Mar 19, 2024

Lysis of diatom bulk samples

DOI
https://dx.doi.org/10.17504/protocols.io.x54v9ddkpg3e/v1

Till Macher[1]

[1]University of Duisburg-Essen, Aquatic Ecosystem Research

Till Macher
University of Duisburg-Essen, Aquatic Ecosystem Research

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9ddkpg3e/v1

Protocol Citation: Till Macher 2024. Lysis of diatom bulk samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9ddkpg3e/v1

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

Created: November 04, 2022

Last Modified: March 19, 2024

Protocol Integer ID: 72294

Keywords: lysis of diatom bulk sample, diatom bulk sample, lysis before dna extraction, dna extraction, protocol of the leeselab, diatom, gedna project, steps of sample preparation, sample preparation, leeselab, extraction, dna, metabarcoding protocol

Funders Acknowledgements:
Umweltbundesamt
Grant ID: FKZ 3719 24 2040

Abstract

This protocol describes the steps of sample preparation and lysis before DNA extraction for the diatoms metabarcoding protocol of the LeeseLab. The protocol was established in the GeDNA project.

Guidelines

Reagents are potentially damaging to the environment. Dispose waste responsibly.

Materials

Materials required:
Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:
Protk: 200 mL Proteinase K working solution (10 mg/mL Proteinase K **7BioScience Catalog #**RP100B)
TNES buffer ([M] 50 Molarity (M) TRIS , [M] 400 Molarity (M) NaCl , [M] 20 Molarity (M) EDTA

see 'Sample preparation and lysis of homogenized malaise trap samples' (dx.doi.org/10.17504/protocols.io.dm6gpjrmjgzp/v1) for details on the stock solutions.

Labware:
1 mm dia zirconia beads Zirconia Beads 1 mm dia **BioSpec Products Catalog #**11079110zx
2 mm dia zirconia beads Zirconia Beads 2 mm dia **BioSpec Products Catalog #**11079124zx
2 mL screwcap tubes 2 mL screwcap tube **Sarstedt Catalog #**72.693
50 mL Falcon tube Easy Reader Conical Polypropylene Centrifuge Tube **Catalog #**11512303

Sample collection (short description)

1 Perform multi-habitat diatoms sampling (depending on the protocol).

2 Prepare one sterile 50 mL Falcon tube for each sample. Label the Falcon tube with EtOH resistant labels (e.g. printed labels).

3 Add 10 mL diatom sample (e.g. scrape sample) to the Falcon tube.

4 Add 40 mL EtOH (96%) denatured to the Falcon tube.

5 Mix sample by inverting the Falcon tube.

6 Store samples under dark conditions at Room temperature or at 4 °C until lysis.

Sample concentration 7m

7 Centrifuge 50 mL falcon tubes containing the diatom samples 4000 x g, 00:10:00 10m

8 Discard supernatant EtOH (can be poured from the falcon tube). 1m

9 Add 10 mL fresh EtOH (96%) denatured to each sample (in falcon tube). Vortex samples to elute pellets again. 1m

Sample lysis 5m

10 Prepare each one 2 mL twist-top tube with each one spoon of 2.0 mm (ca. 10) and 1.0 mm (ca. 30) Zirconia beads. Label tubes both on the side and the lid.

11 Add 1 mL sample to each twist-top tube.

Note

Either use wide-bore tips or cut off the tips. Otherwise tips tend to clock when pipetting the sample.

12 Centrifuge samples 6000 x g, 00:05:00 . 5m

13 Discard supernatant EtOH (use 1000 µL tips).

14 Add 900 µL TNES and 100 µL ProtK to each sample.

Note

Depending on the amount of samples this can be prepared as premix. We usually prepare TNES + Proteinase K in batches for 24 samples. Proteinase K tends to self-digest if the time for samples preparation takes too long.

15 Bead-beat for 00:02:00 at 2400 rpm . 2m

16 Incubate samples at 55 °C for 00:20:00 at 1400 rpm . 20m

17 Store samples at -20 °C until DNA extraction.