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

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National Marine Sanctuaries as Sentinel Sites for a Demonstration Marine Biodiversity Observation Network (MBON)
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Environmental DNA (eDNA) 12S Metabarcoding Illumina MiSeq NGS Protocol with size selection

Forked from SEQUENCING Protocol Template

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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 2x250bp 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]

MIOP Term	Value
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

2

PREPARED BY All authors known to have contributed to the preparation of this protocol, including those who filled in the template	AFFILIATION	ORCID (visit https://orcid.org/ to registe
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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 NASA grant NNX14AP62A 'National Marine Sanctuaries as Sentinel Sites for a Demonstration Marine Biodiversity Observation Network (MBON)' funded under the National Ocean Partnership Program (NOPP RFP NOAA-NOS-IOOS-2014-2003803 in partnership between NOAA, BOEM, and NASA), and the U.S. Integrated Ocean Observing System (IOOS) Program Office.

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 2x250bp 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.	QUANTITY Provi
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	

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 2x250bp 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 server 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+ACGACATGGTTCTACA
FL1-CS2	read2	T+AC+GGT+AGCAGAGACTTGGTCT
FL2-CS1rc		T+GT+AG+AACCATGTCGTCAGTGT

Sequencing Primer Name	Direction	Sequence (5’ -> 3’)
FL2-CS2rc	index	A+GAC+CA+AGT CTCTGCTACCGT A

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

BASIC TROUBLESHOOTING GUIDE

REFERENCES

APPENDIX A: DATASHEETS