Simple DNA Extraction for Phytoplankton Using Chelex 100

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Abstract

This is a modification of the origianl Chelex 100 extraction described by Walsh, Metzger and Higuchi (1991 BioTechniques 10(4):506). Adding PVPP facilitates working with environmental samples as well as pure cultures hi in phenolics or other contaminants.

Citation: G Jason Smith Simple DNA Extraction for Phytoplankton Using Chelex 100. **protocols.io** dx.doi.org/10.17504/protocols.io.hinb4de
Published: 31 Mar 2017

Protocol

REAGENT PREPARATION

Step 1.

Extraction Reagent: *10% Chelex 100, 1%PVPP, 0.1X TE pH8*

Make in 50 mL batches in sterile Falcon Tubes - Stable at RT > 6 months

a. Weigh out and add to 50 mL Falcon Tube

5.0 gm Chelex 100 (Sigma C7901-100G)

0.5 gm Polyvinylpolypyrrolidone (PVPP, Sigma P6755)

b. Resuspend in 50 mL nuclease free H_2O by vortexing to hydrate and wash for 10 min. Collect suspention by centrifugation (3000xg 10 min

c. Decant supernatant and repeat NF H_2O wash.

d. Decant supernatant and resuspend pellet in 50 mL 0.1X TE buffer, pH8, vortex and incubate with mixing 10 min. Centrifuge to collect suspension

e. Decant, and resuspend pellet in 50 mL 0.1X TE buffer, pH8.

DNA EXTRACTION

Step 2.

Be sure to completely resuspend extraction buffer prior to aliquoting

a. ***For filtered (25mm polycarbonate) samples*** aliquot 300 µL of mixed suspension into 1.5 mL tube

b. Add filter (do not fold) into extraction tube, seal and vortex to resuspend cell sample.

c. Heat suspended sample at 65 °C for 5 min, vortex and centrifuge (10000xg, 3 min) to sediment particulates

d. Generally dilute DNA extract supernatant 1:10 with TE pH8 for PCR assays

NOTES:

i. ***For cell pellets*** - add 300µL Chelex suspension directly to pellet and proceed as above.

ii. Volume of Chelex can be adjusted for biomass of samples, but 300 µL good starting point.

iii. Crude Chelex extracts are stable at 4°C for at least 12 months.

iv. DNA concentration and quality can be increased using the Zymo Genomic DNA Clean & Concentrator-10 (Zymo D4011) spin columns following the manufactures protocol