

Dec 22, 2023

Version 2

🌐 Environmental DNA (eDNA) 12S Metabarcoding Illumina MiSeq NGS Protocol with size selection V.2

🔗 Version 1 is forked from [SEQUENCING Protocol Template](#)

📁 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2lyj1kelx9/v2>

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Protocol status: Working

We use this protocol and it's working

Created: December 18, 2023

Last Modified: February 07, 2024

Protocol Integer ID: 92455

Keywords: environmental dna, metabarcoding pcr protocol, dna 12s rRNA gene, sequencing protocol, mitochondrial dna, metabarcoding illumina miseq ngs protocol with size selection, rRNA gene in eukaryote, rRNA gene, metabarcoding illumina miseq ngs protocol, pcr products of the protocol, dna, sequencing, hypervariable region of the mitochondrial dna, paired end format, pcr product, platinum superfi ii taq, illumina miseq v2, pcr

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NOAA

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Abstract

This sequencing protocol is intended to directly follow and use the PCR products of the protocol:

"Environmental DNA (eDNA) 12S Metabarcoding PCR Protocol (with Platinum SuperFi II Taq)" which amplifies the hypervariable region of the mitochondrial DNA 12S rRNA gene in eukaryotes.

This protocol creates a pooled library which is then size selected using a Blue Pippin or Pippin HT to select for the vertebrate/fish band (~350 bp) and remove the co-amplified bacterial band (~435 bp). Then the pooled product is sequenced on an Illumina MiSeq v2 in a 2×250bp paired end format.

MIOP: Minimum Information about an Omics Protocol

1

MIOP Term	Value
methodology category	omics analysis
project	Marine Biodiversity Observation Network (MBON)
purpose	taxonomic diversity assessment by targeted gene survey [OBI:0001960]
analyses	DNA sequencing assay [OBI:0000626]
geographic location	Monterey Bay [GAZ:00002509]
broad-scale environmental context	marine biome ENVO_00000447
local environmental context	oceanic epipelagic zone biome [ENVO:01000033]
environmental medium	PCR product [OBI:0000406]
target	Actinopterygii [NCBITaxon:7898]
creator	Jacoby Baker, https://orcid.org/0000-0002-0673-7535
materials required	Illumina MiSeq Blue Pippin
skills required	
time required	
personnel required	1
language	en
issued	2023-11-14
audience	scientists
publisher	Monterey Bay Aquarium Research Institute, Chavez Lab
hasVersion	V.3
license	CC BY 4.0
maturity level	Mature

See <https://github.com/BeBOP-OBON/miop/blob/main/model/schema/terms.yaml> for list and definitions.

AUTHORS

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RELATED PROTOCOLS

3

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE / ACCESS DATE
https://mbari-bog.github.io/MBON-Protocols/eDNA_12S_SupFi2_PCR_V3.html	Jacoby Baker	2023-11-07
Environmental DNA (eDNA) 12S Metabarcoding PCR Protocol (with Platinum SuperFi II Taq)	Jacoby Baker	2023-11-07

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

4

ACRONYM / ABBREVIATION	DEFINITION
eDNA	environmental DNA

GLOSSARY

5

SPECIALISED TERM	DEFINITION

BACKGROUND

6 Summary

This sequencing protocol is intended to directly follow and use the PCR products of the protocol:

"Environmental DNA (eDNA) 12S Metabarcoding PCR Protocol (with Platinum SuperFi II Taq)" which amplifies the hypervariable region of the mitochondrial DNA 12S rRNA gene in eukaryotes.

The primers (MiFish-U-F & MiFish-U-R) used in the PCR protocol were developed by Miya et al., 2015 for metabarcoding environmental DNA (eDNA) from fishes.

This work was supported by the David and Lucile Packard Foundation, and NASA award 80NSSC21M0032 and NOAA award NA22NOS0120184 in support of the CeNCOOS MBON.

7 Method description and rationale

This protocol creates a pooled library which is then size selected using a Blue Pippin or Pippin HT to select for the vertebrate/fish band (~350 bp) and remove the co-amplified bacterial band (~435 bp). Then the pooled product is sequenced on an Illumina MiSeq v2 in a 2×250bp paired end format.

8 Spatial coverage and environment(s) of relevance

This protocol has been used to sequence extracted DNA from filtered sea water samples taken from marine coastal stations off the western coast of North America (primarily off

of California).

sea water [ENVO:00002149]

http://purl.obolibrary.org/obo/ENVO_00002149

9 PERSONNEL REQUIRED

1 technician

10 Safety

Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure

11 Training requirements

12 Time needed to execute the procedure

EQUIPMENT

13

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
Durable equipment		
Illumina MiSeq	Illumina MiSeq	Illumina
TapeStation	Agilent 4200 TapeStation HS DNA1000	Agilent
Blue Pippin	Blue Pippin	SageScience
Consumable equipment		
Invitrogen SequalPrep Normalization Plate	Invitrogen SequalPrep Normalization Plate	ThermoFisher Scientific
Chemicals		
Library Quantification Kit	Invitrogen Collibri Library Quantification qPCR assays	Invitrogen
Pippin HT kit	HTC2010	Sage Science

STANDARD OPERATING PROCEDURE

- 14 In the following SOP, please use the exact names of equipment as noted in the table above.

Provide a step-by-step description of the protocol. The identification of difficult steps in the protocol and the provision of recommendations for the execution of those steps are encouraged.

PREPARATION

- 15 Follow steps in the protocol "Environmental DNA (eDNA) 12S Metabarcoding PCR Protocol (with Platinum SuperFi II Taq)" through secondary amplification and QC of 12S PCR product.

Pool Library

- 16 1. After secondary PCR, products were run through Invitrogen SequalPrep Normalization Plate (ThermoFisher Scientific) using manufacturer's protocol to create pooled library.
- 17 2. The library pools were QC'd and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA1000 and Invitrogen Collibri Library Quantification qPCR assays.

Size selection of final library

- 18 1. After the pooled library was QC'd, the library was size selected with either a Blue Pippin or Pippin HT to select for the vertebrate/fish band (~350 bp) and remove the co-amplified bacterial band (~435 bp).
- 19 2. After size selection, the pooled library was QC'd again to confirm selection of the correct band and new amplicon concentrations.

SEQUENCING

- 20 1. The pooled product for the genetic locus was loaded on a standard MiSeq v2 flow cell and sequenced in a 2×250bp paired end format using a v2 500-cycle MiSeq reagent cartridge.
- 21 2. The MiSeq run was performed with a 20% PhiX spike added.
- 22 3. Primers complementary to the Fluidigm CS1 & CS2 oligomers were added to appropriate wells of the reagent cartridge to serve as sequencing and index read primers.

12S Sequencing primers (5' to 3' direction):

■ FL1-CS1(read1)

A+CA+CTG+ACGACATGGTTCTACA

■ FL1-CS2(read2)

T+AC+GGT+AGCAGAGACTTGGTCT

■ FL2-CS1rc

T+GT+AG+AACCATGTCGTCAGTGT

■ FL2-CS2rc(index)

A+GAC+CA+AGTCTCTGCTACCGTA

	Sequencing Primer Name	Direction	Sequence (5' → 3')
	FL1-CS1	read1	A+CA+CTG+ACGACATGGTTCTAC A
	FL1-CS2	read2	T+AC+GGT+AGCAGAGACTTGGTC T
	FL2-CS1rc		T+GT+AG+AACCATGTCGTCAGTGT
	FL2-CS2rc	index	A+GAC+CA+AGTCTCTGCTACCGT A

- 23 4. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.20.0

QUALITY CONTROL

- 24 None

BASIC TROUBLESHOOTING GUIDE

25 None

REFERENCES

26 None

APPENDIX A: DATASHEETS

27 None