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Environmental DNA (eDNA) COI Metabarcoding PCR Protocol

Forked from 18S V9 PCR

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Better Biomolecular Ocea…

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

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Abstract

This protocol is aimed at amplifying the cytochrome c oxidase subunit I (COI) mitochondrial gene in eukaryotes. The primers (forward: mlCOIintF, reverse: HCO2198) utilized in this protocol are based on the primers utilized in Leray et al. 2013 (forward) and Folmer et al. 1994 (reverse).

Primers used: Fluidigm CS1+mlCOIintF, Fluidigm CS2+HCO2198
Amplicons generated using this protocol can then be sequenced using the Illumina platform.

Primary PCR amplicon products are then sent to Michigan State University's (MSU) Research Technology Support Facility (RTSF) for indexing, pooling, and sequencing.

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.

Minimum Information about an Omics Protocol (MIOP)

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MIOP Term	Value
methodology category	omics analysis
project	Monterey Bay Time Series
purpose	time series design [OBI:0500020]
analyses	amplicon sequencing assay[OBI:0002767]
geographic location	Monterey Bay [GAZ:00002509]
broad-scale environmental context	marine biome [ENVO:00000447]
local environmental context	upwelling [ENVO:01000005]
environmental medium	sea water [ENVO:00002149]
target	Mitochondrial Cytochrome C Oxidase Subunit 1 [NCIT:C128943]
creator	Jacoby Baker
materials required	Thermal Cycler [OBI:0400116]
skills required	laboratory technician with experience in PCR
time required	
personnel required	1
language	en
issued	
audience	scientists
publisher	Monterey Bay Aquarium Research Institute, Chavez Lab
hasVersion	
license	
maturity level	Demonstrated

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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PROTOCOL REVISION RECORD

3 Version numbers start at “1.0.0” when the protocol is first completed and will increase when changes that impact the outcome of the procedure are made (patches: 1.0.1; minor changes: 1.1.0; major changes: 2.0.0). Please store all versions in the gDrive folder designated to your institute.

VERSION	RELEASE DATE This is the date when a given protocol version was finalised	DESCRIPTION OF REVISIONS Please include a brief description of what was changed relative
1.0.0	2022-04-25	Initial release

RELATED EXTERNAL PROTOCOLS

4 This is a list of other protocols that are not in your folder which should be known to users of this protocol. These include, e.g., kit manuals. Please upload all relevant external protocols to Appendix A and link to them here.

	EXTERNAL PROTOCOL NAME AND LINK	ISSUER / AUTHOR Please note who authored the protocol (this may also be a company name)	ACCESS DATE This is the date you downloa
	Environmental DNA (eDNA) COI metabarcoding Illumina MiSeq NGS PCR Protocol V2 https://mbari-bog.github.io/MBON-Protocols/eDNA_COI_PCR_V2.html	Collin Closek, Anni Djurhuus, Katie Pitz, Ryan Kelly, Reiko Michisaki, Kristine Walz, Hilary Starks, Francisco Chavez, Alexandria Boehm, Mya Breitbart	yyyy-mm-dd

ACRONYMS AND ABBREVIATIONS

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	ACRONYM / ABBREVIATION	DEFINITION
	MBARI	Monterey Bay Aquarium Research Institute
	PCR	polymerase chain reaction
	NTC	no template control

GLOSSARY

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	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

7 This protocol is aimed at amplifying the cytochrome c oxidase subunit I (COI) mitochondrial gene in eukaryotes.

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.

Summary

8 This protocol is aimed at amplifying the cytochrome c oxidase subunit I (COI) mitochondrial gene in eukaryotes. The primers (forward: mlCOIintF, reverse: HCO2198) utilized in this protocol are based on the primers utilized in Leray et al. 2013 (forward) and Folmer et al. 1994 (reverse).

PCR reactions for COI were run with Fluidigm two-step amplification protocol for each sample

Method description and rationale

9 This method is applied because of its ability to amplify the target region (COI) across many different groups of organisms, the target region's ability to discriminate between different taxa, and the common research application of this primer set allowing the data to be compared to a reference database and other published environmental datasets.

Spatial coverage and environment(s) of relevance

10
- ocean [ENVO:00000015]
- freshwater lake [ENVO:00000021]

PERSONNEL REQUIRED

11 1 technician

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			
ultraviolet light source [OBI:0002900]			
PCR instrument [OBI:0000989]			
electrophoresis system [OBI:0001053]			
fluorometer [OBI:0400143]	FMAX Fluorometer	Molecular Devices	
Consumable equipment			
Agarose gel			2
Agencourt AMPure XP bead system		Beckman Coulter, USA	
Quant-It Picogreen ds DNA Assay		Life Technologies	
Chemicals			
10% Bleach			
70% Ethanol			
RNase Away			
Amplitaq Gold Fast PCR mastermix			
molecular-biology grade water			
forward and reverse primers (5 µM)			

Preparation

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1. Disinfect work surfaces with 10% bleach, followed by 70% ethanol.
2. RNase Away and pipets with RNase Away
3. UV pipets, molecular grade water, and tube racks for 20 minutes prior to starting protocol.

PCR

14 PCR reactions were run in single 75ul reactions for each sample using the 26bp primers (forward: mlCOIintF, reverse: HCO2198) utilized in this protocol are based on the primers utilized in Leray et al. 2013 (forward) and Folmer et al. 1994 (reverse) with Fluidigm adapters CS1 & CS2. All primers listed in the 5' to 3' direction.

PCR Primer Name	Direction	Sequence (5' -> 3')
Fluidigm CS1 and **mlCOIintF**	forward	ACACTGACGACATGGTTCTACA**GGWACWGGWTGAACWGTWTAYCCYCC**
Fluidigm CS2 and **HCO2198**	reverse	TACGGAGCAGAGACTTGGTCT**TAAACTTCAGGGTGACCAAAAAATCA**

PCR reactions were run in 96-well plates with a NTC run in singleton for each plate

COI thermal cycling parameters (note: this is a touchdown PCR protocol)

- These parameters use a normal ramp speed

PCR step	Temperature	Duration	Repetition
denature	95° C	10 minutes	1

PCR step	Temperature	Duration	Repetition
16 Cycles of t he folowing th ree steps			
denature	94° C	10 seconds	16
anneal	62° C (this ch anges -1°C for each subsequ ent cycle)	30 seconds	16
extension	68 °C	60 seconds	16
25 Cycles of t he folowing th ree steps			
denature	94° C	10 seconds	25
anneal	46° C	30 seconds	25
extension	68 °C	60 seconds	25
extension	72° C	10 minutes	1
hold	4° C	infinity	1

15 **Reaction Mixture**: PCR reagents, volumes, initial and final concentrations
Total volume per reaction 75 µl

Reagent	Volume	Initial Concentration	Final Concentration
Amplitaq Gold Fast PCR mas termix (Applie d Biosystems)	37.5 µl	2X	1X
Forward Prim er (mlCOlintF)	3 µl	5 µM	0.2 µM
Reverse Prime r (HCO2198)	3 µl	5 µM	0.2 µM
molecular-biol ogy grade wat er	28.5 µl		
Template DN A	3 µl	1 - 20 ng/µl	0.04 - 0.8 ng/µl

Quality control, PCR clean-up

16 After PCR amplification of the marker region, PCR products were run through an agarose gel to confirm the presence of target bands and absense of non-specific amplification across environmental samples as well as the absence of amplification in no-template controls (NTCs).

1. PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA).

2. A second agarose gel was run to confirm primer removal and retention of target amplicons after purification.

3. Purified products were then quantified using Quant-It Picogreen dsDNA Assay (Life Technologies) on an fmax Molecular Devices Fluorometer with SoftMaxPro v1.3.1

Next Steps

17 From here, the amplicon products will move onto the indexing PCR step, normalization, pooling, and sequencing.

REFERENCES

18 **Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ. (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in zoology, 10(1),1-4.**

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3, 294–299.

APPENDIX A: DATASHEETS

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