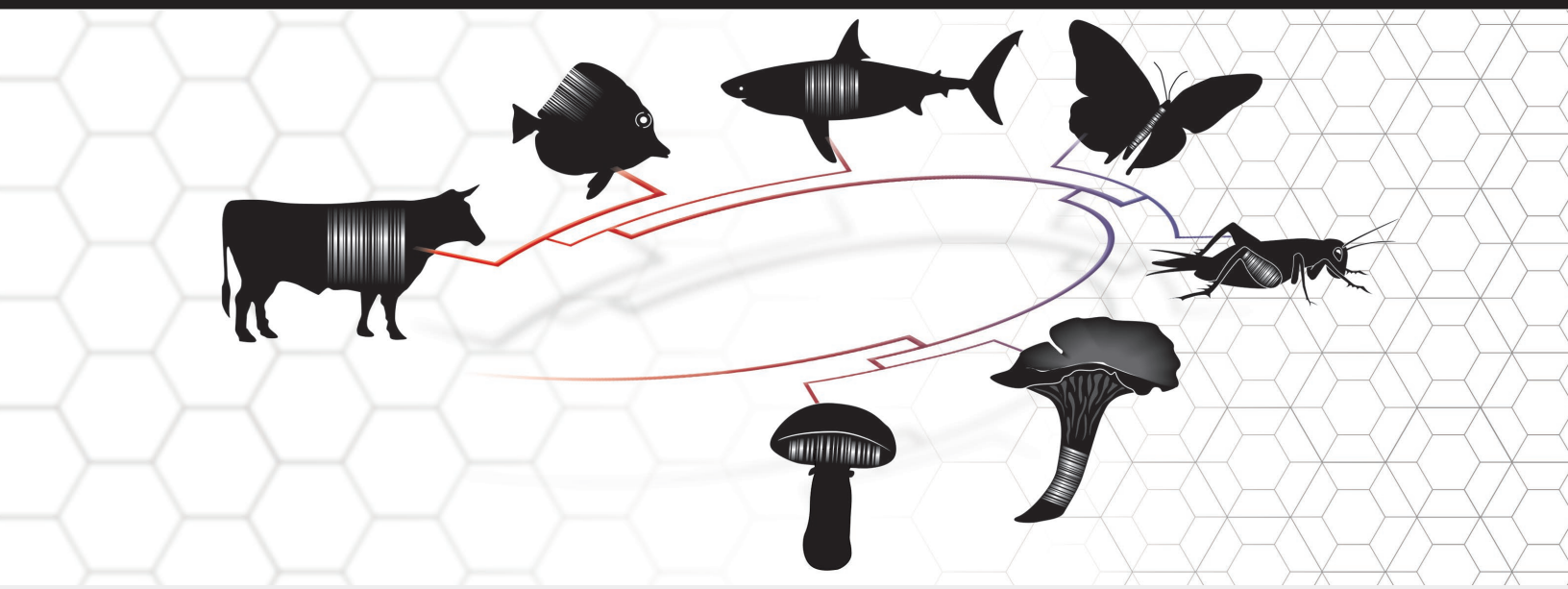
BIO-RAD Explorer



DNA Barcoding Kit

Catalog #17007432EDU, 17007366EDU, and 17007154EDU

Student Guide

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BIO-RAD

What Is DNA Barcoding?

It can be difficult to identify a species simply by analyzing its physical characteristics. The common method of grouping organisms according to shared physical characteristics is known as Linnaean taxonomy. This method of grouping species has been around for 250 years, but that does not mean this method is always easy or accurate. Convergent evolution, where two species starting from very different ancestors develop similar traits due to environmental pressures, can make identification difficult. For example, both bats and birds have wings, porcupines and echidnas each have quills, and nontoxic viceroy butterflies resemble poisonous monarch butterflies.

Even after 250 years of collection, analysis, and categorization of species using physical characteristics, fewer than two million of Earth's estimated 10–50 million plant and animal species have been formally described and cataloged. With the increases in human population, habitat destruction, pollution, and overharvesting, the rate of species loss threatens to outpace the rate of species discovery using this traditional classification system.

The explosion of faster and cheaper technology to isolate, purify, amplify, and sequence DNA has spurred the development of new methods to help identify different species. Using DNA-based technologies, a multinational alliance of scientists is now cataloging life using what is called a DNA barcoding system in order to accelerate the discovery of species and develop powerful new tools to monitor and preserve Earth's vanishing biodiversity.

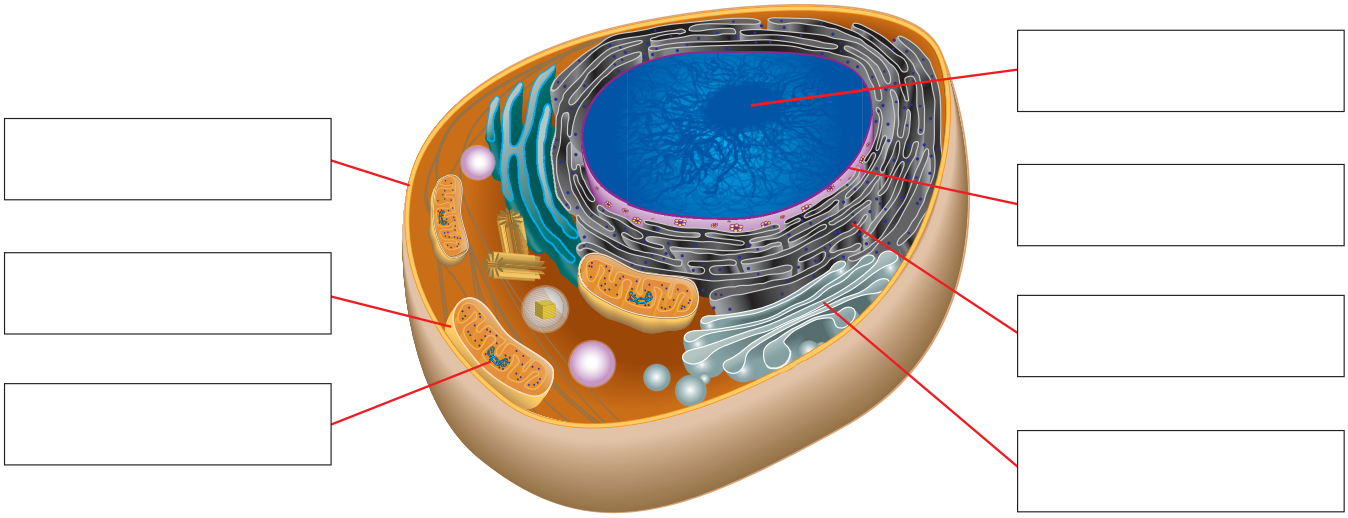
In much the same way that a UPC (universal product code) barcode can differentiate a carton of milk from a bag of carrots when scanned at the grocery store, DNA sequences can be used to uniquely identify different species. This is the basis of DNA barcoding.

Lesson 1
DNA Extraction

Background

In this lesson, you will extract DNA from a tissue sample. Ask your instructor which sample you will be using. You will begin by estimating or weighing out the proper amount of tissue to use and grinding it as finely as possible. After depositing it into a microcentrifuge tube, you will add a series of buffers to your sample in order to release DNA from individual cells. You will then bind the DNA to solid particles within the matrix suspension in a spin column, wash away the impurities present in the sample extract, and finally recover the purified DNA by elution into distilled water. The DNA you've extracted will be used in the next laboratory as your target DNA for polymerase chain reaction (PCR) amplification.

PCR, which you will perform in the next lesson, involves amplification of DNA. It is critical to use proper technique to avoid any cross-contamination between samples during DNA extraction. Do not reuse cutting implements, pipet tips, or containers. If using gloves, change them in between handling different samples.



Focus Questions

A. In the cell diagram above, label the indicated features.

B. Where is the DNA found in eukaryotic cells?

C. What parts of the cell must be broken down to extract DNA?

D. It is important to keep track of the location of the DNA at each stage of purification. For the following steps of the protocol, state whether DNA is in the pellet, in the supernatant, bound to the column, or in the flowthrough:

D1. After centrifuging down the neutralized tissue lysate (pellet or supernatant).

D2. After centrifuging the supernatant through the column (column or flowthrough).

D3. After centrifuging the wash solution through the column (column or flowthrough).

D4. After centrifuging the elution solution through the column (column or flowthrough).

E. Why is it important to use a new cutting utensil for every sample?

Materials Needed

Student Workstation

Materials	Quantity
Resuspension buffer (**R**)	500 µl
Lysis buffer (**Lys**)	600 µl
Neutralization buffer (**N**)	600 µl
Matrix (**X**)	500 µl
Wash buffer (**Wash**)	2 ml
Distilled water (**dH2O**)	300 µl
Empty 2 ml microcentrifuge tubes with caps	2
Empty 2 ml microcentrifuge tubes with caps removed	4
Spin column	2
Bulk tissue sample	2
Empty, clean weigh boat	2
Razor blade, plastic knife, or other clean cutting utensil (**Note:** it is critical to use one utensil per sample)	2
100–1,000 µl adjustable-volume micropipet and tips	1
Marking pen	1

Common Workstation

Materials	Quantity
Water bath or dry bath set to 55°C	1
Microcentrifuge	1–2

Protocol

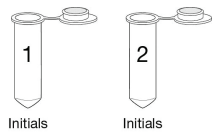
Prepare Samples

1. **Label one capped 2 ml microcentrifuge tube 1 and another 2 to be used for your two samples. Add your initials.**

   If provided or known, record the ID or a description of the tissue sample you will be using.

   Sample 1 _____

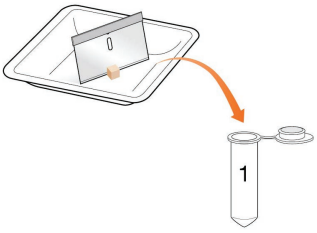
   Sample 2 _____



2. **Cut a piece of tissue sample.**

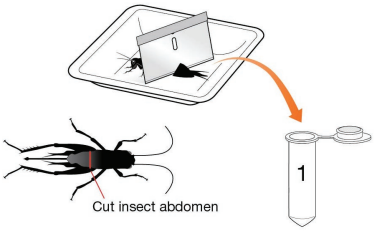
   For fish, mammalian, or fungal tissue samples, cut a piece of tissue up to 100 mg, approximately the size of an eraser-head, from the bulk tissue sample 1 and place it directly into the appropriately labeled microcentrifuge tube.

   Note: Cut a sample from the interior of the bulk tissue to avoid bacterial contamination that may be present on the surface. Avoid fur, scales, or feathers.

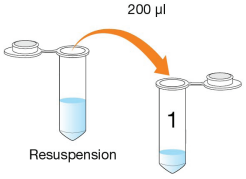


   For insect tissue samples, cut a small piece of tissue approximately half the size of an eraser-head, from the abdomen of your insect. Total mass will vary. Place the piece directly into the appropriately labeled microcentrifuge tube.

   Note: Avoid wings, legs, and other parts of the insect body as they are much less effective for DNA extraction.

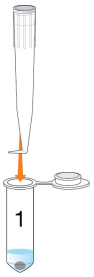


3. **Add 200 µl resuspension buffer (R) to sample 1.**



4. **Take the micro pipet tip in hand and firmly press the narrow end of the tip at a slight angle into the sample 1 weigh boat or a new weigh boat to bend and crush a small portion of the tip.**

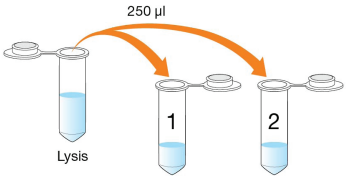
5. **Use the crushed micropipet tip as a pestle to grind the sample in the microcentrifuge tube for at least 30 sec until the sample becomes cloudy. Then discard the tip.**



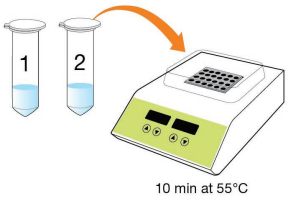
6. **Using a new razor blade or cutting utensil and a new pipet tip, repeat steps 2–5 with tissue sample 2. If wearing gloves, change gloves before handling the next tissue sample. If not, wash your hands thoroughly.**

STOP

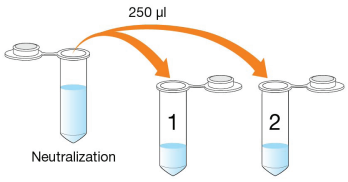
Stop. Ask your instructor whether to proceed now or later.



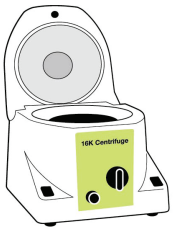
8. **Add 250 µl lysis buffer (Lys) to each tube and mix gently by inverting each tube 10 times. DO NOT VORTEX. Vortexing may shear genomic DNA, which can inhibit PCR amplification.**



9. **Incubate samples at 55°C for 10 min in a water bath or dry bath.**



10. **Add 250 µl neutralization buffer (N) to each tube and mix gently by inverting each tube 10 times. DO NOT VORTEX. A cloudy precipitate may form.**

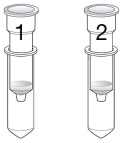


11. **Centrifuge the tubes for 5 min at 12,000–14,000 x g. A compact pellet will form along the side of the tube. The supernatant contains the DNA.**

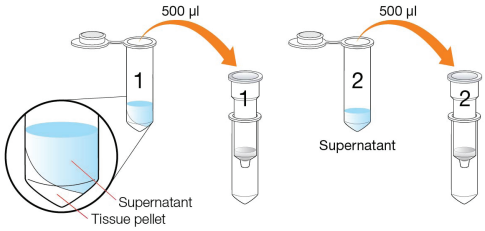
    If there are many particulates in the supernatant after centrifugation, centrifuge the tubes for an additional 5 min.

12. **Snap off (do not twist!) the bottoms of the two spin columns. Insert each column into a capless 2 ml microcentrifuge tube.**

13. **Label one spin column 1 and the other 2. Add your initials to the columns.**



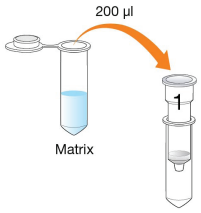
14. **Using a new pipet tip for each sample, transfer ~500 µl supernatant from each sample to the appropriately labeled spin column.**



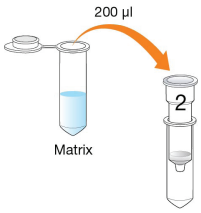
⚠ **Do not disturb the tissue pellet.**

15. **Vortex or repeatedly shake the matrix (X) to make sure the resin is completely resuspended.**

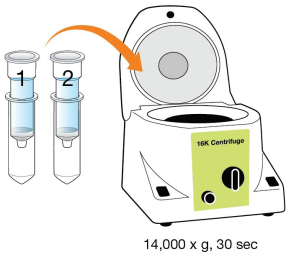
16. **Quickly add 200 µl thoroughly resuspended matrix (X) to the first spin column. Pipet up and down to mix.**



17. **Using a new pipet tip, repeat step 14 with the second sample.**



18. **Centrifuge the spin columns with the capless microcentrifuge tubes at full speed (14,000 x g) for 30 sec.**

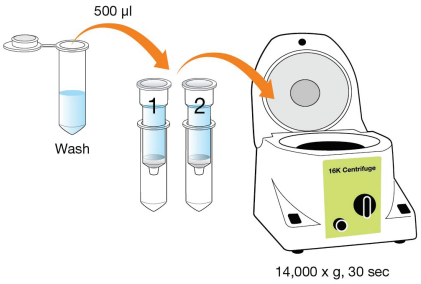


⚠ **Centrifuge for only 30 sec! Completely drying the matrix at this point will result in DNA loss.**

19. **Remove the spin columns from the capless 2 ml microcentrifuge tubes, discard the flowthrough, and replace the spin column into the same tubes.**

20. **Add 500 µl wash buffer (W) and wash the matrix by centrifuging for another 30 sec.**

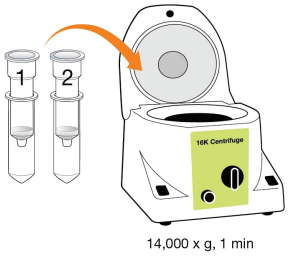
    Centrifuge for only 30 sec! Completely drying the matrix at this point will result in DNA loss.



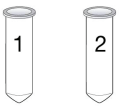
21. **Repeat steps 17–18 to wash the samples again.**

22. **Remove the spin columns from the capless 2 ml microcentrifuge tubes, discard the flowthrough, and replace the spin column into the same tubes.**

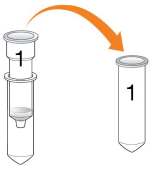
23. **Finally, centrifuge the spin columns with the capless microcentrifuge tubes at full speed for a full 2 min to remove residual traces of ethanol and dry out the matrix.**



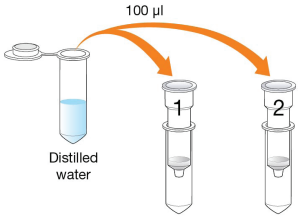
24. **Label two clean 2 ml capless microcentrifuge tubes 1 and 2 with your initials.**



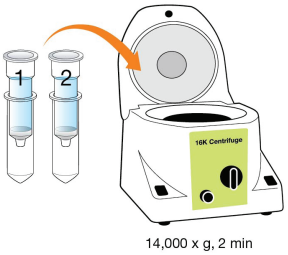
25. **Transfer each spin column into the appropriately labeled new, clean capless microcentrifuge tube. Discard the used capless microcentrifuge tubes along with any liquid in the tubes.**



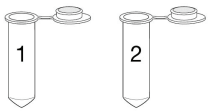
26. **Using a new pipet tip for each sample, add 100 µl distilled water to each spin column, being careful not to touch the resin.**



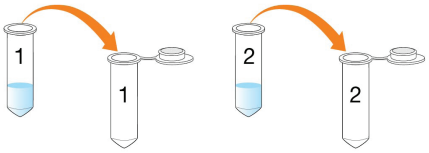
27. **Centrifuge the spin columns with the capless microcentrifuge tubes at full speed (14,000 x g) for 1 min.**



28. Label two new 2 ml capped microcentrifuge tubes 1 and 2 with your initials.



29. Remove the spin columns from the capless microcentrifuge tubes and transfer the flowthrough which contains eluted DNA into the appropriately labeled 2 ml capped microcentrifuge tubes.



Lesson 2
PCR Amplification

In the last lesson, you extracted DNA from tissue samples. In this lesson, you will prepare those samples and some additional experimental controls for the polymerase chain reaction (PCR). PCR is a process to replicate, or amplify, DNA in a test tube. PCR allows you to amplify specific regions of DNA and make millions of copies of the target sequence. Your experiment in this lab is to make enough copies of the target barcoding sequence that the resulting PCR product can be visualized by gel electrophoresis and be submitted for DNA sequencing.

For this lesson, you will set up a PCR reaction for each DNA extract you generated in the last lesson as well as two control PCR reactions, one positive control and one negative control. The positive PCR control sample will contain DNA with a known barcode gene sequence. The negative PCR control sample will contain water which has no DNA. Since water should not have any DNA in it, there should be no amplification of any target sequence in this sample. By having a known control that should not amplify the target sequence, you can tell if your PCR reactions have been contaminated by DNA containing the target sequence.

Focus Questions

A. How do researchers target the portion of the DNA to be amplified during PCR?

B. What is an aspect of primer design that has been used in this laboratory to ensure successful DNA amplification from a wide variety of samples?

C. Do you expect the positive control to generate a PCR product? What about the negative control? Why or why not?

D. Why is it important to use aerosol barrier tips when setting up a PCR reaction?

Materials Needed

Student Workstation

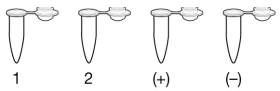
Materials	Quantity
Positive PCR control (**+**)	10 µl
Negative PCR control (**–**)	10 µl
DNA extract samples from Lesson 1	2
Appropriate reaction mix (**FishRM**, **MIRM**, or **FunRM**)	150 µl
PCR tube	4
2 ml microcentrifuge tube with cap	7
2–20 µl adjustable-volume micropipet and aerosol barrier tips	1 1
20–200 µl adjustable-volume micropipet and aerosol barrier tips	1
Ice bath with crushed ice	1
Marking pen	1

Common Workstation

Materials	Quantity
Thermal cycler with at least 32 wells	1

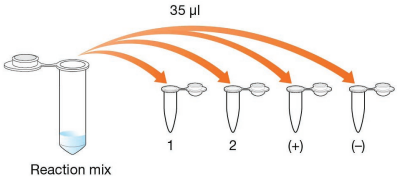
Protocol

Set up PCR reactions



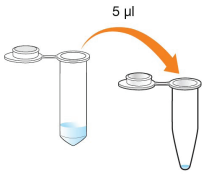
1. Label four PCR tubes **1**, **2**, **(+)**, and **(–)** with your initials.

   Keep your tubes on ice for the remaining steps.



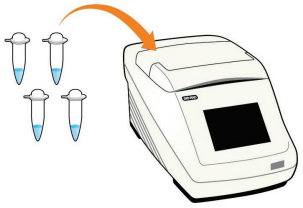
2. Using a new aerosol barrier pipet tip, add 35 µl reaction mix (**FishRM**, **MIRM**, or **FunRM**) to each PCR tube.

   Cap each tube immediately after adding the reaction mix.



3. Using a new aerosol filter pipet tip for each tube, add 5 µl of the appropriate DNA sample directly into the reaction mix solution in each PCR tube. Pipet up and down to mix. Recap each tube immediately after adding the DNA.

PCR tube label	Final contents
1	35 µl reaction mix 5 µl DNA sample 1
2	35 µl reaction mix 5 µl DNA sample 2
(+)	35 µl reaction mix 5 µl positive control sample (+)
(–)	35 µl reaction mix 5 µl negative control sample (–)



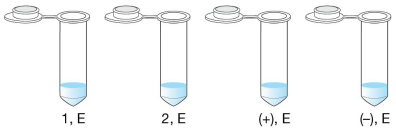
4. When instructed, place your PCR tubes in the thermal cycler.

5. **Run the program with the following conditions:**

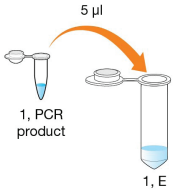
Step	Temperature (°C)	Duration
Initial denaturation	**94**	**2 min**
35 cycles of:		
Denaturation	**94**	**30 sec**
Annealing	**55**	**2 min**
Extension	**72**	**1 min**
Final extension	**72**	**10 min**
Hold	**12**	**(∞)**

Store tubes at 4°C after the cycling program is complete.

6. **Label four 2 ml microcentrifuge tubes 1E, 2E, +E, and –E, each with your initials. The E stands for electrophoresis.**

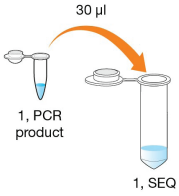


7. **Transfer 5 µl of each PCR reaction to the appropriately labeled 2 ml microcentrifuge tube.**



8. **Label three 3 ml microcentrifuge tubes 1SEQ, 2SEQ, and +SEQ. SEQ stands for sequencing. You will not be sequencing your negative control sample.**

9. **Transfer 30 µl of each PCR reaction to the appropriately labeled 2 ml microcentrifuge tube.**



10. **Store all samples at 4°C until you are ready to proceed with electrophoresis and sequencing.**

Lesson 3
Gel Electrophoresis

In the previous lesson, you sought to amplify a target sequence from your DNA samples and included control PCR reactions to aid in the analysis of the gel electrophoresis results you will obtain in this lesson. Gel electrophoresis will allow you to determine the success of your PCR reactions by visualizing the size of your amplified DNA.

The expected band size that corresponds to your successfully amplified PCR product is approximately 650 base pairs (bp). You may also notice an additional band less than 100 bp in size. This band corresponds to unincorporated primers from your PCR reaction, which can stick to each other in what is known as a primer dimer. A molecular weight ruler (DNA standard) has been provided so that you have a reference sample containing several DNA molecules with known molecular weights. Using this standard for comparison, you can estimate the size of your PCR product.

The UView 6x loading dye and stain that you will add to each of your samples contains a fluorescent compound that binds to DNA. During gel electrophoresis, it will co-migrate with your DNA and allow your DNA to be visualized with UV light. No additional staining of the gel is required for visualization of your results.

Ask your instructor about the use of personal protective equipment (PPE) prior to using any ultraviolet light source.

Focus Questions

A. What is the purpose of the agarose gel?

B. What purpose(s) does the UView 6x loading dye and stain serve?

C. What do you think the results should look like for each sample?

Materials Needed

Student Workstation

Materials	Quantity
Electrophoresis samples from Lesson 2 (**1E**, **2E**, **+E**, **–E**)	4
Molecular weight ruler (**MWR**)	25 µl
UView 6 x Loading Dye and Stain (**LD**)	15 µl
Sterile water (**sH2O**)	40 µl
1% agarose gel	1
TAE running buffer	250 ml
2–20 µl adjustable-volume micropipet with aerosol barrier tips	1
Horizontal gel electrophoresis chamber	1
Power supply (may be shared)	1
Marking pen	1

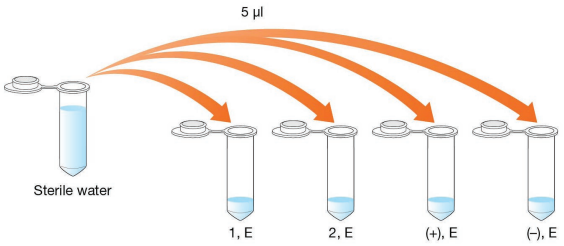
Common Workstation

Materials	Quantity
UV transilluminator or imaging system	1

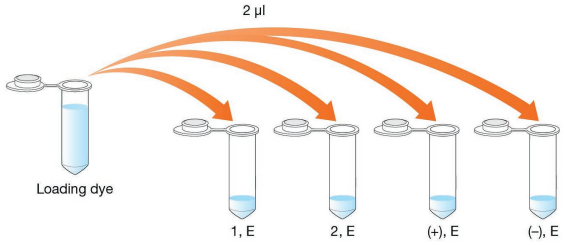
Protocol

Electrophorese samples

1. Using a new pipet tip each time, add 5 µl sterile water (**sH2O**) to each of your electrophoresis (**E**) samples.

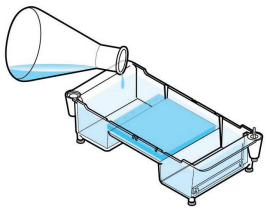


2. Using a new pipet tip each time, add 2 µl UView 6x loading dye and stain (**LD**) to each sample. Flick to mix thoroughly and pulse-spin.

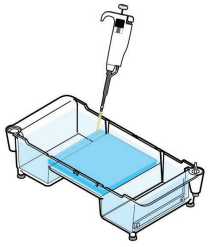


3. Place a TAE agarose gel into the electrophoresis chamber. Be sure the gel is oriented so that the wells are closest to the black (–) electrode, or cathode.

4. Fill the electrophoresis chamber with enough TAE running buffer to cover the gel by about 2 mm.

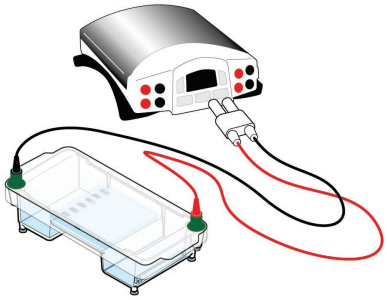


5. Using a new pipet tip for each sample, load samples into the wells according to the table below:



Lane	Sample	Volume, µl
1	Empty	—
2	Empty	—
3	Molecular weight ruler (**MWR**)	20
4	**+E**	12
5	**–E**	12
6	**1E**	12
7	**2E**	12
8	Empty	—

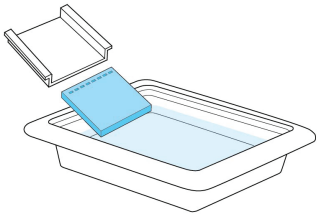
6. **Replace the lid on the electrophoresis chamber and connect the leads to the power supply, red to red and black to black.**



7. **Ask your instructor for the run conditions. Turn on the power and run the gel.**

Visualize samples

8. **Carefully remove the gel from the chamber and transfer it to a gel staining tray (optional).**



9. **Ask your instructor how you will be visualizing you agarose gels.**

Lesson 4
Sequencing

You will be submitting your PCR products to a sequencing facility to be purified and sequenced. Sequencing reactions, like PCR, rely on the basic principles of DNA replication and require primers to initiate DNA replication. However, sequencing is performed in just one direction. So, instead of a primer pair, sequencing makes use of a single primer per reaction. To aid in getting as much data as possible from each PCR product, the sample PCR product will have a sequencing reaction run in each direction. One sequencing reaction will run in the forward direction from the beginning of the target gene, and a second sequencing reaction will run in the reverse direction. Ideally, these sequencing reactions will yield the same results, with one being the reverse complement sequence of the other.

Once you obtain these sequences, you will use bioinformatics tools to analyze the quality of your data, assemble a consensus sequence from your high-quality sequencing results, and search the Barcode of Life Database (BOLD) to determine what is the closest match for your sample.

Focus Questions

A. Why is it necessary to have PCR products purified before sequencing? (Hint: Think about how sequencing works and what might interfere with it if left over from PCR).

B. What primers will be used to sequence your PCR product? Where are those sequences on your PCR product? How did they get there?

C. What steps of the process so far may impact the quality of the sequence that will be generated? How would each step impact the sequence quality?

Materials Needed

Student Workstation

Materials	Quantity
Sequencing samples from Lesson 2, (**1SEQ**, **2SEQ**, **+SEQ**)	3
Marking pen	1
Parafilm pieces	3

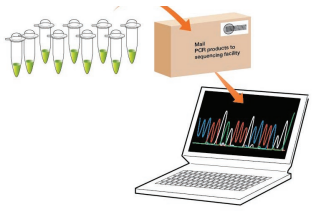
Protocol

Sequence samples

1. **Wrap your samples in parafilm to prevent leakage while shipping.**

2. **Record the sample names exactly as written on your tubes. Give the sample names to your instructor. Make sure these match the names your instructor submits to the sequencing facility. This is the only way you can identify the correct sequencing data file for each sample.**

3. **Give your samples to your instructor for shipment to the sequencing facility.** ➜



Glossary

Aliquot — verb form: to divide a quantity of material into smaller, equal parts. Noun form: one of a number of small, equally divided parts.

Annealing — binding of single-stranded DNA to complementary DNA sequences. Oligonucleotide primers bind to single-stranded (denatured) template DNA.

Base call — reading a DNA sequencing chromatograph and assigning a base to each peak.

Base pairs — complementary nucleotides held together by hydrogen bonds. In DNA, adenine is linked by two hydrogen bonds with thymine (A-T) and guanine is linked with cytosine by three hydrogen bonds (G-C). Because of the three H-bonds between G and C (compared to the two between A and T), the G-C bonding is stronger than the A-T bonding.

Biorepository — a repository (place or building where things are stored) for biological materials. Biorepositories collect, process, preserve, store, and distribute specimens to support future scientific investigation. They may also manage and retain collections of specimens from many diverse organisms.

Chromatogram — also known as electropherogram, or trace file. A visual representation of the signal peaks detected by a sequencing instrument. The chromatogram contains information on the signal intensity as well as the peak separation time.

COI — abbreviation for cytochrome c oxidase subunit I. This gene is located in a cell's mitochondrial DNA and it encodes a protein within an enzyme complex that is involved in the electron transport chain of cellular respiration. This is the process by which organisms harvest energy, in the form of ATP, from food sources.

Consensus sequence — a sequence derived from the alignment of similar DNA, RNA, or protein sequences. Normally, each position in the consensus sequence is determined by the base or amino acid that predominates in that position in the majority of the aligned sequences. Consensus sequences can be used to design primers for PCR.

Contig — a sequence that has been constructed by comparing and merging the information from sets of overlapping DNA segments.

Degenerate primers — a mixture of PCR primers that are similar but not identical. They may be designed based on a consensus sequence derived from similar organisms, with substitutions of different bases at one or more location in the primers.

Denaturation — with respect to DNA, separation of complementary strands of DNA into single-stranded DNA. Denaturation of DNA is also sometimes referred to as "melting." In vivo, DNA is denatured by enzymes. But in PCR, DNA is denatured by heat.

DNA barcode — a short, standardized gene region represented by its constituent nucleotide sequence. DNA barcodes exhibit fewer nucleotide differences among members of the same species and larger differences between members of different species groups. A 650 bp segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene is the standard barcode region for animals, whereas a segment of the nuclear ribosomal internal transcribed spacer region (ITS) is the accepted barcode region for fungi. Nucleotide sequences from two chloroplast genes — the ribulose-1,5-bisphosphate carboxylase (rbcL) and maturase K (matK) genes — are used as standard barcode regions to identify land plants. A query DNA barcode is a barcode sequence that is unknown or unverified and is obtained from a tissue sample or food product of unknown origin. A reference DNA barcode is a barcode sequence from a known source that has been extensively verified through numerous criteria, including taxonomic verification and vouchering processes (see Voucher definition for more information).

dNTPs — commonly used abbreviation for all four deoxynucleotide triphosphates (dATP, dTTP, dGTP, and dCTP) used in synthesizing DNA.

ddNTPs — commonly used abbreviation for dideoxynucleotide triphosphates (ddATP, ddTTP, ddGTP, and ddCTP), which are modified nucleotides that serve as chain terminating inhibitors of DNA polymerase during DNA sequencing. When used as part of Sanger Sequencing, ddNTPs are linked to fluorescent dyes that are unique to each type of base.

Electropherogram (AKA trace file) — see chromatogram.

Electrophoresis — a technique for separating molecules based on their relative migrations in an electric field. DNA and RNA are usually separated using agarose gel electrophoresis, and proteins are separated using a polyacrylamide matrix (PAGE or SDS-PAGE).

Elute — to remove adsorbed material from an adsorbent (that is, a column filter or matrix) by the addition of a solvent.

Extension — the phase of PCR amplification during which the DNA polymerase synthesizes a new DNA strand that is complementary to the template strand by incorporating dNTPs that are complementary to the template DNA.

Genomic DNA (gDNA) — all of the chromosomal DNA found in a cell or organism.

ITS — abbreviation for internal transcribed spacer, a locus within the region of the genome that contains genes for ribosome subunits.

Lysis — the process of rupturing a cell to release its contents. Once lysed, the mixture of the cell and lysis solution is called a lysate.

Master mix — a premixed reagent solution for chemical or biological reactions. A PCR master mix contains all components needed for PCR (dNTPs, primers, buffer, salts, DNA polymerase, and Mg^{2+}) except for the template DNA.

Matrix — for the purposes of this kit, the matrix suspension contains particulates that will bind any DNA present in the supernatant of the centrifuged tissue lysates and will allow other impurities present in the lysate to be washed away.

Neutralization — a step during DNA extraction that entails the addition of a neutralizing salt solution that counteracts the effects of an alkaline lysis solution.

Nucleotide — a fundamental unit of DNA and RNA. Molecules comprising a sugar, a phosphate group, and one of four bases: adenine, guanine, cytosine, and thymine (DNA) or uracil (RNA).

Oligonucleotide (oligo) — a short segment (often 10–30 bases) of DNA or RNA that is usually made synthetically. Frequently used as primers for PCR or sequencing.

PCR — polymerase chain reaction. A technique for rapidly creating multiple copies of a segment of DNA using repeated cycles of DNA synthesis.

Pellet — the insoluble precipitate that occurs on the bottom or side of a tube following centrifugation.

Primer — a short, single-stranded oligonucleotide designed to bind DNA template strands at the end of the sequence of interest and serve as the starting point for DNA synthesis. Primers can be single strands of either DNA or RNA.

Primer dimer — in a PCR reaction, primers with enough complementary sequences may stick to each other, causing bands of approximately 100 base pairs when visualized by electrophoresis.

Quality score (or value) — a numerical value used in DNA sequencing data indicating the confidence level for base calls. A higher quality value means higher confidence that the base call is correct. A lower quality value indicates the base call is less reliable.

Sanger Sequencing — a method of DNA sequencing in which fluorescent tagged dideoxynucleotide triphosphates (ddNTPs) are used as part of an amplification reaction to determine DNA sequence.

Sequence — the ordered list of bases that make up a DNA strand. When linked with a chromatogram, this would be considered a read.

Supernatant — the liquid that remains above a solid residue or precipitate following precipitation, centrifugation, or other process.

Taq DNA polymerase — a DNA polymerase that is stable at high temperatures. Taq DNA polymerase is commonly used in PCR. The enzyme was originally isolated from the thermophilic bacterium *Thermus aquaticus*, which can tolerate high temperatures.

Template DNA — the target DNA that contains the sequence to be amplified by PCR.

UPC — abbreviation for universal product code. A UPC contains a unique combination of bars and spaces that distinguishes each product sold by a company. No two products share the same barcode.

Voucher — a specimen archived in a permanent collection (usually in a museum, biorepository, or other institution with a mandate to preserve the materials indefinitely).

Vortex — induction of a jarring circular motion using a vortexer machine (#1660610EDU). Vortexing is typically used to aid in the resuspension of insoluble material within a liquid suspension.

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