

Name:

Wildlife Forensics

Using biotechnology with traditional law enforcement techniques

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Written and developed for MdBioLab, a mobile bioscience laboratory by Dr. Mary Stapleton.

As a technician at the U.S. Fish and Wildlife Laboratory, your department has received a package containing dried fins from unidentified species of sharks confiscated by U.S. Customs. U.S. Customs agents suspect the samples are from a great white shark, a protected species, after finding a hidden label inside with “blanco.” They are requesting that genetic analyses be conducted to confirm or reject their suspicion.

The technicians used polymerase chain reaction (PCR) to amplify target regions of DNA from each fin. PCR creates identical copies of DNA. The reaction requires a DNA template (target to be copied), “starter DNA molecules” or primers, deoxyribonucleotides (building blocks of DNA), and DNA polymerase (enzyme responsible for the catalysis of the reaction). Once these four components are present, the reaction can be started. This involves separating the two strands of DNA in the sample DNA through increasing the temperature, lowering the temperature to allow the primers to bind to target region, and increasing the temperature to optimize the action of DNA polymerase in which a new copy of the DNA target is made. Thus, at the end of the first cycle, one copy of the target has been made from each sample DNA. The entire “cycle” is repeated again, typically 30-50 times, resulting in an amplification of the target DNA.

Technicians have already extracted DNA and amplified regions of DNA specific to all sharks and great white sharks in each of the tissue samples using polymerase chain reaction (PCR). The technicians used two different sets of primers for the PCR reactions. A primer is a small segment of complimentary DNA that flanks the region to be amplified. The polymerase added to the tubes amplifies the region of DNA between the two flanking primers. One of the primer sets amplifies a region of DNA found in all sharks. The second set of primers amplifies a region of DNA found only in great white sharks.

You will analyze the results of PCR for at least two different fins using agarose gel electrophoresis. Gel electrophoresis uses an agarose gel and electricity to separate DNA fragments according to their size (number of bases, or As, Cs, Ts and Gs). Compare the fragments in the unknown samples to the great white and porbeagle shark DNA controls to determine if any of your unidentified fins are from a great white shark. One control is DNA from a great white shark and will contain both fragments – a fragment of DNA found only in great white sharks (580 bp) and a fragment of DNA found in all shark species (1340bp). The other positive control is DNA from a porbeagle shark and will contain only one fragment, a piece of DNA found in all shark species (1340bp).

IDENTIFY THE PROBLEM

What is the problem you are trying to solve?



<http://news.nationalgeographic.com/news/bigphotos/15848060.html>

Shark fins confiscated by US Customs you will be testing. Label on the outside of the bag said “porbeagle” but a hidden label inside said “blanco.” It is suspected that the fins are actually from a US protected species, great white sharks.

GENETIC ANALYSIS OF SHARK FINS

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*, law enforcement officials suspected the fins belonged to the great white shark, *Carcharodon carcharias*. Porbeagle is a species of shark that may be legally harvested. However, 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 team developed a genetic technique that allowed them to identify any tissue from great white sharks.

One of the most exciting components of the technique developed by Dr. Shivji and his team 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 to meet 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 results are presented as evidence 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.

You and your partner will analyze the PCR fragments amplified by the technicians in your department. You will use agarose gel electrophoresis. How will you know what the different fragments look like?

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.

To do the experiment, you will need several materials. The following is a list of the materials you will be using for the experiment:

Control: Porbeagle	Control: Great white shark
Unidentified fins	Loading Dye
Pipette and tips	Electrophoresis equipment

The following steps are your protocol for analyzing the PCR fragments. Work with your partner and follow the directions carefully – be sure to check off each step as you complete it.

SAFETY FIRST! Your agarose gel contains a suspected mutagen called ethidium bromide. This chemical associates itself with the backbone of DNA. Wear your gloves at all times, respect the agarose gel, your equipment and your other classmates.

1. Locate the samples in your colored tube rack. Your instructor will assign you a gel box and a set of well numbers. Write your well numbers in the table.

Sample	Sample Identification Code	Well Number
Control: Porbeagle		
Control: Great White Shark		
Unidentified Fin		
Unidentified Fin		
Unidentified Fin		

Compare the five samples to each other. Can you tell a difference between them?

2. Add 5 μ L of loading dye to each sample. Centrifuge all samples to collect the contents at the bottom of the tube. Make sure to balance your samples in the centrifuge!
3. Load 15 μ L of each sample into the appropriate wells.

- 4. Locate the beaker labeled “electrophoresis buffer.” Slowly pour enough buffer into the bottom chamber of the electrophoresis box until the liquid flows over the gel and fills the upper chamber. The gel should be covered entirely. Notify an instructor when you have finished adding your electrophoresis buffer. They will instruct you how to finish setting up the electrophoresis box.
- 5. Run the gel for at least 10 minutes at 200 volts. While your gel is running, complete the gel picture with your instructor and answer the following questions.

Write which sample is in which well

Wells →

— **Write what the charge is closest to the wells.**

— **Write what the charge is closest to the bottom of the agarose gel.**

Draw an arrow on this line to show what direction the DNA will travel in the agarose gel.

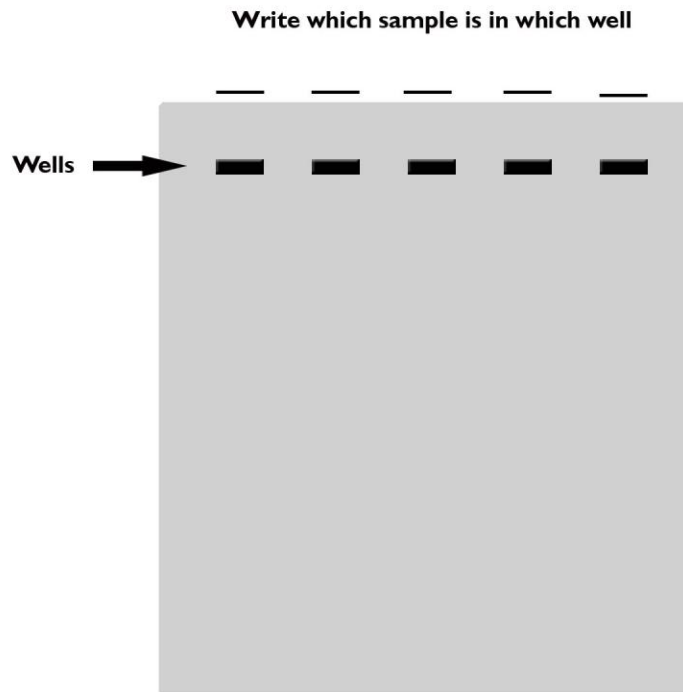
How are you using the agarose gel to compare the DNA samples?

What is the comb used for?

Why did you add electrophoresis buffer to the electrophoresis box?

What direction does the loading dye migrate? Why?

- 7. Your instructor has added ethidium bromide to your gel. This is a DNA stain that associates itself with the DNA backbone; it glows orange when exposed to UV light. Follow your instructor's guidance to place your gel on the UV light box and view the DNA fragments. Draw your results below. Be sure to label which samples you drew in which wells, the direction the samples traveled, and the charge at the top and bottom of the gel:



DATA ANALYSIS

It is time to analyze the results of your test. Observe the banding patterns on your gel. Do you see differences or similarities between the two unknown samples and the positive control? Can you identify what type of shark any of the unidentified fins may or may not be?

CONCLUSION

Think about what conclusions you can make from the experiment. When making a conclusion, scientists have to interpret the results of the test. You can use the controls to make a determination about the unidentified fins.

Based on the results of your experiment, which of the following is true (check your answer)?

- All unidentified fins were from a great white shark.
- None of the unidentified fins was from a great white shark.
- Only some of the unidentified fins were from a great white shark. Which fin(s)? _____

Make your own conclusion. Write a statement about the unknown samples when compared to the controls. Include your determination for the US Customs officials and an explanation of the results. This will be the statement that may be used in court if necessary.