



Jun 20, 2024

Version 1

Environmental DNA Metabarcoding- Full Pipeline Collection V.1

DOI

dx.doi.org/10.17504/protocols.io.eq2lywxwpvx9/v1

Hakai

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Protocol status: Working

We use this collection and it's working

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Keywords: dna extraction from sterivex filter, seawater sample, seawater filtration, filtering seawater, seawater on sterivex filter, environmental dna, plankton communities in the northern salish sea, dna metabarcoding, dna extraction, plankton community, hakai institute ocean observing program, part of the hakai institute ocean observing program, coi mitochondrial gene, diversity of marine invertebrate, diversity of fish, rrna mitochondrial gene, marine invertebrate, biomolecular sample, rrna nuclear gene, northern salish sea, robust phenol chloroform extraction protocol, bioinformatic analysis for the different gene, sterivex filter, analyzing different marker gene, diversity of eukaryote, mitochondrial gene, fish, prokaryotes to eukaryote, eukaryote, prokaryote, different gene, dna

Abstract

This is a collection of protocols used to analyze environmental DNA (eDNA) of seawater samples. 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. These protocols are developed to work across all domains of life, from viruses to prokaryotes to eukaryotes, allowing for both amplicon sequencing and shotgun sequencing.

This pipeline includes the following steps:

1. Seawater filtration: Our standard protocol for filtering seawater on sterivex filters. Alternatives may be used.

2. DNA extraction from sterivex filters: Here we offer two alternatives:

- The robust Phenol Chloroform extraction protocol is used in our long-term monitoring program.
- A kit alternative requires less handling of toxic chemicals, and are used for stand-alone projects.

3. DNA metabarcoding, library prep, and sequencing: With the options of analyzing different marker genes:

- 18S rRNA nuclear gene: Diversity of Eukaryotes. (Balzano et al 2015)
- COI mitochondrial gene: Diversity of marine invertebrates. (Leray et al 2013)
- 12S rRNA mitochondrial gene: Diversity of fish. (Miya et al 2015)

4. Bioinformatic analyses

Examples bioinformatic analysis for the different genes can be found on our GitHub page:

<https://github.com/hakaigenomics>

More information to be found under each protocol:

Troubleshooting



Files

 SEARCH

Protocol



NAME

Seawater Filtration for Microbial or Environmental DNA

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DNA Extraction from 0.22µm Sterivex Filters - Phenol-Chloroform

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DNA Extraction from 0.22µm Sterivex Filters - Qiagen Blood and Tissue Kit

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18S rRNA-Gene Metabarcoding Library Prep: Dual-PCR Method

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COI-Gene Metabarcoding Library Prep: Dual-PCR Method

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12S rRNA-Gene Metabarcoding Library Prep: Dual-PCR Method

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