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DNA Barcoding for Identifying Zooplankton

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Abstract: DNA barcoding is still a relatively new field of study that is gaining popularity as it becomes more available. But there is no single protocol that works consistently and efficiently. While there are protocols that have been developed and proven to be successful, they are for specific organisms. In this article, I took the approach of focusing on a zooplankton species, *Daphnia magna*, to develop a protocol that worked consistently for DNA barcoding freshwater zooplankton. A series of experiments was run to optimize each step of DNA barcoding until a control protocol was developed that could be applied to other species of zooplankton. I expect the final optimized protocol to be robust and capable of being applied to different organisms.

Keywords: Zooplankton, DNA Barcoding, *mCO1*

Introduction

We are able to determine the species present through DNA barcoding, which is a technique that uses the sequence of the mitochondrial cytochrome c oxidase subunit 1 (*mCO1*) gene to identify the individual(s) through searching the sequence through a DNA barcoding library. Over 500,000 species have been barcoded and added to libraries so far (Jinbo et al. 2011). DNA barcoding is becoming a fast and affordable to technique to use for the identification of organisms because of the development of technology and equipment (Jinbo et al. 2011). Other than being more affordable, DNA barcoding is becoming an efficient way to identify organisms without having to be an expert on using the taxonomic identification technique. Once basic lab techniques, such as using pipets and running PCR, are known and can be used efficiently and effectively, DNA barcoding can be very robust, and even freshman undergraduates can do it. Zooplankton are freshwater crustaceans that are found in lakes and ponds around the world. They can communicate information about the health of an ecosystem through the species present and the size of the population.

Methods

To begin we isolated an individual zooplankton of the species *Daphnia magna* from a lab culture (Carolina, Burlington, NC) using a microscrope (Leica DM500) and placed it in a 2 mL micro centrifuge tube. Next, we followed the protocol for the DNeasy Blood & Tissue DNA Extraction Kit (Qiagen, Hilden, Germany), but modified the incubation time to 24 hours and the volume of elution buffer to 50 µL and incubated it at room temperature for 15 minutes before completing the final centrifuge step.

Table 1: PCR master solution for Phusion High-Fidelity polymerase as suggested by Invitrogen by ThermoFisher

Reagent	Volume
UltraPure Water	Up to 50 µL
5X Buffer	10 µL
10 µM dNTPs	1 µL
10 µM Forward Primer	2.5 µL
10 µM Reverse Primer	2.5 µL
Template DNA (~50 ng)	Variable
Phusion High-Fidelity DNA Polymerase	0.5 µL

After extracting the DNA we ran Polymerase Chain Reaction (PCR) with each sample. We used Phusion High Fidelity

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Table 2: PCR cycle for Phusion High-Fidelity polymerase as suggested by Invitrogen by ThermoFisher. Annealing temperature was determined by using the Tm calculator provided on ThermoFisher's website. Extension time was determined by number of base pairs.

PCR Step	Temperature	Duration	
Initial Denaturation	98°C	30 (sec)	
Denaturation	98°C	10 (sec)	
Annealing	55°C	30 (sec)	35 Cycles
Extension	72°C	30 (sec)	
Final Extension	72°C	600 (sec)	

polymerase (Invitrogen, Carlsbad, California) and protocols for PCR (Table 1). We determined how much of the template DNA by using a Nanodrop machine (Thermo Scientific™, Waltham, MA). to measure the concentration (ng/µL) of the DNA in the sample. For our primers we used zooplankton specific primers (Forward: tgtaaaacgacggccagtTCTASWAATCATAARGATATTGG, Reverse: caggaaacagctatgacTTCAGGRTGRCCRAARAATCA) developed by Prosser *et. al* (Prosser et al. 2013). All PCR master solutions samples are run in the optimized PCR conditions (Table 2)

Following PCR, we checked the PCR product using 1% agarose gel with 1 kb Gene Ruler DNA ladder (Thermo Scientific™, Waltham, MA). The gel was stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, California). If we saw a band that was about 720 base pairs long, we cleaned the PCR product using Invitrogen by ChargeSwitch Pro PCR Cleanup Kit (Invitrogen, Carlsbad, California). The cleaned PCR product was submitted to OSU Biochemistry and Molecular Biology Recombinant DNA and Protein Core Facility for Sangre Sequencing. Once we received the sequence results they were searched in Bold Systems (Identification Engine, 2019) for an identification down to the species level. With the samples that Bold Systems did not give us a species level identification, we then searched the sequence results in GenBank (Blast, 2019).

Results

We were able to successfully optimize the protocols for the extraction, PCR amplification of the *mCO1* gene and identification of the organism. Multiple protocols for DNA barcoding were tested over the course of this research using different polymerase and cycles, however the most robust protocol developed used Phusion polymerase. Using the optimized protocols we were able to identify 8 freshwater crustacean individuals (Table 3). Individuals identified came from lab cultures, environmental samples, and preserved environmental samples.

Discussion

The similarities provided through the DNA barcoding databases help to determine the likelihood that our sample is of the species that the database claims. If it is above a 98% similarity, we are confident that the identity is that which is given by the database.

Table 3: Identification of individuals from lab cultures, environmental samples, and preserved samples. South Carolina (lab culture) samples came from Carolina Biological Supply Company. Keystone Lake samples came from environmental samples collected from Keystone Lake in Oklahoma. Similarity percentage was provided through Bold Systems and GenBank.

ID	Location	Number of Organisms	Percentage Similarity
Daphnia magna	South Carolina (lab culture)	2	100%
Daphnia sp. AS1b3	Keystone Lake, OK	1	92.55%
Arctodiaptomus cf. dorsalis3	Keystone Lake, OK	1	100%
Haylella sp. Clade8	South Carolina (lab culture)	1	86.81%
Hayaella azteca	South Carolina (lab culture)	2	98.31%, 97.27%
Hygrotus novemlineatus	Keystone Lake, OK	1	96.5%

If it is below 98% we may need to go back through pictures taken of the individual before the DNA extraction so that we can do a taxonomy ID using the physical features of the individual.

Now that we have an optimized protocol we are able to efficiently identify individuals of freshwater crustaceans. The readily available *Daphnia magna* will be used as a positive control for quality control purpose. With the optimized protocol, identification of an individual can be completed within three days. Multiple samples can be processed at the same time by one person. In the future we plan to expand the protocol to be used for identifying all organisms in a mixed culture or sample by using Next Generation Sequencing. The protocol that was optimized and described in this paper have also been modified to work for different invertebrates as well.

Literature Cited

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