UNIT 3. Generating DNA Barcodes

Overview: In this unit, students are introduced to what DNA barcoding is, why it works, and how it works. In this unit we provide information about the science and theory of DNA barcoding, scientific papers using DNA barcoding methods, as well as protocols that have been modified for use in high schools.

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.

2. *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*

3. *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 articles that you think would be most interesting to your students. Some articles can be downloaded from the Urban Barcode Project website. 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.

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).

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."

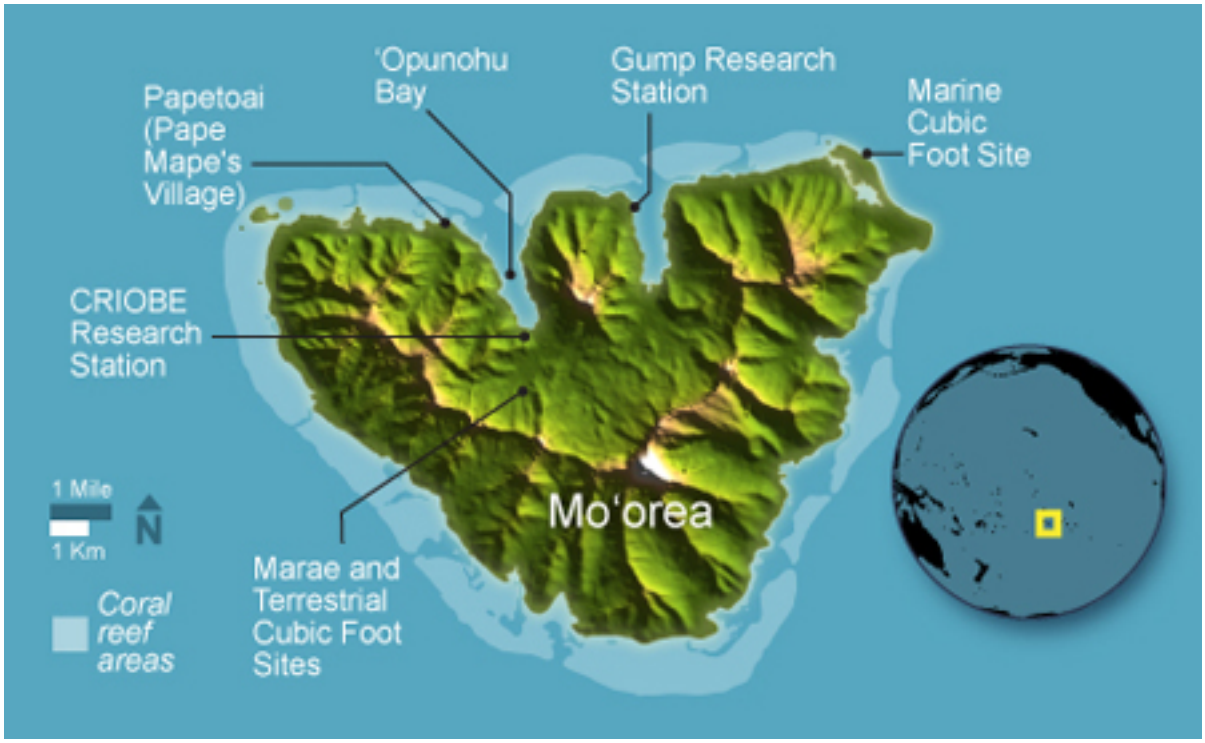

Illustration by Stephen Rountree

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
1-10ul micropipette										
20-200ul micropipette										
100-1000ul 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 100ul 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 500ul of nuclei lysis solution	
6. Incubate at 65°C for 15 minutes	
7. Add 3ul 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 200ul of Protein Precipitate solution, Vortex for 20 seconds	
11. Centrifuge for 3 minutes at max speed (13,000-16,000g)	
12. Carefully transfer 600ul of supernatant containing your DNA into a new labeled tube - discard tubes with pellet	
13. Add 600ul 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 600ul 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 100ul 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 5ul 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 5ul of the DNA ladder to the first well of the top and bottom	
15.	Add 5ul 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

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Thinking about the DNA Extraction:

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

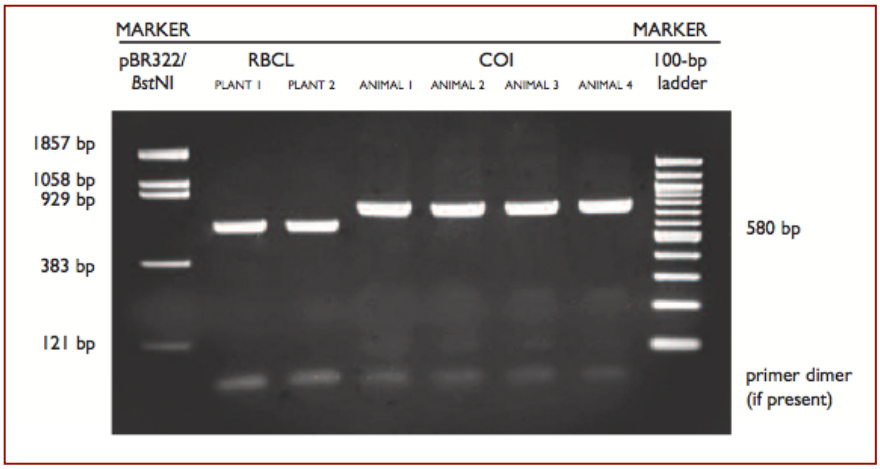
2. Why is it necessary to only use a small piece of tissue?

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
1-10ul micropipette										
20-200ul micropipette										
100-1000ul 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 100ul 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 80ul of PBS buffer	
6. Using the pestle, continue to break up the tissue	
7. Add 20ul proteinase K and 200ul of Buffer AL	
8. Vortex and incubate for 10 minutes	
9. Add 200ul 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 500ul 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 500ul 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 100ul Buffer AE directly into the DNeasy membrane	
21. Incubate at room temperature for 1 minute	
22. Centrifuge for 1 minute	
23. Add 100ul 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 1.5ul 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 5ul of the DNA ladder to the first well of the top and bottom	
15. Add 5ul 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?

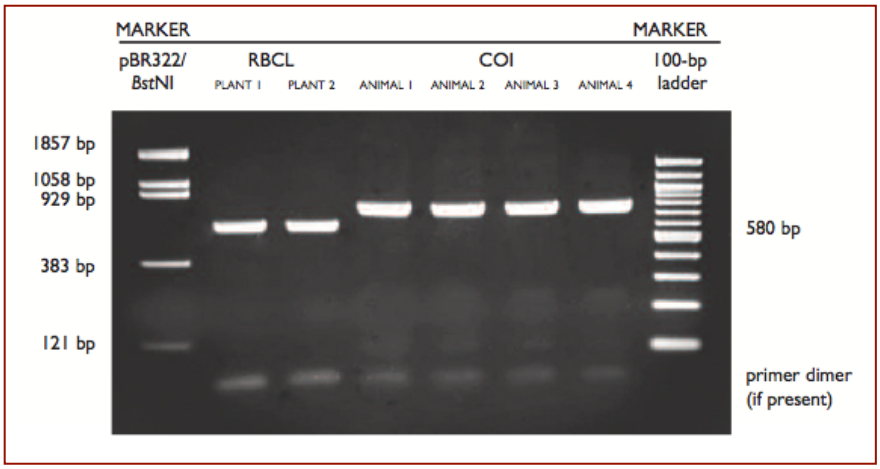
6. How does a spin column work?

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 30ul elution (instead of 100ul).

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.		