Do Hidden Park Foreigners have Different DNA Barcodes than Native Homebodies?

Grace Ye[1] and Kaitlyn Yang[2] Kathleen A. Nolan, Ph.D.[3], (Faculty mentor)
[1]Stuyvesant H.S.,[2]Hunter College High School [3]St. Francis College

Funded by:
The Pinkerton Foundation
SCIENCE Simons Foundation SANDBOX

Abstract

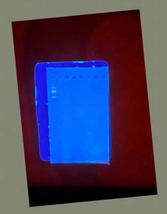
DNA was extracted, isolated, amplified, and analyzed from leaves collected from Central Park and Battery Park. The efficacy of frozen versus fresh, and deciduous versus evergreen samples was also compared. We did not obtain PCR products, so we were not able to obtain DNA barcodes. We repeated this work with additional plant species from the SFC laboratory, in addition to fish and shellfish species to test our reagents and techniques. We still obtained no results This work is important in the face of a changing climate and species loss. Also, as urban students, it is important for us to learn more about our environment.

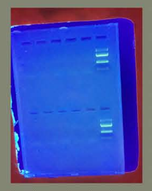
Introduction

DNA barcoding offers a way to easily study and identify urban organisms. For example, Marizzi et al. (2018) found high levels of biodiversity in Marine Park in Brooklyn, New York, from DNA barcoding experiments. We would like to replicate some of those experiments in different parks. Florio et al. (2018) outlines the procedures and methods we will be duplicating in this project. Harris and Bellino (2013) thought it was especially important for urban students to go outside and observe the world around them.

We also think it is important, especially in these times of climate change, to know more about the world around us. As plants are the basis of all food webs, they are extremely crucial, and sometimes overlooked. We aim to learn more about this "hidden world". Realizing that parks are somewhat artificial environments, we want to see if there are high numbers of invasive species versus native in these parks. We also want to see if there is a difference between trees and shrubs, and DNA quality between fresh and frozen samples.

Our hypothesis was that the DNA sequences for invasive plants might be different from those of native plants. Furthermore, we are interested in learning overall how to identify invasive versus native plant species. We are also interested in how climate change might alter species distributions. We began our DNA isolations with known plants and were hoping to confirm their ID with DNA barcoding. Upon getting no discernable results, we tried plants that were in the SFC teaching laboratories, in addition to a few fish species that were used to test out reagents and primers. Tilapia, cod, salmon, New Zealand mussels, and clams were tested. We were still unable to visualize results on a gel.





Gels that show no results---
primer dimer in gel on right

Materials & Methods

Plant specimen, permanent marker, pipette and pipette tips, lysis solution [6 M Guanidine Hydrochloride], incubator, centrifuging machine, tubes, silica, wash buffer with ethanol, distilled water, DNA polymerase, Taq polymerase (12.5 μl) & rubisco-chloroplast or rbcl primer mix (10 μl) or 23 μl primer mix to PCR beads, ice, thermocycler, agarose gel (agarose powder, 1x TAE buffer, dye), marker-PBR 322, gel electrophoresis.

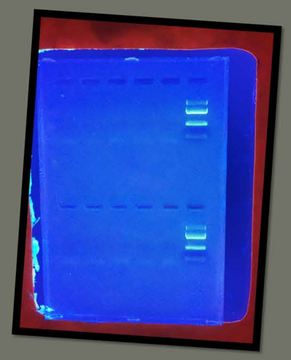
We collected plants from Central Park and Battery Park. We took pictures of the whole plants and recorded the GPS from where we collected them. We had wanted to compare the DNA barcoding efficiency of frozen versus fresh samples (when possible). We also wanted to see if there is a difference in DNA patterns in trees versus shrubs.
We followed a DNA extraction protocol that comes with the Carolina Biological DNA barcoding kit, in which DNA was extracted through mashing, lysing with guanidine hydroxide; then combining the DNA with silica, (DNA sticks to silica), washing with ethanol (DNA is repelled by ethanol); then, after two washes, we elute the DNA with water or a buffer. We used scissors to cut the leaves into tiny pieces (after we noted that the procedure did not work the first time we did it0.

Next we performed the polymerase chain reaction (PCR) by adding primers (from chlorophyll DNA), buffers, and Taq polymerase (a special DNA polymerase isolated from *Thermophilus aquaticus*, a bacterium that was isolated from hot springs in Yellow Stone National Park. We set a thermocycler to heating and cooling cycles (35) to make more copies of the DNA.
Then we run an electrophoresis gel, and if there were positive bands, after staining with Sybrsafe, we would have then sent the samples to

GeneWiz for sequencing. (ATCGTCCCCCGGGGTATA is an example of a DNA sequence.)

Little neck, quahog, and
New Zealand mussel

Salmon, tilapia, cod



Gel from seafood PCR products. Faint bands are "primer dimers"---that is, primers sticking to each other rather than to your DNA samples

Discussion

ReferPlants were collected from Central Park and Battery Park in an attempt to learn more about their ecology, and the differences between native and invasive species. We collected both evergreen (pine) and deciduous leaves from plants and attempted to isolate DNA from them in order to identify them against a known database. In our readings, we learned that invasive species are opportunistic and can take over native species. Furthermore, we learned that native species are usually more resilient in the face of an emergency such as Hurricane Sandy. As climate change might cause additional perturbation in the environment, our interest in plants, both native and invasive, has been greatly piqued. However, the PCR amplifications did not work for an as yet unknown reason. Our suspicion is that either the Taq DNA polymerase in the PCR beads or the primers were denatured. But this was an educational and a learning experience. We improved our pipetting, lab notebook keeping, record keeping, and gel electrophoresis techniques. For this we are grateful, and would like to continue with designing and hypotheses testing about DNA barcoding in the future.

References

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Acknowledgements
Christine Marizzi, Kathleen Nolan, Marlon D. Joseph, Noemi Rivera, Leah Kovenat and Allen Burdowski.

A plant phylogeny
(Kress, 2017)