May 23, 2018

NYC/NJ Aquatic Vertebrate eDNA Project: Protocols I

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.p9gdr3w

Mark Stoeckle[1]

[1]Rockefeller University

Mark Stoeckle

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.p9gdr3w

External link: https://doi.org/10.1371/journal.pone.0198717

Protocol Citation: Mark Stoeckle 2018. NYC/NJ Aquatic Vertebrate eDNA Project: Protocols I. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.p9gdr3w**

Manuscript citation:
Stoeckle MY, Mishu MD, Charlop-Powers Z (2018) GoFish: A versatile nested PCR strategy for environmental DNA assays for marine vertebrates. PLoS ONE 13(12): e0198717. doi: **10.1371/journal.pone.0198717**

Protocol status: In development
We are still developing and optimizing this protocol

Created: May 21, 2018

Last Modified: May 23, 2018

Protocol Integer ID: 12296

Keywords: environmental DNA, eDNA, aquatic eDNA, metabarcoding, vertebrates, bony fish, cartilaginous fish, marine mammals, New York City, New York Bight, urban ecology, MiSeq, aquatic vertebrate edna protocol, aquatic vertebrate edna project, nj aquatic vertebrate edna project, access to essential molecular biology laboratory equipment, essential molecular biology laboratory equipment, cross contamination, including dna isolation, isolate dna, dna isolation, filter water sample, sequencing protocol, filtration, persons familiar with basic molecular biology technique, unfiltered pipette tip, laboratory, laboratory component, basic molecular biology technique, sequencing, pcr, dna, other procedure

Abstract

We are still developing and optimizing these protocols!

The aquatic vertebrate eDNA protocols are designed for persons familiar with basic molecular biology techniques and access to essential molecular biology laboratory equipment. To facilitate use, we utilize commercial kits and open source software, and standardized PCR and sequencing protocols.

Overview. From start to finish there are four laboratory components, each of which can potentially be completed in one day. Except for filtration, which we aim to do within 0-2 days of collection, samples can be safely stored for weeks to months between steps at -20 °C. All procedures are done on an open bench wearing gloves; wipe down work area and equipment after use with 10% bleach. We change gloves a lot to minimize cross contamination. Separate sets of pipettors and reagents are used for pre- and post-PCR work. We do one component at a time; in particular when filtering do not perform other procedures including DNA isolation and PCR. Unfiltered pipette tips are employed; after each procedure used tips are discarded and collection containers rinsed with 10% bleach. To reduce potential for cross-contamination, we do PCR in GE Illustra 8-tube strips (i.e., not 96-well plates), and other steps in individual 1.5 ml microfuge tubes.

LABORATORY COMPONENTS

1. Filter water samples
2. Isolate DNA
3. Amplify vertebrate 12S
4. Add Nextera tags

Attachments

nycnj aquatic verteb...

19.5MB

Troubleshooting

1