eDNA Fish Metabarcoding PCR; 12S & cytB

Harriet Johnson

Abstract

One-step metabarcoding PCR. Used to amplify either mitochondrial 12S or cytochrome B regions from environmental DNA sampled from freshwater to identify fish species. PCR product contains the sequencing primers ready for sequencing on the Illumina MiSeq (custom oligos required for sequencing).

Citation: Harriet Johnson eDNA Fish Metabarcoding PCR; 12S & cytB. **protocols.io**
dx.doi.org/10.17504/protocols.io.fckbiuw
Published: 21 Jul 2016

Guidelines

References to primer sequences and their use with this method:

Kocher, Thomas D., et al. "Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers." *Proceedings of the National Academy of Sciences* 86.16 (1989): 6196-6200.

Riaz, Tiayyba, et al. "ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis." *Nucleic Acids Research* (2011): gkr732.

Kelly, Ryan P., et al. "Using environmental DNA to census marine fishes in a large mesocosm." *PloS one* 9.1 (2014): e86175.
Hänfling, Bernd, et al. "Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods." *Molecular ecology* (2016).

Protocol

Step 1.

Dual indexed primers:

16 forward primers, 24 reverse primers: FA, FB, RA, RB. Can mix and match to create 384 unique combinations to identify multiplexed samples. See 'guidelines' for references to primer sequences.

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Dilute primers to 10 uM concentration with water.

Plate primers in matrix on 96 well plate or strip tubes to create unique combinations:

eg.

RA701 ----> RA712

FA501

|

FA508

NOTES

Harriet Johnson 20 Jul 2016

Mulitple dilutions are recommended for making working concentration primers to minimise handling of stock primers and to avoid contamination.

Expected amplicon size:

Cytochrome B: 460 bp region (F primer: 74, R primer: 70), total: 604 bp

12S: 106 bp region (F primer: 67, R primer: 62), total: 235 bp

Harriet Johnson 20 Jul 2016

Positive control: *Rhamphochromis esox*, a pelagic species in its natural habitat and isn't common in UK aquariums (according to Alan Smith). Info from Lynsey Harper.

SEPA Jan/Feb 2016: 2 µl 1/100 dilution DNA.

EA, April/May 2016 LFC project: 0.5 ul 1/500 dilution DNA.

Negative control: Molecular grade water

Harriet Johnson 20 Jul 2016

No. of cycles were reduced from 40x to 35x, told of 'updated' protocol when I started, do not know when this was changed.

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All rxns I have performed have been 35x.

Step 2.

PCR reaction setup

20 ul rxn: Windermere samples:

10 µl Q5 2x mastermix

1 µl F primer

1 µl R primer

2 µl DNA

6 µl H2O

20 ul rxn: Scottish Loch samples:

10 µl Q5 2x mastermix

1 µl F primer

1 µl R primer

4 µl DNA

4 µl H2O

35ul rxn: LFC samples:

17.5 µl Q5 2x mastermix

1.75 µl F primer

1.75 µl R primer

3.5 µl DNA

10.5 µl H2O

PCR strip tubes with individual lids, instead of open plates.

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Step 3.

Thermal cycling parameters:

Initial denaturation: 98°C 5 min;
35X of
1. 98°C 10 sec
2. 58 °C 15 sec
3. 72°C 20 sec;

Final extension 72°C 5 min
Store at 10°C

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Step 4.

Visualise PCR product via gel electrophoresis:

large gel:

1.5g agarose

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100 ml 0.5x TBE

10 ul GelRed

Run at 90V 40 mins.

2-3 ul product

1.5ul Easy Ladder

Step 5.

Triplicate repeat

SEPA Jan/Feb 2016:

20 ul rxns, performed in triplicate repeat and then pooled.

Warnings

Custom oligos are required for Illumina sequencing of this PCR product. In order to also sequence PhiX on the run, spike in the custom oligos to the supplied standard Illumina sequencing primers.

The sequencing library preparation protocol will also be added here. I have put a link to the Schloss library prep protocol for now (see links).

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