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# Seawater Filtration for Microbial or Environmental DNA

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## Abstract

**This protocol describes water filtrations onto 0.22µl sterivex filters using a peristaltic pump**. 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 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 | AFFILIATION | ORCID | DATE |
|---|---|---|---|---|
| | Colleen Kellogg | Hakai Institute | https://orcid.org/0000-0003-4048-5316 | 2017 |

**RELATED PROTOCOLS**

| | PROTOCOL NAME AND LINK | ISSUER / AUTHOR | RELEASE / ACCESS DATE |
|---|---|---|---|
| | Suckrose Lysis Buffer | 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*

This water 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*

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. We work across all

domains of life, from virus to prokaryotes to eukaryotes, employing 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*
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.

## Troubleshooting

## Before start

Read Minimum Information about an Omics Protocol (MIOP) and other recommendations under the "Guidelines" tab.

Water samples should be collected into clean (acid-washed, MilliQ-rinsed) 2 L bottles that have been triple-rinsed with sample water before filling.  Fill beyond the 2 L mark so that some of the water can be used for rinsing tubing and cell count samples.  After collection, samples should be kept in the dark and cold (in a cooler, at the very least, and on ice, if possible, especially on warm, sunny summer days).  Each 2 L water sample yields one Sterivex filter (0.22 µm). For samples intended for DNA extraction, filters are stored in lysis buffer at -80°C. Although the procedure is simple, the time requirement can be extensive depending on the concentration of biomass or debris in the sample being filtered. RNA samples, however, should not be filtered for more than 25 minutes, regardless of whether or not the entire sample volume has been filtered.

It is imperative to wipe down benches and pumps with a damp towel after use to remove any lingering traces of salt water that would otherwise corrode equipment.

## PREPARATION

1 Before starting, ensure to have sucrose lysis buffer prepared.

Protocol

NAME

Sucrose lysis buffer

CREATED BY

Andreas Novotny

Preview

2

Note

**Important:** To prevent biofilm formation between sampling trips, all filtration equipment must be rinsed with dilute HCl and deionized water immediately after use.

**Materials:**

- Peristaltic pump with attached pump heads
- Tubing setup: autoclaved tubing with attached male luer connection
- Sterivex filters (1 per DNA sample, 1 per RNA sample)
- 0.2 µm filtered Sucrose Lysis Buffer (SLB; 1.8 mL per DNA sample)
- Bench covers (“diapers”)
- Sterile 25 mL pipets
- Forceps
- 2 L bottle (1 per water sample collected, so if both DNA and RNA samples are collected then this would require 2 bottles)
- P1000 pipet
- Sterile or autoclaved pipet tips for P1000 pipet
- Parafilm, cut into squares
- Sterivex luer lock caps
- Gloves
- Squirt bottle with 70% ethanol
- Sharpies, Lab Markers and Pencils
- Shop towels
- Cooler (to keep samples in after collection)

- Labels
- 2% HCl
- MilliQ water
- Dry ice or liquid nitrogen
- Kim wipes
- Repair kit

## SETUP OF FILTRATION APPARATUS

3 Line counter with lab diapers.

4 For extra cleanliness, you can place an autoclave or otherwise plastic bin on one side of the pump.

5 Place the waste flask or container on the other side of the pump.

6 Then place a full sample bottle in the autoclave bin; however, do not remove the cap of the bottle yet.

7 Pass the tubing through the peristaltic pump so that the luer end of the tubing is in the waste collection container. The luer end may need to be held in place by draping it through a clamp but do not pinch the tube with clamp.

8 Using tweezers, remove the cotton from a sterile 25 ml pipette, and attach the pipette to the other end of the tubing.

## FILTRATION OF SEAWATER

9 To begin filtering seawater, invert bottle several times to mix sample.
Open the bottle and insert the pipette.
Turn on the pump and set the flow speed to 1, if use old Cole-Parmer peristaltic pump, or 40-50 if using newer Cole-Parmer peristaltic pumps. Watch to ensure that water is being pumped in the proper direction.

10 If your filtration station in the direct sunlight, it is recommended to cover or drape the sample bottles with a dark bag while filtering. Alternatively, it is also possible to tape a dark garbage bag up on window to prevent direct sun exposure.

11 Rinse the tubing: Allow ~100 ml of water to pass through the entire tubing. If you fill a 2 L polycarbonate bottle, and can just rinse down to the 2 L mark and then turn off the pump.

12 Attach filters: Turn off the pump. Attach a Sterivex filter to the male luer fitting at the end of the tubing.

13 Turn back on pump and continue filtering until no water is left in the bottles.
If the filter(s) clogs prior to complete sample filtration, stop pump and change necessary filter, labeling the first filter as “Filter A” and recording the filtered 3 volume and storing as described below, before attaching a new filter and resuming filtration.

## STORAGE OF FILTERS

14 To store the sterivex filters, first expel any remaining water with a 30-60 ml syringe. Alternatively, you can shake the sterivex filter, forcing excess water out of the inlet (this is what colleen tends to do...wastes less plastic).

15 Seal the bottom of the Sterivex filter with Parafilm.
Using a new pipette tip each time you add anything to the sterivex, add 1.8ml (2 shots of 900 µl) sucrose lysis buffer (SLB) to the Sterivex filter, keeping ~ 200 µl of space in the filter for later addition of reagents.
Be sure to switch the pipet tip between each addition of 900 µl of SLB.

16 Then, seal the top of the Sterivex filter with a small piece of Parafilm or luer lock cap, and label the filters with the date and sample identification [alternatively, you can label the filters before beginning filtration to avoid getting them mixed up if filtering more sample at a time].
Be sure to record the amount of sample filtered on the filter and/or in a field notebook.
Depending on equipment available, freeze on dry ice, in liquid nitrogen or at -80°C

17 Store -80°C

## CLEAN-UP

18 Do not disassemble tube filter units before cleaning.

19 Insert the 25 ml pipette into a flask containing 2% HCl, and switch on the pump.
We tend to rinse 4 sample tubing units at one time with about 1-2 L of 2% HCL total (so each tubing unit gets about 250-500 mL of acid washed through it).

20 After the HCl wash, rinse the each tubing with 500 mL or more [autoclaved] MilliQ water.
Excess water can be removed by whipping around tubing (best done outside).
Allow tubing set-up to dry before re-autoclaving.

21 Sample collection bottles should be similarly rinsed.

Add ~100 mL 2% HCl to sample bottle, shake well, pour off HCl.
HCl can be used 3x before disposal, so save what you pour off for another round of bottle washing at a subsequent date (or transfer from bottle to bottle and use the same HCl to rinse 3 bottles).
Add ~100-200 mL of autoclaved MilliQ water, or otherwise sterile water, and shake shake shake.
Pour off.
Repeat water rinse one more time to make sure all acid is removed.
Leave bottles uncapped on counter and allow to dry completely before closing.
This drying process will take >1 day.

## Protocol references

Adapted by Colleen Kellogg from JOVE video article entitled **Small volume (1-3 L) filtration of Coastal Seawater Samples** by David Walsh, Elena Zaikova, and Steven Hallam https://doi.org/10.3791/1163