**Instructor Play by Play Instructions: DNA Barcoding project**

**Logistics (2 weeks before; Time: 5 minute)**

Point of contact information:

Contact Bruce Nash

nash@cshl.edu

Contact Bruce to request reagents and sequencing costs from Gene Wiz. Please provide the sampling date, number of students, number of samples, and the type of organism to barcode. Be sure to provide a mailing address and the reagents will be sent 1-2 weeks prior to activity happening.

You should receive:

1. PCR beads
2. PCR mix

Provide background material to students (1 week before in-class activity; 10 minutes)

1. This is a video which introduces students to the concept of using DNA as method for exploring biodiversity and the process of DNA barcoding:

<https://www.youtube.com/watch?v=swrEP3NcGVw&feature=emb_logo>

1. This video shows students how to use the BOLD database student portal

<https://www.youtube.com/watch?time_continue=2&v=k6FEtQ9haRA&feature=emb_logo>

1. This publication discusses the use of DNA barcoding to uncover biodiversity in urban areas via education

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0199015

**In the field (45 minutes)**

1. Designate a sampling location for your class. You will need the following equipment:
	1. Falcon tubes (smaller ones would work)



* 1. 70% ethanol (to preserve organisms)
	2. Optional items to maximize obtaining a wide diversity of insects include:
		1. Malaisse trap (set up overnight with light source)
		2. D-net (to use in freshwater streams to capture macroinvertebrates)
		3. Sweep nets (to use in and around vegetation for terrestrial insects)
1. Have students label their tube using a pen (do not use Sharpie’s as ethanol wipes away!) and take a picture of the organism in the field with the picture uploaded to iNaturalist class page
2. Have students place organism in tube and cover specimen with ethanol

**Documenting images (1 hour)**

* Number your samples and create an excel sheet designating each sample to the student
* Take pictures of each specimen with a phone camera or better camera with a ruler in the image for size reference
* Upload your images to iNaturalist
* Make a shared Google drive folders where you also upload pictures for student access

**Laboratory processing (6 hours)**

*DNA extraction (2 hours)*

 Items you need:

1. Bunsen burner
2. Flame starter
3. Scissors
4. Gloves
5. Tweezers
6. 100% Ethanol
7. 1.8 ml microtubes
8. Small plastic pestels
9. Incubator
10. Fume hood

Steps to follow:

1. Number 1.8 ml microtubes with your samples
2. Flame your scissors and tweezers
3. Let these cool
4. Remove your insect and cut off the tail (in some cases, e.g. spiders put entire organisms if small)
5. Place tail in tube and add Proteinase K and ATL buffer
6. Grind sample with pestel to break up tissue for 1 min
7. Vortex and place in incubator at 56 degrees overnight (minimum 12 hours)

Follow DNA extraction procedure (<https://www.qiagen.com/us/resources/resourcedetail?id=cabd47a4-cb5a-4327-b10d-d90b8542421e&lang=en>) \*note half volumes of each yields sufficient DNA

*PCR (2 hours)*

 Items you need:

1. PCR beads
2. PCR mix
3. Extracted DNA
4. Thermocycler with cycling conditions

*COI barcoding markers*

LCO1490 / HC02198

*Thermocycler conditions for COI marker*

* Initial step: 94°C 1 minute
* 35 cycles of the following profile:

\* Denaturing step: 95° C 30 seconds

\* Annealing step: 50° C 30 seconds

\* Extending step: 72° C 45 seconds

* One final step to preserve the sample: 4° C ad infinitum

Steps to follow:

1. Number your strip tubes 1-X
2. To set up the PCR reaction, you will add to the following volumes to each PCR bead tube:

23 ul of PCR mix

2 ul of DNA

Total volume: 25 ul

1. Place the tubes in thermocycler

*Gel electrophoresis (2 hours)*

Items you need:

1. Microwave
2. Agarose
3. Sybr safe
4. Scale
5. Weighing paper
6. Erlenmeyer Flask
7. 1X TAE buffer
8. Gel rig
9. Gel cast
10. Gloves
11. Power supply
12. Stainless steel spatula

Steps to follow:

1. Weigh out 3 grams of agarose by removing powder with spatula and weighing on scale on weighing paper
2. Add powder to Erlenmeyer flask and mix with 200ml of TAE buffer and mix
3. Add flask to microwave for 2 minutes and 30 seconds
4. Let cool (~ 5-7 minutes) and add 3ul Sybr safe
5. Pour gel in assembled gel cast and let solidify for 20 minutes (must be white and not clear)
6. Remove gel cast and place in gel rig
7. Load 5 ul of PCR product and run for 140 Volt for 30 minutes
8. Image your gel and document samples that worked

**Sequence submission (15 minutes)**

Items you need:

1. Styrofoam ice chest
2. Ice pack
3. cardboard
4. Shipping box and label
5. Samples that worked
6. Sample list

Steps to follow:

1. Create spreadsheet for each of your samples (https://docs.google.com/spreadsheets/d/1qqV9dtOAbZpAKSfXqDPXiWq3rswbi-AOpf\_e0iYUF4c/edit?usp=sharing) and email Bruce Nash this information (nash@cshl.edu)
2. Bruce will send you a packing slip confirmation that you will add to your shipping package
3. Place tubes on a piece of cardboard and tape them
4. Obtain a small styrofoam cooler and an ice pack to keep samples cool and ship

**Sequence processing (2 hour)**

1. You will receive a confirmation email from Bruce once samples are processed, which will have an order number.
2. Create an account in Cyverse <https://www.cyverse.org/>
3. You then need to go DNA Subway (<https://dnasubway.cyverse.org/>) and login with same credentials
4. Select the DNA Subway Blue Line, set up a new project, select project type, then click “Upload from DNALC.” That will give you a pop-up window where you can select or search by tracking number (near the top of the screen). If you need the trace files, you can download them with the green arrows under Sequence Viewer. You can process all samples by following this tutorial (<https://docs.google.com/document/d/11RrIaEzdYIFA2Is8dh9eUU0S8Lo7HxX2OUFvX2GwpVQ/edit?usp=sharing>) Be sure to download the cleaned sequences, which you will provide to students via Google Drive folder

**Prepping documents for final activity**

1. You will need to create an answer sheet to your findings (see an example here https://docs.google.com/spreadsheets/d/1NSzIKghRONRyF3u636fcIVf5AkauMKm\_GTkHF2fDrDM/edit?usp=sharing)
2. Match the samples collected by your students to the sequence number for student processing
3. Create a class account for BOLD Student portal (<http://v3.boldsystems.org/index.php/SDP_Login/instructor>) by creating an account, you will receive your class username and password. Log in and add the class roster.

**In class activity**

1. Set up two projectors, one will be for the tutorial and the other for the you to work through the exercise as a model for the students
2. After the presentation, you will guide students through each step, with Teaching Assistants, Learning Assistants and co-instructors assisting students if they get stuck
3. Each student is responsible for submitting information onto worksheet but it is good if they work together
4. The final worksheet will look something like this (https://docs.google.com/spreadsheets/d/1lJzQRG0GNdOTSCkGwUCSsXW2nKszPoJrjsQK5Bi9gog/edit?usp=sharing)

**Additional support**

Here are all of the protocols to follow (<https://dnabarcoding101.org/lab/index.html>), with the left being the tabs for each section

