Worden Lab
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DNA Extraction - CTAB Method

We use this method for extracting genome sequencing quality (i.e. unsheared) DNA that can be used for large insert libraries. It was used to extract material for the *Micromonas* RCC299 complete genome sequencing project, and the *Micromonas* RCC472 genome sequencing project. This protocol originally came to us from Evelyne Derelle at Observatoire Océanologique, Banyuls sur Mer. It was adapted from Winnepenninckx B. *et al*., 1993, TIG: 9 (12), 407 (Technical Tips).

Potential problems with this protocol: no phenol step is used – this could lead to higher protein contamination than desirable (although we have not found this to be an issue).

We use certified DNAse/RNase free plastics and tips - the tips are Aerosol resistant tips.

Cell Harvesting
Micromonas cells are harvested from up to 700 mL of culture by 2 centrifugation steps at 4,500-8,000 x *g*, 20 min at 4°C. First spin 50-85 mL sterile centrifuge tubes, remove most supernatent and pipet the rest into 1.7 mL tubes; spin again and remove supernatant (all of it). These cell pellets can than be stored at -80°C or immediately processed as below.

DNA Extraction
- Cells are resuspended in 0.8 mL of pre-warmed (60°C) CTAB extraction buffer

> *CTAB buffer*
> 2% CTAB (hexadecyltrimethylammonium bromide)
> 100 mM TrisHCl [pH=8]
> 20 mM EDTA,
> 1.4 M NaCl
> 0.2% β-mercaptoethanol [added just before use]
> 0.1 mg/mL proteinase K [added just before use])

and incubated at 60°C for 1 hour. We make the reagents in sterile 18.2 MΩ H_2O (e.g. MilliQ or Nanopure) and filter sterilize (push solution through a 0.2 μm filter on a sterile syringe) the solution (before adding β-mercapto and ProK).
- **Gently** mix by inverting the microtube from time to time.
- After 1 hr add 0.8 mL of chloroform/isoamylalcohol (24:1) solution - work in the fume hood.
- **Gently** mix for 2 min by inverting the tube.
- Spin 10 min at maximum speed (14000 x *g*) at 4°C.
- Carefully transfer the aqueous phase (above the white interface layer) to a clean microtube (then discard the rest). To do so rapidly (before the interface declines), prepare a p1000 with tip on and a p200 with tip on (hanging out off the bench, as you should never do post-contact with solutions for safety/chemical hazard reasons) - take the larger volume off with the p1000 and then you can more gently get near the interface (conservatively near - so as not to disturb) with the p200.

- Add 1 µL RNase (DNase-free) and incubate for 30 min at 37°C. Watch where you do this - if you work with RNA in the lab try to physically separate the areas, pipettors used etc.

- Add 0.6 mL of isopropanol (2/3 of the recovered volume). Gently invert the microtube to be sure mixing is complete. Leave to precipitate for 2 hr to overnight at room temperature to allow the formation of the "DNA jellyfish" (really! very cool looking).

- Spin 15 min at 14000 x *g* at 4°C to pellet the DNA (when placing the tubes in the centrifuge it is helpful to do so 'directionally - so you know where to expect the pellet).

- Remove the supernatant carefully,then wash the pellet once or twice with cold EtOH, for example:

we've done once in with 70% EtOH or
1x in 76% EtOH/10 mM $AcNH_4$; then a second wash 1x70% EtOH

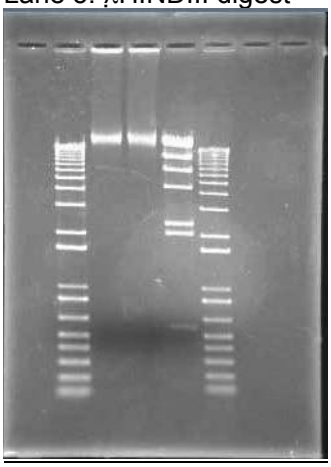
- Spin 15 min at max speed, 4°C.
- Remove supernatant and dry the pellet by leaving tube open @ rt.

- Resuspend pellet in sterile H_2O or TE (we use TE pH 8.0), aliquot & store at -20°C.

Example of resulting 'quality' of DNA

Below is an example of DNA extracted via this method - the gel shown is a 1% agarose gel w/ HINDIII digest of λ (0.5 μg loaded); 1 μl of sample loaded.

Lanes 2 and 6 1Kb Plus ladder;
Lane 3: RCC299 DNA Sample A; Lane 4: RCC299 DNA Sample B;
Lane 5: λHINDIII digest



Ordering info for chemicals used

REAGENT	VENDOR	CATALOG NUMBER
CTAB	Sigma	H6269
Chloroform	Sigma	C2432
Isoamyl alchohol	Sigma	I9392
Isopropyl alcohol	VWR	PX-183514
Rnase (100mg/ml Dnase free)	Qiagen	19101
TE (10 mM tris, 1mM EDTA)	Ambion	9858
Proteinase K	Qiagen	19131
NaCl	Sigma	S3014
β-Mercaptoethanol	Sigma	M3148
Ammonium acetate	Sigma	A1542