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Extraction of genomic DNA from diatoms by the modified method described in (Jacobs et al., 1992)

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Artyom M. Marchenkov[1], Darya P. Petrova[1], Alexey A. Morozov[1], Yulia R. Zakharova[1], Michael A. Grachev[1], Alexander A. Bondar[2]

[1]Limnological Institute of Siberian Branch of Russian Academy of Sciences;
[2]Institute of Chemical Biology and Fundamental Medicine of Siberian Branch of Russian Academy of Sciences

Artyom Marchenkov

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

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Abstract

A protocol for diatom DNA extraction used in the Limnological Institute SB RAS for freshwater diatoms.

Cells collecting

1 The cells were sedimented on polycarbonate filters (5-µm pores) (Whatman, USA), briefly rinsed with cold Diatom Medium (DM), harvested by centrifugation for 2 min at 16,100 g at 4 °C and then stored at −70 °C.

Cells lysis

2 A 4.5 mL volume of lysis buffer (20 mM Tris-HCl, pH 8.0; 100 mM NaCl; 50 mM Na_2EDTA), 20% SDS (250 µL) and proteinase K (250 µL, 10 mg/mL) were added to 1 g of frozen cells, which were then incubated for 2 h at 60 °C with intermittent mixing. Then 250 µL of proteinase K (10 mg/mL) were added again, and the cells were incubated for 40 min under the same conditions.

Extraction

3 The lysate was mixed for 10 min with 8 mL of phenol saturated with TE buffer, pH 8.0 using the tube rotator. The extract was centrifuged (10 min, 16,100 *g*, 20 °C), then the aqueous phase was taken and again mixed with an equal volume of phenol: chloroform: isoamyl alcohol mixture (25:24:1). It was then mixed and centrifuged under the same conditions. The aqueous phase was mixed with a chloroform: isoamyl alcohol mixture (24:1), mixed and centrifuged under the same conditions. Aqueous phase was used for the subsequent stages.

DNA precipitation

4 DNA was precipitated by adding 1/10 volume of 3 M sodium acetate, pH 5.2, and 1 volume of isopropanol to the aqueous phase. The solution was matured at 0 °C for 1 h and then centrifuged (30 min, 16,100 *g*, 4 °C). The supernatant was removed, and the precipitate was twice rinsed with 70% ethanol, dried in air and dissolved in sterile TE buffer (10 mM Tris-HCl; 1 mM Na_2EDTA, pH 8.0).