Sep 04, 2024

Environmental DNA (eDNA) indexing PCR protocol at MSU

Forked from Environmental DNA (eDNA) 12S Metabarcoding PCR Protocol (with Platinum SuperFi II Taq)

DOI
dx.doi.org/10.17504/protocols.io.yxmvme1z5g3p/v1

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Better Biomolecular Ocea…

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Protocol Citation: Jacoby Baker, Kathleen Pitz 2024. Environmental DNA (eDNA) indexing PCR protocol at MSU. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvme1z5g3p/v1

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

Created: September 03, 2024

Last Modified: September 04, 2024

Protocol Integer ID: 106884

Keywords: jbaker@mbari.org

Funders Acknowledgement:
National Marine Sanctuaries as Sentinel Sites for a Demonstration Marine Biodiversity Observation Network (MBON)
Grant ID: NASA grant NNX14AP62A

Abstract

This sequencing protocol is intended to directly follow and use the PCR products of a primary PCR amplification protocol (e.g., 12S rRNA, 18S rRNA V9, cytochrome c oxidase subunit I (COI) mitochondrial gene). Primary PCR products were produced at MBARI and sent to Michigan State University's (MSU) Research Technology Support Facility (RTSF) Genomics Core for indexing, pooling, and sequencing.

Secondary PCR primers used: PE1-BC-CS1, PE2-BC-CS2

This indexing PCR protocol is used to uniquely index the amplified DNA products of each sample. This is necessary so the sequencing results can be demultiplexed and the reads can be properly identified and assigned to each individual sample.

MIOP: Minimum Information about an Omics Protocol

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MIOP Term	Value
methodology category	omics analysis
project	Marine Biodiversity Observation Network (MBON)
purpose	PCR [OBI:0000415]
analyses	PCR [OBI:0000415]
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	sea water [ENVO:00002149] | DNA extraction [OBI:0000257] | PCR product [OBI:0000406]
target	PCR product [OBI:0000406]
creator	Jacoby Baker, https://orcid.org/0000-0002-0673-7535
materials required	agarose gel electrophoresis system [OBI:0001134] | PCR instrument [OBI:0000989]
skills required	sterile technique | pipetting skills
time required	360
personnel required	1
language	en
issued	2024-09-04
audience	scientists
publisher	Monterey Bay Aquarium Research Institute, Chavez Lab
hasVersion	V.1
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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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 re
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Francisco Chavez	MBARI	

MBARI : Monterey Bay Aquarium Research Institute, Moss Landing, CA

RELATED PROTOCOLS

3 Example protocols for primary PCR, secondary PCR, and bead cleanups:

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE DATE This is the date corresponding to the version listed to the left
https://mbari-bog.github.io/MBON-Protocols/eDNA_COI	Jacoby Baker	2023-11-07

	PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE DATE This is the date corresponding to the version listed to the left
	_PCR_V2.html		
	https://mbari-bog.github.io/MBON-Protocols/Bead_cleanup.html	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

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	ACRONYM / ABBREVIATION	DEFINITION
	eDNA	environmental DNA
	NTC	No Template Control
	PCR	polymerase chain reaction

GLOSSARY

5

	SPECIALISED TERM	DEFINITION
	amplicon	A piece of DNA or RNA that is the source and/or product of amplification or replication events (https://en.wikipedia.org/wiki/Amplicon)

BACKGROUND

6 **Summary**

The PCR protocol is used to uniquely index PCR amplicon products from the primary taxa-targeting PCR step using gene-targeting PCR primers (e.g., 12S rRNA, 18S rRNA V9, cytochrome c oxidase subunit I (COI) mitochondrial gene).

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 is performed at Michigan State University's (MSU) Research Technology Support Facility(RTSF) Genomics Core (**https://rtsf.natsci.msu.edu/genomics/**) to prepare amplicon projects for MiSeq sequencing.

8 **Spatial coverage and environment(s) of relevance**

This protocol has been used to uniquely index amplified 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**

Sterile technique, pipetting skills.

12 **Time needed to execute the procedure**

Total time is 6 hours.
PCR preparation and running the PCR protocol takes 1 hours. Running the following gel is 1 hour, bead cleanup setup preparation and process takes 2 hours, and then sample normalization and pooling takes 2 hours.

EQUIPMENT

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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
Durable equipment			
Agarose gel electrophoresis system			
PCR Thermal Cycler			
Consumable equipment			
PCR plates	SuperPlate PCR Plate, 96-well, semi-skirted	Thermofisher Scientific	
Plate seals	PCR Plate Seals	Bio Rad	
Chemicals			
OneTaq® Hot Start 2X Master Mix with Standard Buffer	OneTaq® Hot Start 2X Master Mix with Standard Buffer	NEB	
molecular-biology grade water			
Illumina compatible dual indexed adapters with barcodes			
Amplicon product from the primary PCR reaction			

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 BEFORE STARTING

Disinfect work surfaces with 10% bleach or RNase Away followed by a MilliQ / DI water rinse and 70% ethanol wipe. Clean pipet surfaces with RNase Away and ethanol wipe.
UV pipets, molecular grade water, and tube racks for 30 minutes prior to starting protocol.

Primary PCR

16 eDNA template & PCR processing were performed at the Monterey Bay Aquarium Research Institute (MBARI). PCR reactions were performed with a two-step amplification protocol for each sample using the gene-targeting primers (e.g., 12S MiFish, 18S V9, COI, or 16S V4-V5) with Fluidigm adapters CS1 & CS2. The resulting amplicon product from the primary PCR was bead cleaned, quantified, then sent to MSU for indexing. Below are the steps for the secondary amplificaiton. Library normaliztion, pooling, and sequencing are described in a complementary protocol.

Secondary Amplification

17 **The following steps are performed by *MSU's RTSF Genomics Core***

1. Secondary amplification and NGS were performed at Michigan State University's Research Technology Support Facility (RTSF). An aliquot of 20 µL from each purified primary PCR product was sent to RTSF Genomics Core at MSU for secondary PCR amplification with primers which targeted the CS1/CS2 ends of the primary PCR products and added dual indexed, Illumina compatible adapters with barcodes.

- PE1-BC-CS1 (forward):

```
AATGATACGGCGACCACCGAGATCT-[i5-BC(index 2)]-ACACTGACGACATGGTTCTACA
```

- PE2-BC-CS2 (reverse):

```
CAAGCAGAAGACGGCATACGAGAT-[i7-BC(index 1)]-TACGGTAGCAGAGACTTGGTCT
```

	PCR Primer Name	Direction	Sequence (5' -> 3')
	PE1-BC-CS1	forward	AATGATACGGCGACCACCGAGATCT-[i5-BC(index 2)]-ACACTGACGACATGGTTCTACA
	PE2-BC-CS2	reverse	CAAGCAGAAGACGGCATACGAGAT-[i7-BC(index 1)]-TACGGTAGCAGAGACTTGGTCT

18 **Secondary Indexing Thermal Cycling Parameters:**
The secondary/indexing PCR amplifications were carried out in 15 µL reactions, using 1 µL of primary PCR product.

	PCR step	Temperature	Duration	Repetition
	denaturation	95°C	3 minutes	1
	denaturation	95°C	15 seconds	15 cycles
	annealing	60°C	30 seconds	15 cycles
	extension	72°C	1 minute	15 cycles
	final extension	72°C	3 minutes	1
	HOLD	25°C	HOLD	1

19 **Reaction Mixture**: PCR reagents, volumes, initial and final concentrations
Total volume per reaction 15 µl

	Reagent	Volume	Initial Concentration	Final Concentration
	OneTaq® Hot Start 2X Master Mix with Standard Buffer	6 µl	2X	1X
	Primer Mix (6 uM)	1 µl	6 µM	0.4 µM
	molecular-biology grade water	7 µl	-	-
	primary eDNA PCR product	1 µl	variable	< 1,000 ng

QUALITY CONTROL

20 An agarose gel was run after secondary PCR to confirm the presence of target bands and absence of non-specific amplification across environmental samples as well as the absence of amplification in NTCs.

Products from the protocol are then used to create a pooled library and sequenced following a separate sequencing protocol.

REFERENCES

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1. Kozich, J. J. et al. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology 79, 5112–5120 (2013).
2. https://www.neb.com/en-us/protocols/2012/09/10/onetaq-hot-start-2x-master-mix-with-standard-buffer-m0484

APPENDIX A: DATASHEETS

22 Link templates (e.g. preformatted spreadsheets) used to record measurements and report on the quality of the data as well as any documents such as manufacturer specifications, images, etc that support this protocol. Please include a short note describing the document's relevance.