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

Getting started

Students guide

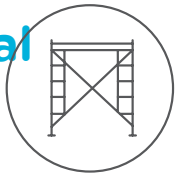
Instructors guide

1. Synopsis

DNA fingerprinting has long been used to distinguish species and to identify individuals within a species. The power of DNA as a tool for identification has been harnessed in a broad range of human applications, from forensic science to paternity testing, as well as in ecology and conservation. In this DNA gel electrophoresis investigation, students will be helping marine biologists understand the source of shark attacks that have been frightening Australian beachgoers. Is it a single shark? This guided-inquiry activity exposes students to the fundamental principles of DNA analysis by comparing patterns of cleaved DNA separated by gel electrophoresis. Students will gain familiarity with DNA gel electrophoresis techniques and engage in determining DNA fragment size.

- ***Techniques utilized***: DNA gel electrophoresis, DNA fingerprint analysis
- ***Time required***: One 45 min. class period
- ***Suggested skill level***: Intended for any student seeking familiarity with DNA gel electrophoresis and fingerprinting, from middle school to college

Additional supports -



Help your students build proficiency in pipetting, PCR, and gel electrophoresis with additional instructional videos, worksheets, and activities available at: https://www.minipcr.com/tutorials/.

For answers to the lab study questions and extensions, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Materials needed

Supplied in Kit (KT-1500-03)

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
DNA Samples • Crown Beach DNA • Doom Cove DNA • Wave Crest DNA • Hoppa-Hoppa DNA	**150 µl each**	**15 µl each**	Freezer	
Gel loading dye	**1000 µl**	**20 µl**	Freezer	
DNA Ladder	**150 µl**	**15 µl**	Freezer	

Sold Separately in Learning Lab Companion Kit (KT-1510-01)

This lab requires reagents for running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). The Learning Lab Companion Kit provides enough electrophoresis reagents for 8 groups when using the blueGel™ electrophoresis system. Gels can also be prepared with agarose tabs or agarose powder. Refer to https://www.minipcr.com/agarose-gel/ for detailed instructions.

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
All-in-one agarose tabs	**8**	**One tab per agarose gel** (2% agarose gel)	Room temp., protected from light	
TBE electrophoresis buffer • 1X working solution	**Supplied as liquid concentrate or powder** Sufficient to prepare 600 ml of 1X working solution	**30 ml of 1X solution** per blueGel™ system	Room temp.	
PCR tubes (0.2 ml)	**100**			
1.7 ml plastic tubes	**50**	**6**		

Materials needed (cont.)

Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed per lab group	Teacher's checklist
Horizontal gel electrophoresis apparatus: *e.g.,* blueGel™ electrophoresis system	**1** Can be shared between groups	
Blue light transilluminator *Note: A blue light transilluminator is integrated in the blueGel™ electrophoresis system.		
Micropipettes • 2-20 µl adjustable volume	**1**	
Disposable micropipette tips	**At least 9 per group**	
Distilled water for making agarose gels and diluting TBE buffer	**60 ml per gel**	
Flask or beaker to dissolve agarose		
Microwave or hot plate to dissolve agarose		
Other supplies: • Disposable laboratory gloves • Protective eyewear • Permanent marker • Cup to dispose of tips		

3. Lab setup

Planning your time

This lab is designed to be completed in a single 45-min period (using blueGel™)

Preparatory activity: Dispense reagents (before class)

Experiment:

A. Cast agarose gel (before or during class)
B. Load DNA samples (during class)
C. Separate DNA by gel electrophoresis (during class)
D. Interpret results (during class)

Visual guide

Preparatory activity

Dispense reagents and prepare equipment

- *10 min*

Experimental stage

A **Prepare agarose mix and pour gels**

- ~15 min until gel solidified

B **Load DNA samples**

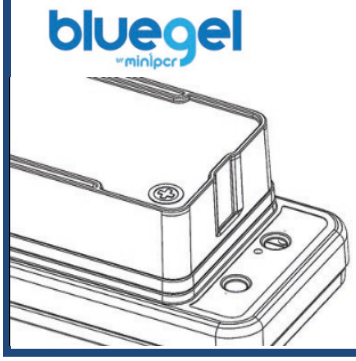
- 5 min

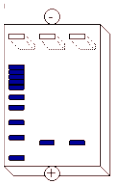
C **Run gels and visualize DNA**

- 20 min run
- Visualization during run

D **Size determination & interpretation**

- 5 min
- Discuss results





Preparatory activity: Dispense reagents (before class)

- Each **Lab Group** will analyze 4 "shark attack" DNA samples. For each group, label and dispense four 1.7 ml microtubes:
  - **Crown Beach** DNA Sample **15 µL**
  - **Doom Cove** DNA Sample **15 µL**
  - **Wave Crest** DNA Sample **15 µL**
  - **Hoppa-Hoppa** DNA Sample **15 µL**

- In addition, label and dispense for each lab group the following reagents:
  - **DNA Ladder** **15 µL**
  - **6X Gel loading dye** **20 µL**

Before aliquoting kit components, make sure to collect DNA at bottom of tubes by briefly spinning in a small centrifuge, or by tapping tubes against the lab counter

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

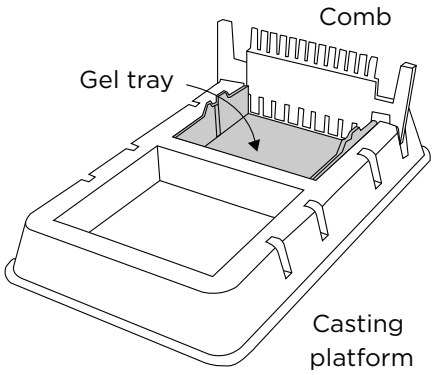
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

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**

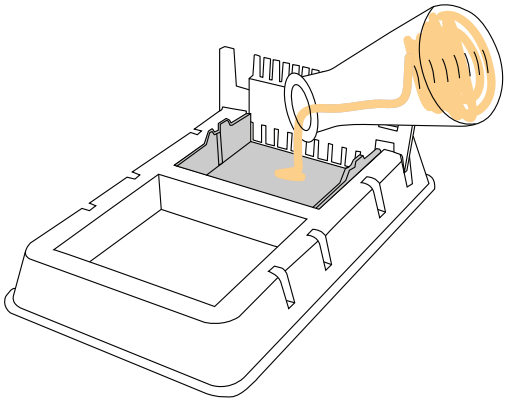
IMPORTANT NOTE: There are several ways to prepare agarose gels

- 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).

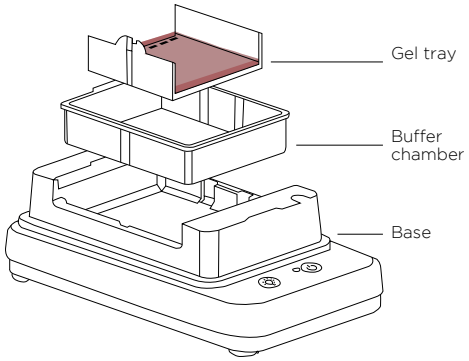
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



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

Gel electrophoresis - Running the gel

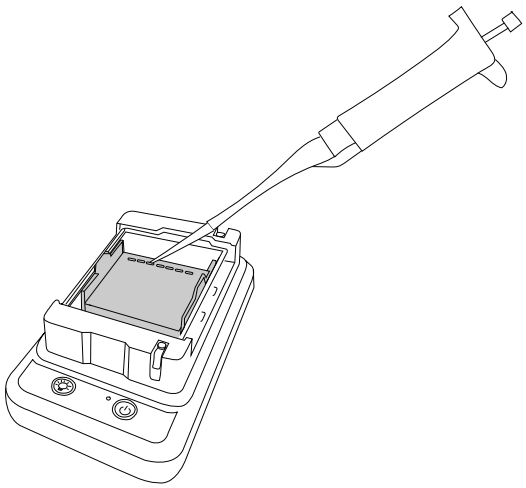
These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.



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).

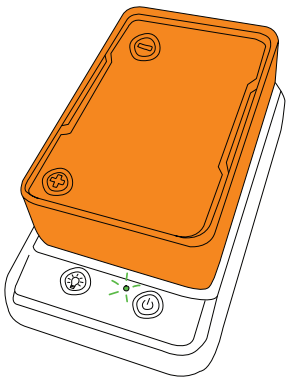
3. **Add 3 µl of gel loading dye to each sample**
   - Dip the pipette tip directly into the DNA solution, and pipette up and down to mix
   - Note: Change pipette tips between samples to prevent contamination.



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.

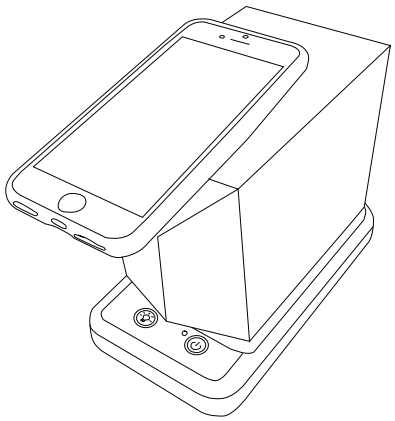
5. **Place the orange cover on the blueGel™ electrophoresis system**
   - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
   - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative 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.

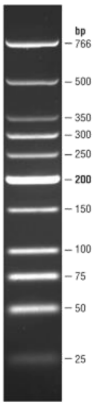
Gel electrophoresis – Visualizing results



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

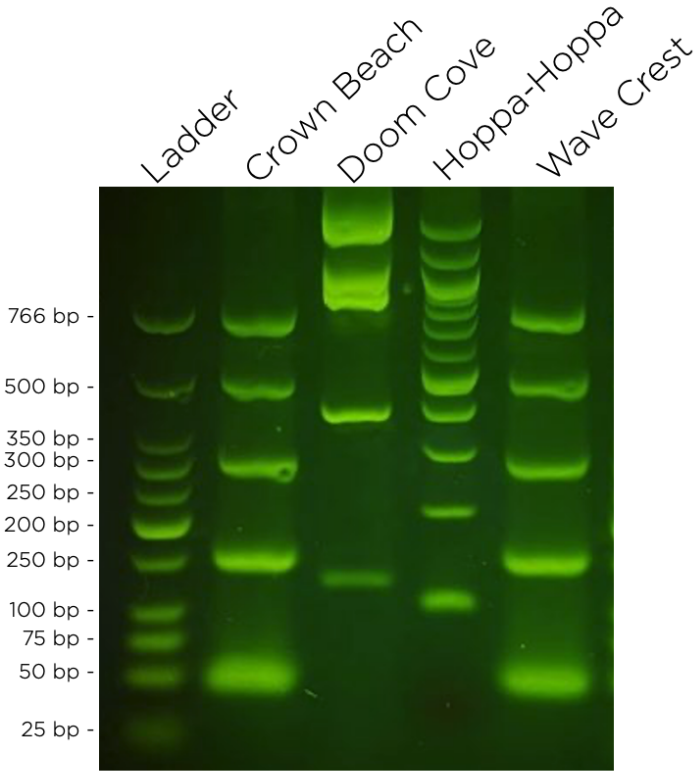
1. Why is there more than one DNA band within each sample analyzed?

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

7. Expected results



This gel represents a 45 minute run using a blueGel™.

8. Additional resources and extension activities

External extension activities

Create a DNA Fingerprint (via PBS NOVA):
http://www.pbs.org/wgbh/nova/education/body/create-dna-fingerprint.html

Simulated DNA Fingerprinting activity (via Holy Trinity Academy):
http://www.holytrinityacademy.ca/documents/general/DNA_Fingerprinting_Activity.pdf

Additional teacher resources

Gel electrophoresis (via Utah Genetic Science Learning Center):
http://learn.genetics.utah.edu/content/labs/gel/

RFLP analysis (via Wikipedia):
https://en.wikipedia.org/wiki/Restriction_fragment_length_polymorphism

Shark species identification using PCR-RFLP
http://www.ibb.unesp.br/Home/Departamentos/Morfologia/Laboratorios/LaboratoriodeGenomicaIntegrativa/7-2009ConsGenResRhizo.pdf

How to use environmental DNA analysis to find elusive sharks.
https://www.sciencenews.org/article/shark-dna-census-oceans

About sharks:

Global Shark Conservation (via Pew Trust)
http://www.pewtrusts.org/en/projects/global-shark-conservation

Shark attack facts (via National Geographic):
http://natgeotv.com/ca/human-shark-bait/facts

Shark Week (via Discovery TV):
http://www.discovery.com/tv-shows/shark-week/

9. Learning goals and skills developed

Student Learning Goals:

- Deepen understanding of the chemistry and structure of DNA
- Develop an understanding of the basic techniques used to study genetic polymorphisms encoded in DNA
- Gain familiarity with Restriction Fragment Length Polymorphisms (RFLPs) and their use in the study of biodiversity
- Apply RFLP analysis to genetic fingerprinting and individual identification
- Use critical thinking to solve problems through DNA analysis

Scientific Inquiry Skills:

- Students will create hypotheses and make predictions about results
- Students will compare experimental results to their predictions
- Students will generate graphics and tables to present their results
- Students will make conclusions about their hypothesis based their experimental results
- Students will follow laboratory safety protocols

Molecular Biology Skills:

- Micropipetting
- Preparation of agarose gels
- DNA agarose gel electrophoresis
- RFLP analysis
- Staining, visualization, and molecular weight analysis of DNA fragments

Disclaimer:

- This is a simulated investigation of shark attacks. It uses the same scientific principles and experimental techniques as real-world investigations, but does not require blood or real animal tissue. No humans or sharks were harmed in the making of this lab. At no point was anyone attacked by a shark. The attacks are fictional to simulate a real life application of DNA analysis. We hope you will gain a new understanding of these ancient vertebrates…. And that you will not be discouraged to bathe at your favorite beach!

10. Standards alignment

Next Generation Science Standards – Students will be able to …

HS-LS3-1	Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
HS-LS3-2	Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
HS-LS4-1	Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.

Common Core English Language Arts Standards – Students will be able to …

RST.11-12.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks; analyze the specific results based on explanations in the text.
RST.11-12.7	Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.
RST.11-12.9	Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
WHST.9-12.1	Write arguments focused on discipline-specific content.
WHST.9-12.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
WHST.9-12.7	Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.
SL.11-12.4	Present claims and findings, emphasizing salient points in a focused, coherent manner with relevant evidence, sound valid reasoning, and well-chosen details; use appropriate eye contact, adequate volume, and clear pronunciation.

Ordering information

To order miniPCR® DNA Fingerprinting Lab kits, you can:

Call (781)-990-8PCR

email us at orders@minipcr.com

visit https://www.minipcr.com

miniPCR® DNA Fingerprinting Lab (catalog no. KT-1500-03) contains the following reagents:

- 4 DNA samples (Crown Beach, Doom Cove, Wave Crest, Hoppa-Hoppa)
- Gel loading dye
- DNA Ladder

Materials are sufficient for 8 lab groups

All components should be kept frozen at -20°C for long-term storage

Reagents must be used within 12 months of shipment

Other reagents needed

- Agarose (electrophoresis grade)
- Fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized water (to dilute TBE buffer concentrate)

Note: Agarose, DNA stain, and TBE buffer are available at minipcr.com as part of the Learning Lab Companion Kit (KT-1510-01)

12. About miniPCR Learning Labs™

This Learning Lab was developed by Amplyus (the makers of miniPCR™ and the blueGel™ electrophoresis system) in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM.

We develop Learning Labs to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The guiding premise for this lab is that a real-life biotechnology application that can be conducted in a single class period provides the right balance between intellectual engagement, guided inquiry, and discussion. The design of this lab has simplified certain elements to achieve these goals. For example, we provide amplified and pre-digested DNA samples ready for classroom use. The DNA fingerprint patterns have been selected to enable direct visual interpretation of the results.

Starting on a modest scale working with Massachusetts public schools, miniPCR™ Learning Labs have been received well, and their use is growing rapidly through academic and outreach collaborations.

Authors: Ezequiel Alvarez Saavedra, Ph.D., Sebastian Kraves, Ph.D., and Alexander Connor