DNA extraction of Chlamydomonas using CTAB

1. **CTAB/phenol extraction**
2. Preheat aliquot of CTAB buffer to 65°C.

CTAB buffer	**Stock**	**Vol**	
50 mM Tris-HCl (pH 8.0)	1M	10ml	
20 mM EDTA	0.5M	4ml	
1.4 NaCl	5M	28ml	
2% CTAB		2g	
1 % PVP 40,000		1g	
Nuclease-free H_2O			
	Total	100ml	(autoclave, store @ RT)

3. Resuspend cells in 1ml of lysis buffer (Prepare before use).

SDS-EB lysis buffer	**Stock**	**Vol**	
50 mM Tris-HCl (pH 8.0)	1M	50ul	
200 mM NaCl	5 M	40ul	
20 mM EDTA	0.5 M	40ul	
Nuclease-free H_2O		620ul	
2% SDS	10%	200ul	
Proteinase K (20mg/ml)		50ul	1mg/ml
	Total	1000ul	

4. Mix well by inversion. (Do not vortex: to avoid potential shearing of DNA)
5. Add 1X volume of hot CTAB extraction buffer to cell pellet and incubate for 30mins at 65°C.
6. Add 1X volume of phenol: chloroform: isoamyl alcohol (25:24:1). Mix well.
7. (Use phase-lock gel for all extraction; spin condition 14000g, 5mins, RT).
8. Add 5ul RNase (100mg/ml) and incubate at RT for 20 minutes.
9. Extract 2X chloroform: isoamyl alcohol (24:1).
10. Transfer aqueous to a new microcentrifuge tube.
11. Add 2X volume cold ethanol to precipitate nucleic acid. Mix well.
12. Leave sample at -80°C for at least 30mins.
13. Centrifuge 13200rpm, 5mins at 4°C.
14. Wash pellet 2X with 1ml cold 70% ethanol.
15. Discard supernatant. Air dry pellet.
16. Resuspent pellet in 200ul TE/ EB overnight at 4°C.

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