Genomic DNA extraction from the pennate diatom Asterionella formosa optimised for next generation sequencing.

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Abstract

Asterionella formosa is a freshwater pennate diatom that forms star-shaped colonies, whose mitochondrial DNA was recently sequenced [1]. This diatom is of interest for genetic studies [2,3] and for the study of photosynthesis [4,5]. However, extraction of genomic DNA (gDNA) from *A. formosa* is difficult because of its silica cell-wall and the presence of photosynthetic pigments that contaminate the DNA. Here, we present an optimised protocol for gDNA extraction from *A. formosa* that overcomes those two problems. We also assessed its efficacity in yielding high-purity gDNA compared to a standard protocol using hexadecyltrimethylammonium bromide (CTAB).

References

[1] Villain A. et al. (2017) Mitochondr. DNA. 2 (1), 97-98.

[2] De Bruin A. et al. (2004) J. Phycol. 40, 823-830.

[3] van Den Wyngaert S. et al. (2015) Mol. Ecol. 24, 2955-2972.

[4] Boggetto N. et al. (2007) J. Phycol. 43, 1227-1235.

[5] Erales J. et al. (2008) J. Phycol. 44, 1455-1464.

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Materials

Chloroform:Isoamyl alcohol 24:1 C0549 by Sigma Aldrich

Hexadecyltrimethylammonium bromide H6269 by Sigma Aldrich

Protocol

Solutions to prepare in advance

Step 1.

Prepare the following solutions:

- 1 M Tris-HCl at pH 8.0.
- 0.5 M EDTA at pH 8.0.
- 5 M NaCl.
- 7.5 M Ammonium acetate.

Preparation of the hexadecyltrimethylammonium bromide (CTAB) buffer

Step 2.

Prepare 10% CTAB (hexadecyltrimethylammonium bromide) solution by dissolving it in DNase- and RNase-free (molecular grade) water.

NOTES
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Slowly add the powder and microwave until complete dissolution. Keep the solution at 37°c to avoid precipitation.

Step 3.

Right before use, make the buffer by mixing together:

- 3 ml of 10% CTAB solution (final concentration of 3%).
- 2.8 ml of 5 M NaCL (final concentration of 28%).
- 0.4 ml of 5 M EDTA (final concentration of 4%).
- 1 ml of Tris-HCl at pH 8.0 (final concentration of 10%).
- 2.78 ml of molecular-grade, DNase- and RNase-free water.
- 0.02 ml of β-mercaptoethanol.

SAFETY INFORMATION
β-mercaptoethanol should be handled in a chemical fume hood and with the appropriate safety gear.

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The CTAB buffer should be made shortly before use, as it is unstable, especially after addition of β-mercaptoethanol.

Before starting

Step 4.

Heat up a water bath to 65°C and another one to 37°C.

Step 5.

Keep 100% isopropanol at -20°C and the 7.5 M ammonium acetate solution at 4°C.

Step 6.

Pre-heat the CTAB buffer to 65°C in order to prevent precipitation and to ensure better protein denaturation.

Extraction

Step 7.

Harvest 90 mg of *Asterionella formosa* cells by centrifuging 250 ml of culture in stationary phase (OD 0.1).

Step 8.

Re-suspend the cells in 1.5 ml of freshly-made, pre-heated CTAB buffer.

Step 9.

Keep at 65°C for 1 hour.

NOTES

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The sample can stay in the hot water bath (at 65°C) for several hours, if necessary.

Step 10.

Centrifuge the sample in order to collect all the liquid that may have stuck to the wall of the tube.

Step 11.

Split the sample into 3 aliquots of 500 µl, each in a DNase- and RNase-free 1.5 ml eppendorf tube.

Step 12.

Add 500 µl of chloroform:isoamyl alcohol (24:1) solution to each tube.

SAFETY INFORMATION

The chloroform:isoamyl alcohol (24:1) solution should be handled in a chemical fume hood and with the appropriate safety gear.

Step 13.

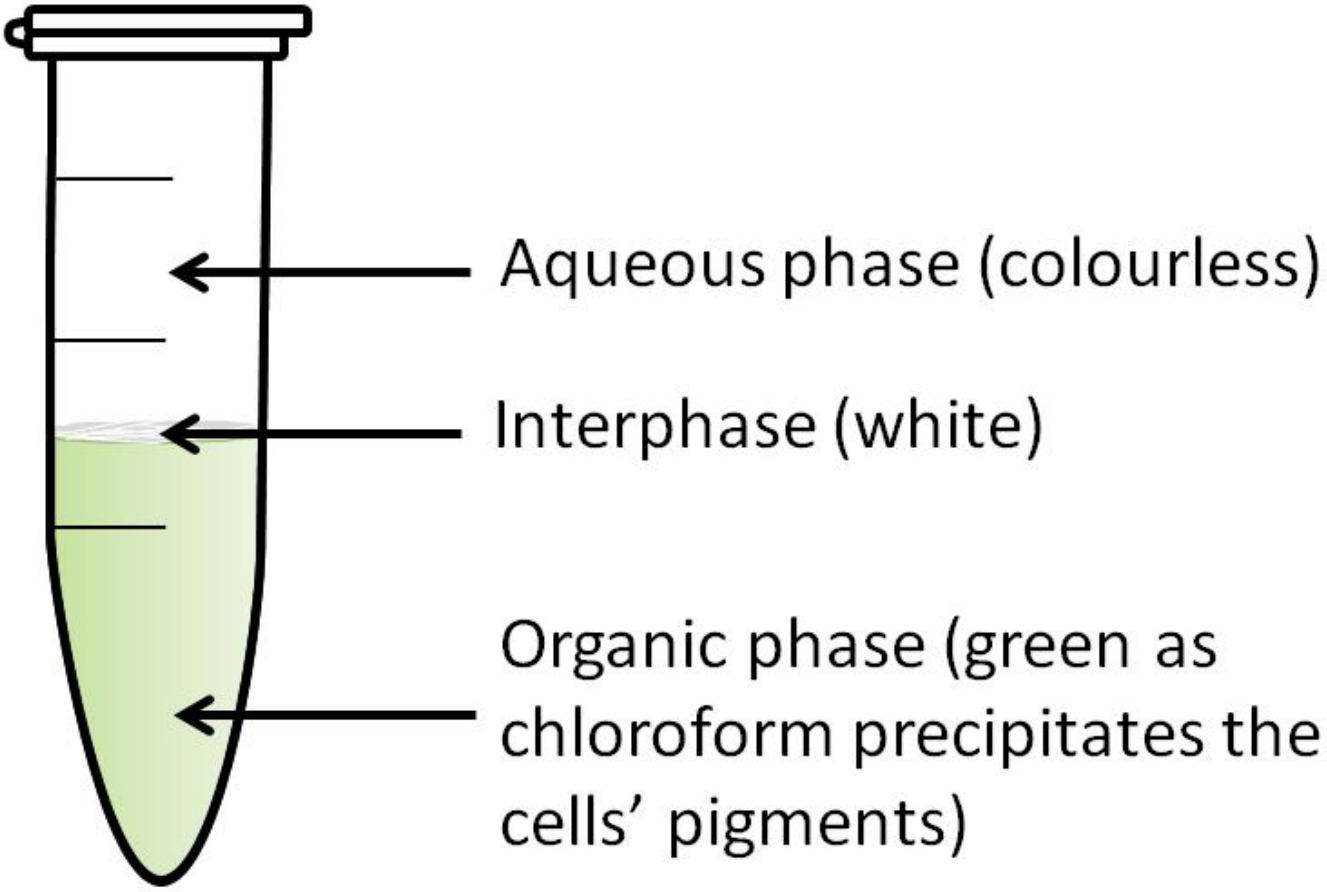
Mix by inversion for 5 min.

Step 14.

Centrifuge for 10 min at 11,000 x g at room temperature.

Step 15.

Pipette the upper (aqueous) phase with a seringe and put 500µL of the phase in each tubes



NOTES

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This first precipitation step allows to separate the nucleic acids (in the aqueous phase) from the cells' pigments and hydrophobic components (in the organic phase) and the denatured proteins (at the interface between the two phases).

Step 16.

Add 8 µl of RNase (4mg/mL), mix thouroughly by pipetting up and down.

Step 17.

Split each tube in 250 µl aliquots, in order to maximise thermal exchanges and thus RNase digestion.

Step 18.

Keep at 37°C for one hour.

Step 19.

Pour the aliquots back together in order to reach a final volume of 500 µl.

Step 20.

Add 500 µl of chloroform:isoamyl alcohol (24:1) solution to suppress RNase activity.

Step 21.

Mix by inversion for 5 min.

Step 22.

Centrifuge for 10 min at 11,000 x g at room temperature.

Step 23.

Transfer the upper (aqueous) phase with a syringe into an eppendorf tube. Combine the aqueous phase from each tube as much as possible, but without exceeding a volume of 750 µl per eppendorf tube.

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Combining the aqueous phases from each sample serves to concentrate the extracted genomic DNA (gDNA).

DNA precipitation

Step 24.

Measure the volume of the liquid obtained from the previous step.

Step 25.

Add a volume of cold 7.5 M ammonium acetate that is equivalent to 8% of the volume measured in the previous step.

Step 26.

Add the same volume of cold 100% isopropanol (kept at -20°C) as the volume measured two steps earlier.

Step 27.

Mix thoroughly by inversion.

Step 28.

Keep in the freezer for 30 minutes to 1 hour.

Step 29.

Centrifuge at x 11,000g at 4°C for 10 min.

Step 30.

Carefully discard the supernatant without touching the pellet.

Step 31.

Add 700 µl of 70% ethanol without re-suspending the pellet.

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The point of this step is to wash the pellet.

Step 32.

Invert the tube 5-10 times.

Step 33.

Centrifuge at 11,000 g at room temperature for 3 min.

Step 34.

Discard the supernatant (without touching the pellet).

Step 35.

Wash the supernatant as previously: add 700 µl of 100% ethanol (without re-suspending the pellet), then invert the tube 5-10 times and centrifuge at 11, 000 g at room temperature for 3 min before finally discarding the supernatant.

Step 36.

Let the DNA dry in chemical flow hood, bearing in mind that it can be a very fast process.

Step 37.

Re-hydrate the DNA with 30-50 µl of molecular-grade water (the exact volume depending on the size of the pellet).

Step 38.

Keep the tube overnight at 4°C.

Assessing the extraction

Step 39.

The following day, re-suspend the DNA and homogenise the solution by pipetting up and down.

Step 40.

Measure the quantity of DNA e.g. with a nanodrop instrument.

Step 41.

Run a 1% agarose gel at 100 V for 20 min.

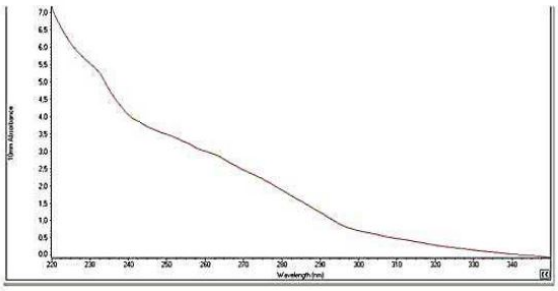
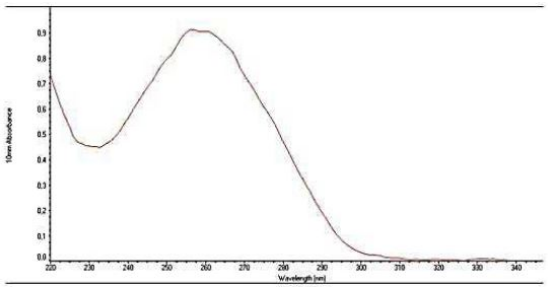
EXPECTED RESULTS

Array

Comparison with standard CTAB protocol

Step 42.

To assess the efficiency of this protocol compared to the standard CTAB protocol by Promega, we have performed the extraction of gDNA from *Asterionella formosa* using both protocols. The results are shown below:

	Standard CTAB protocol	Optimised protocol
Absorption spectrum		
A260:A280 ratio	1.92	1.98
A260:A230 ratio	0.8	2.14
Gel	A unique band is present, no smear, no band near 200pb (contamination by RNA)	

Step 43.

Test the DNA with a PCR reaction

Warnings

Some of the reageants used (e.g. β-mercaptoethanol, chloroform) are toxic and, as such, require careful handling, appropriate safety gear and the use of a chemical fume hood.