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Subsampling Ethanol Preservative from Zooplankton Museum Collections for DNA Extractions

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Hakai Genomics

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

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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/ to register)	DAT
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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 Step 5

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