Genomic DNA extraction from the diatom *Pseudo-nitzschia multistriata* for Illumina sequencing V.5

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ABSTRACT

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1 Grow cells as described in: dx.doi.org/10.17504/protocols.io.bgudjws6

2 Detect the presence/absence of bacteria and collect the cultures as described in: dx.doi.org/10.17504/protocols.io.btt5nnq6

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

4 Add:
- 400 mg of 0.2-0.3 mm diameter zirconia/silica beads
- 500 µL phenol (pH 7.8).

5 Mix with vortex 3 times at 30 Hz for 85 seconds (Each time put sample in ice for 60 seconds before vortex)

6 Centrifuge at 11000 g for 5 minutes at 4°C. 5m

7 Recover aqueous phase in new 1.5 mL Eppendorf tubes (about 600 µl)

8 Add 500 µL of P.C.I (Phenol:Chloroform:Isoamyl alcohol 25:24:1 v/v) and mix by inversion

9 Centrifuge at 11000 g for 5 minutes at 4°C 5m

10 Move the aqueous phase in a new Eppendorf tube and add 5 µL of RNase-A 10 mg/mL

11 Incubate at 37 °C for 30 minutes. 30m

12 Add 500 µl di P.C.I (Phenol:Chloroform:Isoamyl alcohol 25:24:1 v/v) and mix by inversion.

13 Centrifuge at 11000 g for 5 minutes at 4°C. 5m

14 Move the aqueous phase in a new 2 mL Eppendorf tube and add:
- 50 µL of 3 M NaAc (pH ± 5)
- 1 mL of ethanol 96% (- 20 °C)
- 2 µL glycogen (- 20 °C)

15 Incubate over night at -20°C. 12h

16 Centrifuge the samples at 13000 g for 30 minutes at 4°C 30m

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17 Discard aqueous phase

18 Add 1 mL ethanol 70% and mix gently by inversion

19 Centrifuge at 13000 g for 10 minutes at 4°C 10m

20 Discard aqueous phase

21 Add 1 mL ethanol 70% and mix gently by inversion

22 Centrifuge at 13000 g for 10 minutes at 4°C 10m

23 Remove aqueous phase and dry pellet at R.T. for at least 20 minutes 20m

24 Add 50 µL of preheated (55°C) TE 1X (pH 8) or sterile MilliQ water

25 Incubate at 55 °C for 20 minutes 20m

26 Quantify DNA concentration by Nanodrop or Qubit

27 Check DNA integrity.
Run a small amount of DNA with 1% agarose gel

28 Store DNA at +4 °C, or -20°C for longer storage times

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