Jun 09, 2018

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

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.qh6dt9e

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.qh6dt9e

External link: https://doi.org/10.1371/journal.pone.0203161

Protocol Citation: Artyom M. Marchenkov, Darya P. Petrova, Alexey A. Morozov, Yulia R. Zakharova, Michael A. Grachev, Alexander A. Bondar 2018. Extraction of genomic DNA from diatoms by the modified method described in (Jacobs et al., 1992). **protocols.io** **https://dx.doi.org/10.17504/protocols.io.qh6dt9e**

Manuscript citation:
Marchenkov AM, Petrova DP, Morozov AA, Zakharova YR, Grachev MA, Bondar AA (2018) A family of silicon transporter structural genes in a pennate diatom *Synedra ulna* subsp. *danica* (Kütz.) Skabitsch. PLoS ONE 13(8): e0203161. doi: 10.1371/journal.pone.0203161

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

Created: May 28, 2018

Last Modified: June 09, 2018

Protocol Integer ID: 12574

Keywords: diatom, DNA extraction , protocol for diatom dna extraction, diatom dna extraction, freshwater diatom, sb ras for freshwater diatom, extraction of genomic dna, dna extraction, extraction, genomic dna, dna

Abstract

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

Troubleshooting

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).