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DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform

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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 extract genomic DNA from 0.22µl sterivex filters using Phenol-Chlorophorm. As part of the Hakai Institute Ocean Observing Program, biomolecular samples have been collected weekly, from 0 m to near bottom (260 m), to genetically characterize plankton communities in the Northern Salish Sea since 2015. This protocol is developed to work across all domains of life, from viruses to prokaryotes to eukaryotes, allowing for both amplicon sequencing and shotgun sequencing.

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	Colleen Kellogg
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
Seawater filtration	Hakai Institute	

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 extract genomic DNA from 0.22µl sterivex filters using Phenol-Chlorophorm.

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 is developed to work across all domains of life, from viruses to prokaryotes to eukaryotes, allowing for both amplicon sequencing and shotgun sequencing.

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

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 The protocol assumes that water has been collected on a sterivex filter and preserved with Sucrose lysis buffer. See the following protocol:

Protocol

NAME
Seawater Filtration for Microbial or Environmental DNA

CREATED BY
Andreas Novotny

PREVIEW

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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 follows:

Protocol

NAME
Sucrose lysis buffer

CREATED BY
Andreas Novotny

PREVIEW

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- Thaw filters on ice, if applicable.
- Replace Parafilm on each sample if needed.
- Prepare extraction negative - add 1800 µL of SLB (filter-sterilized and UVed) into a new Sterivex.
- Seal the bottom with parafilm and the top with cap or parafilm, treat the same as the rest of the samples.

LYSIS AND INCUBATION

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- 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 filter.
- Reseal the top with Parafilm or a luer cap.
- Incubate Sterivex in a rotating incubator at 37°C for 1h (in labeled 50 ml falcon tubes).

5
- Add 100 µL Proteinase K and 100 µL (20%) SDS to each filter.
- Reseal using Parafilm or a luer cap.
- Incubate at 55°C for 1-2 hours in a rotating incubator (in 50ml falcon tubes; be sure to keep the same falcon tube for the same sterivex throughout these incubation steps).

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- Remove lysate from sterivex filter using a 10cc syringe into a labeled 15 ml falcon tube, use a new syringe for each sample.

> Note
>
> *Do not pull up on* the plunger *while sterivex is attached. You will likely need to do multiple plunges to empty the Sterivex, so unscrew the syringe from the filter, pull back the plunger, reattach to Sterivex, and push and hold to plunge liquid out of Sterivex.*

- Repeat as needed until (nearly) all liquid and foam are out of Sterivex.
- Add 1mL sucrose lysis buffer (SLB), rotate Sterivex with your hands several times to rinse the filter out, and then plunge out the liquid as described above.
- Pool this with the lysate in the 15 ml tube.

EXTRACTION & CENTRIFUGATION

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- 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.

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- 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).

9
- 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).

10
- Add 2 mL TE buffer to Amicon and spin at 3500 g for 6 min.
- Remove filtrate.

11
- 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

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- 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.

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

DNA NORMALISATION

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Note

Optional, but does tend to yield good success during amplification

15 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

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

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

Protocol references

This protocol is modified from **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