© 2016-2018 by Amplyus LLC

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.

© 2016-2018 by Amplyus LLC

Instructor's Guide Contents

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
- ***Reagents needed***: 'Shark Attack' reagents kit (available from miniPCR), gel electrophoresis reagents (see Section 8)
- ***Suggested skill level***: Intended for any student seeking familiarity with DNA gel electrophoresis and fingerprinting, from middle school to college

© 2016-2018 by Amplyus LLC

2. 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!

© 2016-2018 by Amplyus LLC

3. 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

© 2016-2018 by Amplyus LLC

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.

© 2016-2018 by Amplyus LLC

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.

© 2016-2018 by Amplyus LLC

4. Laboratory set-up manual

Reagent	Volume needed per lab group	Storage	Teacher's checklist
DNA samples for analysis • DNA from Crown Beach • DNA from Doom Cove • DNA from Wave Crest • DNA from Hoppa-Hoppa	15 µl	-20°C freezer	
DNA Ladder • Molecular weight marker	15 µl	-20°C freezer	
Gel loading dye • 6X concentrate	20 µl	-20°C freezer	
GelGreen™ DNA stain • 10,000X concentrate	2 µl per gel (based on blueGel™)	Room temp.	
Agarose, • Electrophoresis grade	2% gel (0.4 g of agarose based on blueGel™)	Room temp.	
1X TBE Electrophoresis buffer • Tris-borate EDTA	Depending on gel apparatus (total of 50 ml for blueGel™)	Room temp.	

Supplied in Shark Lab Kit

blueGel Starter Kit available at www.miniPCR.com

© 2016-2018 by Amplyus LLC

Equipment and Supplies	Teacher's checklist
DNA gel electrophoresis apparatus: *e.g.*, blueGel™ which includes: • **Gel casting system** • **DC power supply** • **Blue-light transilluminator**	
Micropipettes: One 2-20 µL per lab group	
Disposable micropipette tips	
Plastic tubes: 1.5 or 1.7 mL tubes to aliquot reagents (6 tubes per group)	
Scale for weighing agarose	
250ml flask or beaker to dissolve agarose gel	
Microwave or hot plate	
Microcentrifuge (optional, to spin down reagents before use)	
Cell phone camera for gel documentation	
Other supplies: • Disposable laboratory gloves • Permanent marker	

© 2016-2018 by Amplyus LLC

Planning your time

This lab is designed to be completed in a single 45-min period (using blueGel™), and it has four stages:

A. Cast agarose gel (before or during class)
B. Dispense reagents (before or during class)
C. Separate DNA by gel electrophoresis (during class)
D. Visualize DNA and interpret results (during class)

OPTIONAL activity before lab: practice micropipetting (see Appendix)

Visual guide

Preparatory activity

Dispense reagents and prepare equipment

- *10 min*

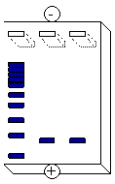
Experimental stage

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

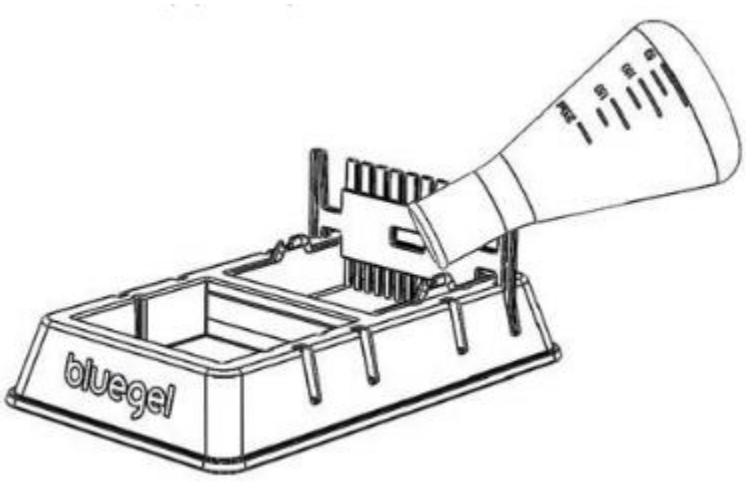
D Size determination & interpretation
- 5 min
- Discuss results



© 2016-2018 by Amplyus LLC

5. 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 agarose to 20 ml of **1X TBE electrophoresis buffer** in a glass beaker or microwave-safe plastic container and swirl
   - If pouring several gels, **multiply quantities accordingly** (e.g. 160 ml = 8 gels)
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 Gel Green™** 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)

© 2016-2018 by Amplyus LLC

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

⚠ *Before you start: This preparatory activity can be done before the class period or while the agarose gel solidifies.*

- Thaw tubes containing Shark Lab reagents by placing them on a rack or water bath at room temperature
- 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*

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

© 2016-2018 by Amplyus LLC

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

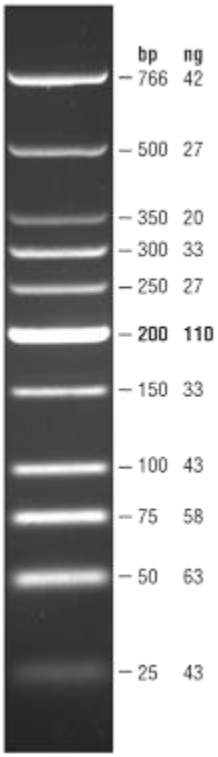
7. Removing the orange cover during the run will interrupt the current flow and stop the run
    - To restart the run, replace the orange cover and press the POWER button

© 2016-2018 by Amplyus LLC

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:



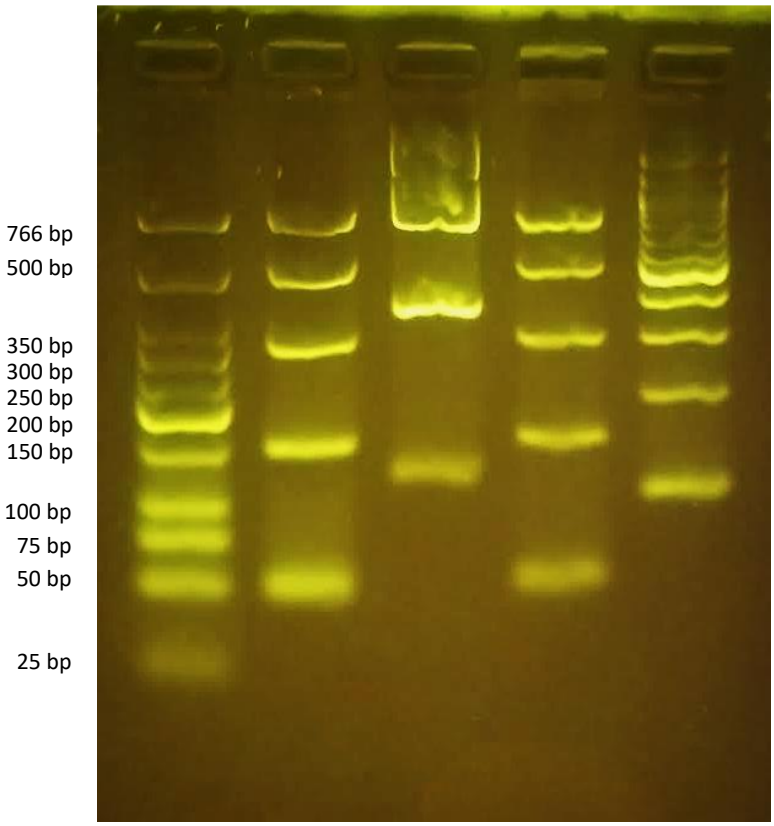
Source: New England Biolabs

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)
    - Capture an image with a smartphone camera

© 2016-2018 by Amplyus LLC

5. Expected results:



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?

© 2016-2018 by Amplyus LLC

6. 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?
    - A sugar-phosphate backbone held together by phosphodiester bonds (covalent)
    - Nitrogen bases (adenine, cytosine, guanine, thymidine) held together by hydrogen bonds (non-covalent)
- Why can DNA be used in personal identification?
    - Polymorphisms create individual differences in DNA sequence. We can analyze these differences using molecular biology, for example, through restriction fragment length polymorphism (RFLP) analysis
- What is a "genetic fingerprint"
    - We typically refer to the unique RFLP pattern of an organism as its "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?
    - DNA molecules are negatively charged. Hence they will migrate towards the positive pole (anode)
- What size fragments (large or small) would you expect to move faster towards the opposite electrode? Explain why.
    - The relative speed of migration of a molecule in gel electrophoresis is determined by two factors: its size (molecular weight) and its charge
    - Size: The larger a molecule is, the harder it will be for it to pass through the agarose gel matrix. Smaller molecules will encounter lower resistance and migrate faster through the matrix
    - Charge: size being equal, two molecules will migrate differentially through the gel depending on the strength of their charge. Higher

© 2016-2018 by Amplyus LLC

charge molecules will be attracted more strongly to the electrodes and "pulled" faster through the agarose gel
    - Because DNA molecules all share the same number of charges per nucleotide, only size determines their speed of migration, with smaller DNA fragments running faster than larger DNA fragments

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?
    - Blue light: excitation wavelength tuned to the fluorophore that's bound to DNA
    - Orange filter: subtracts the excitation wavelength, allowing only the excitation (fluorescence) wavelength to pass, thus enhancing contrast
    - Chemical: GelGreen™ is the fluorescent molecule that intercalates with DNA allowing us to visualize it

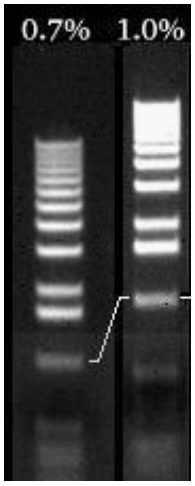
© 2016-2018 by Amplyus LLC

7. Additional resources and extension activities

Agarose concentration and its effect on "Shark DNA" resolution

Agarose gels are commonly used in concentrations of 0.6% to 3% depending on the size of bands that need to be separated. By using gels with different concentrations of agarose, one can resolve different sizes of DNA fragments. Higher concentrations of agarose facilitate separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.

"Shark DNA" samples will display different banding patterns as they are electrophoresed over different agarose concentrations, under the same voltage and for the same length of time. The image below shows an example. Notice how the larger fragments are much better resolved at 0.7% concentration, and how the 1,000 bp band (marked with a white line) migrates different distances.



- Prepare different agarose gels:
  - 2%, 1.5%, 1.0% concentration
  - Calculate how to adjust agarose mass in each case
- Make predictions:
  - How will different DNA samples migrate?
  - Draw expected banding patterns
- Compare and discuss:
  - How did your predictions match observed migration?
  - Based on this table, which agarose concentration would be ideal for each Shark sample?

Agarose (w/v)	*Optimal separation range*
0.6%	**1,000 – 20,000 bp**
0.9%	**500 – 7,000 bp**
1.2%	**400 – 6,000 bp**
1.5%	**300 – 3,000 bp**
2.0%	**100 – 2,000 bp**

© 2016-2018 by Amplyus LLC

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/

© 2016-2018 by Amplyus LLC

8. Ordering information

To request miniPCR™ Shark Attack Lab kits:

- Call 781-990-8PCR
- E-mail us at orders@minipcr.com
- Visit www.minipcr.com

miniPCR™ Shark Attack Lab kit (Catalog No. KT-1500-01) contains:

• DNA sample from Crown Beach	150 µl
• DNA sample from Doom Cove	150 µl
• DNA sample from Wave Crest	150 µl
• DNA sample from Hoppa-Hoppa	150 µl
• DNA Ladder	150 µl
• 6X Gel Loading Dye, Purple	1 ml

Materials are sufficient for 8 lab groups, or at least 32 students

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 (available from www.minipcr.com/store)

• Agarose (electrophoresis grade)	RG-1500-02
• GelGreen™ DNA Stain	RG-1550-01
• 20X TBE gel electrophoresis buffer	RG-1502-02

Available in quantities sufficient for 100 gels as:

blueGel™ Starter Kit *RG-1510-01*

- Distilled water (for TBE dilution to 1X)

© 2016-2018 by Amplyus LLC

9. 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

© 2016-2018 by Amplyus LLC

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.

© 2016-2018 by Amplyus LLC

11. 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