4. Background

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 rare. 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.

Laboratory guide

Protective gloves and eyewear should be worn for the entirety of this experiment.

Gel electrophoresis - Pouring gels (before or during class period)

Important Gel Pouring Notes

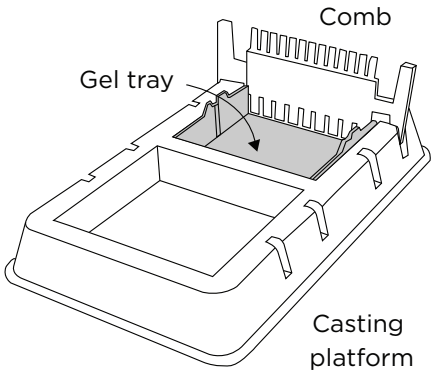
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need four lanes plus one lane for ladder per group. If groups are sharing gels, a single lane for ladder is sufficient.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

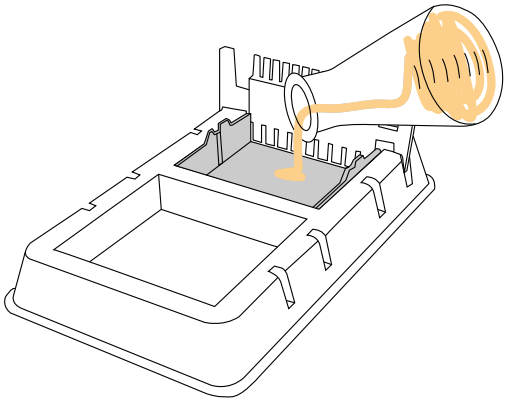
IMPORTANT NOTE: There are several ways to prepare agarose gels

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

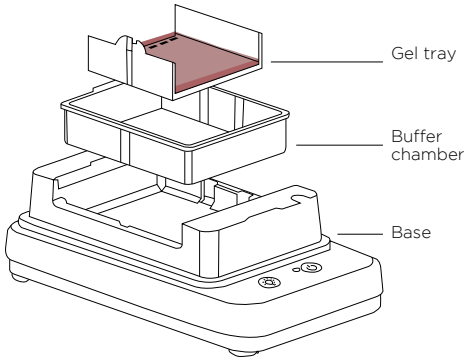
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



Protective gloves and eyewear should be worn for the entirety of this experiment.

Gel electrophoresis - Running the gel

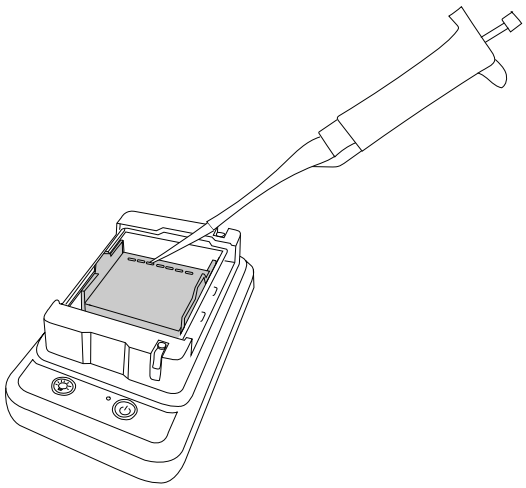
These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.



1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.

2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

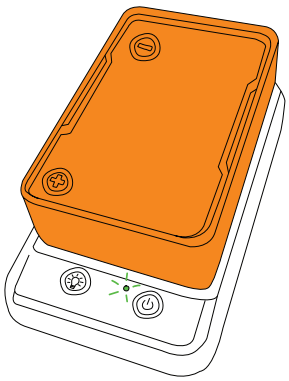
3. **Add 3 µl of gel loading dye to each sample**
   - Dip the pipette tip directly into the DNA solution, and pipette up and down to mix
   - Note: Change pipette tips between samples to prevent contamination.



4. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl DNA Ladder
   - **Lane 2:** 10 µl Crown Beach DNA
   - **Lane 3:** 10 µl Doom Cove DNA
   - **Lane 4:** 10 µl Hoppa-Hoppa DNA
   - **Lane 5:** 10 µl Wave Crest DNA

   Note: Change pipette tips between samples to prevent contamination.

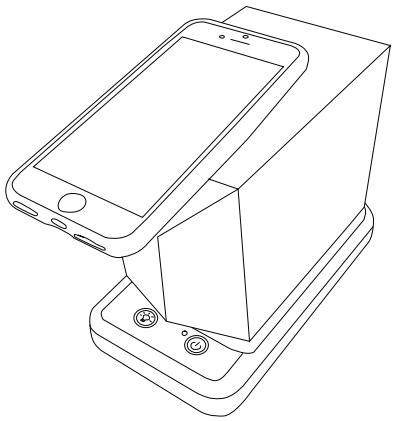
5. **Place the orange cover on the blueGel™ electrophoresis system**
   - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
   - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
   - The electrodes of the lid should be aligned with the metal leads on the base.
   - The orange lid should sit flush with the blue base using little force.



6. **Press the "Run" button**
   - Check that the green light beside the power button remains illuminated.

7. **Conduct electrophoresis for 30-45 minutes**
   - Note: Check the gel every 10 minutes to monitor sample migration.
   - Longer electrophoresis times will result in better size resolution. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

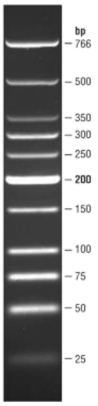
Gel electrophoresis – Visualizing results



1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
   - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
   - Gels may be viewed at the end of the run or periodically throughout the run.
   - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient band separation**
   - Run the gel longer if needed to increase resolution.

3. **Document your results**
   - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
   - Compare the bands from the DNA samples to the ladder to obtain size estimates.



Ladder

6. Study questions

Pre-Lab: Questions before experimental set-up

1. What is the structure of DNA? What are its main building blocks and what types of chemical bonds hold them together?

2. Why can DNA be used in personal identification?

3. What is a "genetic fingerprint"

Lab: Questions during blueGel™ run

1. 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?

2. What size fragments (large or small) would you expect to move faster towards the opposite electrode? Explain why.

Post-lab: Questions after DNA visualization

1. Why is there more than one DNA band within each sample analyzed?

2. What caused the DNA to become cleaved (broken up) in small fragments?

3. 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)

4. 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