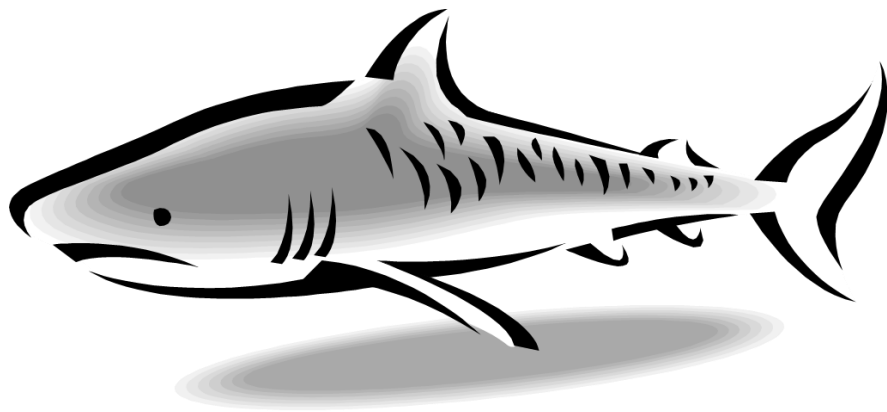


the Wildlife Forensics Lab

A Biotechnology Application for Protecting
an Endangered Species



Developed by MdBioLab
Foundation and adapted by
Towson University.

www.towson.edu/cse/beop

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Loaner Lab Overview

The Wildlife Forensics Activity explores the use of biotechnology in the field of wildlife forensics. Students act as technicians in a genetic lab, testing samples of shark fins confiscated by customs agents that are suspected of being from a great white shark. Great white sharks are a protected species in the US and importation of fins from these sharks is prohibited. Students use the molecular technique of gel electrophoresis to identify the species of shark the fins are from.

The Wildlife Forensic Lab has two parts, a pre-laboratory set of activities that introduce students to concepts and skills and a laboratory activity that allows them to apply the concepts and skills learned in the pre-laboratory activity.

Pre-laboratory Activity

- Model the use of agarose gel electrophoresis for separation of DNA fragments amplified by PCR.

Laboratory Activity

- The Wildlife Forensic Laboratory is an activity in which students will use gel electrophoresis to separate DNA fragments that were amplified using PCR from tissue samples of confiscated shark fins of unknown origin, but suspected to be illegally imported fins of the great white shark. Students will then analyze their gel electrophoresis results to determine the species of the fins.

Equipment and Supplies

Supplied by Maryland Loaner Lab Program

Description	Quantity	Comments	Must Be Returned?
Teacher Binder	1	Contains all info necessary for completing lab	Yes
Gel Electrophoresis box	1	With lid	Yes
1 power supply		For use with gel electrophoresis box	Yes
Gel Trays	6	To make 5 gels + one extra	Yes
Gel Combs	6	To make 5 gels + one extra	Yes
Gel Tray Ends	12	2 per tray	Yes
Pyrex Bottle with orange cap	1	Used to make agarose gels	Clean, dry and return
Agarose Powder (1.2g)	1 baggie	Used to make agarose gels	Return empty baggie
20ul micropipettes	10	1 per group	Yes

Micropipette tips	5 boxes	1 box per 2 groups	Yes
10X TAE Buffer	3 tubes (150 ml)	Follow Teacher Prep for dilution	Rinse and return
2-liter Container	1	Used to Mix 10X TAE and H ₂ O	Rinse and return
Staining trays	2	Used for staining gel	Rinse and return
Non-latex gloves	1 pair	Use with gel stain	No
Carolina Blu™ Stain Kit	1	Dropper bottle and Stain bottle	Yes
1.5-ml microcentrifuge tubes	10	Used for micropipette challenge	No
Foam microtube racks	10	1 per group	Yes
Class set of DNA “Wildlife Forensics DNA Samples”	1 per class	(10) SF odd # (10) SF even # (10) C Refrigerate until use	No
Class set of “Wildlife Forensics Reagents”	1 per class	(10) LD (10) Prac. Dye Refrigerate until use	Yes
Class set of “Micropipette Challenge” Reagents	1 per class	(10) blue water (10) yellow water	Yes
Practice gels	10	1 per group Refrigerate until use	Empty and return
Microcentrifuge	1	Used to spin samples down in tubes	Yes
Graduated cylinder (100ml)	1	Used to pour gels	Yes
Spatula	1	For removing gels from staining trays.	Yes
Disinfectant wipes	1 container	Use to disinfect equipment	Yes
Pre-Lab Envelopes	10 per class	1 per group	Yes

Supplied by the Teacher:

Description	Quantity	Comments
Student worksheets	1 set per student	
Distilled water	2000 ml	For making gels and running buffer, and destaining gels
Tape	10 rolls	1 per station for pre-lab activity
Lab Microwave or Hot Plate	1	To melt agarose
Gloves	1 pair per student	For student use with main laboratory activity
Goggles	1 pair per student	For student use with main laboratory activity
Waste containers	1 per group	

SAFETY: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

Maryland Science Core Learning Goals

Goal 1.0 Skills and Processes	
1.4.2	<i>The student will analyze data to make predictions, decisions, or draw conclusions.*</i>
1.2.6.	The student will identify appropriate methods for conducting an investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size, etc.)
1.2.8	The student will defend the need for verifiable data.
1.3.1	The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques. ^{NTB}
1.3.2	The student will recognize safe laboratory procedures.
1.3.3	The student will demonstrate safe handling of the chemicals and materials of science. ^{NTB}
1.3.4	The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction. ^{NTB}
1.4.2	The student will analyze data to make predictions, decisions, or draw conclusions.
1.5.1	The student will demonstrate the ability to summarize data (measurements/observations).
1.7.1	The student will apply the skills, processes and concepts of biology, chemistry, physics, or earth science to societal issues.
1.7.2	The student will identify and evaluate the impact of scientific ideas and/or advancements in technology in society.
1.7.5	Students will investigate career possibilities in the various areas of science.
1.7.6	The student will explain how development of scientific knowledge leads to the creation of new technology and how technological advances allow for additional scientific accomplishments.
Goal 3.0 Concepts of Biology	
3.4.2	<i>The student will estimate degree of relatedness among organisms or species.*</i>
3.5.1	The student will analyze the relationships between biotic diversity and abiotic factors in environments and the resulting influence on ecosystems.

* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.

General Laboratory Background

Trade in illegally obtained wildlife is a multi-million dollar business. Although there are laws in place that prohibit the killing and trade of endangered and protected species, the parts of the organism that are being bought or sold are often physically unidentifiable, complicating the efforts of wildlife conservation officers and other agencies responsible for conservation and management of protected species. Thus, the uses of molecular techniques that can unambiguously assign tissue to a particular organism are increasingly being employed to help identify and control the trade of protected plants and animals.

The shark fin trade is a prime example of the challenges facing the agencies assigned to conserve and manage protected species. Shark fins are considered a delicacy in the Asian food market. Shark Fin Soup can sell for as much as \$100 a bowl. The huge demand for shark fins and consumer's willingness to pay large sums of money for the fins are fueling the illegal trade and trafficking of sharks. While the trade of some species of shark fin is legal, that of others is forbidden or strictly monitored through permits. However, it is often impossible to visually identify the species from which a shark fin has been taken, especially after it has been dried or otherwise processed.

Recently, the National Oceanic and Atmospheric Administration (NOAA) enlisted the help of a team of scientists led by Dr. Mahmood Shivji¹, the director of the Guy Harvey Research Institute in Florida, to aid them in determining the origin of a suspicious shipment of 21 sets of shark fins seized from an East Coast seafood dealer. Although the outside of the confiscated bag read "porbeagle" (a species of shark that may be legally harvested) law enforcement officials suspected the fins belonged to the great white shark (*Carcharodon carcharias*) since they found a hidden label on the package that said "blanco" (which is white in Spanish). The great white shark is a protected species and regulations prohibit unauthorized sale or trade. Dr. Shivji and his colleagues developed a molecular technique that allowed them to unambiguously identify any tissue originating from great white sharks.

One of the most exciting components of the technique developed by Dr. Shivji and colleagues is the ability to detect the presence of great white shark DNA from extremely small tissue samples, even in the presence of DNA from up to 10 other species of sharks. This technique provides enough certainty in their identification of great white shark tissue to hold up to the burden of proof required in a court of law. Results from the tests performed by Dr. Shivji and his colleagues confirmed that all confiscated tissue was indeed from great white sharks. The genetic results will be presented as evidence in court in the case against the seafood dealer. This example illustrates the importance of cooperation between scientists and law enforcement agencies in the conservation and management of protected species.

¹ Shivji, M.S. et al. 2005. Genetic profiling reveals illegal international trade in fins of the great white shark, *Carcharodon carcharias*. *Conservation Genetics* 6 (6) 1035-1039.

Wildlife forensics is an emerging field that combines cutting edge biotechnology with traditional law enforcement techniques to halt the trade of endangered and protected species. Further advances in the bioscience field will allow identification of a greater number of protected and endangered species, facilitating the detection and prevention of illegal wildlife trade. Advancement of the wildlife forensics field is dependent upon scientific research within the bioscience community that will continue to develop new and efficient means of screening and identifying organisms that are illegally hunted, bought and sold.

Background information on PCR and electrophoresis

The goal of this lab is to test whether the tissue confiscated by the U.S. Customs officials comes from a great white shark. Students will run PCR products out on an agarose gel to determine if the banding pattern matches that expected from a great white shark. As an instructor you should be familiar with the following procedures.

DNA Extraction

Students will not be extracting the DNA themselves. We provide the DNA as PCR products (amplified regions of DNA). However, the following information provides background knowledge that is useful for the educator.

DNA extraction is the process of removing DNA from the nucleus of a cell. This is the first step researchers must perform in order to examine the genetic makeup of an organism. In this case, DNA is extracted from the cells that make up shark fin tissue. This extracted DNA will be used as the template DNA during the PCR testing for species identification. Note that students do not perform this step in the lab, but you may discuss how this process works so they know where the DNA came from.

Polymerase Chain Reaction (PCR)

Note that students do not perform PCR, but are running out fragments of DNA that represent PCR products. As an educator, it is useful to understand the process of PCR, perhaps in more detail than you will normally teach to students so you can answer questions if they arise.

The polymerase chain reaction (commonly referred to as PCR) is a technique that was developed in 1983 by Kary Mullis, an American chemist/molecular biologist. This technique revolutionized the field of genetic research by giving scientists the ability to study specific regions of an organism's genetic code. PCR targets a very specific region of the genome using a short sequence of DNA (primers) and makes millions of copies (amplifies) of that region using an enzyme (polymerase) that facilitates the copying of DNA. PCR is like photocopying a single page of a book millions of times. Millions of copies of a piece of DNA are needed in order to visualize a specific region of DNA using a technique called gel electrophoresis. Therefore, without the use of PCR, there would not be enough DNA copies of the region of interest (i.e. a gene) for scientists to study or to test. PCR is a technique with wide ranging applications in all kinds of scientific research, medical testing, forensics, paternity/pedigree testing, etc.

The PCR technique works because of the components in the PCR mixture and temperature cycling. Once the PCR mixture is made with the necessary reagents (i.e. template DNA, distilled

water, DNA primers, dNTPs (nucleotides) and thermophilic polymerase), the mixture is placed in an instrument referred to as a thermal cycler (so called because it “cycles” a number of times through a range of specified temperatures). These temperature changes facilitate the PCR cycle and its three composite steps: denaturation, annealing and extension. During denaturation, DNA fragments are heated at very high temperatures and the double stranded template DNA becomes single stranded. This single stranded DNA is now accessible to the primers. The next step of annealing involves a cooling down in temperature of this reaction mixture. DNA primers anneal (or “stick”) to the complementary regions of the single stranded template DNA and double strands are then formed in these specific regions. In the last step of extension at high temperatures, the DNA polymerase enzyme synthesizes a complementary DNA strand. The polymerase reads the opposing strand sequence and extends the primers by adding nucleotides in the order in which they can pair. This process is likened to a zipper closing and filling in of the missing teeth until a complete closed zipper is formed. One cycle involves all three of these steps and is done many times to exponentially increase (or amplify) the number of copies of a specific DNA sequence of interest.

In this activity two different sets of primers were used to generate the DNA samples that will be run on the agarose gel. This first set of primers will amplify all species of shark DNA and will serve as an internal positive control. The next step is the key diagnostic step in our forensic testing; a second PCR test that uses species-specific primers. This second set of primers are specific to great white sharks and will only make copies if the DNA we are working with comes from a great white shark. Thus, if the DNA is from another species of shark, the copying process of PCR with the second set of primers will not take place.

Gel electrophoresis

Gel electrophoresis is a technique that allows separation of molecules based on net charge and size by the use of an electric field through a porous matrix (gel made from agarose). Students will be performing gel electrophoresis in this lab. DNA, a negatively charged molecule, migrates toward the positive pole when placed in an electric field. The rate of movement depends on the length of the DNA fragment; small pieces migrate faster than larger pieces. The positive electrode is colored red and electrophoresis of DNA is always “Running towards Red”. The sizes of the DNA fragments can be determined by comparing them to a DNA standard. The size of a DNA fragment is denoted by the number of base pairs or “bp”. DNA standards can be a DNA “ladder” which has standard DNA fragments of known size and when run on a gel appear as a ladder with many rungs. By comparing the resulting pattern of the DNA fragments on the gel (looking at both the number of bands and the corresponding sizes) the different DNA molecules may be differentiated. You can also run DNA without a ladder and compare to a control sample, as we are doing in this lab. In the Wildlife Forensics Lab, the DNA standard is a sample of DNA from a great white shark.

Controls

The use of controls in scientific experiments and testing is universal. Controls verify that a procedure or technique was done properly (because it produced the expected results) and allow for the results of unknown samples to be trusted. Controls are particularly important in genetic work where the results of the experiment are dependent on the proper completion of a series of

steps. Thus, in a test that relies simply on the presence or absence of a PCR product (specific fragments of DNA) the use of a positive control is crucial.

Positive controls allow scientists to be sure that all steps have been performed correctly and allow them to verify the results of unknown samples. For example, by including a positive control in a PCR reaction (e.g. a sample that we know contains the target DNA) we can test that the reactions have been properly set up and that the reaction worked. If at the end of testing a positive control does not yield a result (e.g. at least one PCR product visualized as a band on the agarose gel) you can conclude that something in your protocol or procedure was not correct. Therefore you cannot trust the results of your experimental sample and you would need to repeat the experiment. In our case, we are using a positive control and it is the region of DNA that amplifies in all species of sharks as well as a region of DNA that only amplifies in great white sharks. We use this positive control as a comparison for the experimental results.

Vocabulary

Biotechnology: Using living organisms (or things from living organisms) to make products or create processes that improve our lives. Applications of biotechnology can be found in areas of forensics, medicine, agriculture, food industry, marine environments, biodefense, and for environment science.

DNA: Deoxyribonucleic acid. A series of deoxyribonucleotides (adenine, guanine, cytosine, thymine) that encode the genetic material of an organism. The structure of DNA is a double helix.

DNA Marker: A series of DNA fragments of known size used to determine the size of unknown DNA fragments (e.g. PCR products) by comparison. Often called a “DNA ladder”.

dNTPs: Deoxynucleotide triphosphates (“nucleotides”). A term referring to the nucleotide bases that the DNA polymerase uses to copy a piece of DNA during the last step of the PCR process called extension.

Gel Electrophoresis: A method of separating molecules based on their size and electric charge. Molecules are forced to run through a gel (a porous matrix) by placing them in an electric field. The speed at which they move depends on their size and charge. DNA fragments separate out based on a difference in size since they are all negatively charged.

Nucleotides: The structural units or building blocks of DNA and RNA. The four nucleotides that make up DNA are adenine (A), thymine (T), cytosine (C) and guanine (G) (Note: uracil (U) replaces thymine (T) in RNA). Nucleotides are used in PCR in the form of dNTPs (deoxynucleotides). In a PCR reaction mixture, dNTP’s are needed to copy the template DNA.

PCR: The Polymerase Chain Reaction. A method of making millions of copies of a specific region of an organism's genome (amplifying) through alternating cycles of specified temperatures.

PCR Mixture: Often called the PCR “cocktail”. The components (ingredients) used in a PCR reaction. The specific components of the PCR mixture are template DNA, distilled water, primers, polymerase and dNTPs.

PCR Cycle: Involves the three steps of the polymerase chain reaction that rely upon changes in temperature. Step 1: Denaturation at 94 °C, Step 2: Annealing between 55-65 °C (dependent on the primers used) and Step 3: Extension at 72 °C.

PCR Product: The result of the polymerase chain reaction (PCR). After successfully completing PCR, the millions of copies of the targeted region of the template DNA are referred to as PCR product.

Polymerase: An enzyme that catalyzes the synthesis of nucleic acids on pre-existing nucleic acid templates. The polymerase used for PCR must be able to withstand the high temperatures needed during the extension step in the PCR cycle. Hence, thermophilic (“heat –loving”) polymerases are generally used for PCR.

Positive Control: A sample that demonstrates whether your manipulation of the variable produced the desired effect. In this lab, your positive control is the amplification of a region of a shark genome that occurs in all species of sharks. Thus, if there is any shark template DNA present in your PCR, you should see a band on your agarose gel verifying that the PCR and gel electrophoresis processes worked.

Primer Set: Small pieces of single stranded DNA (usually ~ 10 - 20 bp long) that anneal (stick to) to a specific region of DNA. Primer sets consist of both a forward and reverse primer that are designed and synthesized by researchers to frame the area of the genome that is of interest, essentially defining the area that will be copied millions of times.

Template DNA: A sample of DNA being tested. Template DNA usually consists of an organism's entire genome and provides the initial sequence for amplification using PCR

Thermal Cycler: A machine that cycles among different temperatures and is used to carry out PCR.

Web Resources

Additional Information on Dr. Mahmood Shivji’s DNA Application for Shark Fin Identification:

http://www.nova.edu/ocean/ghri/flseagrant_mag.pdf

http://www.sharkinfo.ch/SI2_02e/mahmood2.html

http://www.sharkinfo.ch/SI1_00e/molecbiol.html

<http://www.nova.edu/ocean/wcs803.html>

Information on Shark Conservation and Management:

<http://www.nova.edu/ocean/ghri/sharkresearch.html>

<http://www.sharks.org/>

<http://www.wcs.org/73133/73304>

<http://www.bite-back.com/>

Wildlife Conservation and Management Programs that Utilize DNA Technology:

<http://www.wdnas.com/>

<http://www.earthtrust.org/dnaproj.html>

<http://www.theriondna.com/fact-wild.htm>

<http://www.pgc.state.pa.us/pgc/cwp/view.asp?a=482&q=170656>

http://www.wildlifetrustofindia.org/html/news/2003/030514_dna.htm

<http://www.ifaw.org/ifaw/general/default.aspx?oid=208726>

<http://www.conbio.org/CIP/article30711.cfm>

<http://www.durrell.org/About-Durrell/Durrell-News/Durrell-discover-unique-DNA/>

http://www.eurekalert.org/pub_releases/2007-04/wcs-umg042007.php

PCR: Basics of Polymerase Chain Reaction:

<http://people.ku.edu/~jbrown/pcr.html>

<http://www.accessexcellence.org/RC/VL/GG/polymerase.html>

<http://allserv.rug.ac.be/~avierstr/principles/pcr.html>

<http://www.pcrstation.com/pcr-cloning-alternative/>

http://www.contexto.info/DNA_Basics/polymerase_chain_reaction.htm

<http://irc.igd.cornell.edu/MolecularMarkers/PCR%20basics.pdf>

Gel Electrophoresis: Basics Concepts and Applications

http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis

<http://gslc.genetics.utah.edu/units/biotech/gel/>

<http://www.life.uiuc.edu/molbio/geldigest/electro.html>

<http://arbl.cvmbs.colostate.edu/hbooks/genetics/biotech/gels/agardna.html>

Material Preparation Before the Students Come

1XTAE Buffer Preparation

1XTAE Buffer, and not water, must be used to make and run the gels. The buffer supplies the necessary ions to conduct the electricity. The buffer received in the kit is 10X Tris-Acetate-EDTA (TAE) with 150 ml in three conical tubes, and needs to be diluted with distilled water (dH₂O) to make a 1X concentrate solution. To dilute to **1 X TAE**:

1. Add the entire 150 ml of 10X TAE buffer to 1350 ml of distilled water in the larger container provided, mix well. From this now diluted 1X TAE buffer, 120 ml will be used to make the agarose gels and ~1000 ml will be used as the electrophoresis running buffer. Keep this buffer cold in a refrigerator. Depending on how many classes you have requested supplies for, you may have more buffers.
2. This buffer can be made days in advance of the lab and stored in a sealed container to prevent evaporation.

Prepare a set of five 1.0 % agarose gels

Agarose gels can be made up to 2-3 days before use. To store, place in sealed container with a small amount of dH₂O to prevent desiccation. The following instructions will make 6 small gels. Each gel will have 8 wells and will accommodate two groups of students with 3 samples each group. You will have one extra gel if needed (i.e. if a gel gets broken or damaged). You may need to make more gels depending on the number of classes you requested.

Preparing Casting Trays

1. Place the rubber dams onto the ends of each gel tray. See Figure 1. It is easiest to lay the rubber dam on a table and holding the gel tray carefully press it into one corner then using your weight to “roll” the gel tray into the second corner and repeat with the other rubber dam. Use caution to prevent breaking the gel tray.
2. Place the gel trays with rubber dams onto a flat surface.
3. Position the comb, with the small teeth down (Use the large teeth only, as it will create 8 wells of the needed size).
4. Pour the contents of the plastic bag marked “1.2 g agarose” into the empty glass bottle labeled “Agarose”
5. Add 120 ml of the diluted 1X TAE buffer and shake gently to mix.
6. Dissolve the agarose in a microwave or on a hot plate. The orange bottle cap must be removed before heating. The power of the microwave may vary, but to prepare 100 ml of agarose it generally takes 1.5-2 minutes on high power. For best results place the bottle in the microwave for one minute, gently swirl and heat for 30 more seconds, gently swirl and heat another 30 seconds only if needed. Do not over heat as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and to mix, then continue heating. The agarose must be completely in solution and well mixed. No particular matter should be visible.

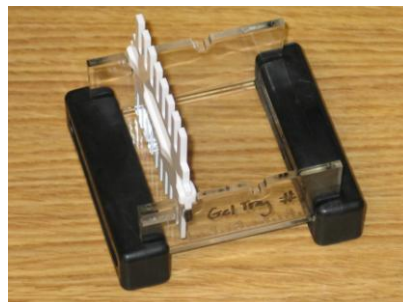


Figure 1 Loading Tray with Comb and Dams

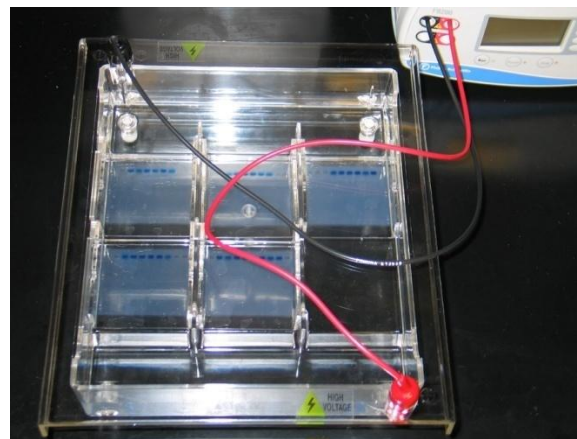
7. Cool the agarose solution to about 60°C by placing the agarose bottle in a 60°C water bath or by allowing it to stand at room temperature for several minutes. Swirl occasionally while it is cooling to avoid rapid cooling of the agarose in the bottom of the bottle, in which it starts to solidify (or reheating will be necessary). The bottle of melted agarose solution is ready to be used when it is warm to the hand, but not too hot to handle (if it's too hot it can warp the comb and gel tray).
8. Add 5 drops of Carolina Blu™ stain (from the dropper bottle) to the bottle of 120 ml of the cooled agarose and swirl to mix. This stain is not toxic; however, it will stain your hands so wear gloves.
9. Swirl the mixture and slowly (to avoid air bubbles) pour 20 ml cooled agarose solution into the 6 casting trays using a graduated cylinder (use a pipette tip to pop any air bubbles).
10. After the gel has hardened (about 30 minutes) gently remove the comb. It is important that the gels have completely solidified before the comb is removed.
11. Being very careful so that the gel does not slide off the gel tray, remove the two rubber dams from each end of the gel tray.
12. The gels may be stored for several days by placing them in a zip-lock bag or other plastic container with a small amount of moisture (dH₂O). Refrigeration is best but not required.
13. Gels stored longer than two to three days tend to lose their stain and the DNA may not be visible during electrophoresis. These gels are still usable, but will require longer staining after electrophoresis.

Prepare Electrophoresis Running Buffer

Measure out 1000 ml of 1X TAE buffer from Step 1, and add 49 drops of Carolina Blue and mix well. This now is the electrophoresis running buffer that will be used to run the gels. The gel boxes require approximately 1000 ml of running buffer. The buffer may be stored in a refrigerator. It is very important to keep the running buffer cold in the refrigerator until used. Cold 1XTAE running buffer is needed due to the heat that is generated while running the gels at 200 volts.

DNA Samples

The student DNA samples come pre-aliquoted. Before distributing to students, you must centrifuge all of the samples to ensure the very small amount of DNA (17 µl) is in the bottom of the tube. During class, if a group of students are having difficulty pipetting, you may need to re-centrifuge to get the entire sample back to the bottom.



Setting up 10 student Workstations and Shared Student Equipment

Each of the 10 student workstation should contain the following:

Materials

- Student Worksheets (1 per student)
- Envelopes with pre-lab materials (1 envelope per station)
- Tape (1 per station)

Reagents

- DNA samples (3 total)
 - Positive control “C”
 - Shark Fin Samples “SF#” *Note that odd numbers denote samples that are from a shark that is not a great white and even numbers denote samples that are from great white sharks. You can choose to give each student both an odd and even number, or distribute randomly.*
- Practice loading dye
- Loading Dye
- Practice Gel
- 5 agarose gels (1 gel per two groups)

Equipment

- 1 micropipette
- box of yellow tips (1 box shared by 2 groups)
- 1 waste container for used tips and tubes
- 1 foam Microtube rack
- 1 tube yellow water
- 1 tube blue water
- 1 empty 1.5 ml microcentrifuge tube for micropipette challenge

Shared Equipment Workstations:

- Centrifuge (teacher use)
- Electrophoresis box with power supply
- 2 staining trays
- 1 pair non-latex gloves (for teacher when handling Carolina Blu Stain)
- Bottle of Carolina Blu Stain

Pre-Laboratory Lesson Plan and Facilitation Guide

The purpose of the Pre-Laboratory activity is to provide students with the opportunity to model the process of agarose gel electrophoresis for the purpose of identifying different species of shark. In this activity, students will conduct a paper version of DNA gel electrophoresis to evaluate the results from a PCR reaction. Students will need background information on the basics of PCR (amplifies exponentially targeted regions of a genome) and gel electrophoresis (method for separating and visualizing DNA of different sizes). In-depth information can be found in the background section of this lab.

The objectives of the Pre-Laboratory are:

- Identify a need for PCR and agarose gel electrophoresis
- Model gel electrophoresis

Materials Needed:

- 1 copy of the Student Pre-Lab Worksheet- each student
- 1 envelope containing paper pieces of DNA for each group of students (total 10 student groups)

Natural Stopping Points. If you will be completing this lab over several class periods, there are several places that are natural stopping points. You may choose to complete the micropipette challenge and/or the practice loading gel activity in a separate session. You may also break up the lesson by having students load and run (or begin running) their gels during a single class period. The instructor can then take care of removing gels from gel box once the run is complete, staining and destaining the gels and then store them in a ziplock bag (with about a tablespoon of buffer to prevent drying out of the gels) for up to a week. Next time you see your students, they can then analyze and interpret the results.

Pre-laboratory Engagement (15 minutes)

Divide students into 10 groups. Have all students read the laboratory introduction found on page S-1 of the student handout. You may choose to have one member of the class read each paragraph aloud. After each paragraph, ask the students to answer the questions that are in italics.

At this time introduce the concepts of PCR and gel electrophoresis. Student should understand that PCR is a technique that allows researchers to target specific regions of a genome and make millions of copies of that particular genetic sequence. Students should understand that gel electrophoresis is a molecular technique that separates DNA according to its size. It is a way for researchers to visually see the results of a PCR.

Pre-laboratory Exploration (15 minutes)

1. Each group will receive 1 envelope, which contains 3 lanes worth of paper DNA pieces each in a separate envelope. These pieces represent PCR products (the samples to be ‘loaded’ into three lanes on a gel). One envelope will contain the positive control sample (containing two bands of DNA), and the other two envelopes will contain DNA from two different shark fin samples (number of bands will vary, from 1-2 depending on whether their samples is from a great white shark or not).

	Well 1
580bp	Control

Examples of piece of DNA found in envelope. The front of the piece of paper has a black band and numbers representing the size of the DNA fragment. The back of the paper has information about which well the band should be put in and the type of sample (control or sample of shark fin).

Included on the student worksheet are numbers along the side of the gel that represent where DNA samples of specific lengths would migrate to during electrophoresis. This is necessary in this simulation to help students know how ‘far’ a piece of DNA would run if an actual current were run through the gels. In the actual lab, the DNA bands will be allowed to migrate for a specific period of time (15 minutes) and the distance they travel will become evident upon staining the DNA.

2. Ask the students to label the positive and negative electrodes, and sample names on the gel picture found on page S-2 of the Student Pre-Lab Worksheet. Ask them to draw an arrow in the direction that the DNA will migrate. Remember, because the DNA is negatively charged, it will be pulled toward the positive electrode. Therefore, the wells of the gel should be next to the negative electrode (opposite end of the positive electrode). DNA “runs to the red”.

3. Each student group will open their own set of envelopes. Have each student group arrange their sample bands in the appropriate order on the drawing of an electrophoresis gel and to attach with tape. All the bands from each envelope need to be in a single lane. A lane is the area directly underneath each well. Remind the students to keep the bands from each envelope separate (they can always check the label on the back of each piece to figure out which lane the piece of DNA is meant for). This would be similar to preventing contamination of individual samples while testing in the lab. They should use number guides on the left side of the paper to assist them in figuring out how far down the paper each piece of DNA would travel. For example, if one of their bands is 1340 bp, it should be taped on the paper below the 1500 bp level, but above the 1000 bp level.

Pre-laboratory Explanation (30 minutes)

After each group has finished putting the DNA fragments on their gel drawing on the Student Pre-Lab Worksheet, ask each group to analyze their results and to be able to present their findings to the class. Have each group draw their results on the board. Each group will have different results that represent a different scenario, depending on the DNA samples that were in their envelopes. Lead a class discussion regarding their conclusions and the process they employed to determine whether their fin samples belonged to a great white shark.

Pre-laboratory Elaboration and Evaluation

Ask each student to write a report to the Custom's officials who confiscated the shark fins. This report should explain the two molecular techniques used to generate these results (PCR and gel electrophoresis) and the results of the genetic analysis performed by your class: how many samples were from great white sharks and how many samples were not from great white sharks.

Laboratory Lesson Plan and Facilitation Guide

The purpose of the laboratory activity is to apply the concepts developed in the pre-laboratory activities to determine the genetic identity of tissue extracted from confiscated shark fins using gel electrophoresis.

The Objectives of the Laboratory Activity are:

- Practice Using Micropipettes and Loading Gels
- Perform gel electrophoresis using PCR products
- Analyze the results of gel electrophoresis
- Determine whether the experimental samples are from great white sharks

The classroom teacher must instruct students with basic laboratory safety rules and provide goggles for student use with the laboratory activity.

Suggested lesson plan

1. Organize the students into 10 workgroups. Students should have read the story on page S-1 of the handout during the pre-laboratory activity. If they have not completed the pre-laboratory activity, have students read the story on page S-1 of the student handout. You may choose an individual to read out loud. After each paragraph, stop reading and ask the students to answer the questions in italics. You may ask for a student volunteer to share their answers.

2. After students have identified the problem that is to be solved during the lab, turn their attention to their workstations. Introduce students to the equipment and reagents in front of them (and shared among groups). These include:

- DNA samples (C, SF odd #, SF even #)
- Micropipette Challenge tubes (yellow and blue water and empty tube)
- Loading dye (LD)
- Practice loading dye (prac. dye)
- Pipette
- Agarose gel (shared by two groups)
- Electrophoresis box
- Box of micropipette tips (shared by two groups)
- Waste container

3. Demonstrate to the students the proper use of the Micropipette. See Student Technical Skills section on page 23 for a detailed explanation of how to work a micropipette.

4. Ask the students to complete the Micropipette Challenge on page S-4 of their student worksheets.

5. Next, demonstrate for the students how to load the wells of their practice gels. See Student Technical Skills section on page 23 for detailed explanation of how to load gels. Then have the

students practice loading agarose gels using the round practice gels at their stations. You may want to draw Figure 3 from page 24 on the board to help demonstrate proper loading techniques.

6. Once students have practiced using a micropipette and practiced loading a gel, they are now ready to complete Step 1 of the student protocol. After they have filled out their table, ask a volunteer to share their observations. They may have noticed that the sample tubes appear to contain “nothing” or “water” or a “clear liquid”. At this point, as a facilitator, you can point out that the sample tubes actually contain PCR products. PCR products are many copies of very specific regions of DNA. In this case, if the DNA samples came from a shark of any species, there are many copies of a region of DNA that is 1540bps. If the sample is from a great white shark, it will contain two bands, one present in all species of sharks (1340 bp) and one band that is only present if the sample came from a great white shark (580 bp). Emphasize that these pieces of DNA are not visible and that the process of DNA gel electrophoresis is what will allow us to separate and visualize these DNA bands.

7. Next, you may choose to let them work their way through the protocol on their own, or walk them through step-by-step.

8. In Step 2 of the Student Protocol, students are adding 5 μ l of loading dye to their samples. Students are asked to add it to the bottom of the tube and gently pipette up and down to mix the DNA and dye. If the samples get stuck on the sides of the tubes, you may need to centrifuge the samples to bring all the liquid to the bottom of the tube. Loading dye is added to the samples to help visualize the samples while loading into the wells of the gels and the loading dye also increases the sample density and keeps the samples in the wells while adding the running buffer. When the gels start to run, the loading dye will start to separate into three separate colors. The blue the largest, purple in the middle, and the yellow is the smallest. It is important for the students to understand that these colored bands seen in the gel are not stained DNA, they make up the loading dye.

9. In Step 4 of the student protocol, each group will be assigned to three wells in a gel. You can pass out the gel trays to each group, but remind them not to pick them up (they are slippery and may slide off the gel tray if not handled carefully). Before they load their samples into the gel, you must assign each group to one of the 5 gels (remember you have one extra gel in case of damage) you have already poured (numbers are written on the sides of the plastic gel trays) and then to specific wells within the gel (students will have to imagine the wells are numbered, 1-8 from left to right). One group can be assigned wells 1-3 and the other group can be assigned wells 5-8. This will leave two empty wells in the middle of the gel. Remind the students to write this information in the data table on their worksheets.

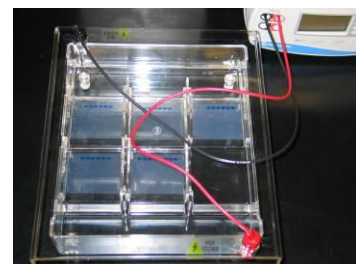


Figure 2. Gels properly oriented in gel box.

10. In Step 3 of the student protocol students are loading 20 μ l of their samples into the wells they were assigned in the agarose gel.

11. In Step 5 of the student protocol the educator must go around and collect all of the gels and place them in the gel electrophoresis box. Be sure to place the gel trays in the gel box so the ends containing the wells are closest to the black electrode, or the samples will run backwards (see Figure 2). Be careful not to let gels slip off the gel trays during this process. Slowly pour 1000 ml of the 1X TAE COLD running buffer into the bottom chamber of the gel box (nearest the red electrode). Do not pour the buffer directly onto the gel or the samples may come out of the wells. The gels in the trays need to be completely submerged to run, but the top of the trays (sides) will be exposed out of the buffer while running. Put the lid on the gel box and connect to power source. Bubbles rising from the electrodes in the box will confirm that power is running through your gel. Run for 15 minutes at 200 volts. The agarose gels act as a sieve to separate the different sized DNA fragments. The DNA samples in the gels are invisible but they will become stained with the Carolina Blu™, which is mixed into both the agarose gel and the electrophoresis running buffer. A final Carolina Blu™ staining procedure may need to be performed to darken the bands in the gel and to allow the loading dye to fade in the gel, allowing for the gels to be analyzed for results.

12. While the gels are running you can ask students to label the diagram on page S-7 of their student worksheets. Labels will include names of samples loaded, where the positive and negative electrodes were and what direction the DNA will migrate.

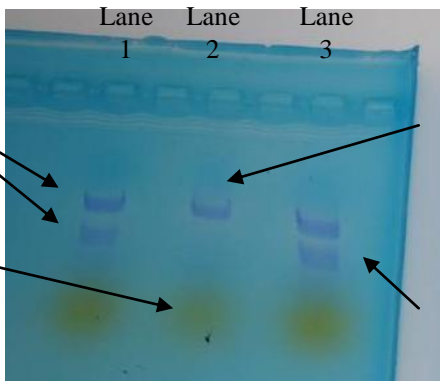
13. After the gel has run for 15 minutes, turn off the power and open the gel electrophoresis box. At this point, staining and destaining may or may not be necessary depending on how well the bands absorbed the stain that was present in the gel and running buffer. If you need to stain and destain follow the following procedure. Remember to use gloves when handling Carolina Blu Stain as it will stain you and your clothes.

14. To stain the gels, gently remove the gels from the trays and place them into the two staining trays and cover them with the Carolina Blu™ Final Stain (large bottle split between two staining trays) for 15 minutes. After staining and with gloves on, remove the gels and place those on a plastic surface or plastic wrap. The used final stain should be poured back into its bottle and returned with the equipment (please mark on the bottle if you used it). Place the gels back into the staining tray and rinse them off with distilled water and pour it out. Destain them by adding distilled water to the staining tray and allow them to sit for 15 minutes. Change the distilled water and repeat for a minimum of 15 minutes. It is best to destain the second time for longer than 15 minutes or the DNA bands may be difficult to see with the loading dye still present in the gels. Gels can be left overnight in distilled water and viewed the next day; the loading dye will be gone and the DNA bands easier to see. Water from the destain process can be poured down a sink drain. The DNA bands are best visualized when viewed against a white background or even better on a light box.

15. Allow the students time to view their gels and copy the results into the diagram on page S-7 of their student worksheets. Students should be reminded that they are looking for the blue bands and should ignore the colors from the loading dye (blue and/or yellow dye). Figure 4 shows a gel loaded with a positive control in lane one and a sample from a shark (not a great white shark).

This is the control sample from a great white shark. It contains two bands, one 1340 bp and one 580 bp.

These yellow bands are from the loading dye and should be disregarded when interpreting the gel.



This single 1340 bp band represents a sample from a shark, but NOT a great white shark.

This single 1340 bp band and 580 bp represents a sample from a great white shark.

Figure 4. Results of gel containing a positive control in Lane 1 and unknown Shark Fin samples in lanes 2 and 3.

16. Have students complete the Analysis questions on their student worksheets. Have the students share their answers with each other or turn in their papers for assessment.

17. You may choose to follow up the laboratory activity by completing one or more of the extension activities on page 25 of the teacher instruction manual.

Answers to Student Worksheet Questions

From Page S-1:

What is the problem that needs to be solved? Write your answer below.

Customs official suspect they have confiscated fins belonging to a federally protected shark, the great white. But they are unable to visually determine what species the dried fins are from. They need a way to determine what type of species these fins are from.

What two molecular techniques were done by other members of your team before they gave the samples to you? Write your answer below.

DNA extraction was performed to obtain DNA from each of the confiscated shark fins.

PCR was then performed using the extracted DNA. The PCR technique allows researchers to make millions of copies of specific regions of DNA. In this case, scientists are making copies of the genome that is present in ALL sharks and also making copies of a region of a genome that is only present if the shark is a great white.

What molecular technique are you going to perform? Write your answer below.

Students are performing gel electrophoresis. Gel electrophoresis allows DNA to be separated according to size and visualized on an agarose gel. Students will be running the results of the PCR reaction that was performed for them.

Page S-4

How many microliters (μ l) should you have in the test tube when you are done?

25 μ l

How many milliliters (ml) should you have in the test tube when you are done?

0.025 ml

Pages S-6 and S-7

1. What was the purpose of the Control Sample?

The control sample provided a comparison for students to compare their samples to. Without the control, they would not have known what the results from a great white shark would have looked like.

2. How and why are you using the agarose gel to compare your DNA samples?

Just looking into a tube of DNA, you cannot see the DNA. It needs a medium on which the DNA can be separated. The agarose gel provides a medium through which differently sized pieces of DNA can be separated and visualized. The porous matrix of the gel allows smaller sized pieces of DNA to move quicker through the gel and therefore migrate a farther distance than larger pieces of DNA.

3. What allows the DNA samples to move through the agarose gel? Explain how the size of a piece of DNA relates to how fast and how far it moves through a gel.

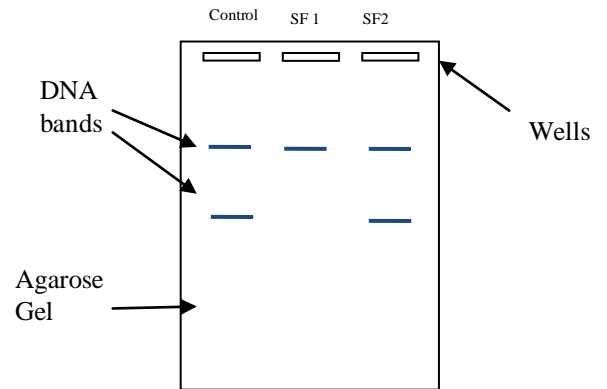
DNA has a negative charge and is attracted to the positive electrode on the opposite side of the gel electrophoresis box. As the electrical current pulls the DNA through the agarose gel the DNA with smaller numbers of base pairs (bp) have a lower molecular weight. These smaller DNA fragments will move through the gel at a faster rate than the DNA fragments that have many base pairs. DNA fragments are differentiated and seen on the agarose gel as bands.

4. Why was a genetic test needed to verify the identity of the shark fins? What molecular techniques were used?

Some species of shark are able to be legally imported into the United States and some are not. Customs officials were unable to visually identify what species of shark the dried shark fins they confiscated belonged to. So they must look at the DNA to determine what species of shark the fin belonged to. The results from these genetic tests may become evidence in a criminal trial if the shark fins are found to be from a great white shark. Two molecular techniques were performed by scientists before giving the samples to the students: DNA extraction and PCR (polymerase chain reaction). Students then performed a third genetic technique, gel electrophoresis.

5. Draw your results in the picture below. Label the wells with the name of the sample you loaded, where the positive and negative poles are, which direction your DNA traveled and how the DNA pattern of your samples look.

See drawing to the right for sample of a student response. Responses will vary depending on which shark fins samples were run (odd numbered samples will have a single band (not great white shark) and even numbered samples will have two bands (great white shark)).



Example of a labeled student gel.

6. Observe the banding patterns on your gel. Do you see differences or similarities between the SF samples and the control sample you loaded?

Answers will vary depending on samples loaded by students.

All control samples should have two bands (one 1340 bp and one 580 bp).

Odd numbered samples will contain one band (1340 bp) and are not from a great white shark, although they do come from another species of shark.

Even numbered samples will contain two bands (one 1340 bp and one 580 bp). These samples are from a great white shark.

7. Can you identify what type of shark any of the fins may or may not be? Why or why not? If you can identify them, what type of shark were they from?

See above. Students should state that they needed to compare their results to the control sample to determine if they were from a great white shark.

Student Technical Skills

As the facilitator, it is the classroom educator's responsibility to demonstrate to the students how to properly use the equipment and reagents in this lab. Proper use of the equipment and reagents is necessary to obtain accurate results from the experiment. Educators should read through the following material and present this information to their students before students use the equipment to complete the laboratory activities.

Micropipettes

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1 μl .

1 liter = 1,000 ml = 1,000,000 μl .

You can imagine that it would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Micropipettes differ in the volume of liquid they can accurately measure. They are expensive and must be used with care. Their accuracy is dependent upon their proper use.

Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume. **Directions for the specific brand of micropipette contained in your kit are provided in the bag containing the micropipettes.** Even if you have used micropipettes before we strongly urge you to read through the directions provided as some windows have very different ways of representing volumes (such as 100 mean 1000 and 10 meaning 1.0).

Before students perform the laboratory activity, discuss with students how to use micropipettes properly. Because loading the wells of agarose gels can be difficult at first, students will be given an opportunity to practice loading dye samples into a practice agarose gel by performing the Practice Gel Loading Exercise.

Micropipettes have 2 stops as you depress the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip depress the plunger control only to the first stop. If you go to the second stop you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume). When you are letting the liquid out of the tip then you go to the second stop. It is worthwhile to check each student for correct technique before beginning laboratory procedures requiring use of the pipettes.

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.

3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To draw liquid into the micropipette tip:
 - a) Depress the plunger to the first stop to measure the desired volume and hold in that position.
 - b) Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
 - c) Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
5. To dispense the liquid:
 - a) Place the tip into the container where the liquid is to be released, near the bottom.
 - b) Slowly depress the plunger to the second stop to blow out all of the liquid in the tip. Be careful to not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
 - c) Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depends on the brand of micropipette).

Practice Gel Loading Exercise

“Loading gels”, or filling the wells of a gel, can be a challenging task especially if one has never done it before. This is an opportunity for your students to practice before they are asked to load the actual samples involved in the laboratory activity. Encourage them to take their time, figure out how they feel most comfortable doing this (example: some people like to rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remind them that this is for practice so don't get frustrated if they spill out of a well or if they accidentally tear the edge of the agarose gel.

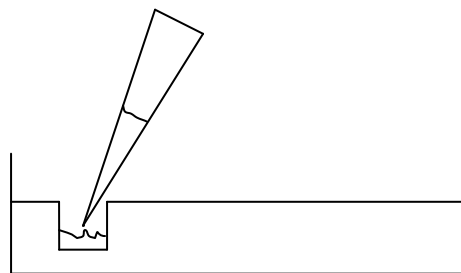


Figure 3. Correct position of tip when filling well.

An important thing to note about gels: the wells appear as holes, but they really aren't. They are more like indentations that do not go through completely to the bottom of the Petri dish. This is why it is so important not to poke the micropipette tip through the bottom of the well, or else the liquid will seep into the bottom of the dish and not stay in the well. Figure 3 shows the correct positioning of the pipette tip when filling a well.

Extension Activities

The following extension activities may be used to reinforce the concepts introduced during the Pre-Laboratory activity and the Laboratory activity.

1. Summarizing and Reporting Data

In this activity, students will summarize the class data collected during the laboratory. They will then prepare a report to send to the U.S. Customs agents. The report will 1) explain how they obtained the results, 2) briefly summarize the use of species-specific primers, PCR and gel electrophoresis 3) conclude whether their data confirms or refutes U.S. Customs suspicions.

2. Ecosystem Stability

In this activity, students will draw a diagram illustrating the importance of great white sharks in the marine ecosystem. In order to address the main issues, students must supply the following information:

- Niche: What role does a shark play in an ecosystem? Name an animal that plays the same role as a shark in a terrestrial ecosystem.
- Trophic level: What does the great white shark eat? What eats great white sharks?
- Energy level: Diagram the flow of energy in this ecosystem. Is total energy increasing or decreasing as you work your way to the top of the food web?
- Diversity: What effect would the removal of great white sharks have on the diversity of a marine ecosystem?

3. Species at risk, conservation and biotechnology

Have the students explore why protecting the great white shark is important. Break the class into 3 groups. Group 1 will list potential reasons for preserving a species based on intrinsic (e.g. a shark is valuable in and of itself, that value does not depend on external factors) as well as extrinsic (e.g. monetary value, for the enjoyment of humans, etc.) values. Group 2 will discuss the great white sharks' role in the ecosystem (top predator), its life history traits that make it susceptible to over harvesting (low reproductive rate, large body size). Group 3 will discuss the role biotechnology can play in protecting nature. How would development of new biotechnology that would allow rapid identification of species affect wildlife that is often traded on the black market? Each group will record the main points made during their discussions and share them with the entire class. As a class, synthesize the ideas from all groups. Students should understand that conservationists need to take into consideration a broad range of problems and use many tools to develop solutions.

Wildlife Forensics Student Data Worksheet

A group of customs agents suspect that someone is trying to illegally smuggle great white shark fins into the U.S. They have taken a bag of dried up fins, which appear to be from sharks, but they cannot tell which species of shark. The suspect claims they are from porbeagle sharks, which are a species of shark that can be legally imported into the U.S. But customs agents found a label in the bag that read “Blanco” (which means white in Spanish). This label made the customs agents suspect the fins were actually from a great white shark, which is a protected species and is not allowed to be imported into the U.S.



Bag of shark fins confiscated by US Customs agents. Agents suspect the fins are from great white sharks, a federally protected species in the US.

What is the problem that needs to be solved? Write your answer below.

You are working in a wildlife forensics lab and are a member of the team assigned to identify these shark fins. Other members of your team have extracted DNA from the shark fins and performed PCR on the samples. It is your job to run out the resulting DNA from the PCR samples using gel electrophoresis to determine if they are from a great white shark or not. You will include a positive control (DNA from a great white shark) so you can compare your samples and determine whether or not they are from great white sharks.

What two molecular techniques were done by other members of your team before they gave the samples to you? Write your answer below.

What molecular technique are you going to perform? Write your answer below.

Paper Gel Electrophoresis Activity

1. On page S-3 of your worksheet, you have a drawing of a virtual agarose gel. You will use this gel to run out the pieces of DNA that are contained in the envelope you have labeled “DNA Samples from PCR reaction of Unidentified Shark Fin Samples.
2. Open the envelope you have received. Inside you will find 3 more envelopes. Each of these envelopes represents pieces of DNA from a PCR reaction. The three envelopes are labeled:
 - Control Samples, Well 1
 - Shark Fin 1, Well 2
 - Shark Fin 2, Well 3
3. Label the following on your paper agarose gel:
 - Wells
 - Sample Names
 - Positive electrode
 - Negative electrode
 - Direction samples will move through gel (using an arrow)
4. Tape the pieces of DNA from each envelope under the appropriate well. Use the numbers along the left hand side of the gel to determine how far down each sample has moved.
5. Your teacher will ask you to summarize your results and present them to the class. Discuss with your partner how you are going to explain the results.

Fill in the sample names.
(Hint: check the front of the envelope)

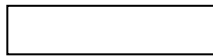
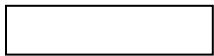
Envelope 1

Envelope 2

Envelope 3



What are these?

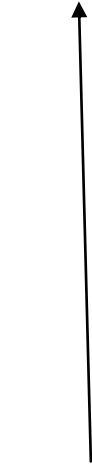


1500 bp

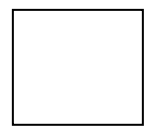
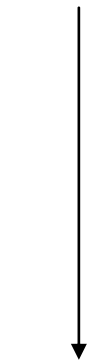
1000 bp

500bp

200 bp



Label which end of the gel is the positive electrode which is negative electrode



Use these numbers to help you determine where to tape your DNA bands.

Wildlife Forensics Student Data Worksheet

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated $1\mu\text{l}$.

$$1 \text{ liter} = 1,000 \text{ ml} = 1,000,000 \mu\text{l}$$

You can imagine that it would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Micropipettes differ in the volume of liquid they can accurately measure.

Complete the Micropipette Challenge by adding the following amounts to an empty tube. All of the amounts will be added to the same tube.

Amount to add to tube	Color	Record the setting as it appears in the window
12 μl	yellow	
5 μl	blue	
8 μl	yellow	

How many microliters (μl) should you have in the test tube when you are done?

How many milliliters (ml) should you have in the test tube when you are done?

1. Locate the following samples at your station. Pick up each tube and write what you see in the table below. Leave well number blank for now.

Sample Name	What do the initials stand for?	What does it look like?	Gel # _____ Well number
C			
SF__			
SF__			

2. Add 5 μ l of loading dye to bottom of your control sample (C) and shark fin samples (they begin with the letters SF). Use a new tip each time. Gently pipette up and down to mix sample.
3. Your teacher will assign your group to a gel and assign specific well numbers. Fill in this information in the last column of the table above.
4. Load 20 μ l of each sample into the agarose gel. Write down in the table above what well you added your sample to.
5. After all student samples are added your teacher will come around and pick up your gel. After all gel trays are added to the gel box your teacher will add electrophoresis buffer to the gel box and cover with the lid. Your teacher will connect the gel box to the power supply and the gel will run for about 15 minutes at 200 volts.
6. After the gel is done running your teacher will turn off the power supply and remove the lid of the gel box. The gels will need to be stained to make the DNA bands darker, and your teacher will do this for your group.

Wildlife Forensics Student Data Worksheet

5. Draw your results in the picture below. Label the wells with the name of the sample you loaded, where the positive and negative poles are, which direction your DNA traveled and how the DNA pattern of your samples look.



6. Observe the banding patterns on your gel. Do you see differences or similarities between the SF samples and the control sample you loaded?

7. Can you identify what type of shark any of the fins may or may not be? Why or why not? If you can identify them, what type of shark were they from?