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# DNA Extraction from Sterivex Filters - Phenol-Chloroform

Forked from DNA EXTRACTION Protocol Template

This protocol is a draft, published without a DOI.

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**External link:** https://hakai.org

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**Protocol status:** In development
**We are still developing and optimizing this protocol**

**Created:** November 07, 2023

**Last Modified:** May 24, 2024

**Protocol Integer ID:** 99222

## Disclaimer

Draft!

## Abstract

This protocol is used to extract genomic DNA from 0.22µl sterivex filters using Phenol-Chlorophorm. The protocol is part of the Hakai Institute's pipeline to analyze microbial and environmental DNA from seawater samples and is implemented as a standard procedure for ongoing sampling programs.

## Guidelines

**MIOP: Minimum Information about an Omics Protocol**

| MIOP Term | Value |
|---|---|
| analyses | Nucleic Acid Water Filtration |
| 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 | oceanic epipelagic zone biome [ENVO:01000033] |
| materials required | Peristaltic Pump |
| maturity level | Mature |
| methodology category | Sample collection |
| personnel required | 1 |
| project | Hakai Institutes Marine Biodiversity |
| publisher | Hakai Institute, Genomics Lab |
| purpose | Sea water filtration [CHMO:0001640] |
| skills required | sterile technique \| pipetting skills |
| target | DNA |
| time required | 30 |

**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 |
| | | | |

**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 document describes the required protocol to to filter seawater onto a 0.22 micrometer Sterivex filters using paristaltic pump setup.

*Method description and rationale*

Thiswater filtration is part of the standard best - practice method for analysing microbial and environmental DNA from seawater samples at the Hakai Institutes Genome Lab. The method is part of a pipeline that includes seawater filtration, DNA extraction, and amplicon sequencing.

*Spatial coverage and environments of relevance*

The protocol has been used to analyze water samples (commonly 2L) from all parts of the water columns, nearshore and offshore stations on the coast of British Columbia, Canad. The protocol is also designed to target a broad range of organisms and has been used for the downstream analysis of Bacteria, Protists, Zooplankton, and Fish - using 16S rRNA, 18S rRNA, COI and 12S respectively.

*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*
The set-up, filtration, and clean-up steps each take about 20-30 minutes. If using two peristaltic pumps simultaneously, up to 8 samples may be filtered at the same time.

## Materials

| | DESCRIPTION e.g. filter | PRODUCT NAME AND MODEL Provide the official name of the product | MANUFACTURER Provide the name of the manufacturer of the product. | QUANTITY Provide qua |
|---|---|---|---|---|
| | Durable equipment | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Consumable equipment | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Chemicals | | | |
| | Content Cell | Content Cell | Content Cell | Content Cell |
| | Content Cell | Content Cell | Content Cell | Content Cell |

## 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!

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 top with cap or parafilm, treat thesame as therest of samples.

## Lysis and Incubation

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

3 Add 100 µL lysozyme (125 mg fully dissolved in 1000 µl 1 x TE) and 20 µL 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 01:00:00 (in labelled 50 ml falcon tubes).

1h

4 Add 100 µL Proteinase K and 100 µL [M] 20 Mass / % volume 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).

5 Remove lysate from sterivex filter using a 10cc syringe into a labeled 15 ml falcon tube, use a new syringe for each sample.
*Do not pull up on* the*plunger while sterivex is attached. You will likely need to do multiple plunges to completely 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 1 mL sucrose lysis buffer (SLB), rotate Sterivex with your hands several times to rinse the filter out and then plunge out liquid as described above.
Pool this with the lysate in the 15 ml tube.

## EXTRACTION & CENTRIFUGATION

6

**Safety information**

Phenol:Chloroform is toxic when inhaled and has to be handled in a fume hood.

6m

In the fume hood, add an equal volume (about 3 mL ) 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 00:06:00 or until the aqueous layer is clear. Wait at least 10 minutes before opening the centrifuge.
Transfer aqueous (top) layer into a new 15 ml falcon tube.

7 Add an equal volume (approx 3 mL ) of Chloroform:IAA (24:1) to the tube containing the aqueous layer. 6m
Invert for 10 seconds by hand.
Spin at 2500 g for 00:06:00 or until aqueous layer is clear with no debris. Wait at least 10 minutes before opening the centrifuge.
Transfer aqueous layer into a labeled Amicon Ultra centrifuge tube (UFC801096, EMD Millipore).

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

9 Add 2 mL TE buffer to Amicon and spin at 3500 g for 00:06:00 . 6m
Remove filtrate.

10 Repeat Step 8 twice more (total of 3 washes with 2 mL 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 therest of the sample in the a labelled 1.5mL Eppendorf tube.

## DNA QUANTIFICATION AND STORING

11 If desired, aliquot 50 µL from the final sample volume into a 1.5ml eppendorf tube to use as working stock and place in -20 °C freezer.
Place the remaining DNA stock in the -70 °C freezer for long term storage.

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

## DNA NORMALISATION

13

Note

Optional, but does tend to yield good success during amplification

14 Make a normalized 2.5 ng/µl DNA stock for PCR :
**Calculate 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 volume of water for 2.5 ng/µL stock**

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

15 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