspend cells (the pellet) in 20ml TAP gently using a paint brush.
5.) Transfer into a 30ml ml Sorstrat tube & centrifuge at 3000g for 5' at 4°C (in JA17 or JA20).
6.) Pour off supernatant.
7.) Gently resuspend the cell pellet with a paintbrush in 8ml of "A" buffer (0.1 M NaCl, 50mM EDTA, 20mM Tris-HCl, pH 8.0) at 4°C.
8.) Transfer the suspension to a 50ml Fisher tube.
9.) Add 0.5ml pronase (10mg/ml in 0.01 M Na citrate, pH 5.0 predigested at 37°C for 2hr made the same day.!!) + mix by swirlin
10.) Add 2ml 20% SDS, mix, place tubes in (a 50°C H2O bath.)
11.) After 45' incubation, add 0.5ml more prona
12.) Again, after 45' add 0.5ml more pronase.
13.) 2½ hours later (incubating at 50°C), the cells should appear brownish. Cool on ice & add 20ml phenol. Mix gently by inversion & leave on ice 20'. Transfer suspension to 30ml Sarstedt tub
14.) Centrifuge the phenol-cell mixture at 7000 rpm for 15' at 4°C.
15.) The upper aqueous phase should appear to be a light pink color.
16.) Transfer the upper aqueous phase carefully, trying to avoid the interface, to a new Sarstedt tube

glass pasteur pipet.

17.) Add 2 volumes 100% EtOH, leave in -20°C overnight.

18.) Precipitate the DNA by centrifugation at 8000 x for 10' at 4°C.

19.) Invert the tubes on a kimwipe & let sit ~5' to drain the EtOH, wipe off the outer edge of the tube with a kimwipe.

20.) Dissolve the pellet in 13ml 10mM Tris, pH 7.5.
@ let tubes rock 2 hrs.

21.) Place tubes in a 65°C H_2O bath, 1hr to completely redissolve the DNA.

22.) Spin 5' at 4°C at 5krpm to remove particulate matter that has not resuspended.

23.) Transfer upper aqueous to a new tube.

24.) Add 1.6 ml 1x TEN.

1x TEN: 10mM Tris-HCl
10mM EDTA
150 mM NaCl, pH 8.0.

25.) Add @ 2.4 ml EtBr (0.4mg/ml in 10mM Tris, pH 7.5)

26.) Add 23 ml NaI saturated in 1x TEN.
to saturate a 1x solution of TEN add 29 NaI / 1ml 1x TEN.

27.) Mix by inversion

28.) Take refractive index of each tube & adjust to 1.435!! (do not stray over 1.4385).
@ This is very important!!
@ Otherwise the gradient is not set up correctly & the DNA will not band correctly.

29.) Transfer to quick-seal tubes

30.) Weigh & balance to 0.05 g.

31.) Seal caps

32.) Spin in VTi50 for 44 hrs at 46 krpm, 20°C.
@ Set brake to OFF!!

33.) Pull topmost chloroplast DNA band with an 18g needle.
34.) Extract EtBr with isoamyl alcohol
35.) Dialyze DNA overnight against 4L TE (50/10), change once + dialyze against 4L TE (10/1).
36.) Precipitate DNA with 2 volumes 100% EtOH, 1/10 volume 3M NaOAc, pH 5.2 (overnight at -20°C).
37.) Spin 8K, 10', 4°C.
38.) Drain pellet
39.) Rinse with 1ml 70% EtOH
40.) Spin 8K, 10', 4°C, drain pellet
41.) Dry in speed vac
42.) Resuspend DNA in H2O to 1mg/ml.