Jun 01, 2024

Subsampling Ethanol Preservative from Zooplankton Museum Collections for DNA Extractions

DOI
dx.doi.org/10.17504/protocols.io.j8nlk888wl5r/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.j8nlk888wl5r/v1

External link: http://hakai.org

Protocol Citation: Andreas Novotny 2024. Subsampling Ethanol Preservative from Zooplankton Museum Collections for DNA Extractions. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk888wl5r/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

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 30, 2024

Last Modified: June 01, 2024

Protocol Integer ID: 100973

Keywords: ethanol preservative from zooplankton museum collection, dna from archived sample, dna extraction, dna extractions this protocol, subsampling ethanol preservative, actual zooplankton biomass, archived sample, zooplankton museum collection, dna, ethanol, sample

Abstract

This protocol is used to sample DNA from archived samples preserved in ethanol, without having to subsample or split the actual zooplankton biomass.

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	2017
	language	en
	license	CC BY 4.0
	local environmental context	coastal sea water [ENVO: 00002150]
	materials required	Sterile workbench, Fume Hood, Centrifuge, Incubator
	maturity level	Mature
	methodology category	Sample collection
	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

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 is used to sample DNA from archived samples preserved in ethanol, without having to subsample or split the actual zooplankton biomass.

Spatial coverage and environments of relevance
As part of the Hakai Institute Ocean Observing Program, biomolecular samples have been collected weekly, from 0 to near bottom (260 m), to genetically characterize plankton communities in the Northern Salish Sea since 2015, developing a climatology from which we can begin uncover the physical, chemical and biological drivers of community and functional change in the dynamic coastal waters of coastal British Columbia.

This protocol has been used as an alternative source of genetic information, when it is not practical to remove biomass from the zooplankton samples.

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 h

Protocol materials

Sterivex Filter (0.2 um) **Merck Millipore (EMD Millipore)** **Catalog #**SVGPL10RC

Troubleshooting

Before start

Read background information, MIOP and BePOP-OBON information under the "Guidelines" tab.

PREPARATIONS

1 ***Prepare Sucrose Lysis Buffer (SLB):***

Protocol

NAME
Sucrose lysis buffer

CREATED BY
Andreas Novotny

Preview

ETHANOL SUBSAMPLING

2 To re suspend DNA in the zooplankton sample, invert the sample jar three times.

3 Let settle for 30 minutes

4 Use a serological pipette to remove 50 ml of the ethanol preservative, and transfer to a 50 mL falcon tube.

5 Use a syringe to push the ethanol through a
Sterivex Filter (0.2 um) **Merck Millipore (EMD Millipore) Catalog #**SVGPL10RC .

6 Seal the outflow of the filter with parafilm.

7 Add 1800 µL sucrose lysis buffer (SLB).

8 Seal the inflow opening with parafilm.

9 Label filter units and store them at -80°C for downstream DNA extraction.

DNA EXTRACTION

10 Follow the same extraction procedures as for Environmental DNA:

Protocol

NAME
DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform

CREATED BY
Andreas Novotny

Preview

11 Alternative extraction method:

Protocol

NAME

DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue Kit

CREATED BY

Andreas Novotny

Preview