UNIT 2. Molecular Biology Theory and Techniques

Overview: In order to work with DNA students must be familiar with basic molecular biology technology as well as master specific skills. This unit introduces students to DNA extraction, Polymerase Chain Reaction (PCR) and gel electrophoresis as well as how to work with small volumes using micropipettes.

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 (.5ul – 1000ul)
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. For DNA barcoding those genes are typically CO1 for animals and RBCL for plants. To fully grasp the science and theory of PCR students must understand the importance of *Taq* Polymerase and primer selection.

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.

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 multi-day 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 ____
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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 100μL 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____

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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 ____
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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 2ul 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 10µL 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 ul stand for?
3. How many ul are in 1 ml?
4. How many ml are in 200 ul?
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.