Typed 30 June 2007
Last modified 2001 by JLM

CHLAMYDOMONAS DNA MIDIPREP

1) Grow 50 mL cultures to stationary phase (high cell density does not adversely affect DNA yield).
2) Harvest cells 5K, 2-5 min. at 4ºC (JA18). Drain pellets well.
3) Resuspend pellets in 2 mL water, transfer to 15 mL Sarstedt tubes.

2x Proteinase K buffer:	**stock:**	**vol. for 64 mL**	**vol. for 4 mL**
10 mM Tris-HCl pH 8.0	1 M Tris-HCl pH 8.0	640λ	40λ
10 mM EDTA	0.5 M EDTA	1.28 mL	80λ
10 mM NaCl	3M NaCl	213λ	13.33λ
	H_2O	29.75 mL	1.86 mL
	20% SDS	1.6 mL	100λ
	20 mg/mL proteinase K	640λ	40λ

nb. i) Do not add the SDS until ready to use. Soln. precipitates on standing.
ii) Proteinase K has to stand at least ½ hr. at RT to activate.

4) Add 2 mL proteinase K buffer.
5) Incubate 2 hrs. at 50ºC or until lysate turns brown.
6) Phenol/chloroform extract, shaking gently by hand 1-2 min. Centrifuge 10K, 15 min (JA17 or JA20) and transfer aqueous phases to new 15 mL tubes.
7) Add 5 µL of 5 mg/mL RNaseA incubate at 37ºC for 30 min.
8) Phenol/chloroform extract, shaking gently by hand 1-2 min. Centrifuge 10K, 15 min. (JA17 or JA20) and transfer aqueous phases to new 15 mL tubes.
9) **SLOWLY, mixing vigorously with the pipette tip,** add 0.35 volumes (1.4 mL) of **room temperature** 100% EtOH to the aqueous phases to selectively precipitate polysaccharides. Incubate on ice 15 min., then spin 10K, 10 min (JA17 or JA20).
10) Transfer supernatants to new 15 mL tubes. Add an equal volume of ispropanol (5.4 mL) to precipitate nucleic acids. Incubate 15 min. at room temperature, then spin 12K, 10 min. Dry pellets in speed-vac.
11) Resuspend nucleic acids in 500λ H_2O and transfer to 1.5 mL eppendorf tubes [(Optional) Spin 5K, 5 min. at RT to remove any undissolved material and remove sups to new tubes]. Add 125λ 4 M NACL, 625λ 20% PEG-8000. 30 min. on wet ice, spin down 12K, 20 min. at 4ºC. Rinse pellets with 70% EtOH and dry in speed-vac.
12) Resuspend nucleic acids in 500λ H_2O and transfer to 1.5 mL eppendorf tubes. [(Optional Spin 5K, 5 min. at RT to remove any undissolved material and remove sups to new tubes]. Add 125λ 4M NaCl, 625λ 20% PEG-8000. 30 min. on wet ice, spin down 12K, 10 min. at 4ºC. Rinse pellets with 0% EtOH and dry in speed-vac.
13) Dissolve DNA in 50λ H_2O. Check OD_{260} of 1λ dilution.

This procedure reverses the order of the RNase treatment and PEG-precipitation steps in the Rochaix protocol. It yields 50-100 µg of DNA and works very well for Southern blots and PCR amplifications.