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

Forked from PCR Protocol Template

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ABSTRACT

The 12S protocol is aimed at amplifying the hypervariable region of the mitochondrial DNA 12S rRNA gene in eukaryotes. The primers (MiFish-U-F & MiFish-U-R) used in this protocol were developed by Miya et al., 2015 for metabarcoding environmental DNA (eDNA) from fishes.

This protocol follows an updated version of the MiFish primer PCR protocol. The Platinum SuperFi II was designed to have high fideleity and have increased resistence to PCR inhibitors.

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

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Keywords: jbaker@mbari.org

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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]

MIOP Term	Value
target	DNA extraction [OBI:0000257]
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	420
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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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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MBARI : Monterey Bay Aquarium Research Institute, Moss Landing, CA

RELATED PROTOCOLS

3

PROTOCOL NAME AND LINK	ISSUER / AUTHOR	RELEASE DATE This is the date corresponding to the version listed to the lef
https://mbari-bog.github.io/MBON-Protocols/eDNA_12S_SupFi2_PCR_V3.html	Jacoby Baker	2023-11-07
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

GLOSSARY

5

SPECIALISED TERM	DEFINITION
Content Cell	Content Cell

SPECIALISED TERM	DEFINITION
Content Cell	Content Cell

BACKGROUND

6 **Summary**

The 12S protocol is aimed at amplifying the hypervariable region of the mitochondrial DNA 12S rRNA gene in eukaryotes. The primers (MiFish-U-F & MiFish-U-R) used in this 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 follows an updated version of the MiFish primer PCR protocol. The Platinum SuperFi II was designed to have high fidelity and have increased resistance to PCR inhibitors.

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

This protocol has been used to amplify 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 7 hours.
PCR preparation and running the PCR protocol takes 3 hours. Running the following gel is 1 hour, bead cleanup setup preparation and process takes 2 hours, and then another gel is run for 1 hour.

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			
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			
2X Platinum SuperFi II PCR Master Mix	2X Platinum SuperFi II PCR Master Mix	Thermofisher Scientific	

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.

PCR

16 1. eDNA template & PCR processing were performed at the Monterey Bay Aquarium Research Institute (MBARI). PCR reactions for the 12S locus were performed with a two-step amplification protocol for each sample using the MiFish_U primers (Miya et al. 2015) with Fluidigm adapters CS1 & CS2. All primers listed in the 5′ to 3′ direction. MiFish primers are in bold.

Fluidigm CS1+**MiFish_U (forward)**:
ACACTGACGACATGGTTCTACA **GTCGGTAAAACTCGTGCCAGC**
Fluidigm CS2+**Mifish_U (reverse)**:
TACGGTAGCAGAGACTTGGTCT **CATAGTGGGGTATCTAATCCCAGTTTG**

PCR Primer Name	Direction	Sequence (5′ -> 3′)
Fluidigm CS1 + 12S MiFish_U	forward	ACACTGACGACATGGTTCTACAGTCGGTAAAACTCGTGCCAGC
Fluidigm CS2 + 12S MiFish_U	reverse	TACGGTAGCAGAGACTTGGTCTCATAGTGGGGTATCTAATCCCAGTTTG

17 2. The primary PCR amplifications were carried out in singleton 50 µl reactions using:

- 25 µL 2X Platinum SuperFi II PCR MM
- 3 µL forward primer (10 µM)
- 3 µL reverse primer (10 µM)
- 3 µL eDNA extract template
- 16 µL molecular-grade, nuclease-free water

18 3. PCR reactions were performed in 96-well plates with a no-template control (NTC) for each PCR plate, for a total of 3 PCR negative controls. An artificial community was used as a positive control.

19 4. Primary 12S cycling parameters, using the manufacturer's recommendation with a slight modification. The Platinum SuperFi II is designed to be used with a universal 60°C annealing temperature, however, we found we had better results with an annealing temperature of 62°C to reduce bacterial co-amplification:

PCR step	Temperature	Duration	Repetition
denaturation	98°C	30 seconds	1
denaturation	98°C	10 seconds	38 cycles
annealing	62°C	10 seconds	38 cycles
extension	72°C	30 seconds	38 cycles
final extension	72°C	5 minutes	1
HOLD	4°C	HOLD	1

Quality Control and Product Clean-up

20 1. After primary PCR amplification of the marker region the PCR product was run through a 2% agarose gel to confirm the presence of target bands (~270 bp) and absence of non-specific amplification (bacterial band ~370 bp) across environmental samples.

Primary PCR clean-up (Bead Clean-up Protocol)

21 2. Primary PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA) at 0.9x volume beads to product.

22 3. A second agarose gel was run to confirm primer removal and retention of target amplicons after purification. NTCs were also tested using a Qubit dsDNA 1x high sensitivity kit to ensure no amplification.

Secondary Amplification

23 **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

24 2. The secondary PCR amplifications were carried out in 15 µL reactions, using 1 µL of primary PCR product.
- 6 µl 2.5X HotMaster Mix
- 7 µl DI water
- 1 µl Primer Mix (6uM)
- 1 µl primary eDNA PCR product

25 3. Secondary 12S cycling parameters:

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

QUALITY CONTROL

26 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.

BASIC TROUBLESHOOTING GUIDE

27 Identify known issues associated with the procedure, if any.

Provide troubleshooting guidelines when available.

REFERENCES

28
1. Miya M et al. 2015 MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. R.Soc.opensci. 2: 150088. http://dx.doi.org/10.1098/rsos.150088
2. Kawato, M., Yoshida, T., Miya, M., Tsuchida, S., Nagano, Y., Nomura, M., Yabuki, A., Fujiwara, Y. and Fujikura, K., 2021. Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. MethodsX, 8, p.101238. https://doi.org/10.1016/j.mex.2021.101238
3. Platinum SuperFi II PCR Master Mix User Guide

APPENDIX A: DATASHEETS

29 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.