miniPCR™ Learning Lab: Shark Attack!
DNA Fingerprinting to the Rescue

Free

The DNA Times

Shark Attacks, AGAIN!

Four shark attacks in just one month roil a surfer's paradise. Is a single shark terrorizing Reefpoint, or are there more lurking off the shores? Experts point to warmer waters due to climate change.

Reefpoint, July 10th, 2027. Following a new shark attack on Wednesday, the count rises to four. Reefpoint Bay is a known mating point for spotted seals, a favorite prey for sharks. This had never been a problem prior to last month, when the first attack ever was recorded at the popular surfing beach. Climate scientists and biodiversity experts cite warmer waters and climate change as a possible culprit. Attack survivors reported different shark characteristics as experts scramble for answers in this formerly sleepy town. Incidents share one thing in common: shark teeth as a "souvenir" on the survivor's surfboard. DNA has been extracted, amplified, and cleaved with restriction enzymes. Now marine biologists will rely on students to solve this mystery through DNA fingerprinting.

1. Scenario overview

A series of shark attacks have terrified vacationers at a remote Australian surfers' paradise, Reefpoint Beach. Students will receive DNA samples from four shark attacks all at nearby sites, and use DNA fingerprinting to assert whether the attacks were committed by the same shark or by more than one.

Shark attacks

Shark attacks are quite uncommon. Unprovoked shark attacks, where a shark attacks the swimmer without the swimmer aggravating the shark in any way, are even rarer. The average yearly fatalities from shark attacks worldwide is just two. In 2014 there were only 73 cases of unprovoked human-shark interactions, yet on occasion we hear reports of increased rates of shark sightings or human encounters within a specific geographic area. Sharks tend to prefer warmer waters and some scientists have linked increased sightings in unusual geographies to warming global weather. Others mention erratic climate patterns altering local marine ecosystems. The important thing to remember is that we are a much larger threat to sharks than they are to us, as human activity kills two million sharks a year. Sharks are among the most endangered wildlife on the planet[1].

DNA fingerprinting

Individuals within a species have mostly identical DNA sequences, but some sites in the genome have considerable sequence variation. This genetic variation can help scientists identify individuals based on their unique DNA profiles. *DNA profiling* or *fingerprinting* is generally used to uniquely identify individuals, for example to analyze evidence in forensic law enforcement cases, in paternity testing, and in other applications. A unique pattern of DNA bands in gel

[1] For more amazing facts about shark attacks, visit http://natgeotv.com/ca/human-shark-bait/facts

electrophoresis, or "fingerprint", can be generated for each individual as a result of specific differences in their genomes[2].

The first step in fingerprinting studies is to extract DNA from tissues or bodily fluids and to amplify these minute quantities of DNA using the Polymerase Chain Reaction (PCR). Amplified DNA samples are then cleaved by restriction endonucleases and separated by gel electrophoresis. The relative positions of DNA bands on a gel, resulting from differences in restriction fragment sizes, reflect variations in DNA sequences.

The DNA fingerprint patterns in this investigation are produced from different samples obtained from each of the four shark attacks, *each one already amplified and cleaved by restriction enzymes that result in DNA fragments of various lengths*.

Restriction endonucleases (a.k.a. restriction enzymes)

Restriction enzymes act like molecular scissors, cutting DNA at specific sequences that they have affinity for. Most restriction enzymes cleave double stranded DNA at short, palindromic sequences 4-15 base pairs (bp) long. A palindromic sequence is a sequence that reads the same on the forward strand and the reverse strand when both are read in the 5'-3' direction.

For example, the restriction site **5'TTAA3' is palindromic** (both strands have TTAA if read 5'-3'):

5' TAA**TTAA**CGG 3'
3' ATT**AATT**GCC 5'

[2] Similar techniques can be used to determine whether individuals belong to the same or different species. Through a combination of the Polymerase Chain Reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP), DNA banding patterns on electrophoresis gels can serve to uniquely identify species.

Restriction Fragment Length Polymorphism (RFLP)

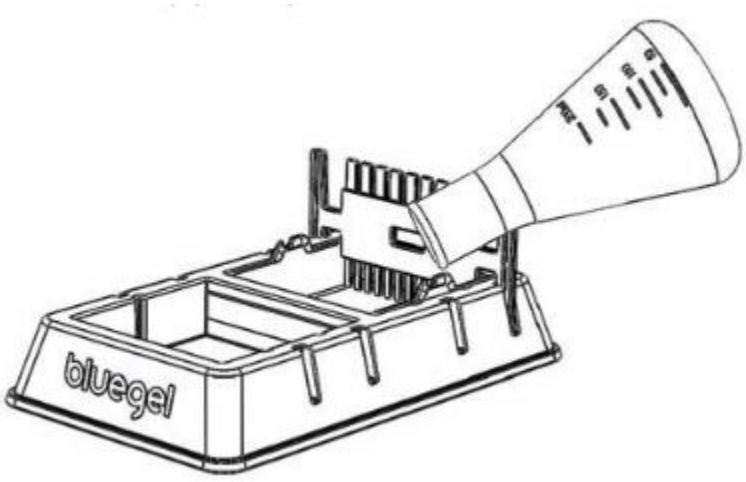
Restriction Fragment Length Polymorphism (RFLP) analysis has been an essential technique in molecular identification for many years. It was first described by geneticist Alec Jeffries in 1985. RFLP analysis relies on variations, or **polymorphisms**, in the genetic sequence, which can create or destroy a site for a restriction enzyme. For humans, each region tested is typically shared by 5-20% of the population. If ten regions are tested for, the probability that two people have the same RFLP pattern is 6 orders of magnitude under 1% (<0.000001%.)

DNA fingerprinting through RFLP analysis does not require sequencing, but relies on generating a unique banding pattern for each individual by digesting DNA with restriction enzymes. The size of the fragments created after digesting DNA with restriction enzymes can be resolved using gel electrophoresis, where DNA fragments will migrate differentially across individuals because of their size differences.

RFLP analysis became the first DNA identification method and was widely used for gene mapping, paternity testing, to determine risk for disease, to identify species, and for other molecular genetic needs. It is a convenient technique that can be simpler to implement than DNA sequencing. But as DNA sequencing becomes faster, cheaper, and more ubiquitous, it is likely to gain more relevance in personal identification applications.

2. Laboratory guide

A. Cast agarose gel (before or during class period) – 15 minutes



1. Prepare a clean and dry **blueGel™ gel casting system** on a level surface
   - Place gel tray in the cavity of the casting platform and insert one comb into the slots at the top of the casting tray, with the 9-well side facing down.

2. Prepare **20 ml of a 2% agarose gel solution** (quantities based on blueGel™)
   - Weigh 0.4 g of **agarose** in a paper or plastic weighing boat
   - Add the agarose to 20 ml of **1X TBE electrophoresis buffer** in a glass beaker or microwave-safe plastic container and swirl

3. **Heat the mixture** using a microwave (in an OPEN container, not capped)
   - 20-30 seconds until fully dissolved and bubbling; the solution should look clear

     ⚠ Use caution, as the mix can bubble over the top and is very hot

4. Let the agarose solution cool for about 2 min at room temperature

5. **Add GelGreen™** DNA staining dye: 2 µL per 20 mL of agarose solution

6. Pour the cooled agarose solution into the gel casting tray with comb

   ⚠ Allow gel to completely solidify (until firm to the touch) before removing the comb (typically, ~10 minutes)

7. Place the gel into the blueGel™ **electrophoresis chamber** and cover it with **1X TBE buffer** (approximately 25-30 ml, or just until the gel is fully covered)

TIME MANAGEMENT TIP

- *Agarose gels can be poured in advance of the class period*
- *Pre-poured gels can be stored in the fridge in a sealed container, or covered in plastic wrap, for ~24 hours*
- *Protect pre-poured gels from light (e.g. using aluminum foil)*

B. Load DNA onto gel – 5 minutes

Prepare DNA samples for gel electrophoresis

1. Add **3 µl of 6X Gel Loading Dye** to each DNA sample, dipping your pipette tip directly into the DNA solution, and pipetting up and down to mix well

2. **Load DNA samples** onto the gel, from left to right:

	Per well
DNA Ladder	15 µL
Crown Beach DNA	15 µL
Doom Cove DNA	15 µL
Wave Crest DNA	15 µl
Hoppa-Hoppa DNA	15 µl

Remember to change tips at each step!

3. **Place the blueGel™ orange cover** on top of the blueGel base
   - Make sure the positive pole (anode) is aligned with the (+) sign

4.  **Press the POWER button** in your blueGel™ to turn it on. The green pilot LED next the power button will illuminate.
   - Make sure the blueGel™ power supply is plugged into the back of the unit, and into the wall outlet.
   - For your safety, the power will not turn on in these situations:
     - There is no TBE buffer in the running chamber
     - The amber cover is not properly in place

5. **Run the gel for ~20 minutes.** blueGel™ runs fast at fixed voltage.
    - Check that small bubbles are forming near the electrode terminals
    - Run until the colored dye has progressed to at least half the length of the gel, or until the DNA bands have achieved sufficient separation.

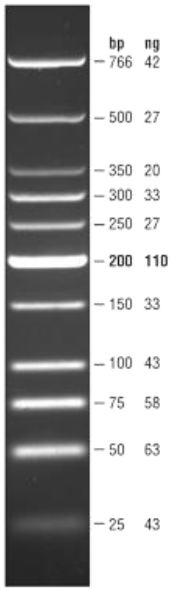
6. **Press the LIGHTS button** during the run to see the DNA as it migrates
    - Blue LEDs in the built-in transilluminator excite the fluorescent dye (GelGreen™) bound to DNA during the run
    - The orange cover filters (subtracts) the excitation wavelength, allowing only the fluorescence from the stain bound to DNA to show through

7. Removing the orange cover during the run will interrupt the current flow and stop the run
    - To restart the run, replace the orange cover and press the POWER button

C. Visualize DNA results (~5 minutes)

1.  Press the Light button on your blueGel™ unit

2. Verify the presence of a DNA banding pattern, ensuring that there is sufficient resolution in the 200-500 bp range of the DNA ladder, a range useful for size determination
    - Run the gel longer if needed to increase resolution
    - DNA Ladder should look approximately as shown:
    - *Source: New England Biolabs*



3. Place the blueGel™ fold-a-view hood over the orange base to subtract ambient light and prepare to document the gel.

4. Place your cell phone or camera directly perpendicular to the amber cover
    - With lights on, take a picture to document the DNA fingerprint patterns
    - Estimate the size of the DNA fragments by comparing the Shark Attack DNA samples to the molecular weight reference marker (DNA Ladder)

5. Capture an image with a smartphone camera

D. Interpret results and size determination (~5 minutes)

1. Capture an image of the gel using your cell phone camera
2. Compare the migration of individual fragments to that of the molecular weight standards (DNA Ladder)
3. Compare the resulting banding patterns
    i. Are there any observable similarities?
    ii. Any observable differences?
    iii. Are there any bands outside of the range of the DNA Ladder? Why?

3. Study questions

Pre-Lab: Questions before experimental set-up

- What is the structure of DNA? What are its main building blocks and what types of chemical bonds hold them together?
- Why can DNA be used in personal identification?
- What is a "genetic fingerprint"

Lab: Questions during blueGel™ run

- The electrophoresis apparatus creates an electrical field with positive and negative poles and the ends of the gel. To which electrode (pole) of the field would you expect DNA to migrate (+ or –)? Why?
- What size fragments (large or small) would you expect to move faster towards the opposite electrode? Explain why.

Post-lab: Questions after DNA visualization

- Why is there more than one DNA band within each sample analyzed?
- What caused the DNA to become cleaved (broken up) in small fragments?
- How many restriction sites do you think existed within each of the samples analyzed? (assume a single linear piece of DNA was the starting material)
- Based on your analysis of the DNA samples, how many sharks were involved in the recent Reefpoint attacks? Explain if one or more sharks were involved, and which attacks if any may have involved the same shark
- Explain the principles behind the visualization of DNA during the run. What's the role of the blue light? What does the amber filter do? What chemical helps make the DNA visible?

4. Appendix: Practice using micropipettes

We recommend that you familiarize your students with proper pipetting techniques

Prior to running this lab, have your students learn how to transfer different volumes of a solution from one tube into another using a micropipette.

Students pay practice using the purple gel loading dye supplied with the Shark Lab kit, other lab dyes, or even food coloring mixed with a sugar or glycerol solution to add density to the samples.

Below is a quick summary of how to use a micropipette:

1. Look at the micropipette plunger or side wall to determine its volume range
2. **Twist the dial** on the top of the plunger to set the desired volume (within the range)
3. **Attach a clean micropipette tip**, paying attention to its volume range
4. **Press the plunger** to the **FIRST** stop (until you feel soft resistance)
5. **Insert the tip** into the solution to be transferred
6. **Slowly release the plunger** while keeping the tip submerged in the liquid
7. **Lift the tip, and insert it into the recipient tube**
8. **Press the plunger** past the first stop onto the second, hard stop to transfer the liquid
9. **Carefully raise the tip** out of the tube, while maintaining the plunger pressed
10. Eject the tip