Genomic DNA extraction from diatom P. multistriata

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ABSTRACT
Genomic DNA extraction from diatom P. multistriata
Grow cells in 250 ml

filter coltures with 1.2 μm nitrocellulose membranes

Centrifuge at 6000 rpm for 5 minutes at 4 °C and remove medium

Resuspend cells with 500 μl of TE buffer (10 mM TrisHCl pH 7.6 and 1 mM EDTA pH 8.0)

Add:
- 400 mg of 0.2-0.3 mm zirconia/silica diameter beads,
- 500 μl phenol.

rinse filter with 1 ml f/2 medium in a falcon and then move cells to a 2 ml eppendorf

Mix with vortex 30 Hz 3 times for 85 seconds, each time put sample in ice for 60 seconds before vortex.
8 · Centrifuge at 10000 rpm for 5 minutes at 4°C.

9 Recover aqueous phase in new 1.5 ml eppendorf (about 600 μl)

10 Add 500 μl of PCI (Phenol:Chloroform: isoamyl alcohol 25:24:1 v/v) and mix by inversion.

11 Centrifuge at 10000 rpm for 5 minutes at 4°C

12 Move the aqueous phase in a new eppendorf and add 5 μl of RNase-A 10 mg/ml

13 Incubate at 37 °C for 30 minutes.


15 Centrifuge at 10000 rpm for 5 minutes at 4°C.

16 Move the aqueous phase in a new 2 ml eppendorf and add:
   ■ 50 μl of 3 M NaAc (pH ± 5)
1 ml of ethanol 96% (-20 °C)
2 μl glycogen (-20 °C)

17 incubate over night at -20°C.

18 Centrifuge the overnight samples at 13000 rpm for 30 minutes at 4°C

19 discard aqueous phase

20 Wash the pellet by adding 1 ml ethanol 70% and mix gently by inversion

21 Centrifuge at 13000 rpm for 10 minutes at 4°C

22 discard aqueous phase

23 Wash the pellet by adding 1 ml ethanol 70% and mix gently by inversion

24 Centrifuge at 13000 rpm for 10 minutes at 4°C
25 discard aqueous phase

26 Remove aqueous phase and dry pellet at RT for at least 20 minutes

27 add 50 μl of Preheated TE 1X (pH 8) or sterile MilliQ water to pellet of DNA

Preheat the TE pH 8 or sterile MilliQ water at 55 °C

28 incubate at 55 °C for 20 minutes

29 quantify DNA concentration by nanodrop or Qubit

30 in order to check DNA integrity, run a small amount of DNA with 1% agarose gel

31 The DNA is ready and store it at -20°C