

Wildlife Forensics

Using Biotechnology with Traditional Law Enforcement Techniques

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Name:

A high demand for shark fins, especially in the eastern part of the world, has resulted in the reduction of many shark populations. In response, the U.S. government has begun to protect the species of sharks that have become threatened due to over-fishing. Customs agents have received a package that contains dried fins from an unidentified species of shark. It is suspected that this package may have fins that belong to the great white, one of the U.S.'s protected species. You will need to use genetic analyses in order to determine if any of the confiscated fins were illegally obtained.

Dr. Mahmood Shivji, a conservation biologist, has devised a test that can rapidly identify a species of shark by using small fragments of the DNA in the shark fin and a technique called Polymerase Chain Reaction (PCR). When examining the great white shark's DNA, Dr. Shivji discovered a sequence of ribosomal DNA that is 1340 base pairs long. Unlike genomic DNA, this exact sequence of DNA is found in all species of shark. In addition, he discovered a species-specific fragment of DNA that is 580 base pairs and is only found in the great white species.

Dr. Shivji can use PCR to make millions of copies of these sequences of DNA. The technique involves a protein called polymerase that copies, or amplifies, specific sequences of DNA as the temperature of the sample changes. Scientists can pinpoint these specific regions of DNA by using primers that tell the polymerase the exact sections of DNA to copy. Dr. Shivji has created primers that target the 1340 base pair sequence of ribosomal DNA found in all sharks and the 580 base pair sequence of DNA found in only great whites.

Technicians have already extracted DNA from the confiscated fins and have used the ribosomal DNA primer and the great white-specific DNA primer to amplify the corresponding DNA. As a technician for the U.S. Fish and Wildlife Laboratory, it is your job to analyze these PCR results. To do so, you will utilize agarose gel electrophoresis, which uses electricity to separate DNA fragments according to their size (i.e. number of base pairs). You will need to compare the DNA fragment(s) in your unknown sample to great white and porbeagle shark DNA controls to determine if your unidentified fin is from a great white shark.



How will PCR help you determine if the confiscated fins come from a great white shark?

MATERIALS

- Control: Porbeagle sample
- Control: Great white shark sample
- Unidentified fin sample
- Electrophoresis equipment
- Micropipette and tips

PART I — Sample Prep

1. Locate the samples in your colored tube rack, and, along with the instructor, fill in the following chart:

Sample	Tube Label	Well Number
Control: Porbeagle		
Control: Great White Shark		
Unidentified Fin		

QUICK CHECK:

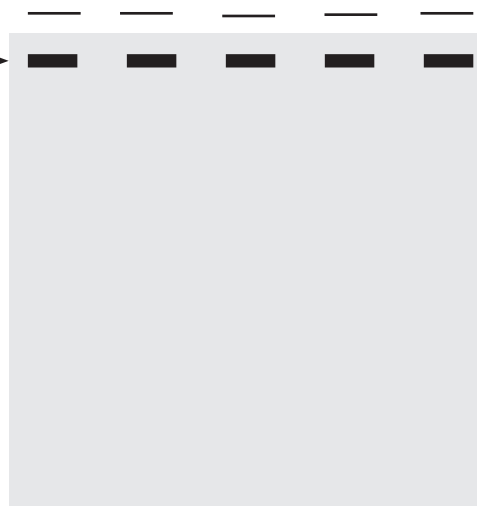
- ? Compare the three samples to each other. Can you tell a difference between them?

2. Add 5 μL of loading dye to each sample.
3. Centrifuge each sample for two (2) seconds to collect the contents at the bottom of the tube. Make sure you balance your centrifuge.
4. Locate the practice gel at your station. Practice loading 15 μL of practice dye into three different wells.
5. After practicing, load 15 μL of each sample into the appropriate wells as assigned in the chart above.
6. Locate the electrophoresis box, and place your sample into one of the six gel slots. The gel should be completely covered by the electrophoresis buffer. Place the lid on the box, ensuring that the black wire is on top.
7. Run the gel for at least 10 minutes at 240 volts. While your gel is running, complete the gel picture with your instructor and answer the questions that follow.

PART II — Agarose Gel Electrophoresis

Write which sample is in which well

Wells →



Write what the charge is closest to the wells.

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

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

- ❓ What affects the rate at which DNA travels through an agarose gel?

- ❓ Which fragment(s) (the 1340 bp ribosomal DNA or the 580 bp great white-specific DNA) will be shown in the Porbeagle DNA control?

- ❓ Which fragment(s) will be shown in the great-white shark control?

- ❓ What does it indicate if no DNA is present on the gel?

PART III — Data analysis

- 8. To visualize your results, slide your gel off of the tray and onto the UV light box.
- 9. Draw your results on the next page.

Write which sample is in which well

Wells →



PART IV — 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.

Write a statement about your unknown sample 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.

The Basking shark is another shark species whose population is threatened. Could your unidentified fin belong to the Basking shark? How would you test this?