

May 30, 2024

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

In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn63oql5d/v1>

Colleen Kellogg<sup>1</sup>

<sup>1</sup>Hakai Institute

Hakai Genomics



Andreas Novotny

University of British Columbia, Hakai Institute, Stockholm U...



## Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.14egn63oql5d/v1>

External link: <https://hakai.org>

**Protocol Citation:** Colleen Kellogg 2024. DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform. protocols.io <https://dx.doi.org/10.17504/protocols.io.14egn63oql5d/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>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

We use this protocol and it's working

**Created:** May 27, 2024

**Last Modified:** May 30, 2024

**Protocol Integer ID:** 100675

**Keywords:** eDNA, marine microbiology, biodiversity, plankton communities in the northern salish sea, sterivex filter, part of the hakai institute ocean observing program, hakai institute ocean observing program, plankton community, dna extraction, biomolecular sample, chloroform this protocol, northern salish sea, using phenol, genomic dna, phenol, chloroform, prokaryote, eukaryote, prokaryotes to eukaryote, dna, chlorophorm, sterivex

## 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 <a href="https://orcid.org">https://orcid.org</a> )        |
| Colleen Kellogg                                                                                                                    | Hakai Institute | <a href="https://orcid.org/0000-0003-">https://orcid.org/0000-0003-</a> |

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

- ☒ Lysozyme from chicken egg white **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L6876**
- ☒ RNase A **Qiagen Catalog #19101**
- ☒ UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1) pH 8.0 **Thermo Fisher Scientific Catalog #15593049**
- ☒ Amicon® Ultra Centrifugal Filter Units **Thermo Fisher Catalog #UFC801096**
- ☒ Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**
- ☒ 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**
- ☒ Chloroform:Isoamyl alcohol 24:1 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C0549**

## 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

- 2

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 follows:

Protocol


NAME  
**Sucrose lysis buffer**

CREATED BY  
 Andreas Novotny

Preview

- 3
  - 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

- 4
- Add 100  $\mu$ L lysozyme (125 mg fully dissolved in 1000  $\mu$ L 1 x TE) and 20  $\mu$ L RNase A (10  $\mu$ g/ml: 1  $\mu$ L in 999  $\mu$ L 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  $\mu$ L Proteinase K and 100  $\mu$ L (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).



- 6
- 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

- 7
- 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.
- 8
- 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  $\mu$ L remain in Amicon (typically 8-10 minutes).
  - Note the final volume and transfer to a labeled 1.5  $\mu$ L Eppendorf tube.
  - Rinse Amicon sides with 50  $\mu$ L of 1xTE and pool with the rest of the sample in a labeled 1.5mL Eppendorf tube.



## DNA QUANTIFICATION AND STORING

- 12
- If desired, aliquot 50  $\mu$ 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.



- 13 Quantify DNA stock using Qubit (following manufacturer's instructions). Use 2  $\mu\text{L}$  of stock DNA when quantifying.

## DNA NORMALISATION

14

Note

Optional, but does tend to yield good success during amplification

- 15 Make a normalized 2.5 ng/ $\mu\text{L}$  DNA stock for PCR :  
**Calculate the volume of DNA for 2.5 ng/ $\mu\text{L}$  stock** (there is a spreadsheet for this)

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

Where **[DNA]** is the DNA concentration from Qubit and **x** is the number of  $\mu\text{L}$  of the DNA stock that you need to add for a 25  $\mu\text{L}$  volume of a 2.5 ng/ $\mu\text{L}$  DNA stock.

**Calculate the volume of water for 2.5 ng/ $\mu\text{L}$  stock**

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

- 16 ■ Place the 2.5 ng/ $\mu\text{L}$  DNA stock in the  $-20^{\circ}\text{C}$  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>