Genetic Insight into Store-Bought Shrimp: A DNA Barcoding Exploration

Jones McNamar, Etiniabasi Inyang and Alexis Hockett

Abstract

In the DNA barcoding project, an investigation was conducted on commercially acquired shrimp from grocery stores with the aim of determining their origin, identifying the species, and detecting potential mislabeling. A prior research explored the mislabeling phenomenon within seafood, encompassing varieties like grouper, pike perch, yellowfin tuna, and bluefin tuna. This study revealed a correlation between mislabeling occurrences and the affordability of certain fish species (Pardo, Miguel, et al., 2018).

Introduction

Determining the species of shrimp being sold in grocery stores is important due to that fact that several shrimp species are at risk of endangerment (Bilgin, R., et al, 2015). Studying their DNA can help determine if the shrimp sold in stores is endangered or not, as well as if there are any health risks in consuming that type of shrimp. DNA barcoding is a source that is useful in identifying species (Grave, S. De, et al, 1970). DNA barcoding will help answer our questions because we can gather the shrimp's DNA and compare it to the DNA of shrimp species already logged on the internet, or potentially even discover new species.

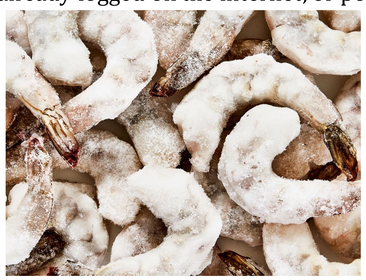

Figure 1. Photo via Bon appetite, Delany, A. (2019, May 21). *How to thaw frozen shrimp and win weeknight dinner*. Bon Appétit. https://www.bonappetit.com/story/how-to-thaw-frozen-shrimp

Materials and Methods

DNA Extraction:

Frozen shrimp samples were obtained for the study. DNA extraction was done using the sample and InstaGene Matrix according to protocol. This method was used to ensure a high-yield of DNA extraction from shrimp samples.

PCR Amplification:

To amplify a large quantity of DNA, COI (Cytochrome Oxidase I) is used. The primers utilized were HC02198 and LCO1490 which are used to amplify invertebrate.

Gel Electrophoresis:

Following PCR amplification, the DNA results were analyzed using gel electrophoresis. Agarose gel (1.5%) was prepared, and the PCR products were loaded along with a DNA ladder.

DNA Extraction

The primary objective of DNA extraction is the isolation of target material through a systematic process. In this extraction procedure, fragments of two different shrimp specimens (0.625 g of shrimp 1 and 0.630 g of shrimp 2) were broken down to achieve a pasty consistency. Subsequently, the paste was transferred to a small collection tube and subjected to heat for an extended duration. Following this, the collection tube underwent processing in a flow tube, incorporating specific buffers to facilitate tissue breakdown. Utilizing a vortex spinner iteratively, the resulting mixture was refined until only DNA remained in the collection tube. Upon calculating the DNA content in each tube, the concentrations were determined to be 9.2 nanograms per microliter for shrimp 1 and 1.74 nanograms per microliter for shrimp 2.

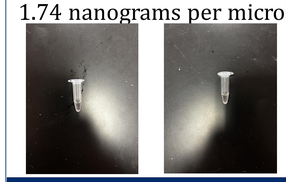

Figure 2. What the shrimp DNA looked like after the buffer and vortex process (left photo is shrimp 1, right photo is shrimp 2)

PCR and DNA Barcoding

After determing remaining DNA post-extraction, insights from Oskar Rennstam Rubbmark, Daniela Sint, Nina Horngacher, and Michael Traugott guided the selection of the necessary primers—HCO2198 and LCO1490. The preparation of the polymerase chain reaction (PCR) for both shrimp specimens involved a blending of ingredients in a microtube, destined for utilization in the subsequent barcoding process. The concoction comprised 1 microliter of each primer, a specific quantity of DNA (2 microliters for shrimp 1, 6 microliters for shrimp 2), 12.5 micrometers of biomix, and water (10.5 microliters for shrimp 1, 66.5 microliters for shrimp 2), culminating in a total volume of 25 microliters for the PCR. Upon completion, the PCR products underwent gel insertion, which was subjected to gel electrophoresis to detect any discernible DNA strands. Regrettably, the analysis revealed an absence of observable DNA strands after running the gel through the machine.

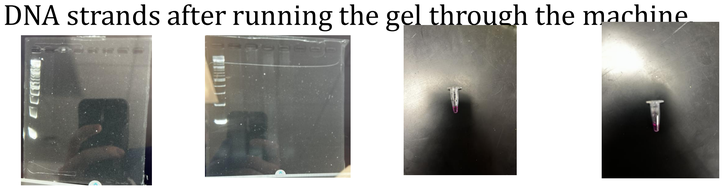

Figure 3. From right to left, two images of the gels after the gel electrophoresis process, the two PRC that were made and placed into the gel.

What Went Wrong?

In the intricate process of DNA extraction or PCR creation and barcoding, the likelihood of errors occurring in the PCR creation is notably minimal. Potential errors, such as using incorrect primers or incorrectly mixing the PCRs, were ruled out, as the primers were verified for accuracy. The most likely source of the issue points toward the DNA extraction phase, where there is a considerable chance that an insufficient amount of shrimp tissue was utilized or that the tissue was inadequately broken down. Additionally, the introduction of human error during the buffering and vortexing steps of the extraction process could have contributed to the challenge.

Conclusions

In conclusion, our DNA barcoding project, aimed at determing the origin, species identification, and potential mislabeling of store-bought shrimp, which encountered a setback during gel electrophoresis. While the error likely originated from the DNA extraction phase, where insufficient tissue or human errors might have occurred, the detailed PCR creation process minimized the chances of mistakes. The selection of primers, guided by the research of Oskar Rennstam Rubbmark, Daniela Sint, Nina Horngacher, and Michael Traugott, ensured accuracy in the barcoding proccess. Despite facing a challenge in gel electrophoresis with no observable DNA strands, this provides valuable insights for conducting future experiments and spotlights the complexity of the genetic processes.

Figure 4. Photo via Fisher Scientific: *Eppendorf pipette pick-a-pack sets - pipette products, pipettes*. Eppendorf Pipette Pick-a-Pack Sets:Pipette Products:Pipettes | Fisher Scientific. (n.d.). https://www.fishersci.com/shop/products/eppendorf-pipette-pick-a-pack-sets-5/p-4344412

Sources

Bilgin, R., et al. "DNA Barcoding of Twelve Shrimp Species (Crustacea: Decapoda) from Turkish Seas Reveals Cryptic Diversity." *Mediterranean Marine Science*, ejournals.epublishing.ekt.gr/index.php/hcmr-med-mar-sc/article/view/12494. Accessed 11 Oct. 2023.

Pardo, Miguel, et al. "DNA Barcoding Revealing Mislabeling of Seafood in European Mass Caterings." *Food Control*, Elsevier, 23 Apr. 2018, www.sciencedirect.com/science/article/pii/S095671351830207X.

Rennstam Rubbmark, Oskar, et al. "A Broadly Applicable COI Primer Pair and an Efficient Single-Tube Amplicon Library Preparation Protocol for Metabarcoding." *Ecology and Evolution*, U.S. National Library of Medicine, 11 Dec. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6308894/#:~:text=It%20combines%20DNA%E2%80%90based%20identification,et%20al.%2C%202012).

Acknowledgments

Thanks to Pardo, Miguel, Bilgin for the great articles, as well as Dr. Rob Denton for the help and support throughout the whole research process!