Sep 17, 2019 Version 1

Genomic DNA extraction from diatom P. multistriata V.1

DOI
dx.doi.org/10.17504/protocols.io.7dghi3w

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Protocol Citation: Francesco Manfellotto, Monia Teresa Russo, Rossella Annunziata, Mariella Ferrante 2019. Genomic DNA extraction from diatom P. multistriata. **protocols.io https://dx.doi.org/10.17504/protocols.io.7dghi3w**

Protocol status: Working
We use this protocol and it's working

Created: September 16, 2019

Last Modified: September 17, 2019

Protocol Integer ID: 27784

Abstract

Genomic DNA extraction from diatom P. multistriata

1 Grow cells in 250 ml

2 filter coltures with 1.2 µm nitrocellulose membranes

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

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

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

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

7 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. 3m

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

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. 1h

18 Centrifuge the overnight samples at 13000 rpm for 30 minutes at 4°C 3m

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 1m

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 1m

25 discard aqueous phase

26 Remove aqueous phase and dry pellet at RT for at least 20 minutes 2m

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 2m

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