May 28, 2024

Sucrose lysis buffer

DOI
dx.doi.org/10.17504/protocols.io.j8nlk8op1l5r/v1

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Protocol Citation: Colleen Kellogg 2024. Sucrose lysis buffer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk8op1l5r/v1

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

Created: May 27, 2024

Last Modified: May 28, 2024

Protocol Integer ID: 100673

Keywords: eDNA, marine microbiology, biodiversity

Abstract

This protocol describes the preparation of sucrose lysis buffer to preserve DNA on sterivex filters. 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. 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	AFFILIATION	ORCID	DATE
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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.
seawater

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

Protocol materials

1M Tris-HCl (pH 8.0) **Thermo Fisher Scientific Catalog #**15568025 Step 1
UltraPure™ 0.5M EDTA, pH 8.0 **Thermo Scientific Catalog #**15575020 Step 1
sucrose **Fisher Scientific Catalog #**BP220-1 Step 1

Preparations

1

Note

Wear gloves and sterilize work area

You will need:

- 1M Tris-HCl (pH 8.0) **Thermo Fisher Scientific Catalog #**15568025
- UltraPure™ 0.5M EDTA, pH 8.0 **Thermo Scientific Catalog #**15575020
- sucrose **Fisher Scientific Catalog #**BP220-1

- MilliQ Water
-500 mL bottle top filtration unit

Final concentrations of chemicals in SLB:
EDTA: 40 mM
Tris: 50 mM
Sucrose: 0.75 M

Calculations

2 Calculate how much Sucrose powder you need (using the molecular weight on the bottle, MW or FW) for a 0.75M solution of 500 mL.

Calculation of Sucrose:

$$\frac{Y\ g}{mol} \times \frac{0.75\ mol}{L} \times 0.5\ L = Z \text{ grams of Sucrose to add in step 2}$$

Where Y is the molecular weight of the sucrose (MW or FW) from the bottle.

3 Calculation of Tris or EDTA:
Use the equation C1V1 = C2V2

Eg for EDTA:
(0.5M)(X mL) = (0.04M)(500 mL)
Solve for X
((0.04M)(500 mL)/(0.5M)) = 40 mL of 0.5M EDTA

Methods

4 Add the appropriate amount of Sucrose calculated above and place it in a clean bottle or beaker.

5 Add 40 mL of 0.5M EDTA to the beaker.

6 Add 25mL of 1M Tris to the beaker.

7 Add milliQ water to about the 400 mL line.

8 Add a stir bar and dissolve all the powder.

9 Top up water to 500 mL. (no need to pH this one!)

10 Filter-sterilize and label bottle.