The Use of DNA Barcoding to Teach Students the Importance of Classifying Biodiversity

Antonia Florio, Noemi Rivera, Kathleen Nolan*

Department of Biology and Health Promotions, St. Francis College, Brooklyn NY 11201

**Corresponding author: antonia.m.florio@gmail.com*

INTRODUCTION

Students can develop an awareness and concern for biodiversity loss by learning to identify the organisms around their neighborhoods or campuses. Previous studies have suggested that this can be accomplished by inspiring a connection with nature [1], and by allowing students to develop a relationship with the species that surround them [2].

In response to this, we implemented DNA barcoding curriculum at St. Francis College (SFC) to identify species in New York City (NYC). Classifying species based on morphology can prove challenging for students because they often become frustrated by species descriptions and taxonomic keys. The use of DNA barcoding has been championed as a way to overcome this, while also providing an inquiry-based approach for student-driven research. DNA barcoding, or sequence-based specimen identification, was developed by Paul Hebert in 2003 to identify a broad range of taxa by sequencing a standardized short DNA fragment, the "DNA barcode" [3].

Using the DNA barcoding method, students propose projects, collect samples, extract whole genomic DNA, and use PCR to amplify the appropriate gene for their taxonomic group (plants: chloroplast genes rbcL and matk; animals: mitochondrial COI; fungi: nuclear ITS; and bacteria: 16S rRNA). Successful PCRs (confirmed by gel electrophoresis) can then either be sequenced in-house or sent away to a company for Sanger sequencing. Students clean their data using any sequence editing program, and perform BLAST searches through Genbank to identify their samples. Students can also learn how to resolve evolutionary relationships by generating multiple sequence alignments and phylogenetic trees.

This poster will summarize how we have used DNA barcoding at SFC to introduce high school and undergraduate students to classifying NYC biodiversity. We focused on how DNA barcoding was used to identify plant biodiversity in two NYC wetland localities: Brooklyn Bridge Park and the Jamaica Bay Wildlife Refuge. Our study can be easily expanded to be included in many undergraduate laboratory courses such as genetics, ecology, evolutionary biology, and conservation biology.

STUDY SITES

Brooklyn Bridge Park

- 85 acre post-industrial waterfront site stretching 1.3 miles
- 15 minute walk from St. Francis College!
- Worked with 13 high school students to collect and document samples in July 2016 as part of SFC Summer Science Academy

Image from nycgovparks.org

Jamaica Bay

- Wildlife Refuge in Brooklyn/Queens managed by the National Park Service
- Salt marshes offer prime habitat for migratory birds and other wildlife
- Collected samples with 23 undergraduate students in the SFC Ecology lab in Oct. 2016

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2. Lindemann-Matthies P. The Influence of an Educational Program on Children's Perception of Biodiversity. J Environ Educ. 2002;33: 22–31. doi:10.1080/00958960209600805
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4. Kress WJ, Erickson DL. A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. PLoS One. 2007;2. doi:10.1371/journal.pone.0000508

PROJECT DESIGN AND METHODS

Step 1: Obtain necessary permission for sample collection

City of New York Parks & Recreation
Natural Resources Group
Permit to Conduct Research in Parks
The following individuals are authorized to conduct scientific research at the location(s) and dates listed below:
Personnel: Antonia Florio, Christine Mazza

Sampling permits for collection on city or state lands should be written in advance because approval can take several months. For private lands, you can sometimes obtain permission directly from the owner (i.e., community garden board directors).

Step 2. Sample Collection

At Brooklyn Bridge Park (left), students collected both animal and plant samples by seining. Only the plant samples were used for DNA barcoding (n=20). In Jamaica Bay, students collected leaves that had fallen in the parking lot (n=24).

Step 3. Catalog samples

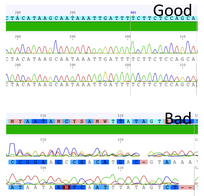
All collected samples need to be cataloged before the wet lab portion of the procedure can begin. All samples should be given an unique identifier code and photos should be taken with a scale bar for later morphological identification.

Step 4. Wet lab
DNA extraction, PCR, Gel Electrophoresis

Whole genomic DNA can be extracted using any protocol. We used a silica-based DNA extraction that can be ordered from Carolina Biological.

We then amplified the chloroplast rbcL gene using previously published primers and conditions [4], with a 54°C annealing temperature. Gel electrophoresis was used to confirm PCR success.

Step 5. Sanger Sequencing



Samples can be sent away to various companies for sequencing. We used Genewiz, Inc. It usually takes a few days to get results.

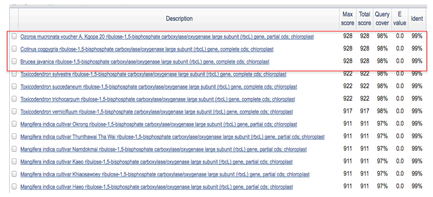
Above are examples of "good" and "bad" sequence quality.

Step 6. Sequence editing

There are many software options for sequence editing including

- Geneious
- MacClade
- DNA Subway is useful for an introduction to sequence editing

Step 7. BLAST searches on NCBI Genbank



Description	Max score	Total score	Query cover	E value	Ident
Cissma mucronata voucher A. Kigoza 210 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	928	928	98%	0.0	99%
Cotinus coggygria ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	928	928	98%	0.0	99%
Bruyas javanica ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	928	928	98%	0.0	99%
Toxicodendron sylvestre ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	922	922	98%	0.0	99%
Toxicodendron succedaneum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	922	922	98%	0.0	99%
Toxicodendron trichocarpum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	922	922	98%	0.0	99%
Toxicodendron vernicifluum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	917	917	98%	0.0	99%
Mangifera indica cultivar Okrong ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%
Mangifera indica cultivar Thunthawai Tha Wai ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%
Mangifera indica cultivar Namdokmai ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%
Mangifera indica cultivar Kaew ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%
Mangifera indica cultivar Khaoseawoy ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%
Mangifera indica cultivar Haeo ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	911	911	97%	0.0	99%

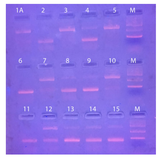
After sequence data is clean and edited, students then directly BLAST their results on Genbank. Students sometimes become frustrated when they cannot get a conclusive result; for example, here you can see that the top BLAST matches are to three different plant genera, all with identical scores. An example like this is discussed below.

DNA barcoding can easily be implemented in a lab course by following the steps outlined here. For the fall semester, I suggest the following timeline:

Obtain permit by end of summer: Step 1
Week 1: Steps 2+3
Week 2: Step 4 (DNA extraction + PCR)
Week 3: Step 4 (Gels) + Step 5 (Send samples)
Week 4: Steps 6+7

RESULTS AND DISCUSSION

Overall Amplification Success



Gel electrophoresis was used to confirm PCR success:

Brooklyn Bridge Park:
8/20 (40%) samples amplified

Jamaica Bay:
18/24 (75%) samples amplified

Jamaica Bay Results

5/18 samples (28%) could be identified to the genus level, while most plants 13/18 (72%) could not be identified to the genus-level, and many not even to the family-level. Those that could be identified to the genus-level included:

- *Ilex* sp.: Holy
- *Lonicera* sp.: Honeysuckle
- *Prunus* sp.: includes plums, peaches, nectarines

Ilex sp.

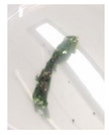
Prunus sp.

Lonicera sp.

Brooklyn Bridge Park Samples

6/8 samples (75%) could be identified to the genus level. 2/8 samples (25%) could not even be identified to the family level. Identified genera included:

- *Lepidium* sp.: pepperwort
- *Oxalis* sp.: "false shamrocks"
- *Parietaria* sp.
- *Ulva* sp.: "sea lettuce"



Ulva sp.

Lepidium sp.

Overall. We found that:

- PCR success rate varied: 40% (BBP) vs. 75% (Jamaica Bay)
- rbcL is not great for identifying species (<50% were identified to even the genus-level).
- The identified genera in the two localities varied
- The projects were successfully completed over 10 days with high school students, and in 4 lab sessions with college students
- All participating students were introduced to taxonomic methods by completing real research

What do you do when identification by rbcL fails?

There were several instances where we were unable to identify samples to the species-level using rbcL. If this happens, you can use another gene (such as matk or ITS) to help identify the plant, or you can rely on morphology to pinpoint the species identity. This is why taking sample photos is so important. For example, sample #7 (below) was identified (with identical NCBI scores) as belonging to potentially three genera, as seen below.

Does sample #7 most closely resemble which of the following genera?

Artemisia sp.?

Chrysanthemum sp.?

Achillea millefolium?

You should be able to see that this sample most closely resembles a *Chrysanthemum* sp.! The next step is to identify the sample down to the species level using a taxonomic guide. As a word of caution, multiple species should be examined for each genus, because plant morphology can differ quite a lot even within a genus.