Biodiversity Between North Shore and South Shore

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Abstract

The purpose of this project is to determine the biodiversity of aquatic organisms from the North and South shore. The advantages of finding the biodiversity is that it can accurately identify unknown species which will be used for biodiversity assessment and ecological studies. The two sites that will be used are Bar Beach and Long Beach. At these two sites, ten invertebrate organisms will be collected from each site and will then be frozen. After this, the DNA will be isolated and amplified in the Cold Spring Harbor Laboratory. After using the PCR machine to amplify the DNA, the gel electrophoresis machine will be used. Although DNA barcoding for identification and taxonomy has been controversial, it is often used by many scientists for biodiversity studies.

Introduction

The biodiversity of Long Island of the aquatic organisms is very divergent in different bodies of water. 70% of our planet is made up of oceans, which has a higher diversity of organisms than terrestrial or freshwater ecosystems. (Briggs, 1994) The purpose of this study is to examine the DNA of different aquatic invertebrates from the North Shore and the South Shore in order to look at the DNA sequences from each shore and to see the number of different species from each site. The two locations being collected from are Bar Beach and Long Beach. The number of different species from each shore will help determine the diversity of organisms on two different parts of Long Island. (Bakker et al, 2016) This study demonstrates the impact of functional constraints and how they can decrease if a protein-coding gene is used as a central idea. When marine organisms that are in embryonic and in larval stages are exposed to trace elements or pollution, their chances of developing successfully are reduced. Additionally, they are more likely to get affected than adults due to the fact that they are more vulnerable. (Smith et al, 2015) DNA barcoding is a powerful tool for identifying and confirming species. In fact, DNA barcoding involves forensics too. DNA barcoding can be used to discover possible taxonomic problems and other sources of error which can show that DNA barcoding can serve to confirm sequencing accuracy and discover problems such as misidentified species, inaccurate taxonomies, contamination, and errors in sequencing. (Shen, 2013) Barcoding has been a recent advancement in the scientific field, for researchers are now able to generate DNA sequences for all named species on the planet. COI, also known as cytochrome c oxidase subunit I gene, is used to identify current and new species of animals, offering more than 1 billion different combinations of bases and patterns of DNA. COI, in some instances, is not enough to analyze the DNA because extant species may have different genetic sequences that COI can miss out on. However, the COI system provides a more reliable and less expensive solution to identify species. Taxonomy is a difficult process, and it may result in the periodic misidentification of species. The COI is efficient in the way that it helps avoid these misidentifications from occurring. Scientists are hoping that it'll help explain the differences in populations of organisms, thus opening new doors in the world of science. It's important that the most effective identification method is used when studying the diversity of an area.

Materials & Methods

First, ten invertebrate organisms were collected from each shore. While collecting the samples from each site, the metadata of the two locations was collected using the GPS app. Each of the specimens were photographed and their phylogenetic characteristics was used to make a determination of possible genus prior to DNA barcoding analysis. Then, they were brought into school and frozen until they were taken into the Cold Spring Harbor Laboratory. The samples were placed in a clean 1.5 mL tubes labeled with an identification number. At Cold Spring Harbor Laboratory, the DNA was isolated from the samples by taking a 10-20 mg or ¼ inch diameter from the sample and then adding 300 microliters of lysis to the solution to each tube with the sample in it. A pestle was then used to grind the tissue for about two minutes. The tubes were incubated in a water bath for 10 minutes at 65 degree Celsius. After that, the samples were placed in a microcentrifuge and then were centrifuged for a minute at maximum speed to pellet debris. 150 microliters of the supernatant was transferred to a fresh tube, making sure that the debris pellet is not disturbed. Next, 3 microliters of silica resin was added to each tube and the tubes are incubated for 10 minutes in a water bath at 57 degrees Celsius. The tubes are then put in a centrifuge machine for 30 seconds at maximum speed in order to pellet the resin. A micropipette was used to remove all of the supernatant. This was done diligently in order to not disrupt the white silica resin pellet at the bottom of the tube. After that, 500 microliters of ice cold wash buffer was added and mixed to the pellet to resuspend the silica resin. The tubes were then centrifuged for 30 seconds at maximum speed to pellet the resin and a micropipette was used to remove all supernatant. These steps will be repeated three times. Then, 100 microliters of distilled water was added and mixed well into the silica resin using the micropipette. The tubes were then incubated at 57 degrees Celsius for 5 minutes and centrifuged for 30 seconds at maximum speed to pellet the resin. 90 microliters of supernatant was transferred to fresh tubes and stored on ice until the PCR machine is ready. The PCR machine will be used to further amplify the DNA using the COI primer. Then, the samples will be placed in gel to determine if enough DNA is produced. The rest of the DNA will be sent for sequencing at Cold Spring Harbor Laboratory. The DNA subway account will be used to trim the sequence and compare the identity of the sequences in order for the relation of the species to be compared.

Results

The gel electrophoresis analysis machine indicated which samples could be used for comparison. The samples used were the ones that appeared clear or slightly faint on the machine. However, to be more accurate DNA Subway and BLAST: Basic Local Alignment Search Tool was used. DNA Subway is an intuitive interface for analyzing DNA barcodes and provided both a phylogenetic tree and a multiple alignment. A multiple sequence alignment is a comparison of three or more DNA or amino acid sequences and can be used for many purposes including inferring the presence of ancestral relationships between sequences. A phylogenetic tree shows evolutionary relationships among various species based upon similarities and differences in their genetic characteristics. DNA Subway approved four of our samples: PJQ-008, PJQ-009, PJQ-017, and PJQ-018. PJQ-008 and PJQ-009 is from North Shore whereas PJQ-017 and PJQ-018 is from South Shore. Each primer sequence has met errors which allows for trimming. Trimming focuses on a specific region of sequence which makes it possible to directly compare the nucleotide bases of DNA from one site to another. Based on the results provided by DNA Subway, PJQ-008 and PJQ-009 are closely related and PJQ-017 and PJQ-018 are closely related which was anticipated since these samples are from the same shore. The multiple sequence alignment can be poor which is seen if there are lots of gaps, indicating that some sequences do not share identity with other sequences. From the multiple sequence alignment provided, similarities between the samples are shown indicating that these species are genetically related and have a common ancestor. This shows that there is diversity in both shores.

Tables & Figures

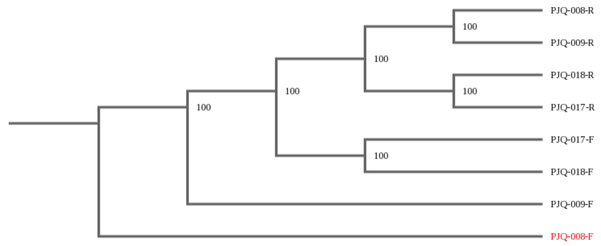

Figure 1: The phylogenetic tree of samples from the North and South Shore.

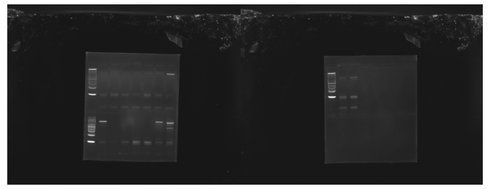

Figure 2: The gel electrophoresis as seen via as transilluminator box shows the 100 bp ladder (far left corner) used as a baseline for size comparison of the mussel sample DNA bands. The samples presented in the images are PJQ-001, 003-012, 016-020. These are bands of samples collected this year, with samples from different species of invertebrate organisms.

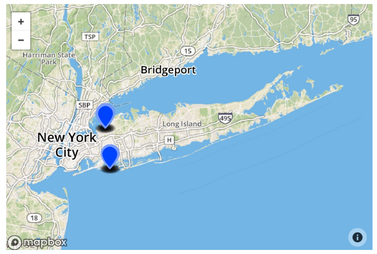

Figure 3: The two locations collected from were Bar Beach, which is located on the North Shore, and Long Beach, which is located on the South Shore. Samples 1-10 were collected from the North Shore, while samples 11-20 were collected from the South Shore.

Discussion

The gel electrophoresis analysis machine showed the samples that could be used to compare to other samples and samples that could not be used. The samples that would end up being used are the ones that either appear clear on the machine or slightly faint. Some faint bands at other positions occur when primers bind to chromosome loci other than the internet locus and give rise to nonspecific amplification products. The COI primers amplify differently sized products that migrate to different positions on the gel and it is set to amplify the same region for a certain range of species. With the use of these samples, we will be able to continue on with our research. This experiment will demonstrate whether or which samples from North Shore and South Shore will have a greater biodiversity based on future research on their effect on pollution. This experiment contributes to both environmental and ecological by improving sequencing accuracy, discovering problems such as misidentified species, inaccurate taxonomies, contamination, and errors in sequencing. Possible sources of error include the isolation of the DNA, since this process needed to be done precisely. The possibility of the results being altered was very high, since the pellet or the supernatant, depending which one held the DNA at the certain process, couldn't be disturbed.

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