



DNA Fingerprinting Lab

Shark Attack



Student guide

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Background information

A series of shark attacks has terrified vacationers at a remote Australian surfers' paradise. Following four shark attacks, you will receive DNA samples collected at each of the sites and use DNA fingerprinting to determine whether the attacks were committed by the same shark or by more than one.

Shark attacks

Shark attacks on humans are rare. Unprovoked incidents where a shark attacks the swimmer without prior shark aggravation are even rarer. In 2025, there were only 65 unprovoked shark bites of humans around the world. Scientists think sharks may bite humans when they mistake them for marine mammals, such as seals, which are a favorite shark meal. From below the water surface, surfers lying on their boards and sea lions do look similar.

DNA fingerprinting

Members of a species have mostly identical DNA sequences, but some locations in the genome have considerable sequence variation across individuals. 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, to conduct paternity testing, and in other applications. A unique pattern of electrophoretic DNA bands, or "fingerprint", can be generated for each individual as a result of specific differences in their genomes.

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.

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

Restriction enzymes act like molecular scissors, cutting DNA at specific sequences that they recognize. 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'-TTAA-3' is palindromic (both strands have TTAA if read 5'-3'):

5' TA**TTAA**CGG 3'

3' ATT**AATT**GCC 5'



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 that can create or destroy a restriction enzyme recognition site. For humans, each region tested is typically shared by 5-20% of the population. If ten polymorphic regions are tested, the probability that two people have the same RFLP pattern across all regions is $<0.000001\%$. This means that the probability of a random match is extremely low, making this a very high-confidence test.

After digesting the DNA with restriction enzymes, the size of the generated fragments can be resolved using gel electrophoresis. Each individual's DNA will migrate differentially because of size differences across their unique RFLP fragments.

RFLP analysis became the first DNA identification method and has been 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.

Today's lab

There have been unusual shark attacks in Australia at four different locations: Crown Beach, Doom Cove, Hoppa-Hoppa, and Wave Crest. Is a single shark responsible for these attacks?

It's time to complete the key step of DNA fingerprinting: you will use gel electrophoresis to determine if more than one shark was involved in these four attacks.



Student lab protocol



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

1. Place the prepared gel into the electrophoresis chamber.
2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
 - You will need 30 ml of TBE buffer for a blueGel or Bandit electrophoresis system. Do not overfill the chamber.
 - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.
3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.
 - Well 1: 10 µl Crown Beach (tube C)
 - Well 2: 10 µl Doom Cove (tube D)
 - Well 3: 10 µl Hoppa-Hoppa (tube H)
 - Well 4: 10 µl Wave Crest (tube W)
4. Run the gel for 15-20 minutes.
 - The blueGel and Bandit electrophoresis systems run at a fixed voltage.
 - If using another gel electrophoresis system, set the voltage in the 70-90 V range.
5. To visualize the DNA samples, turn on the blue light in your electrophoresis system or move the gel to a transilluminator.
6. If needed, continue to run the gel until there is sufficient separation between the bands in the samples to interpret the results.
7. If desired, take a photo to document the results.

Detailed operating instructions for miniPCR electrophoresis systems



blueGel

<https://links.minipcr.com/blueGelRun>



Bandit

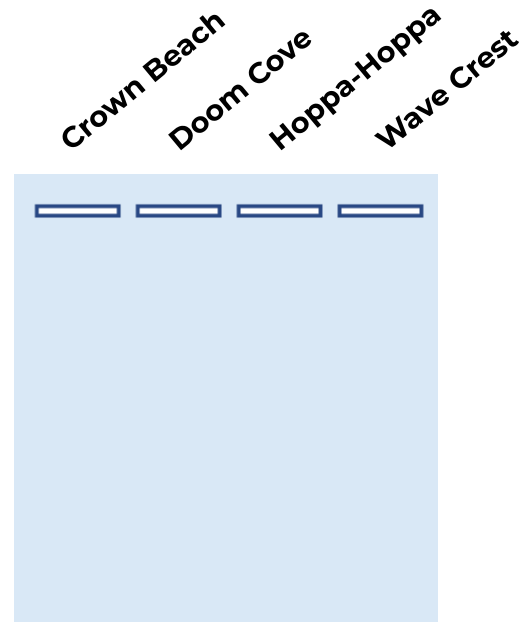
<https://links.minipcr.com/BanditViewit>



Post-lab questions

Interpreting results

1. Use the schematic gel on the right to draw what your results look like. For each sample, draw the bands that you see on your actual gel.
2. How many individual sharks are on this gel?
3. Which samples, if any, are from the same shark?





CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

Question:

Which samples, if any, came from the same individual shark?

Claim

Make a clear statement that answers the above question

Evidence

Provide data from the lab that supports your claim

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.



Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric Score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100