Sep 06, 2021

HMW DNA extraction for diatoms

Raffaela Abbriano[1]

[1]University of Technology Sydney

dx.doi.org/10.17504/protocols.io.qzudx6w

Raffaela Abbriano

DISCLAIMER

ABSTRACT

Protocol for genomic DNA extraction from diatom cell culture without use of phenol/chloroform. Resulting DNA fragments were suitable for nanopore sequencing.

DOI

dx.doi.org/10.17504/protocols.io.qzudx6w

PROTOCOL CITATION

Raffaela Abbriano 2021. HMW DNA extraction for diatoms . **protocols.io** https://dx.doi.org/10.17504/protocols.io.qzudx6w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://doi.org/10.3389/fbioe.2020.00513

CREATED

Jun 15, 2018

LAST MODIFIED

Sep 06, 2021

PROTOCOL INTEGER ID

13076

MATERIALS TEXT

MATERIALS

TAE (TRIS-ACETATE-EDTA) Buffer, 50x **Amresco Catalog #K915**

Ethyl alcohol, Pure 200 proof, for molecular biology **Sigma Aldrich Catalog #E7023**

RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #EN0531**

PBS **Contributed by users**

Agarose **Sigma**

Nuclei Lysis Solution, 50ml **Promega Catalog #A7941**

Protein Precipitation Solution, 25ml **Promega Catalog #A7951**

Isopropanol, molecular grade **Sigma Aldrich Catalog #I9516**

Tris-EDTA buffer, pH 8.0, molecular grade **Sigma Aldrich Catalog #93283**

DISCLAIMER:

Harvest cells

1

1. Harvest approximately 7 x 10^7 - 2 x 10^8 cells from mid-exponential phase by centrifugation (5000xg, 3 minutes)
2. Wash pelleted cells once with 1X PBS (5000xg, 3 minutes) and remove the supernatant

Cell lysis and RNAse treatment

2

1. Resuspend cells in 600 L Nuclei Lysis Solution (Promega #A7941)
2. Freeze in dry ice or liquid nitrogen for 3 minutes and thaw in 65˚C heat block or water bath for 3 minutes (or until completely thawed)
3. Repeat freeze/thaw cycle 3X
4. Heat to 65˚C for 15 minutes
5. Let sample cool to room temperature and add 1.2 µL of 10 mg/ml RNase A (ThermoFisher #ENO531)
6. Incubate for 15 minutes at 37˚C in heat block or water bath
7. Let sample cool to room temperature

Be aware that rapid temperature changes can cause pressure to build up inside epi tubes, causing the lids to pop open. Use safe lock epi tubes or lid locks for the freeze/thaw step.

Citation: Raffaela Abbriano (09/06/2021). HMW DNA extraction for diatoms . https://dx.doi.org/10.17504/protocols.io.qzudx6w

https://us.vwr.com/store/product/4615516/lidlocks-microcentrifuge-tube-locks-sorenson-bioscience

Protein precipitation

3
1. Add 250 µL Protein Precipitation Solution and mix well (Promega # A7951)
2. Centrifuge (16,000xg) for 40 minutes at 4˚C
3. Transfer supernatant to fresh tube

DNA precipitation

4
1. Precipitate DNA by adding 700 µL 100% isopropanol, mix well by inversion
2. Centrifuge (16,000xg) for 30 minutes at room temperature
3. Wash DNA pellet with 600 µL freshly prepared 70% molecular grade ethanol
4. Centrifuge (16,000xg) for 1 minute and remove all ethanol from the pellet (use quick spin and a smaller pipette tip if necessary to remove all residual ethanol)
5. Air dry DNA pellet for 10-15 minutes
6. Resuspend DNA pellet in ~25-50 µL TE buffer, depending on pellet size and desired DNA concentration
7. Let DNA pellet resuspend in TE buffer at room temperature overnight (HMW gDNA takes longer to dissolve)
8. Determine DNA concentration by Nanodrop

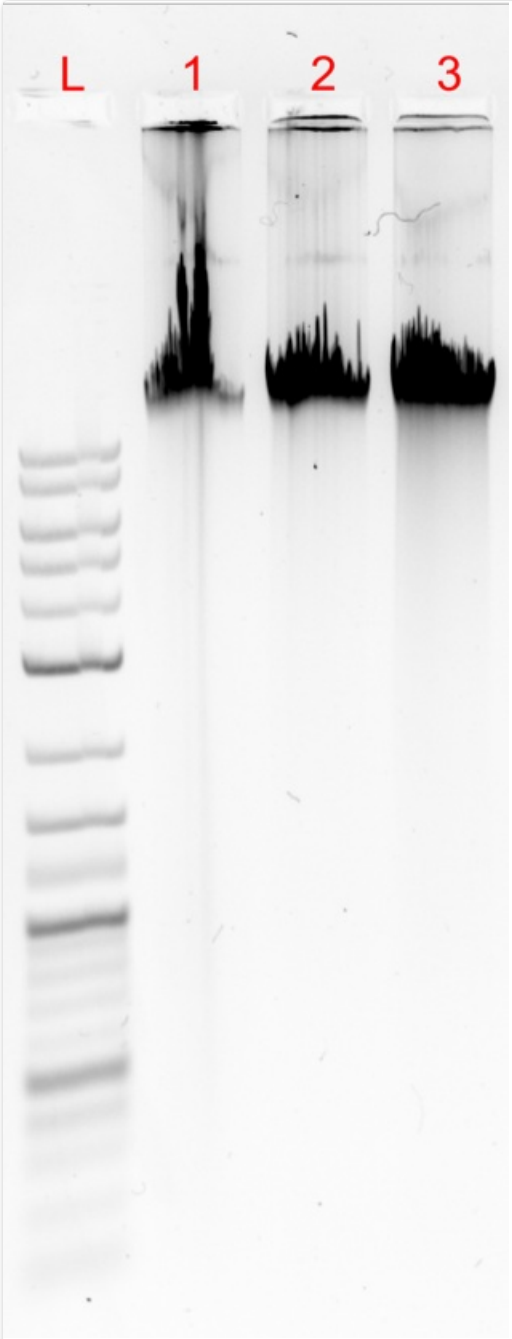
After isopropanol precipitation, DNA pellet may appear as a translucent smear on the side of the tube. Pellet becomes more visible (white) following the ethanol wash step.

Gel electrophoresis

5
1. Run 100-200 ng DNA on a 0.8% agarose gel

Example data:

Citation: Raffaela Abbriano (09/06/2021). HMW DNA extraction for diatoms . https://dx.doi.org/10.17504/protocols.io.qzudx6w



L = ladder (top band = 40kb)
1. Phaeodactylum tricornutum
2. Thalassiosira pseudonana
3. Chaetoceros muelleri

Species	Yield (ng/uL)	260/280	260/230
Thalassiosira pseudonana	1255.8	1.8	1.86
Phaeodactylum tricornutum	673.8	1.85	2.07

Citation: Raffaela Abbriano (09/06/2021). HMW DNA extraction for diatoms . https://dx.doi.org/10.17504/protocols.io.qzudx6w

Chaetoceros muelleri	1066.5	1.89	1.49

Citation: Raffaela Abbriano (09/06/2021). HMW DNA extraction for diatoms . https://dx.doi.org/10.17504/protocols.io.qzudx6w