Jun 01, 2024

DNA Extraction from Ethanol Zooplankton Samples - Phenol-Chloroform

DOI
dx.doi.org/10.17504/protocols.io.n92ld88mnv5b/v1

Andreas Novotny[1], Colleen Kellogg[2], rute.carvalho Carvalho[2], Matt Lemay[2]
[1]University of British Columbia; [2]Hakai Institute

Hakai Genomics

Andreas Novotny
University of British Columbia, Hakai Institute, Stockholm U...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.n92ld88mnv5b/v1

External link: https://hakai.org

Protocol Citation: Andreas Novotny, Colleen Kellogg, rute.carvalho Carvalho, Matt Lemay 2024. DNA Extraction from Ethanol Zooplankton Samples - Phenol-Chloroform. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld88mnv5b/v1

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

Created: May 30, 2024

Last Modified: June 01, 2024

Protocol Integer ID: 100959

Keywords: Zooplankton, Biodiversity, DNA

Abstract

This protocol extracts genomic DNA from bulk zooplankton samples, preserved either in ethanol or frozen - using Phenol/Chloroform extraction. The protocol was developed to be as similar as possible to the standard protocol used for environmental DNA at Hakai Institute Ocean Observing Program, to be used for downstream DNA metabarcoding:

CITATION

Colleen Kellogg. DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform. protocols.io.
LINK
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin

Guidelines

MIOP: Minimum Information about an Omics Protocol

MIOP Term	Value
analyses	Nucleic Acid Extraction
audience	scientists
broad-scale environmental context	marine biome ENVO_00000447
creator	Andreas Novotny
environmental medium	sea water [ENVO:00002149]
geographic location	North Pacific Ocean [GAZ:00002410]
hasVersion	1
issued	2024
language	en
license	CC BY 4.0
local environmental context	Zooplankton
materials required	Sterile workbench, Fume Hood, Centrifuge, Incubator
maturity level	Mature
methodology category	DNA extraction
personnel required	1
project	Biomolecular surveys of marine biodiversity in the Northern Salish Sea, BC
publisher	Hakai Institute, Ocean Observing Program
purpose	DNA Extraction
skills required	sterile technique | pipetting skills
target	DNA
time required	1 day

AUTHORS

PREPARED BY All authors known to have contributed to the preparation of this protocol, including those who filled in the template.	AFFILIATION	ORCID (visit https://orcid.org/ to register)	DAT
Colleen Kellogg	Hakai Institute	https://orcid.org/0000-0003-4048-5316	201 7
Andreas Novotny	University of British Columbia	https://orcid.org/0000-0001-8910-6183	202 4

RELATED PROTOCOLS

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
DNA extraction from 0.22um sterivex filters using phenol chloroform.	Hakai Institute	https://dx.doi.org/10.17504/protocols.io.14egn63oql5d/v1

This is a list of other protocols which should be known to users of this protocol. Please include the link to each related protocol.

ACRONYMS AND ABBREVIATIONS

ACRONYM / ABBREVIATION	DEFINITION

GLOSSARY

SPECIALISED TERM	DEFINITION

BACKGROUND

This protocol extracts genomic DNA from bulk zooplankton samples, preserved either in ethanol or frozen - using Phenol/Chloroform extraction. The protocol was developed to be as similar as possible to the standard protocol used for environmental DNA at Hakai Institute Ocean Observing Program, to be used for downstream DNA metabarcoding:

CITATION

Colleen Kellogg. DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform. protocols.io.
LINK
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin

Spatial coverage and environments of relevance
As part of the Hakai Institute Ocean Observing Program, zooplankton samples have been collected monthly to biweekly, using a 250um bongo net with an opening diameter of 0.5m, towed from 230m-0m. The zooplankton samples have been preserved in formalin for microscopic analysis and in 99% ethanol for genetic analysis.

Personnel Required
1 Technician

Safety
Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure!

Training requirements
Sterile technique, pipetting skills. Work-safe laboratory practices.

Time needed to execute the procedure
1 Day (for 24 samples).

Protocol materials

- UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1) pH 8.0 **Thermo Fisher Scientific Catalog #**15593049 Step 1
- 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0) Step 1
- Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**L6876 Step 1
- Stainless Steel Beads 0.9 – 2.0 mm blend **Next Advance Catalog #**SSB14B Step 1
- Proteinase K, 2mL **Qiagen Catalog #**19131 Step 1
- Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q32854 Step 1
- Stainless Steel UFO Beads 3.5 mm **Next Advance Catalog #**SSUFO35 Step 1
- Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C0549 Step 1
- 500ml SDS [20%] **G-Biosciences Catalog #**786-016 Step 1
- RNAse A **Qiagen Catalog #**19101 Step 1
- Amicon™ Ultra Centrifugal Filter Units **Thermo Fisher Catalog #**UFC801096 Step 1

Safety warnings

Several of the chemicals are harmful to humans and the environment. Make a proper risk assessment before starting this procedure. Fumes from the extraction are toxic and waste must be treated with extreme care!

Before start

Read Minimum Information about an Omics Protocol (MIOP) and other recommendations under the "Guidelines" tab.

PREPARATIONS

1

Safety information

Several of the chemicals are harmful to humans and the environment. Make a proper risk assessment before starting this procedure. Fumes from the extraction are toxic and waste must be treated with extreme care!

Materials needed (OR similar):

- Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**L6876
- RNAse A **Qiagen Catalog #**19101
- 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0) **Contributed by users**
- Proteinase K, 2mL **Qiagen Catalog #**19131
- 500ml SDS [20%] **G-Biosciences Catalog #**786-016
- UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1) pH 8.0 **Thermo Fisher Scientific Catalog #**15593049
- Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C0549
- Amicon™ Ultra Centrifugal Filter Units **Thermo Fisher Catalog #**UFC801096
- Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q32854

Sucrose Lysis Buffer (SLB) is prepared as follow:

Protocol

NAME

Sucrose lysis buffer

CREATED BY

Andreas Novotny

PREVIEW

To be Autoclaved:

- Stainless Steel UFO Beads 3.5 mm **Next Advance Catalog #**SSUFO35
- Stainless Steel Beads 0.9 – 2.0 mm blend **Next Advance Catalog #**SSB14B

ZOOPLANKTON HOMOGENIZATION

2 Subset the bulk zooplankton sample to a to a reasoable volume (here, 1/8th of the original sample):

- Use a plankton net filter to remove the ethanol preservative (keep the preservative).
- Resuspend zooplankton in dH2O
- Use a Fulton splitter to subset 1/8 fraction of the zooplankton sample and add to a 50ml falcon tube.
- Return the remaining fractions to the ethanol preservative.

3

- Centrifuge the falcon tube 5000xg for 5 min to pellet the zooplankton
- Use a pipett to remove all liquid.
- Add SLB to the falcon tube, to a total volume of 6mL, and resuspend.

4

- Add 3 large UFO beads (3.5 mm) to the falcon tube.
- Add ~30 small stainless steal beads (0.9-2.0 mm) to the falcon tube.
- Vortex vigorously at full speed for at least 10 min, or until no visible structures of the zooplankton are left.

5

- For each sample, add 1800 µL SLB to new labeled 15mL falcon tubes.
- Add an aliquot of 100 µL of the homogenized zooplankton sample to the SLB tube. The total volume should be around 1900 µL.

LYSIS AND INCUBATION

6
- Add 100 µL lysozyme (125 mg fully dissolved in 1000 µl 1 x TE) and 20µL RNase A (10 µg/ml: 1µL in 999µL 1 x TE) to each sample tube.
- Incubate samples in a rotating incubator at 37°C for 1h.

7
- Add 100 µL Proteinase K and 100 µL (20%) SDS to each lysate tube.
- Incubate at 55°C for 1-2 hours in a rotating incubator.

8 Add another 1mL sucrose lysis buffer (SLB) to the lysate tube.

EXTRACTION & CENTRIFUGATION

9

Safety information

The following section should be done in a fume hood.

- **In the fume hood**, add an equal volume (about 3mL) of Phenol:Chloroform:IAA (25:24:1), pH 8.0 to the lysate tube.
- Invert for 10 secondsby hand to mix.
- Spin at 2500 g for 6 min or until the aqueous layer is clear. Wait at least 10 minutes before opening the centrifuge.
- Transfer the aqueous (top) layer into a new 15 ml falcon tube.

10
- Add an equal volume (approx 3mL) of Chloroform:IAA (24:1) to the tube containing the aqueous layer.
- Invert for 10 seconds by hand.
- Spin at 2500 g for 6 min or until the aqueous layer is clear with no debris. Wait at least 10 minutes before opening the centrifuge.
- Transfer the aqueous layer into a labeled Amicon Ultra centrifuge tube (UFC801096, EMD Millipore).

11
- Top up Amicon with 1-2mL of 1 x TE buffer.
- Spin at 3500 g for 10-15 minutes.
- Check to make sure there is less than 1ml liquid left in Amicon at the end of this (if not, fill up with 2mL 1 x TE and spin again).

12
- Add 2 mL TE buffer to Amicon and spin at 3500 g for 6 min.
- Remove filtrate.

13
- Repeat Step 8 twice more (total of 3 washes with 2mL TE).
- For the last wash, spin until 200 – 500 µl remain in Amicon (typically 8-10 minutes).
- Note the final volume and transfer to a labeled 1.5 µl Eppendorf tube.
- Rinse Amicon sides with 50 µL of 1xTE and pool with the rest of the sample in a labeled 1.5mL Eppendorf tube.

DNA QUANTIFICATION AND STORING

14
- If desired, aliquot 50 µL from the final sample volume into a 1.5ml Eppendorf tube to use as working stock and place in a -20°C freezer.
- Place the remaining DNA stock in the -80°C freezer for long-term storage.

15 Quantify DNA stock using Qubit (following manufacturer's instructions). Use 2 µL of stock DNA when quantifying.

DNA NORMALISATION

16

Note

Optional, but does tend to yield good success during amplification

17 Make a normalized 2.5 ng/µl DNA stock for PCR :
Calculate the volume of DNA for 2.5 ng/µL stock (there is a spreadsheet for this)

$$\left(2.5\frac{ng}{\mu L}\right)(25\ \mu L) = \left([DNA]\frac{ng}{\mu L}\right)(x\ \mu L)$$

Where **[DNA]** is the DNA concentration from Qubit and ***x*** is the number of uL of the DNA stock that you need to add for a 25 uL volume of a 2.5 ng/uL DNA stock.

Calculate the volume of water for 2.5 ng/µL stock

$$25\ \mu L - x\ \mu L\ of\ DNA = y\ \mu L\ \text{of water}$$

18 - Place the 2.5 ng/µL DNA stock in the -20°C freezer.

Protocol references

This protocol is modified from
Colleen Kellogg. **DNA Extraction from 0.22 micrometer Sterivex filters - Phenol Chloroform**. Protocols.io:
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin
....which is a modified version of:
DNA extraction from 0.22 microM Sterivex filters and cesium chloride density gradient centrifugation. Jody J. Wright, Elena Zaikova, David A. Walsh and Steven J. Hallam
https://doi.org/10.3791/1352

Citations

Colleen Kellogg. DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin

Colleen Kellogg. DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform
https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin