



Jun 01, 2024

DNA Extraction from Ethanol Zooplankton Samples - Phenol-Chloroform

DOI

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

Hakai

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DOI: <https://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>

Manuscript citation:

Andreas Novotny, Caterina Rodrigues, Loïc Jacquemot, Rute B G Clemente-Carvalho, Rebecca S Piercey, Evan Morien, Moira Galbraith, Colleen T E Kellogg, Matthew A Lemay, Brian P V Hunt, DNA metabarcoding captures temporal and vertical dynamics of mesozooplankton communities, ICES Journal of Marine Science, Volume 82, Issue 2, February 2025, fsaf007, <https://doi.org/10.1093/icesjms/fsaf007>

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Protocol status: Working

This version of the protocol is published as a supplementary to: Andreas Novotny, Caterina Rodrigues, Loïc Jacquemot, Rute B G Clemente-Carvalho, Rebecca S Piercey, Evan Morien, Moira Galbraith, Colleen T E Kellogg, Matthew A Lemay, Brian P V Hunt, DNA metabarcoding captures temporal and vertical dynamics of mesozooplankton communities, ICES Journal of Marine Science, Volume 82, Issue 2, February 2025, fsaf007, <https://doi.org/10.1093/icesjms/fsaf007>

Created: May 31, 2024

Last Modified: June 01, 2024

Protocol Integer ID: 100959

Keywords: Zooplankton, Biodiversity, DNA, dna extraction from ethanol zooplankton sample, genomic dna from bulk zooplankton sample, ethanol zooplankton sample, environmental dna at hakai institute ocean observing program, dna extraction, bulk zooplankton sample, environmental dna, downstream dna metabarcoding, chloroform extraction, chloroform this protocol, genomic dna, dna, hakai institute ocean observing program, using phenol, phenol, ethanol, chloroform



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.

<https://protocols.io/view/dna-extraction-from-0-22-m-sterivex-filters-phenol-dejb3cin>^{LINK}

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)
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RELATED PROTOCOLS

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
DNA extraction from 0.22um sterilvex 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.

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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

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

Troubleshooting

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)
- ☒ 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 reasonable 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 steel 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 μ L lysozyme (125 mg fully dissolved in 1000 μ L 1 x TE) and 20 μ L RNase A (10 μ g/ml: 1 μ L in 999 μ L 1 x TE) to each sample tube.
 - Incubate samples in a rotating incubator at 37°C for 1h.
- 7
- Add 100 μ L Proteinase K and 100 μ L (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 seconds by 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 μ L remain in Amicon (typically 8-10 minutes).
 - Note the final volume and transfer to a labeled 1.5 μ L Eppendorf tube.
 - Rinse Amicon sides with 50 μ L 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 μ L 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 μ L 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)

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

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

Calculate the volume of water for 2.5 ng/μL stock

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

- 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

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