Supplementary Materials for

DNA Barcoding from NYC to Belize

Stephen E. Harris and Marissa Bellino

*Corresponding author. E-mail: harris.stephen.e@gmail.com

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The Student DNA Barcoding Project

Curriculum Guide

Marissa E. Bellino[1,2,4] and Stephen E. Harris[3,4]

[1]Science Department, High School for Environmental Studies, 444 West 56th Street, New York, NY 10019, USA.
[2]Program in Urban Education, The Graduate Center, City University of New York (CUNY), 365 Fifth Avenue, New York, NY 10016, USA.
[3]Program in Ecology, Evolutionary Biology and Behavior, The Graduate Center, City University of New York (CUNY), 365 Fifth Avenue, New York, NY 10016, USA.
[4]The Biodiversity Center of Belize (BIOBELIZE), The Petters Research Institute, 1 Citron Street Dangriga Town, Stann Creek District, Belize.

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HANDOUTS

INTRODUCTION

Stephen Harris, a CUNY National Science Foundation (NSF) Graduate STEM Fellows in K–12 (GK-12) graduate student fellow, and Marissa Bellino, a CUNY NSF GK-12 teacher fellow, began working together in 2010 at the High School for Environmental Studies (HSES). HSES had a science research class, but students were not doing genetic work. The initial idea was to develop authentic research projects with students by introducing advanced molecular biology techniques. Our goal was to provide a skill set to high school students that would allow them to more easily succeed in a science-based major at the postsecondary level. We received funding from GK-12, a Toyota Tapestry grant, and Toshiba America Foundation to build a molecular biology laboratory at HSES. Nearly every field in the life sciences currently uses molecular biology techniques to answer genetics-based questions. We are in the age of DNA and genomics, but unfortunately, the necessary lab skills and molecular theory are not taught to science students at the high school or even college level. We believed that students will be more interested in STEM fields and scientific careers by giving them the appropriate skill set as early as possible.

This DNA Barcoding course that we developed is the first year in a multiyear environmental science research program. DNA barcoding is a method scientists can use to help in the identification of species from small unidentifiable fragments (piece of tissue, larval stage) or to potentially identify cryptic species. DNA barcoding has not replaced traditional taxonomy but rather is a new tool that scientists can use in their effort to catalogue biodiversity. It is based on sequences of short fragments of DNA, and it is used as the foundation for introducing students to modern biology questions and research. The full curriculum has five major units. In Unit 1, Sampling Local Biodiversity, students collect local samples of interest as the first step in generating a DNA barcode. Sample collection can be done just about anywhere but what matters most is documenting information about how and where the samples were collected. Sampling can occur in local parks as a way to inventory local biological diversity or at local markets to investigate potential mislabeling. Unit 2, Molecular Biology Theory and Techniques, is used to introduce students to the ideas and laboratory techniques needed to successfully produce and analyze DNA barcodes. Students become comfortable working with small volumes using micropipettes and are introduced to DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis. Units 3 and 4, The Science of DNA Barcoding and Analyzing DNA Barcodes, respectively, both focus on giving students the knowledge and skills necessary to later generate their own research in Unit 5.

In 2011, a colleague saw the molecular work we were doing with students in NYC and asked if we wanted to try to set up something similar in his native country of Belize. He knew of Arlie Petters, a native Belizean and professor at Duke University, who has a nonprofit in Dangriga (The Petters Research Institute, www.pribelize.org). We went to Dangrigra for the first trip last summer 2012. We called the program BioBelize (www.biobelize.org) and worked with ~20 local high school students. After speaking with agricultural professionals and professors from Galen University, we realized Belize does not have a DNA laboratory.

In 2013, we received additional funding support from CUNY's GK-12 program, the European Society for Evolutionary Biology, and NSF's GRFP and have put together the necessary equipment for a portable DNA lab. We ran a 3-day DNA barcoding workshop at Galen University with their faculty and master's students. We anticipate this will be part of an ongoing education program at Galen University and eventually a permanent molecular biology laboratory at BioBelize. Similar to our goals in NYC, we hope to train Belizean students in molecular biology techniques to help them enter into STEM-related fields. We would like to train the next generation of Belizean scientists and establish our own research program in Belize.

COURSE GOALS

Learning Objectives

Research Literacy (RL)

1. Locate printed and online articles from the professional scientific literature
2. Read and interpret data in the professional scientific literature
3. Write a review of scientific literature in the area of interest
4. Critically assess relevance and procedures of primary research

Experimental Design (ED)

1. Explore patterns in biological data that lead to the development of testable hypotheses
2. Understand the significance of relevant variables, controls, materials, and overall experimental design
3. Develop a research plan in the area of interest
4. Explain and perform analytical procedures in the area of interest

Data Collection/Analysis (DC)

1. Apply appropriate field and laboratory techniques to sample collection in the area of interest
2. Summarize and present data in an appropriate format using statistical software such as Excel
3. Interpret and evaluate data as supporting or refuting the hypothesis proposed
4. Maintain organized lab/field notebook

Sharing Results (SR)

1. Write, present and publicly defend a research proposal/report
2. Summarize key points of research concisely and clearly using presentation software
3. Write a final research proposal/report

Relevance and Impact of Research (RI)

1. Connect primary research to everyday problem solving
2. Evaluate the ethical implications of research and acknowledge limitations of research
3. Develop hypotheses using a whole system approach that incorporates various fields of inquiry. Incorporate ecology, biology, and genetics, for example.
4. Apply critical thinking skills outside of the classroom

Research Objectives (RO)

1. Develop a method for sampling local biodiversity
2. Fieldwork and sample collection
3. Extract DNA, Amplify barcode region, check results
4. Analyze DNA sequences using bioinformatics

COURSE OVERVIEW

Unit	Essential Question	Learning Objectives	Research Objectives
1. Sampling Local Biodiversity	How can we calculate the biodiversity of insects at Inwood Hill Park?	ED1, ED3, ED4 DC1, DC2, DC3, DC4 SR1, SR3 RI1, RI2, RI3, RI4	RO1, RO2
2. Molecular Biology Theory and Techniques	How do we work with DNA?	DC1, DC2, DC3, DC4	RO3
3. Generating DNA Barcodes	How can DNA barcodes be used to identify a species?	DC1, DC2, DC3, DC4 RI1, RI2, RI3, RI4	RO3
4. Analyzing DNA Barcodes	How can existing molecular data help us interpret our DNA barcode results?	DC1, DC2, DC3, DC4 RI2, RI3, RI4	RO4
5. Developing new DNA barcoding research questions	How can we generate new research questions based on the science and theory of DNA barcoding?	RL1, RL2, RL3, RL4 ED1, ED2, ED3, ED4 DC1 SR1, SR2, SR3 RI1, RI2, RI3, RI4	RO1, RO2, RO3, RO4

IMPLEMENTATION

Each unit in our DNA Barcoding course is designed to be a stand-alone module that can be modified for use in a variety of environments and with a variety of student age groups. We share here two variations of how the course has been implemented.

New York City: This DNA Barcoding curriculum was developed in New York City with high school freshman as the first year of an environmental science research program. We had students for 45 minutes each day, and we conducted out fieldwork on three Saturday trips in the fall. The first year is very structured and revolves around several class trips to Inwood Hill Park in northern Manhattan. Freshman students are introduced to relevant field ecology topics and learn how to perform ecological surveys. They collect insects and then learn about how to catalogue and identify their samples. Students are introduced to the science of DNA, genetics, and molecular biology. They are taken into the lab where they learn how to extract DNA, amplify specific genes, and sequence the DNA of their samples. At the end of their first year, students learn the computer skills needed to analyze their DNA samples through a bioinformatics unit. The goals for the first year include an understanding of the theoretical background knowledge behind DNA barcoding and how it can be used to answer biological questions. Starting in their second year and for the rest of their time in the science research class, students develop individual projects and compete in local science competitions.

Belize: Our DNA Barcoding curriculum was modified and implemented in the summer of 2012 and the summer of 2013 in Belize. In the summer of 2012, we worked with students ages 12 to 17. For this workshop we had students for 1 week, 6 hours each day. We did not have the equipment for a molecular lab, so we modified our first unit and conducted fieldwork and introduced students to ecological concepts including ecosystem services, biodiversity, and sampling. We took students out and collected insects, taught them how to mount and identify them, and introduced the concept of DNA barcoding. In the summer of 2013, we implemented a variation of unit 3 and unit 4, introducing undergraduate and master's students to molecular technique and DNA barcoding. This was a 3-day workshop that students attended for 6 hours each day. Our DNA barcoding workshop was held at Galen University in San Ignacio, Belize, and was based on a previous report of fish mislabeling in Belize. We presented students with the problem of potentially mislabeled fish from local Belizean markets. This marked the first time in the country's history that Belizean students generated DNA barcodes using methods and equipment in Belize.

INSTRUCTIONAL INFORMATION

UNIT 1. Sampling Local Biodiversity

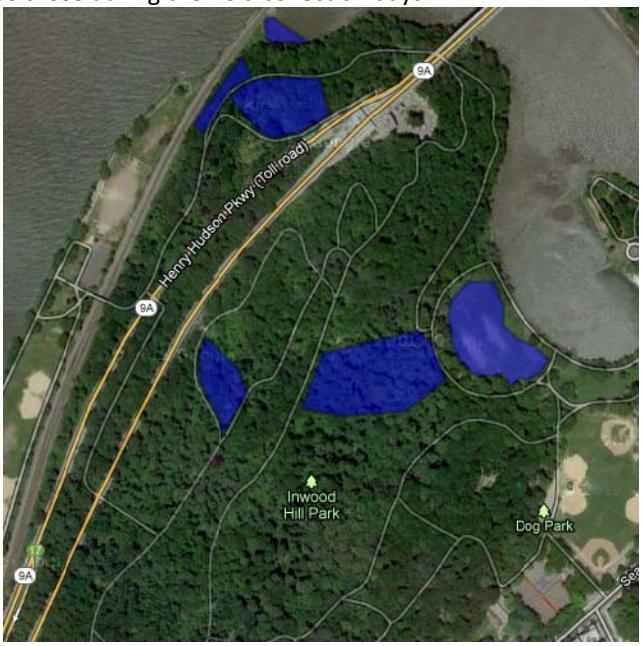
See Appendix 6 for field trip participant guide and list of potential risks

1. *Developing a method for sampling local biodiversity:* Before collecting samples, a general question should be generated as a class. Overarching questions about local biodiversity can include anything related to the collection of biological material from a variety of locations including, but not limited to, parks, markets (and other stores), and zoos. For this curriculum, we asked students to try to determine the biodiversity of insects at a local park in New York City. Students researched the different types of ecosystems in the park, randomly generated plots using Google Maps, determined the best collection method for their ecosystem, and generated data sheets.

Examples of Activities for Developing a Sampling Method:

A. Introduction to Local Ecosystems: Students read about local ecosystems in New York City, the characteristics of that ecosystem, the types of animals and plants that exist there, and local examples. Students create short presentations for each ecosystem and share with the class.

B. Generating Random Samples: Students use Google Maps to find where their ecosystem of interest is located within the park. Students outline the area on graph paper, number the squares, and use a random number generator to select three locations within the park to sample. Students get the GPS coordinates for each location and use those during the field collection days.



C. Developing Collection Methods: Students are given entomology books and collection equipment to become familiar with. Each ecosystem uses their knowledge of their ecosystem and the collection resources to develop two collection methods for insects. Students design their own traps and baits in class before field collection days.

2. *Fieldwork and Sample Collection:* After determining the method for sampling local biodiversity, fieldwork is conducted to collect and preserve samples. Depending on the question being asked, collection and fieldwork will vary. For this curriculum, we brought students to a local park on three weekend days for about 6 hours of fieldwork. The first field day allowed students to become oriented to the park, make observations of the insects in their ecosystem, and locate and flag their sample plots. The second and third trips were for collection of insects.

A. Insect Collection Materials: To collect insects from a local park there are some basic materials that must be used. Additionally, any materials that students need for their own traps should be included.
   - GPS unit
   - Camera
   - Insect nets
   - Collection jars and envelopes
   - Kill jars
   - Acetone for killing
   - Tweezers
   - Hand lens
   - Labeling marker
   - Field guides
   - Materials for traps

B. Data Sheets: Generating good data sheets will make it easier for students to organize their samples while working in the field. We are providing two sample data sheets. The first we used at a local park collecting insects (1.2a) and the second we used for students in Belize collecting fish fillets (1.2b).

C. Killing and Labeling Samples: Killing insects in the field requires the use of acetone in a kill jar. Add a few drops of acetone to the kill jar, close the lid to let the fumes build up, insert the insect. Once the insect has been killed, move into a collection jar. In the field, it is important to make sure that students keep track of samples. All samples must be labeled with the following information:
   - Insect ID Number
   - Location
   - Date
   - Collector Name
   - Collection Method

3. *Post-trip Tasks:* After returning to the classroom with samples, each sample must be processed. For this curriculum, processing includes recording observations of the insect, identification to the lowest level using morphology, pinning of insects, photographing each insect from the top and side view, removing a leg for DNA barcoding, and documenting all of the insects on our class website.
   a. It is important to introduce students to the importance of traditional identification methods in addition to DNA barcoding. Educators should spend sufficient time introducing students to the vocabulary used to identify orders of insects using morphological characteristics. Students should have plenty of time to identify species on their own. There are many Web sites with material at an appropriate level for high school students, and we also use the book, *The Practical Entomologist*, with our students

      http://gk12calbio.berkeley.edu/lessons/less_quickinsect_id.html
      http://www.amazon.com/Practical-Entomologist-Rick-Imes/dp/0671746952

SAMPLE POST-TRIP CHECKLIST:

Task	Completed
1. Upload trip photos to Picasa and share	
2. Transfer data table(s) into notebook	
3. Observe and measure morphology of insects and try to identify to the lowest level - for each specimen keep records in your notebooks of the characteristics/drawings/names	
4. Pin insects	
5. Photograph each insect after it is pinned- top shot with ruler, side shot	

6. Upload all pictures to Picasa account – edit pictures (crop, brighten, enhance)	
7. Remove leg (back right) and place into ethanol tube with DNA barcode label, that is, specimen ID number	
8. Type up and print labels for each specimen	
9. Upload all photos and collection information to class website	

SAMPLE STUDENT DNA BARCODING DATA:

Specimen ID#	Country	City	Latitude	Longitude	Date Collected	Collector Name	Collection Method	Insect Order	DNA Barcode ID
IHPG541	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. E. Bellino	Sweep Net	Hymenoptera	41
IHPG542	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	B. A. Font	Bare Hands	Diptera	42
IHPG543	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	E. McKan	Sweep Net	Diptera	43
IHPG544	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	J. Ramtel	Sweep Net	Lepidoptera	44
IHPG545	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. Poppy	Tweezers	Hymenoptera	45
IHPG546	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. E. Bellino	Under rock	Dermaptera	46
IHPG547	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	E. McKan	Tweezers	Dermaptera	47
IHPG548	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	J. Ramtel	In sand (Kill Jar)	Hymenoptera	48
IHPG549	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	M. Poppy	Sweep Net	Hymenoptera	49
IHPG550	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	M. Poppy	Sweep Net	Diptera	50

SAMPLE INSECT LABELS:

TOP LABEL:	MIDDLE LABEL:	BOTTOM LABEL:
Location: COUNTRY, State, City Latitude, Longitude Date (Day, Month, Year) Collector Name (Last Name, First and middle initials)	Insect Identification	DNA Barcode ID #

Example:

TOP LABEL:	MIDDLE LABEL:	BOTTOM LABEL:
BELIZE, Dangriga 16°58.468'N, 088°13.327'W 24 July, 2012 Rancharan S.	Family Salticidae	DNA Barcode ID #06

4. *Calculating Biodiversity:* Even if students cannot identify all the samples using morphology, it is possible to differentiate between species based on morphological characteristics. Students can use these differences to calculate the biodiversity of insects using the Simpson's Diversity Index (1.4a).

5. *Writing up a final report:* Writing scientifically takes practice. All of the student research is written up into a final paper in the style of a journal article (1.5a, 1.5b). Sample articles are given to students to help with language, style, and formatting. (See Appendix 7 for sample report)

1.2a. INWOOD HILL PARK: DATA SHEET

CHALLENGE: How can we determine the biodiversity of invertebrates at Inwood Hill Park?
This is a sample of what should be in each of your lab notebooks. You can fill this in as a group during your collection and copy into each of your lab notebooks back in the classroom. Remember to take pictures.

Date _____
Weather _____

Group # _____
Group members: _____

Ecosystem: _____

Plot 1: Latitude: _____ Longitude _____
Plot Description: _____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

Plot 2: Latitude: _____ Longitude _____
Plot Description: _____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

Plot 3: Latitude: _____ Longitude _____

Plot Description: _____

_____

_____

_____

_____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

1.2b. DATA SHEET FOR SAMPLE COLLECTION

Type of Study:

- Investigative (Unknown sample/species) or Reference (Species Known)

Identification Code:

- Year-Institution_Code-Initials-Number (example, 13-BIOB-SH-01):

Time Stamp

- Time of Day Collected:
- Date Collected:

Depth/Elevation

- Elevation at collection site (Meters):

GPS Coordinates:

- Latitude:
- Longitude:

Site Name:

- Country/Ocean, City, Street, Name of business:

Photo

- Take a digital photograph if possible (use your phone):
- Include a metric ruler in the photo so the specimen can be measured

Sample Information

- Life Stage (Adult, Immature, Unknown):
- Sex (Male, Female, Hermaphrodite, Unknown):
- Reproduction (sexual, asexual, Cyclic Parthenogen, Unknown):

Species Identification

- Phylum, Class, Order, Family, Subfamily, Tribe, Genus, Species:

Identification Method

- Barcode, Morphology, etc:

Notes

- Collector's name and any important information:

1.4a. CALCULATING INWOOD HILL PARK INSECT BIODIVERSITY

Objectives: Students will be able to:

1. Identify the number of ***different species*** found in your ecosystem using morphological measurements.
2. Identify the number of ***individuals of the same species*** found in your ecosystem.
3. Identify the number of ***different species*** found in Inwood Hill Park.
4. Identify the number of ***individuals of the same species*** found in Inwood Hill Park.
5. Calculate the Simpson's Diversity Index for your ecosystem and Inwood Hill Park.
6. Draw conclusions about their results and how they relate to their original study design.

Part 1: Count up the number of **different species** you have in your ecosystem collection. Use all of the organisms you collected, even if they are not in your immediate collection. For each unique species, count the **number of individuals** you have in your collection.

Example:

Species	**Species Description**	**Number of Individuals (*n*)**
Species A	Fly with blue eyes, about 2cm	3
Species B	Fly with red eyes, 1cm	2
Species C	Wasp, 4cm	1
Species D	Stink bug	1

Group_____

Species	**Species Description**	**Number of individuals (*n*)**

Species Richness = total number of species: _____

Total number of individuals of all species (***N***): _____

Part 2: Walk around to the other collections and using the images on the website as well as the physical collection, count up the number of **different species** in **all** of the collections. For each unique species, count the **number of individuals** you have in your collection.

Example:

Species	**Species Description**	**Tally Number of individuals (*n*)**	**Total Number of Individuals**
Species A	Fly with blue eyes, about 2cm	IIII	4
Species B	Fly with red eyes, 1cm	II	2
Species C	Wasp, 4cm	IIIIII	6
Species D	Stink bug	IIIIIIII	8

Species	**Species Description**	**Tally Number of individuals (*n*)**	**Total Number of Individuals**

Species Richness = total number of species: _____

Total number of individuals of all species (***N***): _____

Biodiversity background Information

Biological Diversity **-** *the great variety of life*

Biological diversity can be quantified in many different ways. The two main factors taken into account when measuring diversity are richness and evenness.

1. Richness

Richness is a measure of the number of different kinds of organisms present in a particular area. For example, species richness is the total number of different species present in a community. Some communities may be simple enough to allow complete species counts to determine species richness. However, this is often impossible, especially when dealing with insects and other invertebrates, in which case some form of sampling has to be used to estimate species richness.

2. Evenness

Evenness is a measure of the relative abundance of the different species making up the richness of an area. A community dominated by one or two species is considered to be less diverse than one in which several different species have a similar abundance.

Simpson's Diversity Index

Simpson's Diversity Index is a measure of diversity. In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present (species richness), as well as the abundance of each species (species evenness). As species richness and evenness increase, so diversity increases. Educators should note that the index is scientifically valid only when an area has been sampled completely. Results obtained through the class are likely misrepresentative of the true value.

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

n **= the total number of individuals of a particular species**
N **= the total number of individuals of all species**

The value of ***D*** ranges between 0 and 1

Simpson's Index of Diversity (I) = 1 - **D**

The value of this index ranges between 0 and almost 1, the greater the value, the greater the sample diversity. The index represents the probability that two individuals randomly selected from a sample will belong to different species.

Part 3: Use the information from Part 1 to calculate the biodiversity of **your ecosystem** using the Simpson's Diversity Index. Use the example below to help you complete the calculation.

Example:

Species	***n***	***n*(*n* – 1)**
Species A	3	6
Species B	2	2
Species C	1	0
Species D	1	0
Total (*N*)	7	
∑ *n*(*n* – 1)		8

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

Show all your calculations here:

Step 1: *D* = 8/7(7-1)

Step 2: *D* = 8/42 = 0.19

Step 3: I = 1 – *D* = 1 –0.19 = .81

Simpson's Diversity Index = .81

Simpson's Diversity Index Calculation

Species	***n***	***n*(*n* – 1)**
Total (*N*)		
∑*n*(*n* – 1)		

Show all your calculations here:

Part 4: Obtain the Simpson's Diversity Index for each of the ecosystems and fill in the table below.

Simpson's Diversity Index	**Group 1: Upper Ridge Forest**	**Group 2: Field**	**Group 3: Lower Successional Forest**	**Group 4: Lower Ridge Forest**	**Group 5: Beach**	**Group 6: Upper Successional Forest**

Part 5: Use the information from Part 2 to calculate the biodiversity of **Inwood Hill Park** using the Simpson's Diversity Index.

Simpson's Diversity Index Calculation

Species	***n***	***n*(*n* – 1)**
Total (*N*)		
∑*n*(*n* – 1)		

Show all your calculations here:

Part 6: Use the information from your original study design and your calculations to answer the following questions:

1. Which ecosystem was most diverse? How do you know? Why do you think?
2. Which ecosystem was least diverse? How do you know? Why do you think?
3. What variables may have influenced these results?
4. What were the limitations (potential errors) of your particular study design? How did this affect your data?
5. If you were to do this again, what changes might you make in your study design? Why?
6. How might you use this information to help increase biodiversity at Inwood Hill Park?
7. Based on these results, what new question(s) could you ask for future experiments?

1.5a. INWOOD HILL PARK SCIENTIFIC REPORT

Directions: Use the format below to begin to develop your final paper on Inwood Hill Park Invertebrate Biodiversity. Your final paper should be typed and you must submit one per group.

TITLE: The title should be descriptive and the reader should know what your study is about by reading the title.

1. **INTRODUCTION**:
   - Paragraph 1: Biodiversity – what is it, why it matters, what it does for us.
   - Paragraph 2: Inwood Hill Park History and diversity of ecosystems
   - Paragraph 3: Your ecosystem – talk about your ecosystem, its characteristics and organisms that live there
   - Paragraph 4: Invertebrate diversity and importance to ecosystems
   - Paragraph 5: Purpose of the study, what you expect to find (Exploring pattern and testing hypothesis), and why this is an important study.

2. **METHODS**: For each of the bullet points below, explain what you did.

Collection

   - Random sampling
   - Site setup (how did you set your plots)
   - Collection methods
   - Preservation methods
   - Identification and organization methods (include information on pinning, labeling, and DNA barcoding)
   - Are there any visuals you could include that might help the reader understand more about what you did (think about maps, photos, diagrams of traps, etc…)? Describe each below.

Analysis

   - Calculating Biodiversity (include information about species richness and Simpson's Diversity Index)

3. **RESULTS**:
   - Visual data – photos
   - Biodiversity data as a bar graph
   - Biodiversity calculation

4. DISCUSSION/CONCLUSIONS
   - Connect your results back to your introduction
   - What are possible errors? How might these have impacted your results?
   - If you were to do this again, how would you improve this study?
   - What new questions can you ask now with this information?

5. **REFERENCES** (APA format)
   - Use http://citationmachine.net/index2.php to help you with citations.

1.5b. INWOOD HILL PARK SCIENTIFIC REPORT RUBRIC

Criteria	Points	Comments
Title: (5 points) • Clearly and concisely describes the nature of the study • Includes pertinent information		
Introduction: (35 points) • Provides sufficiently broad background info • Provides rationale (Why is this important?) • Provides a context (What has already been done?) • Goals and objectives of the study are clearly stated • Hypothesis is clearly stated, specific and testable		
Methodology: (40 points) • Methods are directly aimed at testing the stated hypothesis • Methods are feasible • Pertinent diagrams and/or photos included and are informative • Identifies the study area and data collected • Procedures appear to be replicable • Analysis described		
Results: (40 points) • Where appropriate, data are presented in figures (graphs) and tables • Figures and tables correspond with the stated method • Axes, titles and legends of tables and figures are properly labeled • Figures and tables are professional-looking and easy to interpret • Appropriate types of figures (line vs. bar) are used • All calculations and observations not in figures and tables are included in the text • Each figure and table presented is described in the text • Figures and tables are cited in the text that describe them • Relevant statistics and statistical analysis are presented • Data and data analysis are presented in a logical order		

Discussion and Conclusions: (50 points) • States whether the hypothesis was supported by the results • Presents a logical explanation and interpretation of the results • Explains the significance of all results • No extraneous information is presented • Describes how these results fit into the "big picture" • Discuss the practical applications of the results • Demonstrates creative and critical thinking • Discusses possible reasons for unexpected results • Identifies and discusses all major potential sources of error • Conclusion paragraph concisely summarizes the paper • Conclusion paragraph restates the major findings • Conclusion paragraph restates the significance of the findings • Conclusion paragraph generates ideas and questions to guide future research		
References: (10 points) • Listed in scientific journal format (APA) • Listed alphabetically		
Organization and Style: (20 points) • Uses headings and subheadings to visually organize the material • Few errors in spelling, punctuation and grammar • All required elements are present and additional elements that add to the paper (e.g., graphs, tables, figures, images) • Research paper handed in on time		
Total Points: (200 points)		
General Comments:		

UNIT 2. Molecular Biology Theory and Techniques

See lab certification exam for risks and safety measures

1. *Molecular Equipment:* There is specialized equipment that is required to work with DNA. For each piece of equipment that is introduced to students it is important to explain the purpose of each and the proper usage. While many universities may have access to this type of equipment, most high schools will probably not. We wrote many grants to obtain the necessary materials and continue to write grants to keep the lab up to date. See Appendix 1 for laboratory materials and related costs.

Most Popular Laboratory Equipment and Purpose

Equipment	Purpose
Micropipettes	Allows the transfer of small volumes of liquid (0.5 ul – 1000 ul)
Centrifuge	Separates materials based on density
Vortexer	Mixes materials
Thermocycler	Used for PCR
Heat Block	Allows temperatures to be held for an extended period of time
Gel Electrophoresis	To confirm the presence of DNA after PCR
UV Transilluminator	Allows you to visualize the gel results under UV light

2. *Molecular Technique:* Students should be exposed to the various molecular techniques that are required for successful laboratory work. These techniques include sterilization of the workspace and materials, solutions and dilutions, and proper micropipetting. Teaching this to students usually involves demonstrating the various techniques and giving students time to practice.

Examples of activities for developing an understanding of DNA extraction:

A. *Metric Conversions:* Have students practice converting volumes and mass so they feel comfortable with the small units used in molecular biology. Have students convert between microliters, milliliters, liters, nanograms, micrograms, milligrams, and grams.
B. *Micropipetting Artwork:* Give students different tubes of food coloring and allow them to create images on parafilm. This will help them with the various volumes that each pipette can hold as well as teach them about the different size tips that are associated with the different pipettes.
C. *Make 1X TAE Buffer:* 1X TAE buffer is used to make and run gels. Most TAE buffer comes as 50X. Have students practice diluting the 50X TAE buffer and by making their own bottle of 1X TAE.
D. *Loading a Gel:* Practice loading the gel is essential so that students don't puncture the gel or mislead their sample. Make a gel and allow students to load food coloring into the wells.

3. *Extracting DNA:* The first step in any DNA barcoding work is to extract DNA from the sample. Students should be familiar with the general theory of how DNA extraction works. In our lab we use two different types of DNA extraction, but there are many other ways of doing this.

Examples of activities for developing an understanding of DNA extraction:

A. *Dirty DNA Extraction:* Dirty DNA extraction is a fun way to get students excited about working with DNA and to also teach the types of chemicals that are used to extract DNA. There are many great examples of dirty DNA extractions. We like the Utah Genetics one a lot.
B. *DNA Extraction Virtual Lab:* There are many great online virtual labs that help students understand the process of DNA extraction. We like the Utah Genetics DNA extraction virtual lab.
C. *DNA Extraction Protocol: Promega Wizard Genomic DNA Purification*: The Promega kit extracts DNA through a lysis and purification process. DNA is separated from the cell debris and is precipitated with isopropyl alcohol. This kit is cheaper than the Qiagen and works best when you have an ample amount of starting material. Another benefit of this kit is that all reagents can be kept at room temperature. Have students practice extracting DNA. Use the Promega Genomic DNA purification table (2.3a) to review the materials, techniques, and science.

D. *DNA Extraction Protocol: Qiagen DNeasy Blood and Tissue*: The Qiagen kit extracts DNA using a silica matrix spin column. DNA is first released from tissue using Proteinase K and is then bound to the spin column using buffers. The DNA is then washed with several was buffers and eluted from the spin column with an elution buffer. The QIagen kit is more expensive but it is successful at extracting DNA from small starting samples. Additionally the Qiagen kit requires non-denatured ethanol and this may be difficult for high school laboratories.

4. *Amplifying DNA using PCR:* After DNA extraction, the Polymerase Chain Reaction (PCR) amplifies the specific gene of interest in preparation for sequencing. For DNA barcoding those genes are typically CO1 for animals and RBCL for plants. To fully grasp the science and theory of PCR, gel electrophoresis, and sequencing, students must understand the importance of *Taq* Polymerase, primer selection, and sanger sequencing.

Examples of activities for developing an understanding of Polymerase Chain Reaction (PCR):

A. PCR Virtual Lab and Animation: Virtual Labs are a great way to expose students to the science of PCR before they go into the lab. The Utah Genetics Virtual Lab is a great one. Cold Spring Harbor DNA Learning Center has a great PCR animation as well.

B. Primer Selection for DNA Barcoding: Specific primers are utilized when generating DNA barcodes. For most animals, a region of the CO1 gene in the mitochondria is used. For most plants, a region of the RBCL gene in the chloroplast is used. The Urban Barcode Project website describes the science of designing and selecting primers along with some common primer sequences.

C. PCR Protocol: The illustra PuReTaq Ready-To-Go PCR Beads are an easy way to do PCR in the lab. The beads come with *Taq* Polymerase and the dNTPs. All you need to add are your primers and DNA sample. The amounts of primer and DNA sample you add to the PCR bead will be determined by the concentration of each. Have students practice PCR. Use the PCR table (2.4a) to review the materials, techniques, and science.

D. This is also where we introduce technical details of DNA barcoding. More detail is given in Unit 3, but resources from www.dnabarcoding101.org and http://www.urbanbarcodeproject.org/ are extremely helpful in discussing how a barcode gene was chosen and the limitations of using one gene across taxonomic groups.

5. *Gel Electrophoresis*: Running a gel electrophoresis will confirm the results of your DNA extraction and PCR. A successful extraction and amplification of the DNA barcode region will reveal a band on your gel in the 650-750 base pair region. Depending on your gel electrophoresis system, students will need to know how to make, stain, run, visualize, and interpret a gel.

Examples of activities for developing an understanding of Gel Electrophoresis:

A. Science of Gel Electrophoresis: There are many online videos and presentations will help students understand how a gel electrophoresis separates DNA fragments based on size and charge.

B. Gel Electrophoresis Virtual Lab: There are many great virtual labs that can help students better understand the science of gel electrophoresis. One we like to use is the Utah Genetics Gel Electrophoresis Virtual Lab.

C. Gel Electrophoresis Equipment Description: Each gel electrophoresis system will be different so it is best to introduce students to the types that you will use in your laboratory. Important parts include the power supply, gel box, gel tray, and combs. Students should also understand the purpose of the DNA stain. In the high school we use SYBR Safe or SYBR Green, both safe for student handling. DNA ladder, loading dye, and TAE buffer should also be introduced to students.

D. Gel Electrophoresis Protocol for DNA Barcoding: Have students practice making and running a gel. Use the gel electrophoresis table (2.5a) to review the materials, techniques, and science.

6. *Molecular Laboratory Certification Exam:* As a final assessment to ensure that all students are comfortable with molecular biology and laboratory techniques we give a laboratory certification exam. This is a multiday exam that includes both a written portion (2.6a) and practical portion (2.6b). Students are allowed to utilize all of their resources during this exam.

2.3a. PROMEGA GENOMIC DNA PURIFICATION TABLE

The purpose of DNA extraction is _____

_____

_____

_____

Step #	Step Description	Materials	Technique	Science (what is happening?)
1	Obtain plant or animal tissue ~10-20mg or 1⁄4 inch diameter from your sample. If you are working with more than one sample, be careful not to cross contaminate specimens. (If you only have one specimen, make a balance tube with the appropriate volume of water for centrifuge steps.)			
2	Place sample in a clean 1.5mL tube labeled with an identification number.			
3	Add 100 µl of nuclei lysis solution to tube.			
4	Twist a clean plastic pestle against the inner surface of 1.5 mL tube to forcefully grind the tissue for 1 minute. Use a clean pestle for each tube if you are doing more than one sample.			
5	Add 500μL more nuclei lysis solution to tube.			
6	Incubate the tube in a water bath or heat block at 65°C for 15 minutes.			
7	Add 3μL of RNAse solution to tube. Close cap, and mix by rapidly inverting tube several times.			
8	Incubate the tube in a water bath or heat block at 37°C for 15 minutes. Then stand tube at room temperature for 5 minutes.			
9	Add 200μL of protein precipitation solution to each tube. Vortex tubes for 5 seconds: Securely grasp the upper part of tube, and vigorously hit the bottom end with the index finger of the opposite hand. Use a vortexer if available.			
10	Stand tube on ice for 5 minutes.			
11	Place your tube and those of other groups in a balanced configuration in a micro-centrifuge, with cap hinges pointing outward. Centrifuge for 4 minutes at maximum speed to pellet protein and cell debris.			

Step #	Step Description	Materials	Technique	Science (what is happening?)
12	Label a clean 1.5 mL tube with your sample number. Use a fresh tip to transfer 600μl of supernatant to the clean tube. Be careful not to disturb the pelleted debris when transferring the supernatant. Discard old tube containing the precipitate.			
13	Add 600μL of isopropanol to the supernatant in tube. Close cap, and mix by rapidly inverting tubes several times.			
14	Place your tube and those of other groups in a balanced configuration in a micro-centrifuge, with cap hinges pointing outward. Centrifuge for 1 minute at maximum speed to pellet the DNA.			
15	Carefully pour off the supernatant from tube, and add 600μL of 70% ethanol. Close cap, and flick the bottom of each tube several times to “wash” the pellet.			
16	Centrifuge the tube for 1 minute at maximum speed.			
17	Carefully pour off the supernatant. Use a micropipette with fresh tip to remove any remaining ethanol, being careful not to disturb the pellet.			
18	Air dry the pellet for 10-15 minutes to evaporate remaining ethanol.			
19	Add 100µL of the DNA rehydration solution to each tube, and dissolve the DNA pellet by pipetting in and out several times.			
20	Incubate the DNA at 65°Cf or 45-60 minutes, or overnight at 4°C.			
21	Store your sample on ice or at -20°C until you are ready to begin Part III.			

2.4a. PCR AMPLIFICATION TABLE

The purpose of PCR is_____

Step #	Step Description	Materials	Technique	Science (what is happening?)
1	Obtain PCR tube containing illustra PuReTaq Ready-To-Go PCR Beads. Label the tube with your identification number.			
2	Use a micropipette with a fresh tip to add sterile water to each tube. Allow the beads to dissolve for 1 minute.			
3	Add a forward and reverse primers to the tube			
4	Use a micropipette with fresh tip to add your DNA. Ensure that no DNA remains in the tip after pipetting.			
5	Store your sample on ice until your class is ready to begin thermal cycling.			
6	Place your PCR tube, along with those of the other students, in a thermal cycler that has been programmed for 35 cycles of the following profile: Denaturing step: 94°C, 30 seconds Annealing step: 54°C, 45 seconds Extending step: 72°C, 45 seconds The profile may be linked to a 4°C hold program after the 35 cycles have been completed.			
7	After thermal cycling, store the amplified DNA on ice or at -20 °C until you are ready to continue.			

2.5a. GEL ELECTROPHORESIS TABLE

The whole purpose of gel electrophoresis is _____

_____

_____

_____

_____

Step #	Step Description	Materials	Technique	Science (what is happening?)
1	Seal the ends of the gel-casting tray with masking tape, or other method appropriate for the gel electrophoresis chamber used and insert a well-forming comb.			
2	Make a 2% gel by adding 1g of agarose powder to 50ml of distilled water. Heat up until agarose is dissolved and the gel is clear.			
2	Add 2 µl of SYBR to your cooling gel. Pour the 2% agarose solution into the tray to a depth that covers about one-third the height of the comb teeth.			
3	Allow the agarose gel to completely solidify; this takes approximately 20 minutes.			
4	Place the gel into the electrophoresis chamber and add enough 1x TAE buffer to cover the surface of the gel.			
5	Carefully remove the comb and add additional 1x TAE buffer to fill in the wells and just cover the gel, creating a smooth buffer surface.			
8	Orient the gel so the wells are along the top of the gel. Use a micropipette with a fresh tip to load 10 µl of DNA ladder into the far left well.			
9	Use a micropipette with a fresh tip to load each sample into your assigned wells. Draw picture of how well was loaded			
10	Store the remainder of your PCR product on ice or at-20°C until you are ready to submit your samples for sequencing.			
11	Run the gel for approximately 30 minutes at 120V.			
12	View the gel using UV transilluminator. Photograph the gel using a digital camera.			

2.6a. SCIENCE RESEARCH CERTIFICATION EXAM – PART 1

Part 1: Written – Please explain each answer in as much detail as possible. Please write neatly.

LABORATORY TECHNIQUE:

1. What is the first thing you should do when you pick up a micropipette?
2. What does µl stand for?
3. How many µl are in 1 ml?
4. How many ml are in 200 µl?
5. There are three size tips we use in the lab. What is the difference and how do you know when to use which?
6. The scale reads .03g. How many milligrams is this?
7. Why is it important to wear gloves while working in the lab?
8. Why is it important not to eat or drink in the lab?
9. Why is the general rule that you change pipet tips between every use?
10. What is room temperature in Celsius?
11. What temperature is the refrigerator in Celsius?
12. What temperature is the freezer in Celsius?
13. Why do we use the centrifuge? Draw a tube after centrifuging and labeling the pellet and supernatant?
14. In general, what is the purpose of buffers during experimental protocols?

DNA EXTRACTION

15. How much tissue sample do you need for a successful DNA extraction using Promega?
16. What is the purpose of Proteinase K (Qiagen) and Nuclei Lysis (Promega) solution?
17. What does RNAse (Promega) do during the DNA extraction protocol?
18. What is the purpose of heating samples during DNA extraction?
19. What is the purpose of vortexing samples during various steps of DNA extraction?
20. What does the protein precipitation solution (Promega) do during DNA extraction?
21. What does isopropyl do during DNA extraction?
22. What does rehydrating/eluting of DNA do during DNA extraction?
23. Why is it important to rehydrate/elute DNA at the end of DNA extraction?

POLYMERASE CHAIN REACTION

24. What is the purpose of PCR?
25. What is a thermocycler?
26. What needs to be included in the PCR tube for a successful PCR run?
27. What materials are included in the PCR bead?
28. What are primers, and what do they do?
29. What genes are used in DNA barcoding of plants?
30. What genes are used in DNA barcoding of animals?
31. What genes are used in DNA barcoding of insects?
32. Why are specific genes used for DNA barcoding?
33. Why would one gene work as a barcode for one species but not for another?
34. What happens if you add too much primer?
35. Why do you need a forward and a reverse primer?
36. What are dNTPs, and why are they essential for PCR to work?
37. What is TAQ polymerase, where did it come from, and why do molecular biologists use it?
38. Why does a PCR run have multiple cycles, and what is the goal of PCR (what product are you making)?
39. What are the 3 basic steps of the PCR process and what are the approximate temperature settings and times?

GEL ELECTROPHORESIS

40. How does DNA move through a gel? Why does this happen?
41. How do you make a 2% agarose gel (include the materials and amounts)?
42. What is the charge of DNA and how is the charge of DNA used to aid in the process of moving DNA through a gel?
43. How should the electrical current always run in a gel electrophoresis run?
44. Why is it important to never touch the gel while the power source is on?
45. Why is loading dye using during gel electrophoresis?
46. What is DNA ladder and why is it needed for gel electrophoresis? Give an example of the DNA used in our lab.
47. What is SYBR safe and why is it (and DNA stains in general) important for visualizing DNA?
48. How long do you typically run a gel and at what voltages do you typically run a gel?
49. What form of light, and what machine is used to actually visualize DNA?
50. How is the design of primers, the DNA ladder, and the PCR product in the gel, used together to determine whether the PCR was successful?

2.6b. SCIENCE RESEARCH CERTIFICATION EXAM – PART 2

Part 2: Practical – Please record all information in your lab notebook.

Station 1: Attach a photo to a Google Map and record the photo number and GPS coordinates.

To pass this station you must prepare a Google map with a picture attached and your lab notebook with photo number and GPS coordinates.

Station 2: Cut and weigh out 10-20mg of different samples of: Plant, Insect, Fish. Record the mass data in your field notebook.

To pass this station, you must be able to weigh out a sample of animal and plant tissue and record the data in your lab notebook.

Station 3: Pipet different volumes of liquids into and out of microcentrifuge tubes. Record the different volume pipets and tips in your lab notebook.

To pass this station, you must show that you can identify the 4 different volume pipets and their appropriate tips, change volumes and pipet different volumes between microcentrifuge tubes.

Station 4: Break down a tissue sample using a pestle, homogenizer, and sonicator

To pass this station you must show that you know how to use the pestle and how to dispose of the pestle, use the homogenizer and how to use the sonicator.

Station 5: Centrifuge a bee sample and record the results in your lab notebook. Be sure to label the pellet and the supernatant.

To pass this station you must show that you can balance a centrifuge properly and turn the mircocentrifuge on. You must also identify the pellet and the supernatant.

Station 6: Set the heat block to different temperatures and heat a sample.

To pass this station you must show that you know how to use the heat block.

Station 7: Gel Electrophoresis - Set up a gel box, load a gel. Draw the proper setup of the gel in your lab notebook.

To pass this station you must be able to load a gel and set the gel up properly.

Station 8: Visualize a gel and compare the bands to a DNA ladder. Draw a picture of the gel in your lab notebook and label the different bands with their approximate number of base pairs.

To pass this station you must be able to use the UV transilluminator and identify different size bands compared to the DNA ladder picture.

UNIT 3. Generating DNA Barcodes

All equipment needed and primers used are located in Appendix 1, and Appendices 3 & 4 contain specific instructions for running a DNA barcoding short course

1. *Introduction to DNA Barcoding:* Introduction to DNA barcoding is necessary for students to begin to understand the theory of DNA Barcoding, how it works, and why it works. For this introduction we use presentations and animations offered through Cold Spring Harbor Laboratories Urban Barcode Project and DNA Learning Center. It is also important for students to understand any potential flaws or pitfalls with any given method. While DNA barcoding can answer many questions, there is still potential for the misuse of the method, for example, DNA barcoding by itself can not identify a new species. To address this issue we have the entire class read the papers, *The seven deadly sins of DNA barcoding* and *An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding*, and analyze them using the CREATE methods by Sally Hoskins.

   Collins RA, Zealand N (2013) The seven deadly sins of DNA barcoding: 969–975. doi:10.1111/1755-0998.12046.

   Taylor HR, Harris WE (2012) An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. Mol Ecol Resour. doi:10.1111/j.1755-0998.2012.03119.x.

   Lorenz JG, Jackson WE, Beck JC, Hanner R (2005) The problems and promise of DNA barcodes for species diagnosis of primate biomaterials. Philos Trans R Soc Lond B Biol Sci 360: 1869–1877. doi:10.1098/rstb.2005.1718.

2. *DNA Barcoding in the Scientific Literature*: There are many examples of DNA barcoding in the scientific literature. It is best to do a Google Scholar search for "DNA barcoding" and articles that you think would be most interesting to your students. Some articles can be downloaded from the Urban Barcode Project website. It might be useful to first introduce students to the concept of DNA barcoding by reviewing some popular media writing on the topic. Ones that we use in the course are:
   - Hebert PDN, Cywinska A, Ball SL, DeWaard JR. (2003)**Biological identifications through DNA barcodes. Proceedings of the Royal Society** B: Biological Sciences 270(1512):313-321.
   - Hebert PDN, Gregory TR. (2005) **The promise of DNA barcoding for taxonomy**. Syst Biol 54(5):852-859.
   - Hebert PDN, Penton EH, Burns JM, JAnzen DH, Hallwachs W (2004) **Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator**. Proceedings of the National Academy of Sciences 101(41):14812-14817.
   - Hollingsworth PM. (2007) **DNA Barcoding: Potential Users. Genomics, Society and Policy** 3(2):44-474.
   - Steinke D, Zemlak TS, Hebert PDN. (2009) **Barcoding Nemo: DNA-based identifications for the ornamental fish trade**. PLoS ONE 4(7): e6300.
   - Cox CE, Jones CD, Wares JP, Castillo KD, McField MD, Bruno JF (2013) **Genetic testing reveals some mislabeling but general compliance with a ban on herbivorous fish harvesting in Belize.** Conservation Letters 6(2):132–140.

3. *DNA Barcoding in the Popular Media*: There are many examples of DNA barcoding in the popular media. Some websites that contain news articles are included as well as examples of specific stories. Here is one article we use that discusses how DNA barcoding is being used to inventory biodiversity for long-term conservation on the island of Mo'orea (3.2a). Also included is a handout that helps students summarize the article and connect the ideas to what they are learning (3.2b).
   a. *DNA Barcoding News Sources:*
      - Barcode of Life - http://www.barcodeoflife.org/content/news/general-news
      - Science Daily - http://www.sciencedaily.com
      - New York Times – www.nytimes.com

- BrightSurf: http://www.brightsurf.com/search/r-a/DNA_Barcoding/1/DNA_Barcoding_current_events_and_news.html

b. DNA Barcoding New Stories:

- *Fish Tale has DNA Hook: Students Find Bad Labels*
- *Young Sleuths' Last Target: Sushi. This Time: Tea.*
- *Scanning Life: Biodiversity*
- *The Rockefeller University: DNA Barcoding Projects*

4. *DNA Barcoding Research Questions:* It is important for students to understand the kinds of questions that DNA barcoding can answer. For our curriculum we use literature from various barcoding organizations, popular media, and scientific literature to help students understand the breadth of research areas that DNA barcoding be applied to. Areas of research include:
    - Inventory of Biodiversity
    - Conservation Management (fisheries, lumber)
    - Illegal Trading
    - Mislabeling of Products
    - Cryptic Species Identification or Identification of species at different life stages

5. *DNA Barcoding Protocols:* We have modified the Promega and Qiagen protocols presented in Unit 2 so that they can be done in a high school laboratory during the course of a week. We have included a materials list for the Promega (3.5a) and Qiagen (3.5c) protocols that will help you and your students keep track of all materials. Our protocol handouts also include questions that review protocol technique, help students interpret results, and troubleshoot. The first protocol is a modification of the Promega Wizard Genomic DNA Purification (3.5b) that we used with a basil leaf. This allowed students to practice with a high yield tissue sample and get successful results. The second protocol is a modification of the Qiagen DNeasy Blood and Tissue (3.5d) that we used for the insect legs. We practiced this protocol on the legs of bees and then students used these results to adapt the protocol for their insect legs collected from Unit 1 (3.5e). All of this work is in preparation for sequencing of the PCR product to produce a short string of nucleotides. We have included this work in Unit 4 along with the bioinformatics skills needed to properly analyze the sequence. Appendix 1 discusses the company used to generate DNA sequences. There are many companies across the country and world that sequence DNA for as little at $3.00 per sample. Often, shipping and handling is free and sequences are returned within 48 hours. No educator, at the high school or college level needs a sequencing facility to generate DNA barcodes. They just need to set up an account at Macrogen or Genewiz.

3.2a. DNA BARCODING POPULAR MEDIA READING

A South Pacific Island, Under the Microscope

Mo‘orea becomes a biodiversity lab as researchers catalogue the DNA of its species

Specimens from the Biocode Project at Gump Research Station, Mo'orea, French Polynesia.

Photograph by David Liittschwager, National Geographic

Tasha Eichenseher in Mo‘orea for National Geographic News
Published February 23, 2011

SPECIAL REPORT: BIODIVERSITY AND INDIGENOUS KNOWLEDGE

Portions of the once vibrant reef ringing the South Pacific island of Mo‘orea are now an apocalyptic landscape of gray rubble. Under the rich turquoise-colored surface, dead coral towers lie in pieces, blanketed with a fine layer of decay.

What has caused such trouble in paradise? A nasty invasion of armored starfish. The crown of thorns (*Acanthaster planci* or *taramea* in Tahitian), with menacing poisonous spikes and a voracious appetite, literally sucks the life out of reef communities. The starfish feast on coral polyps, leaving an empty white skeleton and ransacked home for other marine species before moving on to the next meal.

(See before and after photos of the reef.)

But thanks to unique research on this island just 12 miles (20 kilometers) northwest of Tahiti, scientists may be able to predict outbreaks like the crown-of-thorn siege. In fact, Mo‘orea could eventually serve as a model for understanding how ecosystems respond to stresses such as invasive species, climate change, and pollution.

One key is ambitious scientific research called the Biocode Project—a four-year, $5 million effort to collect, document, and genetically sequence the non-microbial biodiversity of the island. When the project wraps up this

year, it will be the first time a complex tropical ecosystem has been catalogued in such detail. Biocode scientists have come from around the world to find and "barcode" the species they specialize in—from fungi, snails, insects and plants, to algae, crabs, marine worms, and coral. DNA bar coding uses genetic markers to identify species and offers a simple, standardized way to analyze lifecycles and interactions.

"The goal is to build a catalogue of digital signatures," explains Chris Meyer, a zoologist and curator at the Smithsonian Institution. Meyer directs the Biocode Project, which will enable other scientists to more efficiently identify species, better understand how they behave and interact, and recognize how many species may actually be at risk.

"Ultimately, we want to answer the question: how much biodiversity is needed to ensure ecosystems continue to function?" says Neil Davies, Biocode's principal investigator. "It should be clear that this is a difficult question to answer if you don't know how much biodiversity you have in the first place."

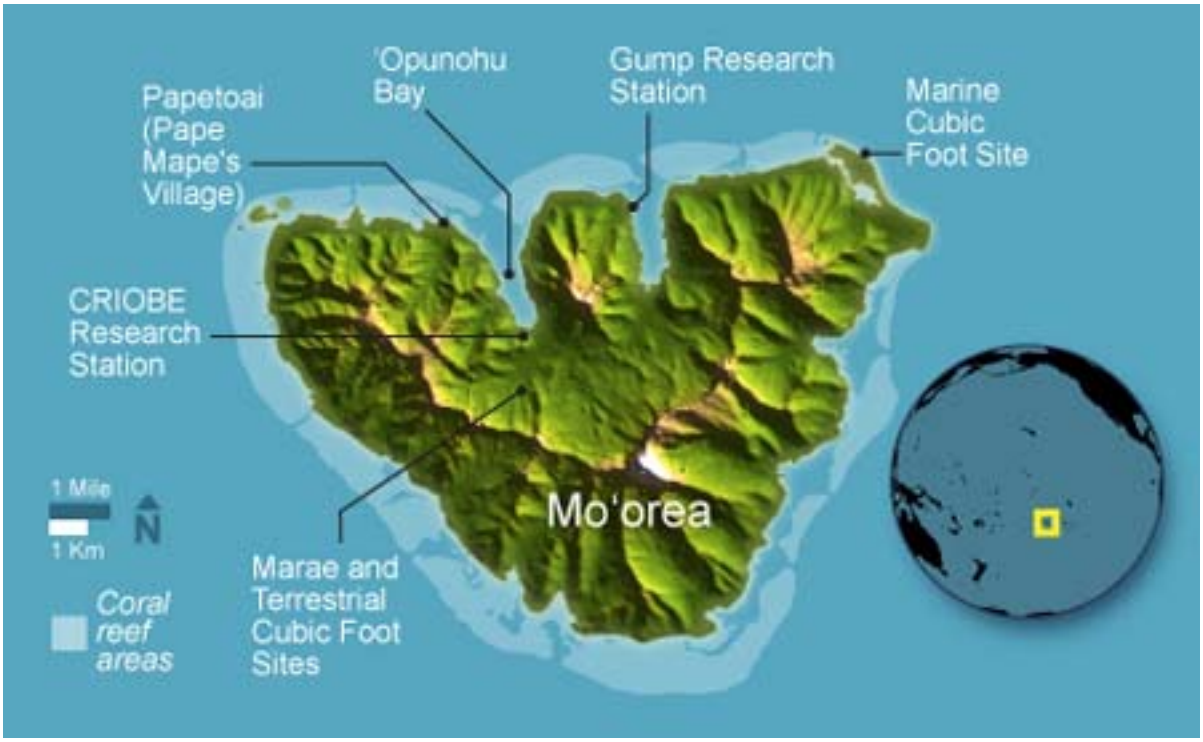


Illustration by Stephen Rountree

DNA on Ice

Mo'orea is smaller than the District of Columbia, but it's home to two prodigious research stations—University of California Berkeley's 25-year-old Gump Station, and the 40-year-old Insular Research Center and Environmental Observatory (CRIOBE) a joint venture of France's National Center for Scientific Research andSchool of Advanced Studies. Working in concert, scientists on the island have already collected more than 37,000 specimens, from which 251 algal species, 200 fungal species, 3,000 marine invertebrate species, 600 marine vertebrate species, 930 plant species, 700 terrestrial invertebrate species, and 21 terrestrial vertebrate species have emerged, so far.

(See photos of what you find in a cubic foot of Mo'orea's tropical forests.)

Meyer has spent countless hours sifting through the reef with a wide variety of sampling methods—plankton nets, baited traps, automated reef monitoring structures, vacuums—and now picking through the rubble by hand. He estimates that at least 30 percent of the marine species they've found are new to science.

Tissue and DNA have been extracted from every specimen. Some of the sample is shipped to universities and museums, and the remainder is stored at Gump. "The entire island is in there," Meyer says as he points to a six-by-

three-by-two-foot freezer, which looks like the kind you keep in your basement full of extra summer berries and frozen fish but is actually -112 degrees Fahrenheit (-80 degrees Celsius).

Why Mo‘orea?

With its forests, lagoons, reefs, and freshwater and marine habitats, Mo‘orea is a typical tropical island, but located toward the eastern end of a natural biodiversity gradient across the South Pacific, and so isn’t overwhelmingly diverse like some western Pacific islands. That’s one reason it is an ideal ecosystem for creating a comprehensive genetic catalogue of species. Biocode is, in some ways, keeping it simple.

The island also is unique in that it now has sophisticated research facilities to complement its tradition of hosting international scientists. Along with that tradition comes a long-term record of the island’s ecological trends.

Forty years ago, a French foundation wanted to send an expedition to the Pacific to study reefs, explains Serge Planes, the French scientist who directs CRIOBE and leads the Biocode team specializing in fish. “That was at the exact time army forces from France started nuclear atoll testing” on the neighboring Tuamotu archipelago, he adds. The scientific outpost was never an official monitoring effort for nuclear testing, explains Planes, but it did pave the road for researchers to come to Mo‘orea.

“Biocode is intended to help develop Mo‘orea as a model ecosystem for environmental research, as the fruit fly or mouse is a model species for biomedical research,” Davies explains. “Model species were the first to have their ‘whole genomes sequenced.’ We want Mo‘orea to be the first ‘whole ecome sequenced’.”

(Read more about the history of Mo‘orea.)

Biocode in Action

Meyer, Planes, and Davies hope the Biocode digital library of genetic barcodes, available to the public, will not only aid other scientists, but also establish Mo‘orea as the testing ground for new technologies in monitoring ecosystems and studying how species interact with each other.

Davies, whose expertise is the genetics of biological invasion, points out that being able to map genes across an entire ecosystem enables scientists to trace and mathematically analyze interactions among Mo‘orea’s species. “For example,” he explains, “food webs reveal energy flows through a system, and network theory provides one way of studying how resilient different systems are to change. We then need real-world observations and experiments to test and refine our theories. Post-Biocode Mo‘orea is a place where we can begin to do this at an appropriate scale: the whole ecosystem.”

Biocode, which is supported by a grant from the Gordon and Betty Moore Foundation, shares the Gump station with the Mo‘orea Coral Reef Long Term Ecological Research (LTER) program. Funded by the National Science Foundation, the Mo‘orea LTER was established in 2004 to determine how the reef will respond to short- and long-term disturbances, and its scientists think the Biocode data could help them better understand the interaction between fish and coral.

For instance, damselfish fertilize the reef with their waste products, and in turn, the corals provide shelter for the fish. But both the coral and the damselfish eat zooplankton. If they are competing for the same species of zooplankton, that symbiotic relationship could be harmed. The problem up until now is that “if you look at much of what’s in a coral’s stomach, or a fish’s stomach, animals that feed on things like zooplankton, the stomach contents looks like oatmeal . . . it is impossible to tell the exact species,” says Andrew Brooks, deputy program director of the Mo‘orea LTER.

But Biocode data would allow scientists to examine the stomach contents and tell if the coral and fish are vying for the same food source. “That is a major advance,” Brooks adds. “Biocode gives you a way to identify the pieces. We put what Biocode does in context.”

With continued monitoring and sampling, the Biocode database may also allow scientists to better understand biological disturbances, whether that’s crown of thorns or an invasive plant, by identifying previously unidentifiable larvae in the water,

or seeds in the soil, before they grow up to become an invasion, Meyer explains. “It allows us to use these digital signatures to see things that aren’t established yet,” he adds.

Biocode data “give us a brand new tool to address why coral reefs behave the way they do,” says Russell Schmitt, lead principal investigator for the Mo‘orea LTER. “We’re just beginning to discover the tremendous opportunities it provides.”

Reef Recovery

As for the starfish-devastated parts of the reef, scientists say they think the coral will come back. Growing populations of herbivorous fish are eating algae off the dead coral, suggesting that the system won’t remain in an algal state like other crushed reefs that have not fully recovered. “Herbivorous fish are going like gangbusters, and that’s a good sign,” Meyer explains. “Moreover there are plenty of smaller animals still living within the nooks and crannies of the reef.”

(See what the reef looked like before the crown-of-thorns invasion.)

If you ask island elders, and scientists like Planes who have been on Mo‘orea for a while, they will tell you that a crown-of-thorns invasion happens every 20 years or so. Stories of the starfish creeping over the reef shelf and into the lagoon are part of ancient island chants. Similar patterns have been recorded in Australia and elsewhere. Recovery can take more than a decade, Planes says.

(Read more about indigenous knowledge of Mo‘orea.)

The chances of recovery this time are muddied by new challenges—climate change, coral bleaching from increasing water temperatures, ocean acidification, and land use changes on Mo‘orea that could load lagoons with nutrient-rich sediments that affects fish nursery productivity. To add insult to injury, Mo‘orea’s north shore, where the starfish had their fill, was hit by Cyclone Oli in 2010, which turned much of the dead coral into rubble.

“In 2006 dead coral heads were hard to find,” Meyer says. “Now it’s ‘How many do you want?’ ” In less then four years the outer reef of the north shore went from as alive as it gets to between 2 and 5 percent live coral.

Adds Davies: “We did Biocode over a very tumultuous four years.”

This report was made possible with funding from the Christensen Fund.

3.2b. DNA BARCODING POPULAR MEDIA REACTION PAPER

Directions: For this assignment you are to find an article about DNA barcoding from a reputable news source. Read the article and construct a SOAPSTone outline using the guidelines on the reverse of this paper. On a separate sheet of paper (written or typed) include a one-page reaction paper about the article using the info you placed in the outline to help. Be sure to mention in your reaction paper the following things:

1. How does this article relate to our Science Research class?
2. Why did you choose this article? What about it interests you?
3. What is your personal reaction to the article? What did it make you think of?

SOAPSTone Outline:

Subject:
Occasion:
Audience:
Purpose:
Speaker:
Tone:

SOAPStone	Questions for Analysis
Subject:	What is the subject of the text (the general topic, content, or ideas contained in the text)? How do you know this? How does the author present the subject? Is it introduced immediately or delayed? Is the subject hidden? Is there more than one subject?
Occasion:	What is the rhetorical occasion (the time and place of the piece or the current situation)? Is it a memory, a description, an observation, a valedictory, an argument, a diatribe, an elegy, a declaration, a critique, a journal entry, or…?
Audience:	Who is the audience (the group of readers to whom this piece is directed)? Does the speaker identify an audience? What assumptions exist about the intended audience?
Purpose:	What is the purpose for the passage (the reason for its composition)? What is the speaker's purpose (the reason behind the text)? How is this message conveyed? What is the message? How does the speaker try to spark a reaction in the audience? What techniques are used to achieve a purpose? How does the text make the audience feel? What is its intended effect?
Speaker:	Who is the speaker (the voice that tells the story/makes the argument/explains the idea)? Is someone identified as the speaker? What assumptions can be made about the speaker? What age, gender, class, emotional state, education, or…?
Tone:	If the author were to read aloud the passage, describe the likely tone of voice. It is whatever clarifies the author's attitude toward the subject. What emotional sense pervades the piece? How does the diction point to tone? How do the author's diction, details, images, language, and sentence structure convey his or her feelings?

3.3a. MATERIALS CHECKLIST FOR PROMEGA GENOMIC DNA PURIFICATION

Item	Monday		Tuesday		Wednesday		Thursday		Friday	
	In	Out	In	Out	In	Out	In	Out	In	Out
1-10 µl micropipette										
20-200 µl micropipette										
100-1000 µl micropipette										
.5-10 pipette tips										
20-200 pipette tips										
100-1000 pipette tips										
Scissors										
Cube rack										
Tweezers										
Timer										
Lab Marker										
Beaker of 1.5 mL tubes										
Box of Kimwipes										
Plastic pestles										
PCR tubes with beads										
PCR tube rack										
RNAse solution										
Microscope slides										
Distilled water										
Acetone										
DNA Away										
Tube rack with all reagents										
Solid/Liquid waste beakers										

3.3b. DNA BARCODING: DNA EXTRACTION PROMEGA GENOMIC DNA PURIFICATION

Goals: By the end you should be able to:

- Extract DNA from the leaf of a plant
- Explain the purpose of the different steps of the Promega protocol

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

DNA Extraction:

STEP	COMPLETED
1. Using the fat end of a large blue tip, cut out a circle of your leaf and place it into a 1.5ml tube	
2. Label your tube with your initials and a sample number (Ex. MEB1)	
3. Add 100 µl of nuclei lysis solution	
4. Use a clean pestle to grind your sample. Grind your sample until you can no longer see any real solid parts	
5. Add 500 µl of nuclei lysis solution	
6. Incubate at 65°C for 15 minutes	
7. Add 3 µl RNase Solution, invert mix the sample 5 times	
8. Incubate at 37°C for 15 minutes, remove, let sit at room temperature for 5 minutes	
9. Label a new 1.5ml tube with your same sample code	
10. Add 200 µl of Protein Precipitate solution, Vortex for 20 seconds	
11. Centrifuge for 3 minutes at max speed (13,000-16,000g)	
12. Carefully transfer 600 µl of supernatant containing your DNA into a new labeled tube - discard tubes with pellet	
13. Add 600 µl of isopropanol to the new tube – gently mix and see if any DNA begins to precipitate out...if it doesn't that is okay.	
14. Centrifuge for 1 minute (the DNA is now in the pellet)	
15. Pipet out and dump supernatant being careful not to touch the sides of the tube (if you see a pellet, do not disturb it)	
16. Add 600 µl 70% room temperature ethanol, invert the tube 5 times	
17. Centrifuge for 1 minute (max speed)	
18. VERY CAREFULLY REMOVE THE SUPERNATANT – DO NOT DISTURB THE PELLET!!!	
19. Allow the pellet to air dry for a few minutes until the alcohol is evaporated	
20. Add 100 µl of DNA Rehydration Solution	
21. Place samples into properly labeled stock box in the refrigerator.	

At the end of the lab:

- Fill out materials checklist return and place all materials back in box top
- Wipe down counter with acetone
- All gloves get thrown out in the TRASH

DNA BARCODING: PCR AND BUFFERS

Goals: By the end you should be able to:

- Describe the PCR process including the specific primers needed for plant DNA barcoding
- Dilute a concentrated buffer solution

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

PCR:

STEP	COMPLETED
1. Obtain PCR tubes containing Ready-To-Go PCR Bead.	
2. Label the tube with your sample – you should be consistent with your numbering (Example: MEB1 (DNA) → MEB1 (PCR)	
3. Use a micropipette with a fresh tip to add 23 µL of plant primer/loading dye mix. Allow the beads to dissolve for 1 minute.	
4. Use a micropipette with fresh tip to add 2 µL of your DNA (from your DNA Extraction) directly into the appropriately labeled PCR tubes. Ensure that no DNA remains in the tip after pipetting.	
5. Pulse the sample in the microcentrifuge set up for PCR tubes for 5-10 seconds	
6. Run the UBP Protocol overnight, samples will be placed in the freezer.	

#

MAKING 1X TAE BUFFER

STEP	COMPLETED
1. Add 10ml of 50x TAE (measured with a large pipet) to the bottle	
2. Add 490 ml of dH20 (distilled water) (fill to the line of the bottle)	
3. Label the bottle using masking tape (Group # 1xTAE buffer made on date)	

At the end of the lab:

- Fill out materials checklist return and place all materials back in box top
- Wipe down counter with acetone
- All gloves get thrown out in the TRASH

DNA BARCODING: GEL ELECTROPHORESIS AND DNA VISUALIZATION

Goals: By the end you should be able to:

- Confirm your PCR product to ensure the amplitude of the plant barcode using gel electrophoresis

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

GEL ELECTROPHORESIS: Making, running, and visualizing the gel

	STEP	COMPLETED
1.	Weigh out 1 gram of agarose into a 250ml beaker	
2.	Add 50 ml 1X TAE from gel buffer jar. Swirl to mix	
3.	Microwave (IN THE LAB MICROWAVE) for 60 seconds, swirling the gel every 15 seconds (YOU MUST BE WEARING THE PROTECTIVE GLOVES)	
4.	Leave the gel to cool on the bench for about 1 -2 minutes	
5.	Add 5 µl of SYBR Safe into the cooling gel, mix in with pipet tip	
6.	Pour the gel slowly into the taped gel tray	
7.	Insert 2 combs and double check they are correctly positioned	
8.	Leave the gel to set for 15 minutes	
9.	Remove the combs and masking tape	
10.	Align your gel tray so the wells are at the black end of the gel box	
11.	Pour 1X TAE Running Buffer into gel tank until the gel is submerged. It should just cover the top of the gel by a few millimeters and the wells should be full of liquid.	
12.	Draw a gel in your notebook and number each well (there are 8 wells) – as you add your ladder and samples, record in your notebook what you add to each well.	
13.	Remove the PCR products and the DNA ladder from the freezer, thaw	
14.	Add 5 µl of the DNA ladder to the first well of the top and bottom	
15.	Add 5 µl of the PCR product into each well (Record which sample went into each well)	
16.	Put the remaining PCR product back into the freezer	
17.	Cover the gel box with the gel cover – make sure it is running from negative to positive (black to red)	
18.	Plug the lid into the power supply and turn on the power supply	
19.	Set the power supply at 120 V. Hit the RUN button on the power supply	
20.	Let the gel run for 30 minutes or until the loading dye is near the bottom of the gel – DO NOT LET THE LOADING DYE RUN OFF THE BOTTOM OF THE GEL	
21.	After 30 minutes turn off the power supply and unplug.	
22.	Remove the gel tray and slide your gel into a plastic container	
23.	Bring your gel and container to the dark room	
24.	Turn on the lamp by the gel and turn off the dark room lights	
25.	Place your gel on the UV transilluminator using the spatula	
26.	Close the lid of the UV transilluminator	
27.	Turn the UV transilluminator on and turn off the lamp	
28.	Take a picture of your gel and send it to yourself. WARNING: DO NOT LOOK AT THE UV LIGHT DIRECTLY – ALWAYS THROUGH THE LID	

DNA BARCODING: RESULTS AND TROUBLESHOOTING

Goals: By the end you should be able to:

- Interpret your gel results
- Troubleshoot possible errors during the extraction protocol

Thinking about the DNA Extraction:

1. Why do we you think it is important to use green plant tissue?

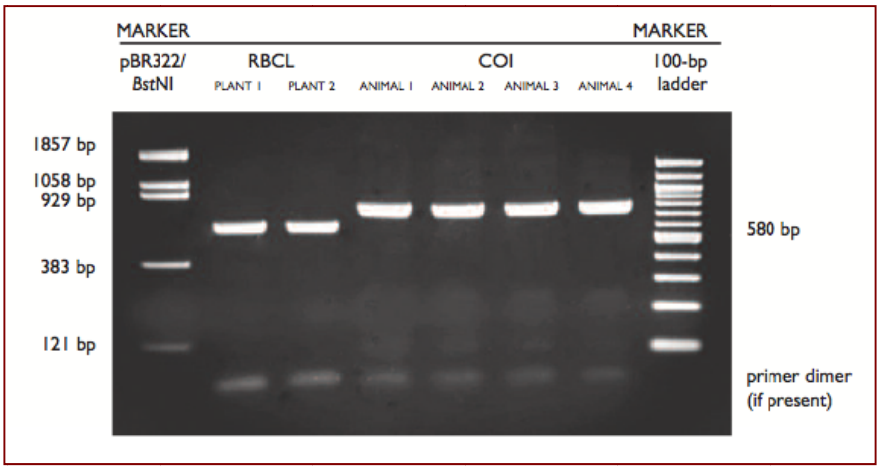
2. Why is it necessary to only use a small piece of tissue?

3. Why is it important to grind your sample very well?

4. What is the purpose of the heating and cooling steps during DNA extraction?

Analysis of Gel

1. Print or draw a picture of your gel in the space below. Compare your gel picture to the sample.



2. Explain what you see in your gel picture.

3. Which samples amplified well? Which ones did not? How do you know?

4. Did you see any primer dimers? Explain why you sometimes see them.

5. Was your extraction and amplification successful? If so, what would you do next with your samples? If not, explain three reasons why you did not get a successful result.

3.3c. MATERIALS LIST FOR QIAGEN DNEASY BLOOD AND TISSUE

Item	Monday		Tuesday		Wednesday		Thursday		Friday	
	In	Out	In	Out	In	Out	In	Out	In	Out
1-10 µl micropipette										
20-200 µl micropipette										
100-1000 µl micropipette										
.5-10 pipette tips										
20-200 pipette tips										
100-1000 pipette tips										
Scissors										
Cube rack										
Tweezers										
Razor blade										
Timer										
Lab Marker										
Beaker of 1.5 mL tubes										
Spin columns and collection tubes										
Box of Kimwipes										
Plastic pestles										
PCR tubes with beads										
PCR tube rack										
Microscope slides										
Distilled water										
Acetone										
DNA Away										
Tube rack with all reagents										
Solid/Liquid waste beakers										

3.3d. DNA BARCODING: DNA EXTRACTION QIAGEN DNEASY BLOOD AND TISSUE KIT

Goals: By the end you should be able to:

- Extract DNA from the leg of a bee
- Explain the purpose of the different steps of the Qiagen DNeasy Blood and Tissue protocol

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

DNA Extraction:

STEP	COMPLETED
1. Remove a leg from the bee and place it on your microscope slide	
2. Add 100 µl of PBS buffer to the slide on the leg	
3. Chop up the leg in the PBS buffer using the razor blade. Chop it into very small pieces	
4. Using a large pipet, suck up all of the liquid and leg bits and place into a labeled 1.5ml tube	
5. Add 80 µl of PBS buffer	
6. Using the pestle, continue to break up the tissue	
7. Add 20 µl proteinase K and 200 µl of Buffer AL	
8. Vortex and incubate for 10 minutes	
9. Add 200 µl ethanol (100%) to the sample, Vortex thoroughly	
10. Pipet the entire mixture into the DNeasy Mini spin column placed in a 2 ml collection tube.	
11. Centrifuge for 1 minute	
12. Discard the flow-through and collection tube	
13. Place the DNeasy mini spin column into a new 2 ml collection tube, add 500 µl Buffer AW1	
14. Centrifuge for 1 minute	
15. Discard the flow-through and collection tube	
16. Place the DNeasy mini spin column into a new 2 ml collection tube, add 500 µl Buffer AW2	
17. Centrifuge for 3 minute	
18. Discard the flow-through and collection tube	
19. Place the DNeasy mini spin column into a new 1.5ml tube	
20. Add 100 µl Buffer AE directly into the DNeasy membrane	
21. Incubate at room temperature for 1 minute	
22. Centrifuge for 1 minute	
23. Add 100 µl Buffer AE directly into the DNeasy membrane	
24. Incubate at room temperature for 1 minute	
25. Centrifuge for 1 minute	
26. Throw out the spin column and freeze your DNA sample	

At the end of the lab:

- Fill out materials checklist return and place all materials back in box top
- Wipe down counter with acetone
- All gloves get thrown out in the TRASH

DNA BARCODING: PCR AND BUFFERS

Goals: By the end you should be able to:

- Describe the PCR process including the specific primers needed for plant DNA barcoding
- Dilute a concentrated buffer solution

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

PCR:

STEP	COMPLETED
1. Obtain PCR tubes containing Ready-To-Go PCR Bead.	
2. Label the tube with your sample – you should be consistent with your numbering (Example: MEB1 (DNA) → MEB1 (PCR)	
3. Use a micropipette with a fresh tip to add 23 µL of insect primer/loading dye mix. Allow the beads to dissolve for 1 minute.	
4. Use a micropipette with fresh tip to add 2 µL of your DNA (from your DNA Extraction) directly into the appropriately labeled PCR tubes. Ensure that no DNA remains in the tip after pipetting.	
5. Pulse the sample in the microcentrifuge set up for PCR tubes for 5-10 seconds	
6. Run the UBP Protocol overnight, samples will be placed in the freezer.	

#

MAKING 1X TAE BUFFER

STEP	COMPLETED
1. Add 10ml of 50x TAE (measured with a large pipet) to the bottle	
2. Add 490 ml of dH20 (distilled water) (fill to the line of the bottle)	
3. Label the bottle using masking tape (Group # 1xTAE buffer made on date)	

At the end of the lab:

- Fill out materials checklist return and place all materials back in box top
- Wipe down counter with acetone
- All gloves get thrown out in the TRASH

DNA BARCODING: GEL ELECTROPHORESIS AND DNA VISUALIZATION

Goals: By the end you should be able to:

- Confirm your PCR product to ensure the amplitude of the plant barcode using gel electrophoresis

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

GEL ELECTROPHORESIS: Making, running, and visualizing the gel

	STEP	COMPLETED
1.	Weigh out 1 gram of agarose into a 250ml beaker (this is a 2% gel)	
2.	Add 50 ml 1X TAE from gel buffer jar. Swirl to mix	
3.	Microwave (IN THE LAB MICROWAVE) for 60 seconds, swirling the gel every 15 seconds (YOU MUST BE WEARING THE PROTECTIVE GLOVES)	
4.	Leave the gel to cool on the bench for about 1 -2 minutes	
5.	Add 1.5 µl of SYBR Green into the cooling gel, mix in with pipet tip	
6.	Pour the gel slowly into the taped gel tray	
7.	Insert 2 combs and double check they are correctly positioned	
8.	Leave the gel to set for 15 minutes	
9.	Remove the combs and masking tape	
10.	Align your gel tray so the wells are at the black end of the gel box	
11.	Pour 1X TAE Running Buffer into gel tank until the gel is submerged. It should just cover the top of the gel by a few millimeters and the wells should be full of liquid.	
12.	Draw a gel in your notebook and number each well (there are 8 wells) – as you add your ladder and samples, record in your notebook what you add to each well.	
13.	Remove the PCR products and the DNA ladder from the freezer, thaw	
14.	Add 5 µl of the DNA ladder to the first well of the top and bottom	
15.	Add 5 µl of the PCR product into each well (Record which sample went into each well)	
16.	Put the remaining PCR product back into the freezer	
17.	Cover the gel box with the gel cover – make sure it is running from negative to positive (black to red)	
18.	Plug the lid into the power supply and turn on the power supply	
19.	Set the power supply at 120 V. Hit the RUN button on the power supply	
20.	Let the gel run for 30 minutes or until the loading dye is near the bottom of the gel – DO NOT LET THE LOADING DYE RUN OFF THE BOTTOM OF THE GEL	
21.	After 30 minutes turn off the power supply and unplug.	
22.	Remove the gel tray and slide your gel into a plastic container	
23.	Bring your gel and container to the dark room	
24.	Turn on the lamp by the gel and turn off the dark room lights	
25.	Place your gel on the UV transilluminator using the spatula	
26.	Close the lid of the UV transilluminator	
27.	Turn the UV transilluminator on and turn off the lamp	
28.	Take a picture of your gel and send it to yourself. WARNING: DO NOT LOOK AT THE UV LIGHT DIRECTLY – ALWAYS THROUGH THE LID	

DNA BARCODING: RESULTS AND TROUBLESHOOTING

Goals: By the end you should be able to:

- Interpret your gel results
- Troubleshoot possible errors during the extraction protocol

---

Thinking about the DNA Extraction:

5. What is the challenge of using the leg of an insect?

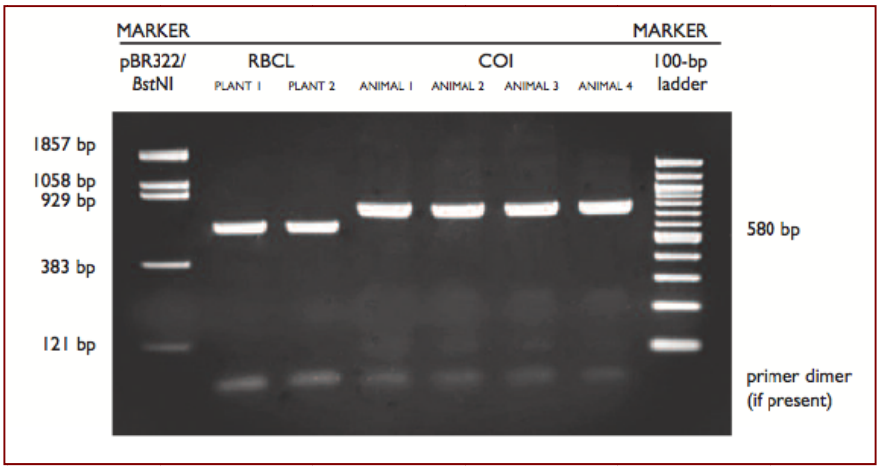
6. How does a spin column work?

7. What does each buffer do?

8. What is the purpose of the centrifugation steps during DNA extraction?

Analysis of Gel

1. Print a picture of your gel and tape it below. Compare your gel picture to the sample.



2. Explain what you see in your gel picture.

4. Which samples amplified well? Which ones did not? How do you know?

4. Did you see any primer dimers? Explain why you sometimes see them.

5. Was your extraction and amplification successful? If so, what would you do next with your samples? If not, explain three reasons why you did not get a successful result.

3.3e. DNEASY BLOOD & TISSUE PROTOCOL MODIFICATION

1. What are the parts of the protocol we can modify? How might these modifications help us extract DNA?

2. We are going to make some modifications as a class. One involves the elution buffer. ALL GROUPS WILL DO 2 ELUTIONS STEPS, EACH STEP WILL BE A 30 µl elution (instead of 100 µl).

3. As a group decide how you are going to modify the protocol and how this might increase our chances of getting DNA.

4. Record all the group modifications.

Group 1:
Group 2:
Group 3:
Group 4:
Group 5:
Group 6:

Post Questions:

Why did we change all of these protocols? How does this represent how scientists actually do their work (as opposed to the labs we sometimes perform in other classes)?

What were some challenges you experienced throughout the protocol? Did you make any mistakes? If so, what were they?

Explain the results. Which samples were successful? Which ones were not?

Based on the results, what recommendations do you have for moving forward? (Remember, our goal is to try and get DNA out of our Inwood legs)

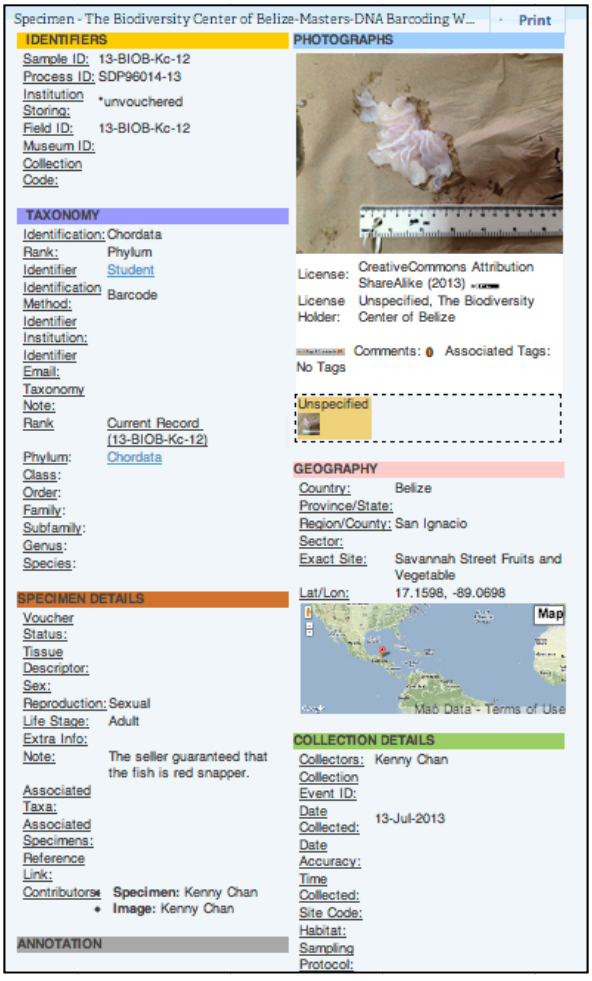
Student Generated Protocol Variations

PROTOCOL	QUIBIT READING	BAND PRESENT?
GROUP 1: BEE HEAD – 10 MIN.		
GROUP 1: BEE HEAD – 24HRS.		
GROUP 2: 10MIN – 10ul DNA, 15ul PRIMER		
GROUP 2: 24HRS. – 10ul DNA, 15ul PRIMER		
GROUP 3: ABDOMEN – 10MIN.		
GROUP 3: ABDOMEN – 24HRS.		
GROUP 4: WHOLE LEG – 10MIN.		
GROUP 4: WHOLE LEG- 24HRS.		
GROUP 4: SMALL BITS – 10MIN.		
GROUP 4: SMALL BITS 24HRS.		
GROUP 5: HYMENOPTERA PRIMER – 10MIN.		
GROUP 5: HYMENOPTERA PRIMER – 24HRS.		
GROUP 6: SONICATE – WHOLE LEG – 10MIN.		
GROUP 6: SONICATE – WHOLE LEG – 24HRS.		
GROUP 6: SONICATE – LEG BITS – 10 MIN.		
GROUP 6: SONICATE – LEG BITS – 24HRS.		

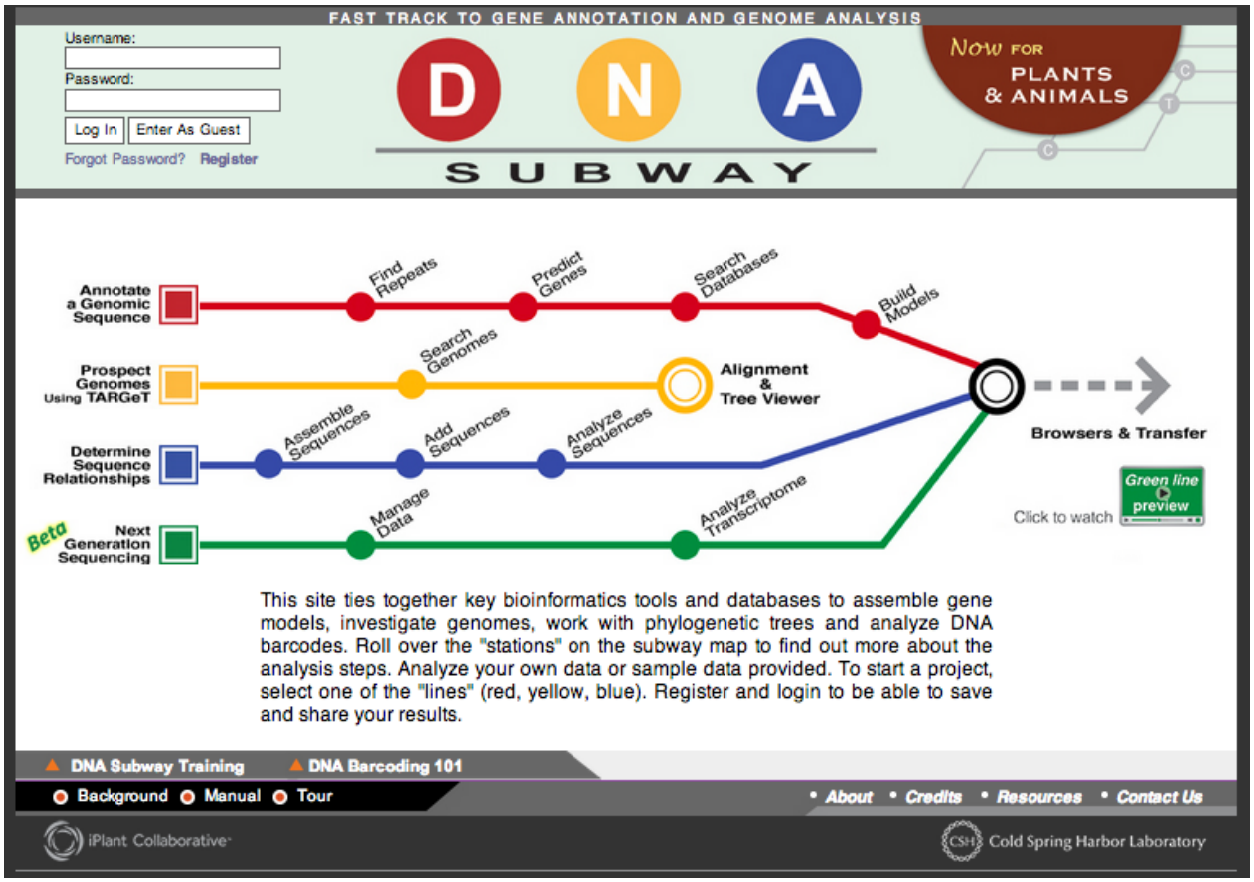
UNIT 4. Analyzing DNA Barcodes

1. *Introduction to Bioinformatics*: In order to analyze the DNA sequences, students must be familiar with the basics of bioinformatics. Bioinformatics uses computer programs to analyze large amounts of data (like DNA sequences). Genbank is the online database that stores all publically available DNA sequences. This first lesson (4.1a) introduces students to bioinformatics through an activity that investigates the phylogenetic relationship of different species of bears based on DNA sequences.

2. *Using The BOLD Student Data Portal*: The Barcode of Life Data Systems Student Data Portal is a powerful tool to store information about your sample. Sample information including location, collector, notes, images, and a map, are all organized on one page. BOLD also allows users to clean up their sequence data and input clean sequences and trace files onto sample pages. BOLD has great video tutorials and a user-friendly guidelines for students and educators.



3. *DNA Subway*: DNA Subway is a bioinformatics pipeline that was developed by Cold Spring Harbor Laboratories. It allows users to create new projects, upload sequence data and trace files, clean up sequences, and BLAST sequences to identify species. DNA Subway can also build phylogenetic trees using Neighbor Joining or Maximum Likelihood methods. DNA Subway's manual is very helpful in moving students through the interface.


FAST TRACK TO GENE ANNOTATION AND GENOME ANALYSIS
Username:
Password:
Log In
Enter As Guest
Forgot Password?
Register
D N A
SUBWAY
Now FOR PLANTS & ANIMALS
Annotate a Genomic Sequence
Find Repeats
Predict Genes
Search Databases
Build Models
Prospect Genomes Using TARGeT
Search Genomes
Alignment & Tree Viewer
Determine Sequence Relationships
Assemble Sequences
Add Sequences
Analyze Sequences
Beta
Next Generation Sequencing
Manage Data
Analyze Transcriptome
Browsers & Transfer
Click to watch
Green line preview
This site ties together key bioinformatics tools and databases to assemble gene models, investigate genomes, work with phylogenetic trees and analyze DNA barcodes. Roll over the "stations" on the subway map to find out more about the analysis steps. Analyze your own data or sample data provided. To start a project, select one of the "lines" (red, yellow, blue). Register and login to be able to save and share your results.
DNA Subway Training
DNA Barcoding 101
Background
Manual
Tour
About
Credits
Resources
Contact Us
iPlant Collaborative
Cold Spring Harbor Laboratory

4.1a. MOLECULAR PHYLOGENETICS

STEP 1: OBTAIN THE FOLOWING SEQUENCES FROM GENBANK

American black bear	Y08520
American brown bear	L21889
Spectacled bear	L21883
Asiatic black bear	L21890
Polar bear	L22164
Giant panda	Y08521

(1) Enter Genbank: www.ncbi.nlm.nih.gov
(2) Select Nulceotide on the pull-down search menu.
(3) Enter accession number (e.g. Y08520) and select Go
(4) Select FASTA to view the data
(5) View the nucleotide sequence for this species in FASTA format
(6) Highlight and copy the entire sequence including the ">"and paste into phylogeny.fr (Step 11)
- Do this for each Accession number and any other sequences you want to add (THEY MUST BE THE SAME GENE!!!!)

STEP2: GET THE NUCLEOTIDE DATA INTO PHYLOGENY.FR SO THAT WE CAN ANALYZE IT
(7) Open Phylogony.fr in a new tab (http://www.phylogeny.fr/)
(8) Go to Phylogeny Analysis and click "Advanced"
(9) Switch the choice from all at once to step by step
(10) Click Create Workflow
(11) Paste the FASTA sequence you copied into the textbox
(12) When done inputting data click submit
(13) Scroll through the Alignment Results to see where in the genetic sequences match and where there are differences
(14) Go down to the bottom of the page and click next step
(15) Do not change any of the Curation settings, just click submit
(16) Scroll through the Curation Results to see where the sequences matched. How is it different from the alignment?
(17) Go down to the bottom of the page and click next step
(18) Leave the settings alone, and click submit
(19) Look at your Phylogeny Results
(20) Scroll down and click submit
(21) Click submit again
(22) What does the Phylogenetic Tree tell you? What can you infer about the evolutionary relationship between the species you have viewed?

UNIT 5. Developing Original DNA Barcoding Research Proposals

1. *Searching the Scientific Literature*: Students can practice searching the scientific literature using Google Scholar. Google Scholar allows students to access articles that are available as a PDF. Practice literature searches help students determine the articles that are most relevant to their research interest. We developed an activity where students brainstorm some of their interests and then use a Google Scholar search to try and find related scientific papers (5.1a). Students are then encouraged to read the abstracts (and more if available) of multiple papers and begin to generate their own research questions (5.1b). From these activities, students can narrow down their interests to one question. From this question they can compile a series of articles to conduct their literature review.

2. *Reading the Scientific Literature*: After students generate researchable questions they can narrow their options down and find articles related to their specific subject. Reading scientific literature is challenging. In our curriculum we have supported student reading with the CREATE method developed by Dr. Sally Hoskins. We have adapted her work to meet the needs of high school students (5.2a). The CREATE method helps students break down the sections of a scientific paper so they can better understand the purpose and information in each section.

3. *Writing a Literature Review:* There are many guides that can help students write up a literature review. One we have found useful is Dr. Roby's Completely Doable 10-Step Plan for Writing a Literature Review.

4. *Developing a Research Proposal*: We end the course with student's developing their own DNA barcoding research questions and writing up a research proposal. Included is the proposal description (5.4a), a proposal rubric (5.4b), and a proposal presentation rubric (5.4c).

5.1 a. SEARCHING FOR SCIENTIFIC ARTICLES

1. What are some general organisms that interest you?

2. What are some general topics (related to the class themes – environmental, molecular) that interest you?

3. What types of ecosystems interest you?

4. Create different combinations of search terms using your ideas from above. Write down 5 different sets of search terms below (Example: spiders and DNA barcoding and temperate forests).

5. Go into Google Scholar and search your terms from above. Find one article for each term that looks interesting and record the citation information (authors, title, journal, year, volume, issue, pages)
Example:
Search terms: Spiders and DNA barcoding and temperate forests
Citation: Rowan D.H Barrett, Paul D.N Hebert, **Identifying spiders through DNA barcodes**, *Canadian Journal of Zoology*, 2005, 83(3): 481-491.

1. **Search Terms**
   Citation:
2. **Search Terms**
   Citation:
3. **Search Terms**
   Citation:
4. **Search Terms**
   Citation:
5. **Search Terms**
   Citation:

5.1b. GENERATING RESEARCH QUESTIONS

Read and scan the abstracts and articles, especially the introduction and conclusion to generate new research questions. Record five of your own questions.

For each question above answer the following:

1. What do you expect? What is your hypothesis?
2. Why does this study matter? Who cares?

Question 1:

Question 2:

Question 3:

Question 4:

Question 5:

5.2a. CREATE PROTOCOL

C.R.E.A.T.E. Step	*What am I doing?*	*I will learn to:*	*What goes in my CREATE Notebook*
***C**onsider*	•***Read the Introduction*** •Construct **CONCEPT MAPS** that identify the major concepts discussed in the introduction •Examine the relationships between the independent and dependent variables	• Relate old and new knowledge • Define what I do and don't know about a topic • Review to fill gaps in my knowledge	• CONCEPT MAP • 10 SENTENCES THAT SUMMARIZE INFORMATION FROM YOUR CONCEPT MAP • GLOSSARY OF RELEVANT VOCABULARY
***R**ead*	•***Read the Methods & Materials*** •**DRAW CARTOONS** that model the methods researchers used to collect their data	• Visualize the experiments by representing "what went on in the field/laboratory" • Link specific methods to specific data • Describe *how* researchers generated the data	• CARTOONS/DRAWINGS THAT ILLUSTRATE THE METHODS AND MATERIALS USED IN EACH EXPERIMENT
***E**lucidate hypotheses*	•Propose a testable hypothesis for each figure/table	• Define, in my own words, the question being asked or hypothesis being tested in experiments related to each figure or table	• HYPOTHESIS FOR EACH FIGURE/TABLE
***A**nalyze and interpret the data*	☐ ***Read Results*** ☐ Analyze data to identify what researchers actually found ☐ Identify interesting findings or results ☐ Predict which results researchers will focus on in the Discussion ☐ ***Read Discussion/Conclusion*** ☐ Identify important findings ☐ Describe how researchers explain their results	• Actively engage with data • Determine the significance of each figure • Determine the logic of each experiment • Define controls and determine their role • Relate data presented to results derived • Debate the significance of the data, defend my own ideas, and intelligently criticize the authors' interpretations	• 10 FIGURE ANNOTATIONS FOR EACH FIGURE/TABLE • COMPLETED ANALYSIS TEMPLATES FOR EACH FIGURE/TABLE • BULLETED LIST OF 10 PREDICTED DISCUSSION POINTS • BULLETED LISTS OF ACTUAL DISCUSSION POINTS
***T**hink of the next* ***E**xperiment*	☐ Design an experiment or study that follows up on the current research ☐ Cartoon the follow-up study's procedure for in-class discussion	• Recognize research as a never-ending process • Exercise creativity in experimental design • Consider that multiple options exist; science is not necessarily linear and predictable	• HYPOTHESIS FOR NEW EXPERIMENT • DESCRIPTION OF NEW EXPERIMENT • DRAW CARTOON OF METHODS

5.4a. DNA BARCODING PROPOSAL DESCRIPTION

For your final project, you will choose a DNA barcoding research topic that you will develop through the end of the semester based on your observations and questions. The first step is to develop a proposal for your research topic. Scientists develop proposals before they begin their research and to find funding for their projects. You will work in teams of 1-3 to write a proposal and prepare a presentation for the class.

IMPORTANT DATES:
Your proposal is due
Your presentation is due
Final presentation will be

PROPOSAL OUTLINE:
Your proposal should be typed, double-spaced, and should use the basic section outline and subheadings highlighted in **bold font** below:

KEYWORDS: List 3 -5 words that describe the topic of your research

TITLE: Use your keywords to clearly and concisely describe the content of your proposal

RESEARCH QUESTION(S): What question(s) are you trying to answer with your research?

HYPOTHESIS: What are your predictions for your research? Why do you expect this result?

INTRODUCTION

a. Why is this research question important? Think Big Picture! What work has previously been done on this topic? Use facts, statistics, and primary literature references to back up your statements.
b. Provide background information and literature to set a context for why you are choosing this project and why it is important to conduct your research project. Use information that relates to your project and helps to explain its importance including information about urban ecosystems, your organism(s), and DNA barcoding.

METHODS

a. Provide details of how you will approach your study. Use the Urban Barcode Protocol as well as the literature to help you organize the sections of your proposal including (Study Site, Sample Collection, DNA Extraction, DNA Amplification, Sample Sequencing and Sequence Analysis.
c. Remember that these methods might change between now and when you finish your project, but do the best you can with what you know now.
d. Is there anyone that you need to contact in order to help you obtain samples? What organization/people are studying what you are interested in studying? Who are the primary researchers working on DNA barcoding of your organism? Where are they located? Where do they conduct their research?

STUDY PLAN (TIMELINE)

a. Give a general plan for each month from June to January.

REFERENCES

a. Find 5 to 8 primary literature references. Cite all references you have used in the proposal. Most of your references will come from the Introduction and Methods sections. Use APA citations for your references.

5.4b. DNA BARCODING PROPOSAL PAPER RUBRIC

Criteria	Points	Comments
Key Words: (5 points) • Used words that are descriptive and informational		
Title: (5 points) • Clearly describes the nature of the study		
Research Question: (10 points) • Original question(s) that is clear and specific		
Hypothesis: (10 points) • Your expected results • Why you expect these results		
Introduction: (30 points) • Provides sufficiently broad background info • Provides rationale (Why is this important?) • Provides a context (What has already been done?) • Uses facts and statistics • Citations within the text in proper format • Goals and objectives of the study are clearly stated		
Methodology: (25 points) • Methods are directly aimed at testing the stated hypothesis • Methods are feasible • Identifies the data to be collected • Identify the primary researchers and organizations • Proposes using an unbiased, quantitative approach • Procedures appear to be replicable		
Timeline: (5 points) • Research schedule identifies when all steps of the project will be completed		
References: (10 points) • Listed in scientific journal format (APA) • Listed alphabetically • Uses primary literature		
Organization and Style: (20 points) • Uses headings and subheadings to visually organize paper • Few errors in spelling, punctuation and grammar • All required elements are present and additional elements that add to the proposal (e.g., graphs, tables, figures) • Proposal handed in on time		
Total Points: (120 points)		

5.4c. DNA BARCODING PROPOSAL PRESENTATION RUBRIC

CATEGORY	4	3	2	1
Presentation Requirements	All required parts to the presentation are present including background, methods, proposed data collection, study plan, and references.	All but one of the required parts to the presentation is present, including background, methods, proposed data collection, study plan, and references.	All but two of the required parts to the presentation are present, including background, methods, proposed data collection, study plan, and references.	More than two parts of the required parts to the presentation are missing.
Research Question	Research question(s) is original, related to the topic and is very specific and clear.	Research question(s) is original, sort of related to the topic and is very specific and clear.	Research question(s) is original and related to the topic but is not specific or clear.	Research question(s) is not original, related to the topic and/or specific.
Hypothesis	Hypothesis is an "educated answer" to the research question based on observations and previous research that includes an explanation as to why you think that will happen.	Hypothesis is an "educated answer" to the research question but not based on observations or previous research that includes an explanation as to why you think that will happen.	Hypothesis is an "educated answer" to the research question based on observations and previous research but does not include an explanation as to why you think that will happen.	Hypothesis is an "educated answer" to the research question but not based on observations and previous research and is does not include an explanation as to why you think that will happen.
Significance of Study	There is a significance and rationale to your study that clearly relates to your topic and is beneficial to society.	There is a significance and rationale to your study that indirectly relates to your topic but is beneficial to society	There is a significance and rationale to your study that relates to your topic but is not necessarily beneficial to society	There is no significance and rationale to your study that relates to your topic and it is not necessarily beneficial to society
Methods	Methods test your hypothesis, are clearly organized, original or based on previous research and repeatable.	Methods test your hypothesis, are not clearly organized but are original or based on previous research and repeatable.	Methods test your hypothesis, are clearly organized, but are not original or based on previous research or repeatable.	Methods do not test your hypothesis, but are clearly organized, original or based on previous research and repeatable.
Graphics and Animation	All graphics are relevant and contribute to the understanding of the topic and animations do not distract from the presentation.	Some graphics are relevant and contribute to the understanding of the topic and animations do not distract from the presentation.	Some graphics are relevant and contribute to the understanding of the topic and some animations distract from the presentation.	The graphics and/or animations are distracting throughout the entire presentation.
Slides	All slides are easy to read as a result of good backgrounds, text length, font size and font color.	Some slides are easy to read as a result of good backgrounds, text length, font size and font color.	Many slides are not easy to read as a result of poor use of backgrounds, text length, font size and/or font color.	Most slides are not easy to read as a result of poor use of backgrounds, text length, font size and/or font color.
Preparedness	Students are completely prepared and have obviously rehearsed.	Students seems pretty prepared but might have needed a couple more rehearsals.	The students are somewhat prepared, but it is clear that rehearsal was lacking.	Students do not seem at all prepared to present.
Volume	Volume is loud enough to be heard by all audience members throughout the presentation.	Volume is loud enough to be heard by all audience members at least 90% of the time.	Volume is loud enough to be heard by all audience members at least 80% of the time.	Volume often too soft to be heard by all audience members.
Speaks Clearly	Speaks clearly and distinctly all (100-95%) the time, and mispronounces no words.	Speaks clearly and distinctly all (100-95%) the time, but mispronounces one word.	Speaks clearly and distinctly most (94-85%) of the time. Mispronounces no more than one word.	Often mumbles or cannot be understood OR mispronounces more than one word.
Posture and Eye Contact	Stands up straight, looks relaxed and confident. Establishes eye contact with everyone in the room during the presentation.	Stands up straight and establishes eye contact with everyone in the room during the presentation.	Sometimes stands up straight and establishes eye contact	Slouches and/or does not look at people during the presentation.
Comprehension	Students are able to accurately answer all questions posed by classmates about the topic.	Students are able to accurately answer most questions posed by classmates about the topic.	Students are able to accurately answer a few questions posed by classmates about the topic.	Students are unable to accurately answer questions posed by classmates about the topic.

REFERENCES

Barcode of Life Data Systems Student Data Portal
http://www.boldsystems.org/index.php/SDP_Home

Cold Spring Harbor Laboratories
http://www.cshl.edu/

Cold Spring Harbor DNA Learning Center
http://www.dnalc.org/

National Geographic News - **A South Pacific Island, Under the Microscope**
http://news.nationalgeographic.com/news/2011/02/110223-biodiversity-moorea-biocode-barcoding-genetic-sequencing-ecosystem/

Urban Barcode Project
http://www.urbanbarcodeproject.org/

Sally Hoskins – CREATE
http://teachcreate.org/

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APPENDIX

APPENDIX 1. Molecular Equipment, Reagents (primers), Costs, and Budget Schedules

For all lab setup options, if there will be any transportation of materials, we highly recommend Pelican cases. They are extremely durable, watertight, and contain foam inserts for snug packing.

Product Name	Company	Product #	Quantity	Cost
Pelican 1620 Case with Foam for Camera (Black)	Pelican	1620 case	2	$217.70

Basic DNA Lab

- Bare minimum for successful DNA extraction, PCR amplification, and sequencing
- Good for group of 10-15 students and ~100 samples
- Everything can be stored and used at room temperature

Product Name	Company	Product #	Quantity	Cost
Vortexer with round attachment	Fisher Science	S96519	1	$413.25
Dry Heat Block	Fisher Science	11-715-125DQ	1	$622.50
Fisher Scientific Isotemp Heating Blocks	Fisher Science	11-718-23Q	2 ($137.50/block)	$275.00
Microcentrifuge	Fisher Science	13-100-675	1	$1,914.00
Edvotek Thermocycler	Fisher Science	S68640	1	$1,950.00
E-GEL GO 2% STARTER KIT EA	Life Technologies	G4402ST	1	$660.00
E-GEL GO 2% GELS (20 Pack)	Life Technologies	G442002	1	$98.00
Illustra Pure Taq Ready-To-Go PCR Beads	Fisher Science	46001014	1 (100 rxns)	$122.31
Promega Wizard Genomic DNA Purification Kit	Promega	A1120	1 (100 rxns)	$161.00
Primers (COI, RBCL)*	Integrated DNA Technologies	Custom DNA Oligos	1 pair forward and reverse primers ($10.00/primer)	~$20.00
DNA Sequencing	Macrogen	EZseq	20-30 ($6.00/sample)	~$150.00
Cryo-Boxes	Fisher Science	03395464	5	$30.85
0.1-10 µl TipOne natural tip, racks, sterile	USA Scientific	1111-3810	1 (960 tips)	$57.50
1-200 µl TipOne natural tip, racks, sterile	USA Scientific	1111-0810	1 (960 tips)	$57.50
1000 µl TipOne natural tip, racks, sterile	USA Scientific	1111-2830	1 (960 tips)	$61.00
Seal-Rite 1.5 ml microcentrifuge tube, natural, sterile	USA Scientific	1615-5510	1 (500 tubes)	$23.75
Powder-free nitrile gloves, cobalt blue, box	USA Scientific	4914-3300	1	$23.25
Pestle for 1.5 ml microcentrifuge tubes	USA Scientific	1415-5390	1 (100 pestles)	$66.00
Isopropanol (Rubbing Alcohol) 70%	Any Drug Store	N/A	1 bottle	~$3.00
Ethyl Alcohol (Ethanol), 70%,	Fisher Science	S25306A	1	$38.00

denatured				
0.2 ml TempAssure PCR tube, attached frosted flat cap, natural. DNase, RNase, DNA, PCR inhibitor, and pyrogen free	USA Scientific	1402-8100	1 (1000 tubes)	$49.25
Dual rotor personal microcentrifuge	USA Scientific	2641-0016	1	$295.00
1.5 ml microcentrifuge tube racks	Bio-Rad	166-0481EDU	1 (5 racks)	$67.15
0.2 ml PCR tube racks with cover	Bio-Rad	TRC-0501	1 (5 racks)	$38.00
Parafilm	Fisher Science	13-374-10	1	$61.79
Sterile Water	Fisher Science	BP5611	1 (1 Liter)	$56.54
Petri Dish for dissection	Fisher Science	08-757-14	1 (100)	$198.80
Chemical resistant lab markers	Fisher Science	22-026-700	1 (12 markers)	$71.28
Dissecting Kit	Fisher Science	S06843	5 ($9.50/kit)	$47.50
Set of Micropipettes (0.5-10, 5-50, 20-200, 100-1000)	Fisher Science	21-377-328	3 ($1,018.00/set)	$3,054.00
			Total Cost	**$10,686.22**

Advanced DNA Lab

- Equipment for a fully functioning DNA lab
- Good for an entire class of student DNA barcoding research (32 students)
- Good speed and efficiency from DNA extraction to sequencing

Product Name	**Company**	**Product #**	**Quantity**	**Cost**
Vortexer with round attachment	Fisher Science	S96519	1	$413.25
Dry Heat Block	Fisher Science	11-715-125DQ	2 (622/unit)	$1,244
Fisher Scientific Isotemp Heating Blocks	Fisher Science	11-718-23Q	4 ($137.50/block)	$550.00
Eppendorf Microcentrifuge 5424	Fisher Science	05-403-93	1	$3.034.50
Analytical Balance	Fisher Science	01-912-401	2 (2,379/unit)	$4,758.00
Microscope	Fisher Science	12-561-3D	5 ($1,391.73/unit)	$6,958.65
S1000™ Thermal Cycler with Dual 48/48 Fast Reaction Module	Bio-Rad	185-2148	1	$6,895.00
E-GEL GO 2% STARTER KIT EA	Life Technologies	G4402ST	1	$660.00
E-GEL GO 2% GELS 20 Pack	Life Technologies	G442002	1	$98.00
Traditional Gel Electrophoresis Kit	Bio-Rad	166-0706EDU	1 (for 32 students)	$2,728.57
Electrophoresis reagent package	Fisher	S94620	5 (108/package)	$540.00
Illustra Pure Taq Ready-To-Go PCR Beads	Fisher Science	46001014	5 (122.31/unit)	$611.55
Promega Wizard Genomic DNA Purification Kit	Promega	A1120	5 (161/unit)	$805.00
Primers (COI, RBCL)*	Integrated DNA Technologies	Custom DNA Oligos	5 pair forward and reverse primers ($10.00/primer)	~$100.00
DNA Sequencing	Macrogen	EZseq	100-200	~$900.00

			($6.00/sample)	
Cryo-Boxes	Fisher Science	03395464	5	$30.85
0.1-10 µl TipOne natural tip, racks, sterile	USA Scientific	1111-3810	2 (960 tips, 57.50/box)	$115.00
1-200 µl TipOne natural tip, racks, sterile	USA Scientific	1111-0810	2 (960 tips, 57.50/box)	$115.00
1000 µl TipOne natural tip, racks, sterile	USA Scientific	1111-2830	2 (960 tips, 61.00/box)	$122.00
Seal-Rite 1.5 ml microcentrifuge tube, natural, sterile	USA Scientific	1615-5510	2 (1000 tubes, 23.75/unit)	$47.50
Powder-free nitrile gloves, cobalt blue, box	USA Scientific	4914-3300	9 (23.25/box)	$209.25
Pestle for 1.5 ml microcentrifuge tubes	USA Scientific	1415-5390	5 (500 pestles, 66.00/unit)	$330.00
Isopropanol (Rubbing Alcohol) 70%	Any Drug Store	N/A	1 bottle	~$3.00
Ethyl Alcohol (Ethanol), 70%, denatured	Fisher Science	S25306A	1	$38.00
0.2 ml TempAssure PCR tube, attached frosted flat cap, natural. DNase, RNase, DNA, PCR inhibitor, and pyrogen free	USA Scientific	1402-8100	1 (1000 tubes)	$49.25
Dual rotor personal microcentrifuge	USA Scientific	2641-0016	3 (295/unit)	$885.00
1.5 ml microcentrifuge tube racks	Bio-Rad	166-0481EDU	2 (10 racks, 67.15/unit)	$134.30
0.2 ml PCR tube racks with cover	Bio-Rad	TRC-0501	2 (10 racks, 38/unit)	$76.00
Parafilm	Fisher Science	13-374-10	1	$61.79
Sterile Water	Fisher Science	BP5611	2 (1 Liter, 56.54/bottle)	$113.08
Petri Dish for dissection	Fisher Science	08-757-14	5 (100, 198.8/pack)	$994.00
Chemical resistant lab markers	Fisher Science	22-026-700	1 (12 markers)	$71.28
Dissecting Kit	Fisher Science	S06843	10 ($9.50/kit)	$95.00
Set of Micropipettes (0.5-10, 5-50, 20-200, 100-1000)	Fisher Science	21-377-328	5($1,018.00/set)	$5,090.00
			Total Cost	**$35,842.32**

* Reference List for Primers

- Kress WJ, Erickson DL (2007) A Two-Locus Global DNA Barcode for Land Plants: The Coding *rbcL* Gene Complements the Non-Coding *trnH-psbA* Spacer Region. *PLoS ONE* 2(6).
- Ivanova et. al. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology* Notes 7, 544-548.
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- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 360: 1847–1857.

Appendix 2. Qubit Protocol

The Qubit is a Fluorometer that allows you to test your DNA extraction prior to running PCR and gels. This is a great piece of equipment for a well-funded laboratory as it saves both time and money. Here is a protocol we developed for our students to check the success of their DNA extractions.

Qubit Protocol and Worksheet

1. Set up 2 tubes for the standards (Standard #1 and Standard #2). Label each.
2. Set up 1 tube for each sample you have. Label each
3. Prepare the Qubit working solution:
    a. Qubit Buffer: Calculate 199 µl x sample number (include the 2 standards)
        i. Total samples = _____ x 199 µl = _____
    b. Qubit Reagent: Calculate 1 µl x sample number (include the 2 standards)
        i. Total samples = _____x 1 µl = _____
    c. Add the volume from (a) and the volume from (b) into a 15ml tube – this is the Qubit working solution
4. Prepare your standards:
    a. Add 190 µl of working solution into your Standard #1 and Standard #2 tubes
    b. Add 10 µl of Standard #1 to the Standard #1 tube
    c. Add 10 µl of Standard #2 to the Standard #2 tube
5. Prepare your samples
    a. Add 199 µl of working solution to each of your labeled tubes
    b. Add 1 µl of your extracted DNA to the properly labeled tube.
6. Vortex all tubes for 2-3 seconds
7. Incubate at room temperature for 2 minutes
8. Turn on the Qubit
9. Follow the directions on the machine to read each standard.
10. Place your first sample in and read. Repeat
11. Record readings for each sample on your group data sheet.

Use the space below to draw out the procedure using the class data (Follow the picture on the Qubit protocol I gave you yesterday, just add in actual numbers:

Appendix 3: Sample Student Proposals

Endangered Meats in Ethnic Markets

Introduction

DNA Barcoding is a way to identify species using a fast and efficient process. In order to do this, we need to take a genetic sequence that comes from the genome. This process gives people the ability to identify animals from different samples using DNA markers. For example, if you took the DNA from a beetle, you will be able to identify which specific type of beetle you took the DNA from. We will be using this process to identify different types of meats from ethnic food markets to see if they are labeled correctly. Many meats have been labeled to be one thing but later have been identified as a species of meat that is in danger of becoming extinct (Pappin). There have been multiple occurrences of this happening within the United States (Pappin).

Many people are unaware of this happening because when they sell these endangered meats in markets they do not label them correctly, or they label them in another language. This is very noticeable within Chinatown and other locations that are known for selling food in other languages. These meats could be very detrimental to the people that consume them because they could carry diseases that are very dangerous to Americans (BBC news). Also we have a right to know exactly what we are eating. When they mislabel the meats that they are selling they are not allowing us to actually know what we consume. When using DNA barcoding we will be able to identify which species of meat we have collected from markets and whether or not they were labeled correctly. Also if we find that the meat was not labeled correctly then we will continue to do further research to see if the meat we collected is endangered or not and also if the meat is legal to be consumed in the United States or not.

In the United States, there have been many other locations that sell endangered meats (Schwartz). For example, in California they marked food as sushi but it actually was whale meat. Many other similar examples have happened. The majority of these endangered species are able to be found in Chinese markets. For example, in a Chinese medicine shop they sell bear claws, dried scorpions, umbilical cords from donkeys, and many other different types of animals that in America seems taboo. Our project is to go to these ethnic food markets to collect as much as we can and see if the products they are selling are legal.

Procedure

In order to do the barcoding for the experiment, we are going to collect samples from different ethnic food markets. All the samples will be collected throughout New York City, as many boroughs as necessary to understand how widespread it is. We'll collect the samples and we will label each sample corresponding to the location where we obtained it. Once we've gotten all of our samples from the markets, we are going to take our samples to the High School of Environmental Studies laboratory.

In the laboratory, we are going to work to extract the DNA from our samples. In order to extract the DNA we would have to crush our samples to a pulp and use the Promega Wizard Genomic DNA purification kit. This protocol breaks apart cellular components and then removes RNA, proteins, and other contaminants until leaving pure DNA for further analysis. We will take the DNA extract and go through the process of PCR, which is taking the DNA and amplifying it. When doing this we will use a specific primer for the COI gene. This gene is similar enough across species to be able to line sequences

up, but different enough to be able to identify individual species. Once this is done we should be able to identify whether the meat was properly labeled by comparing sequences to databases like NCBI and the Barcod of Life.

Timeline

The process of collecting the samples and doing the DNA extraction and PCR will be a very time consuming task. We plan on collecting the samples throughout the fall. Through this time period we should have collected an amount of meat that is satisfactory for our experiment. We hope that this will take little time so that we could start on the DNA extraction process. We should be able to start working on this within the ending of November and December as well. We also expect to work on the PCR as well.

References

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Genetic Diversity of the Bed Bug Species in New York City

Introduction:
The method used to identify, name, and classify organisms is built on a taxonomic system invented 250 years ago by Carl Linnaeus. The identification method has been modified many times over the years, but is largely based on morphology (Stoeckle and Janzen 2003). DNA barcoding is a recent and powerful tool in the science community which is being used for classification. One of its major benefits is ease of use, and it is allowing people not trained in classification techniques the opportunity to identify species. It also facilitates many population genetics and molecular phylogenetic studies, as explained in "DNA barcoding: how it complements taxonomy, molecular phylogenetic and population genetics" (Hajibabaei et al. 2004). DNA barcoding is an efficient way of identifying species and an increasing number of scientists have been using it in their studies exploring biodiversity. To accommodate their experiments, scientists use DNA barcoding to identify the organism's species. With more advanced technology DNA barcoding is being used as a faster, easy to use tool for classifying biodiversity on earth.

Metropolitan areas are dominated by very dense human populations and man-made structures that often replace natural habitats. In urbanized areas, anthropogenic activities are more prevalent, therefore resulting in greater pollution emissions and alteration of the natural environment. It is important to study urban ecosystems because scientists can establish a better understanding of urban environments and human effects on them. Our experiment aims to find the overall genetic variation in the bed bug species, the biodiversity of our collected samples, and the similarities and differences with previous studies on bed bugs. We will use DNA barcoding to distinguish the samples we collect, and then further study if there is a genetic variation within the species. Our experiment also aims to indicate new invasive species of bed bugs in the New York metropolitan area.

Bed bugs have been found to carry 28 human pathogens, but no one has shown that they can transmit them to their hosts (Harlen 2006). They are hematophagous insects and adult bed bugs are 6-7 mm long and oval and reddish-brown (Harold J. Harlen 2006; Szalanski et al. 2008). Bed bugs are closely linked with humans partly because they can be transported very easily by humans through luggage and clothing. Over the course of a few years, bed bug infestations have been on the rise worldwide and are starting to plague New York City (Wang and Wen 2011). NYC is home to about 8 million people and is potentially in danger from too many bed bugs. The city is also one of the most visited tourist destination in the world making it easy for bed bugs to become introduced here. For example, recently there have been outbreaks of bed bugs in New York City hotels and these infestations can scare away many tourists and therefore cause economic problems for the city.

Specific Aims:
We will call Manhattan exterminators to obtain 50 bed bug samples that will be preserved in ethanol to prevent DNA breakdown. We will extract the DNA from the samples and use PCR (polymerase chain reaction) to amplify the DNA. We will then use gel electrophoresis to check if we actually extracted a clean sample of bedbug DNA. Once we determine the quality of our sample, we will send our samples to the Dolan DNA Laboratory for DNA sequencing. The lab will send back the DNA sequences and we will create DNA barcodes for our bed bug samples. Our goal is to indicate any new invasive bed bug species in NYC and discover the biodiversity of our bed bug samples and the overall genetic variation of the species.

Methods:

Sampling Collection – Samples will be collected from Broadway Exterminators (782 Amsterdam Avenue), who agreed to send us 50 specimens. To preserve the collection, samples will be placed in plastic containers filled with ethanol.

DNA Extraction/PCR/Gel Electrophoresis - To extract DNA from the samples, Promega Wizard Genomic DNA Purification Kit will be used. To amplify the DNA samples, polymerase chain reaction (PCR) will be used. The samples will be placed in the Bio-Rad MJ Mini PCR machine. Before placing the samples into the PCR machine, primers LR-J-13007 and LR-N-13398 will be added. Gel electrophoresis will be used to test the quality of the samples. It cuts the DNA samples into fragments that will run through a well. The electric current pulls the negatively charged DNA to the positive end of the well. The smaller fragments travel further and faster towards the positive end of the well, whereas the larger fragments travel slower.

DNA Barcoding Analysis– The DNA samples will be sent to Dolan DNA Laboratory. The laboratory will read the samples and sequence it. The sequenced samples will be used to compare with other gene sequences from Barcode of Life Data System. From the comparison with BOLD, samples will be analyzed by looking at variation between species. Variations within the samples will also be analyzed.

Estimating and Identifying Species of the *Nycticebus Coucang* (Slow Loris) In Southeast Asia

Introduction:

One of the most accurate and reliable processes used within the scientific community to identify a specific species is DNA barcoding (Herbert et al, 2010). Essentially, it is an alternative process that a taxonomist can take in order to label or identity an unknown organism. Renowned for its 95% accuracy in correctly classifying species, it gives both scientists and non-experts the ability to correctly recognize specific species or even identify new ones (Herbert et al. 2005). Despite phenotypic similarities between organisms (cryptic species) – DNA barcoding can often correctly differentiate between them. In addition to species identification, DNA barcoding also provides taxonomists with genetics of the organism as well. While the method may be straightforward enough for anyone to understand, it allows taxonomists to organize specimens and gives novel insights into the evolutionary history of a species.

Identifying species is crucial to our understanding of biology because it provides a clear outlook on the biodiversity in a region. With DNA barcoding, the identification is done rapidly and effectively, and can be used to answer many questions. For example, it can be used to monitor the effects of climate changes, identify invasive species, and it can help us understand how many species of plants and animals there actually are. This last point is important, because according to "Using DNA Barcodes to Identify and Classify Living Things", there are still millions of species that have not been identified.

We plan on using DNA barcoding for identifying various species of slow lorises (*Nycticebus coucan*) in Southeast Asia. Slow lorises are strepsirrhines primates that consist of 5 validated species. They have extremely large eyes and colors that vary from species to species. Anthropogenic activities in this region of the world have caused a decline in the populations of many of the slow loris species. People are currently selling them in the illegal pet trade as well as using them for medicinal purposes that reflect some tribal beliefs. By being able to track individual species of Slow Loris, scientists may be able to help in the conservations of them and identify potential hotspots of illegal activity.

Purpose:

Slow lorises are extremely vulnerable to the effects of deforestation and hunting. Studies have also shown that slow lorises are in high demand in Southeast Asia in terms of domestication as well as in the medical field. Anthropogenic activity in the region due to cultural beliefs of the Southeast Asian people has caused the decrease in slow loris populations. A major issue concerning these animals in Southeast Asia is their illegal trade, with Vietnam being the main center of this system. There are two islands on which the slow loris population is most concentrated, Borneo and Sumatra. There are many cases in which the slow lorises from these two islands are sold and traded on the mainland. When a government official monitoring imports and exports encounters a tradesman with the possession of a slow loris, they are confiscated and released without knowledge of which island they originally came from. This causes a major problem because certain species can only live in the habitat from where they came from, due in part to genetic factors. Due to different adaptations, the slow lorises are not able to survive in other environments. DNA barcoding can help determine the origin of the confiscated species, and this enables us to track their location and transport them to their original habitat to ensure their survival.

Specific Aims:

1. We will develop a protocol that would effectively utilize primers, and incorporate modern DNA barcoding techniques, to efficiently barcode the Slow Loris species.
2. We will identify species of slow lorises from previously collected samples and try to identify any cryptic species.
3. We will also look for genetic variations specific to individual islands and regions in order to aide in conservation efforts.

With the help of our mentor we will collect approximately 50 samples primarily from Southeast Asia. This will include relatively fresh samples and older museum samples. We will then extract the DNA from these samples using the Promega Wizard Genomic DNA Extraction Kit. We will use polymerase chain reaction (PCR) to amplify this DNA for future analyses we use. We will be amplifying the COI gene, a mitochondrial gene commonly used in DNA barcoding. We will also use gel electrophoresis to separate the DNA fragments and identify proper amplification of the slow loris COI gene. Once we contain a positive sample, we will take this DNA and sequence it at the museum with our mentor. This information can help us identify invasive species as well as the origin of these species, and help prevent the population decline occurring due to the illegal trading of these animals in Southeast Asia.

Methods:

Sample Collections: We will request samples with the assistance of our mentor, Ph.D Postdoctoral student, Mary Blair, from the Museum of Natural History. We will also exchange samples with several foreign contacts who could potentially supply us with slow lorises from various other locations, primarily in Southeast Asia. We will collect 50 samples of the specimen. Throughout the project, we will label and take pictures of the samples to ensure proper identification.

DNA Extraction and Data Analysis: We then will proceed to the physical extraction of the DNA, which will be preformed with the Promega Wizard Genomic DNA Purification Kit. In order to amplify (replicate) the samples, polymerase chain reaction, (PCR), will have to be performed on the samples. Primers for COI will be added to the samples. These samples will be placed in the Bio-Rad MJ Mini thermocycler and amplified. Once this is finished, the bands will be visualized through gel electrophoresis, which we will perform at the high school. Finally, we shall have the samples sequenced at the Museum of Natural History. We will analyze samples using the barcode of life database and software recommended by our mentor.

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Appendix 4: DNA Barcoding Workshop Protocol

Goals: By the end you should be able to:

- Extract DNA from a piece of animal tissue

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Gather materials
- Set up your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with 70% Ethanol

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with 70% Ethanol
- Always change your tips

DNA Extraction:

STEP	COMPLETED
1. Add 10–20mg of fresh or thawed tissue and place it into a 1.5ml tube (The size of a pencil eraser)	
2. Label your tube with your initials and a sample number (Ex. MEB1)	
3. Add 100 µl of nuclei lysis solution	
4. Use a clean pestle to grind your sample. Grind your sample until you can no longer see any real solid parts	
5. Add 500 µl of nuclei lysis solution	
6. Incubate at 65°C for 30 minutes	
7. Add 3 µl RNase Solution, invert mix the sample 5 times	
8. Incubate at 37°C for 15 minutes, remove, let sit at room temperature for 5 minutes	
9. Label a new 1.5ml tube with your same sample code	
10. Add 200 µl of Protein Precipitate solution, Vortex for 20 seconds	
11. Centrifuge for 4 minutes at max speed (13,000-16,000g)	
12. Carefully transfer 600 µl of supernatant containing your DNA into a new labeled tube - discard tubes with pellet	
13. Add 600 µl of isopropanol to the new tube – gently mix and see if any DNA begins to precipitate out...if it doesn't that is okay.	
14. Centrifuge for 1 minute (the DNA is now in the pellet)	
15. Pipet out and dump supernatant being careful not to touch the sides of the tube (if you see a pellet, do not disturb it)	
16. Add 600 µl 70% room temperature ethanol, invert the tube 5 times	
17. Centrifuge for 1 minute (max speed)	
18. VERY CAREFULLY REMOVE THE SUPERNATANT – DO NOT DISTURB THE PELLET!!!	
19. Allow the pellet to air dry for a few minutes until the alcohol is evaporated	
20. Add 100 µl of DNA Rehydration Solution	
21. Incubate at 65°C for 45 min or Place samples into properly labeled stock box in the refrigerator overnight.	

At the end of the lab:

- Fill out materials checklist return and place all materials back in box top
- Wipe down counter with acetone
- All gloves get thrown out in the TRASH

DNA Barcoding: PCR and BUFFERS

Goals: By the end you should be able to:

- Describe the PCR process including the specific primers needed for plant DNA barcoding
- Dilute a concentrated buffer solution

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Wipe down countertop with acetone and paper towels
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with DNA Away

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with DNA Away
- Always change your tips

PCR:

STEP	COMPLETED
7. Obtain PCR tubes containing Ready-To-Go PCR Bead.	
8. Label the tube with your sample – you should be consistent with your numbering (Example: MEB1 (DNA) → MEB1 (PCR)	
9. Use a micropipette with a fresh tip to add 18 µL of sterile H20. Allow the beads to dissolve for 1 minute.	
10. Use a micropipette with fresh tip to add 1 µL of your Forward primer directly into the appropriately labeled PCR tubes. Ensure that no primer remains in the tip after pipetting	
11. Use a micropipette with fresh tip to add 1 µL of your Reverse primer directly into the appropriately labeled PCR tubes. Ensure that no primer remains in the tip after pipetting	
12. Use a micropipette with fresh tip to add 5 µL of your DNA (from your DNA Extraction) directly into the appropriately labeled PCR tubes. Ensure that no DNA remains in the tip after pipetting.	
13. Pulse the sample in the microcentrifuge set up for PCR tubes for 5-10 seconds	
14. Run the Protocol through lunch, samples will be placed in the freezer.	

DNA Barcoding: Gel Electrophoresis and DNA Visualization

Goals: By the end you should be able to:

- Confirm your PCR product to ensure the amplitude of the barcode using gel electrophoresis

First steps:

- Put on gloves (you will only get one pair of gloves each day)
- Get box of materials
- Set up materials on your lab bench
- Use the checklist to make sure all of your materials are accounted for
- Wipe down all pipets, tweezers with 70% Ethanol

To reduce contamination:

- Always keep on your gloves
- Always wipe down pipets with 70% Ethanol
- Always change your tips

GEL ELECTROPHORESIS: Making, running, and visualizing the gel

STEP	COMPLETED
29. Remove the gel cassette from the package and gently remove the comb.	
30. Insert the gel cassette into the E-Gel® Go! Base and close the cover. The status indicator LED illuminates with a steady red light if the cassette is correctly inserted.	
31. Open the cover and load 10 µL DNA Ladder in the first well	
32. In a separate tube, add 1 µl of PCR product and 9 µl of steril H20	
33. Load 10 µL of sample into each well	
34. Load 10 µL of deionized water into any empty wells	
35. Close the cover and press the start button. The LED light will turn green	

Prepare for sequencing

Sequencing: Sending sample and Primer to Macrogen

STEP	COMPLETED
1. Obtain a clean 1.5 ml tube and new Macrogen barcode label	
2. In a separate tube, add 5 µl PCR product	
3. Add the total 5 µl of sample to the Macrogen 1.5 ml tube	
4. Add 5 µl of Forward primer to the Macrogen tube	
5. Seal the tube and attach the barcode label and store in the refrigerator	

STEP	COMPLETED
6. Obtain a clean 1.5 ml tube and new Macrogen barcode label	
7. In a separate tube, add 5 µl PCR product	
8. Add the total 5 µl of sample to the Macrogen 1.5 ml tube	
9. Add 5 µl of Reverse primer to the Macrogen tube	
10. Seal the tube and attach the barcode label and store in the refrigerator	

#

DNA Barcoding: Results and Troubleshooting

Goals: By the end you should be able to:

- Interpret your gel results
- Troubleshoot possible errors during the extraction protocol

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Thinking about the DNA Extraction:

1. Why do we you think it is important to use fresh animal tissue?

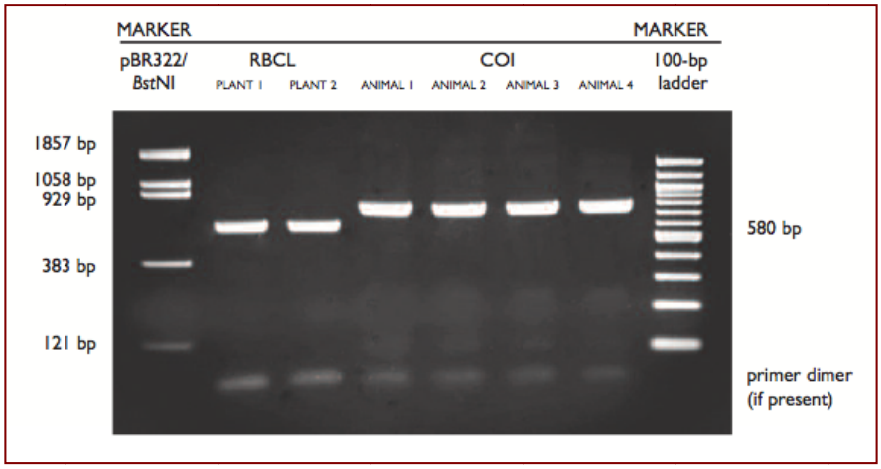
2. Why is it necessary to only use a small piece of tissue?

3. Why is it important to grind your sample very well?

4. What is the purpose of the heating and cooling steps during DNA extraction?

Analysis of Gel

1. Compare your gel picture to the sample below.



2. Explain what you see in your gel picture.

3. Which samples amplified well? Which ones did not? How do you know?

4. Did you see any primer dimers? Explain why you sometimes see them.

5. Was your extraction and amplification successful? If so, what would you do next with your samples? If not, explain three reasons why you did not get a successful result.

Appendix 5: Guide to DNA Yields for DNA Barcoding

Promega DNA extraction

- DNA yield = 15,000 – 20,000 ng
- Elute DNA in 100 µl
- Final concentration ~ 150 ng/µl

Ready-To-Go PCR

- Requires 50 ng – 1,000 ng of DNA
- We should add 5 µl of DNA (750 – 1000 ng)
- Yield from PCR is ~ 12,000 ng – 20,000 ng
- 25 µl final volume, so 480 – 800 ng/µl

Egel GO electrophoresis

- Requires 100 – 500 ng of sample DNA in each well (optimum is 100 ng)
- Dilute PCR product (1 µl PCR product in 9 µl H20)
- Dilute further if gel is smeared

Macrogen Sequencing

- Requires 5 µl of PCR Product at 50 ng/µl (Total 250 ng PCR Product)

Primers

- Make 100 X stock solution
- Then add 10 µl of 100 X stock in 90 µl of H20 to make 10 X working solution (10 pmol/µl)

Appendix 6: Field Work Student Agreement Form

Risk factors and issues to prepare for in the field

- Transportation to and from the site for all students
- Inclement weather the day of trip
- Enough adult supervision for students
- Communication between all groups while in the field (students often work out of eyesight and hearing)
- First aid: Are any students allergic to bees, are there first aid kits for minor scrapes, bruises, or injuries. Wipes to soothe poison ivy.
- Student conflict

I, _____(Name) acknowledge that voluntary participation in collection field trips involves inherent risks and hazards. This may include physical injury from exposure to hazards such as adverse weather conditions, insects and wildlife, poisonous plants, rough and unknown terrain, moderately strenuous exercise, and transport to and from field sites. I acknowledge that all risks cannot be prevented and I am physically capable in undertaking these activities.

Furthermore, I acknowledge that I will abide by the (Your High School) Code of Student Conduct while on this trip. Specifically, I will avoid any behavior that negatively affects the comfort, well-being, or learning environment of myself or others on the trip or the general public.

_____ _____

Participant's printed name Date

_____

Participant's signature

_____

Emergency contact

_____

Contact's phone number

Biodiversity of Invertebrates at Inwood Hill Park's Beach Ecosystem

Abstract:

This study focuses on the biodiversity of insects in New York City. The study was done in Inwood Hill Park which is an urban park in northern Manhattan. Inwood Hill Park is a living piece of New York's history which has the only salt marsh (beach) left in NYC. The ecosystem investigated was the beach which was at the tip of Inwood. The study was to catch as many insects as possible and study them to find out the ecosystem's biodiversity. Before going to Inwood Hill Park, Group 5 expected to find many small insects such as ants and flies. However Group 5 did not expect to find flying invertebrates like butterflies and bees. Four different kinds of traps were set up to catch the insects- pitfall traps, yellow food plates, kill jars and, sweep nets. After the insects were captured, they were brought to the lab to be analyzed, identified, and pinned. After those three tasks were completed, the biodiversity of the beach ecosystem was calculated and recorded. Using the biodiversity calculation, Group 5 came up with questions and a reflection about the invertebrate study.

Keywords:

Biodiversity, Keystone species, Ecosystem, Invertebrates, Transect, Species Richness, Simpson's Diversity Index, DNA barcoding

Introduction:

Biodiversity is important to the environment because it helps people understand how much of a variety of different species of insects and other organisms there are in a specific ecosystem. If there is no biodiversity, a species won't be able to thrive. All the species in an ecosystem depend on one another and if only one species exists, it cannot survive on its own. Biodiversity is beneficial because it allows people to know that there are different varieties of species and that there isn't just one type of species that flourishes in that specific area. There are different species that depend on one another for survival, so if there is a small biodiversity and one species dies out, the others may also be in jeopardy. That's why having a large biodiversity is important, so that if a species dies out, then the whole chain of invertebrates and plants can still prosper.

Biodiversity is important because it could give information on how many organisms are in a particular species. If the biodiversity is very low, people can learn how to protect endangered species that are part of that low biodiversity. They can protect those species by not polluting and not killing them. If the biodiversity is high, people can still protect different species so the biodiversity will remain high. The biodiversity is very important because it determines how stable an ecosystem is and how stable the different species are in that ecosystem.

Inwood Hill Park is a living piece of New York's history. This park has been operated by New York City Department of Parks and Recreation since 1926 in upper Manhattan, New York City. Inwood spreads over 196.4 acres of land from Dyckman Street to the Hudson River (which is located on the tip of Manhattan). Those 196.4 acres have encountered much of New York's history. For example in the

seventeenth century, Native Americans inhabited the eastern edge of the park (*Department of Parks & Recreation*). The Native Americans, also known as Lenape, used the Hudson and Harlem River for food sources and survival. In addition, their fire remains (ashes) left valuable artifacts showing their temporary homes around Inwood. Also, before the New York City Department of Parks and Recreation purchased the park in 1926, post- Revolutionary Wars such as Cox's Hill occurred on the land. Evidence of this is shown in the criss-cross pattern across Dyckman (*Your Guide to Inwood, NY History and Department of Environmental Conservation*).

When it comes to what's inside of Inwood Hill Park (Fauna), it depends on the different ecosystems throughout the park such as the salt marsh and upper successional forest. For example, in the salt marsh, many water birds like Mallards and Ring- billed gulls can be found, in addition to flies, mosquitoes, dragonflies and mollusks. However, in more wooded areas such as the upper and lower successional forest, a more wide variety of birds and insects can be found. Instead of water birds, there are Blue Jays, Cardinals, beetles, and butterflies. In conclusion, Inwood Hill Park is full of a wide variety of insects, birds and bugs.

The ecosystem for insect collecting was the beach at Inwood Hill Park. The beach was located at the very top of the park, bordering the Hudson River. The beach was very small and it had some tall grasses in one area (Figure 1(a)). It led to a railroad where trains occasionally passed by. Another area of the beach consisted of rocks that were halfway in the water. The rocks that were not under the water were covered in kelp and plants. The beach also led to a forest (Figure 1(b) and 2). Most of the insects that lived at the beach were very small like flies and ants. Small shrimps lived under the soil, too. There were also crabs that lived under the rocks near the water. Lastly, there were many birds flying around the water, especially ducks.

If there is a large diversity in an ecosystem, it is very important because there is a variety of organisms that help the environment in many ways. The bigger the diversity of invertebrates, the more the ecosystem thrives because everything depends on one another. An example of an invertebrate that helps the environment is the bumblebee. The bumblebee is a very important insect to the environment because it transports pollen everywhere it goes. If pollen is not transported around the environment, the diversity of plants will not grow. The bumblebee is an example of a Keystone Species because it helps the environment by increasing the biodiversity of plants.

The purpose of this study is to find the amount of diversity of invertebrates at Inwood Hill Park's only beach. This experiment is an important study because it gives information about the different amounts of species at a beach in the New York area. It gives people an insight about the amount of animals that live and thrive there. Group 5 expected to find various insects in different regions at Inwood Hill Park, but important information was found about invertebrates at the beach which was used to calculate the biodiversity and find out what people need to do to protect it as a whole. By gathering invertebrates, Group 5 can calculate how much of them are in a specific area, like the beach. The main purpose is to look at each of the different regions and conclude on what people can do- to stop overpopulation or invasion of habitats, and to protect the environment so different species will be able to survive.

(a)

(b)

Figure 1 (a). *The left side of the beach.* ***(b)*** *The right side of the beach.*

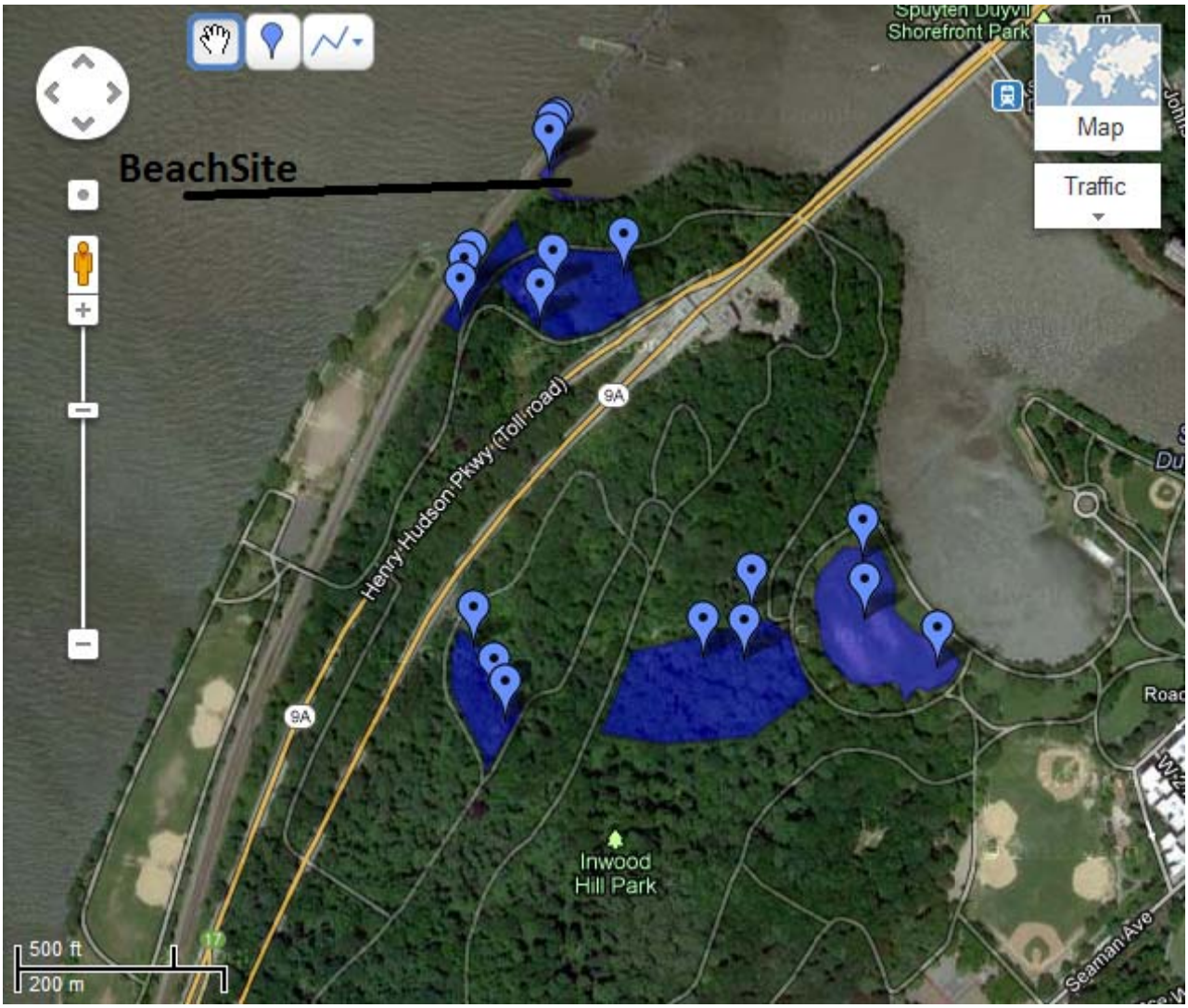


Figure 2. A map of Inwood Hill Park' ecosystems and sampling locations.

Methods:

Collection:

Seeing that the beach ecosystem was smaller than other ecosystems such as the upper and lower successional forests, a transect was determined to be the most appropriate method. The transect extended across the length of the beach, from where the rocks started all the way to a steep hill. In width, the transect extended from the water to the edge of the forest. Within the transect, the group placed a variety of traps.

Once observing the beach environment, four different types of traps were set. Those four traps were sweeping nets, kill jars, pitfall traps, and yellow food plates. The sweeping nets acted like a big strainer that went through the air and bushes around the transect. Once something was caught within the net, the insect was carefully transferred into a kill jar with two or three drops of 99% ethyl acetate (a volatile substance). This substance is a type of poison that suffocates the insects until they are dead. Next up, pitfall traps were put into the ground to catch any crawling insects (Figure 3b). Five blue cups were filled one fifth of the way with Isopropyl Alcohol (Trip 1) or cranberry juice (Trip 2) and placed into the ground at five different distinct locations. When the insects fell into the alcohol or cranberry juice, they would drown in the substance (Figure 3a). Finally, five yellow food plates were set around the beach. These plates were filled with rotting oranges, apples, and bananas sweetened with sugar. When these baits were set in the plate, honey was spread around it. The yellow plates, honey, and fruits were supposed to attract insects because of its eye popping color and sweet smells/tastes (Figure 3a).

After capturing the insects, they were separated into different jars or envelopes with labels (Figure 4) and placed into the freezer. The freezer would stop the decomposition of the insect's deoxyribonucleic acid (DNA) so it could be preserved for DNA barcoding.

When it came time to start organizing and labeling the beach collection, each insect had to be properly identified so the information could be accurate in order to find the diversity of insects at the beach ecosystem. The insects were identified to the level of Order using "The Practical Entomologist". To pin the insects, a pin was placed through the thorax of each given insect leaving ten mm from the top of the pins. Next came labeling- Group 5 made labels for each insect which contained a specific format (Figure 4). Back right legs were removed for future DNA barcoding. This process will allow for more detailed identification of organisms using molecular techniques and will be conducted in the spring.

Calculating Biodiversity:

The biodiversity was calculated using the Simpson's Diversity Index. The Simpson's Diversity Index shows how much biodiversity there is in an ecosystem and how many species there are (species richness). The group first counted up all of the different species in the collection (Figure 7). Then, the number of different species was put in a chart. The total number came out to be 15 (Figure 6). This number represents the species richness of the collection. The Simpson's Diversity Index was calculated by using a formula: $D = n(n-1)/N(N-1)$. The small n represented the total number of organisms of a particular species. The big N represented the total number of organisms of all the species. For Group 5, n was equal to 36 and N was equal to 22. By using the formula, the Simpson's Diversity Index came out to be 0.922 (Figure 5).

(a)


(b)

Figure 3 (a). *These were five yellow food plates and three pitfall traps after being set for three hours.* ***(b)*** *A mosquito (Diptera) caught using the sweeping net and placed in the kill jar.*

Results

USA, New York
40.877467° N, 73.926412° W
Date captured
Who caught the bug
Order
DNA Barcode #

Figure 4. This is how each label for an insect looked like.

Step 1	D= 32/ 22 (22-1)
Step 2	D= 36/ 462= 0.078
Step 3	I= I-D= 1- 0.078= 0.922
Index=	0.922

Figure 5. These were the steps taken to determine the Simpson's Diversity Index.

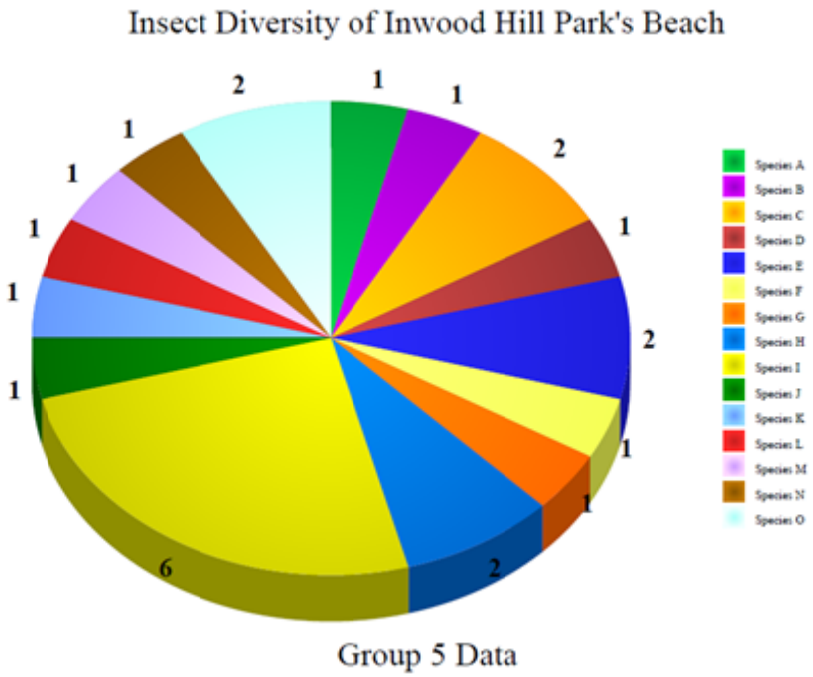


Figure 6. This is a pie chart differentiating the different species and the amount of each species.

Figure 7. The complete collection of insects from Inwood Hill Park's Beach.

Discussion and Conclusions

Biodiversity is very important to all ecosystems because it increases the stability of the ecosystem. Each species has an important role to play in the environment. If only one species exists, then the environment won't thrive.

This study's results were varied because of the different insects that were caught. Many small insects were caught during the two trips. The small insects included ants, flies, and shrimps. Most of these small insects were caught using the yellow food plates and the pitfall traps. There were also large insects that were caught by the sweep nets. These large insects were bees, wasps, and butterflies.

Small insects were caught more than large insects because of the environment that they lived in which was the beach. Many small insects like to hide in the trees and grasses that were at the beach. They also like to hide under small plants on the ground. Since the traps were on the ground, that is why mostly small insects were caught. Not that many flying insects like to live on the beach because of the waves and other large animals that live there. Since most of the insects were small, it was very hard to pin them and not break any of their body parts at the same time. But, all of the insects were eventually pinned and the final collection consisted of over 15 insects.

In this study, there were some errors and limitations. One error was that the traps didn't work to their full potential. Some of the traps didn't catch many insects because of the materials used. Alcohol nor the cranberry juice attracted insects to the pitfall traps. Another error happened during pinning the insects. While pinning, some of the insects' abdomens broke off because of the pressure put on the thorax which makes the pressure push out, causing it to disperse outward.

These errors might have impacted our results because if better materials were used for the traps, more insects may have been caught. Also, if the traps were set up in different places along the beach (under trees, next to rocks), the number of insects caught could have been larger.

If this study were to be improved, there will be many modifications. The number of traps will increase because there will be a better chance of catching more insects. There will also be a more variety of traps because different insects might be attracted to different traps. The transect of the beach will be extended because the insects can live in different parts of the beach. Last, the amount of time waiting for the traps will be shortened because some insects can escape from the traps.

If this study were done again with these modifications, there will be many questions about the outcome. One question might be- What kinds of traps attract insects like butterflies and spiders? Another question can be- Should a person of the group always keep watch of the traps in case any insects escape? The

last question may be about biodiversity- Since some insects, move from place to place, could the biodiversity of many species be affected from the movement of insects? These questions can have many answers to them and they can be answered if this study at Inwood Hill Park were done again and improved on.

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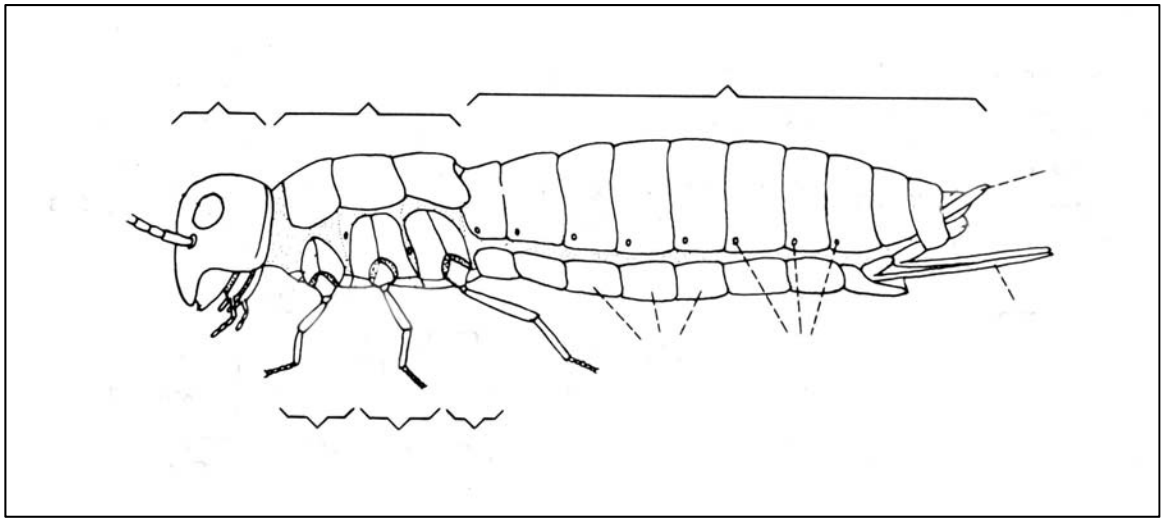
Appendix 8: Insect Morphology Worksheet

Insect Morphology

Name_____

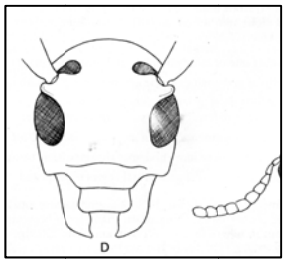
Using the drawing below, write in the three parts of the insect body. Add names of additional parts that you remember.

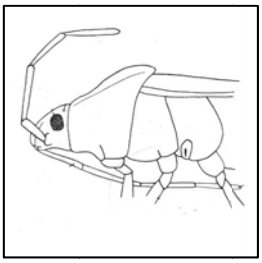
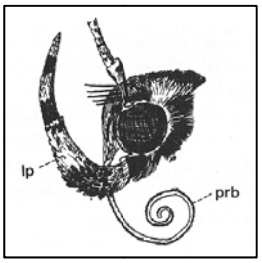


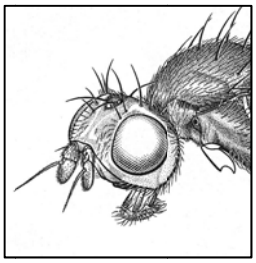
Observe the specimen that you were given. Draw a picture of your organism below.

For your specimen describe:

1) Mouthparts:

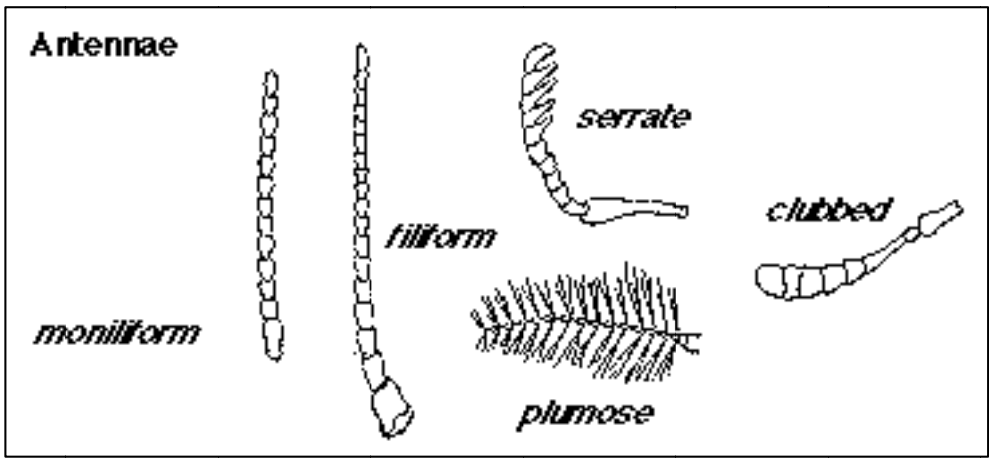




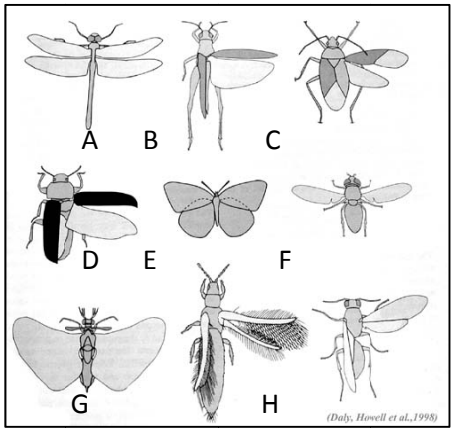
My insect has _____ mouthparts.

2) Antennae



My insect has _____ antenna.

3) Wings



My insect has wings like insect _____ because they are_____
_____
_____
_____

4) Using all of this information, try to determine the order of insect you have.

My insect is in Order _____.