miniOne®
MINILABS

DNA Fingerprinting MiniLab

Student Guide

Cat# M3004

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Laboratory Safety

1. Wear lab coats, gloves, and eye protection whenever possible.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Objectives

To develop an understanding of electrophoresis principles. To obtain data, analyze results, and deduce a probable conclusion concerning parentage using DNA fingerprinting and DNA separation technology.

Background

Every individual is unique, but within your DNA exist genetic clues to your heritage. These clues are found in the non-coding regions of DNA where random mutations are relatively common. Since these minor changes do not usually affect genes essential to survival, the variations pass from parent to offspring. Over generations, these changes accumulate and the DNA regions develop distinct patterns. To analyze these DNA regions, scientists use a method of genetic profiling called DNA fingerprinting. DNA fingerprinting takes advantage of these inherited sequences, and uses them to identify the genetic similarities between certain individuals. Using this technology, family relationships can be identified on a genetic level and uncertainty about lineage can be resolved.

In the most traditional method of DNA fingerprinting, DNA is first collected and isolated. If there is not enough DNA, it can be amplified (replicated) using **PCR** (**P**olymerase **C**hain **R**eaction). Once enough DNA is obtained, it is digested or "cut" by **restriction enzymes**.

These enzymes recognize particular DNA sequences called restriction sites, and the length of the DNA between these **restriction sites** is variable in different people based on their inherited genes. Once the digest is finished, the DNA is then analyzed using a DNA separation technique called gel electrophoresis (for more information on gel electrophoresis, refer to Appendix A).

When separated by gel electrophoresis, a banding pattern for each individual's DNA is created based on fragment size. This pattern can then be compared with the banding patterns of other individuals. The more similar banding patterns are, the higher the probability that individuals are related. DNA fingerprinting techniques are used in many areas, from crime scene forensics and paternity cases, to healthcare and research.

Scenario

For three years, a team of researchers has been tracking and studying three different humpback whale pods during their annual winter migration off the coast of Hawaii. Since humpback whales are an endangered species, the team has been attempting to observe their behavior in order to try and gain insight into their reproductive strategies. Extensive commercial whaling in the first half of the 20th century caused a population bottleneck, significantly depleting the gene pool of these whales. As conservation efforts have helped increase whale numbers, the determination of the genetic diversity of populations has become increasingly more important in order to track the health of the species and ensure its continued survival.

The recent birth of a female whale calf named Luna has given the research team an opportunity to gather more data about the whales' mating behaviors. By observing nursing behavior, they have already been able to match Luna with her mother. They have also narrowed down her father to one of three possible candidates based on observations made during the previous mating season, however they have yet to definitively link Luna to her father. Using a DNA fingerprinting technique, you are going to help them figure out which whale is Luna's father!

Pre-Lab Questions

1. What is DNA and what does it do?

2. What are chromosomes and how many copies do you have in each of your cells? Who did they come from?

3. How much DNA do you share with each of your parents? How much DNA do you share with your siblings?

4. What are some characteristics or properties of DNA?

5. What is DNA fingerprinting?

6. What are the steps to conducting DNA fingerprinting?

7. What do the banding patterns tell you about the relationship between individuals?

8. What is the effect of extensive commercial whaling on the population of humpback whales?

9. What are some results of reduced genetic diversity in a population?

Part I: Electrophoresis

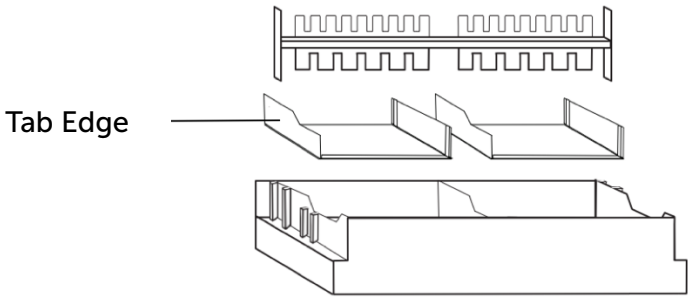
Materials

1 Minione® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1 %)
5 DNA samples
TBE running buffer (135 mL)
1 micropipette (2-20µL)
5 pipette tips

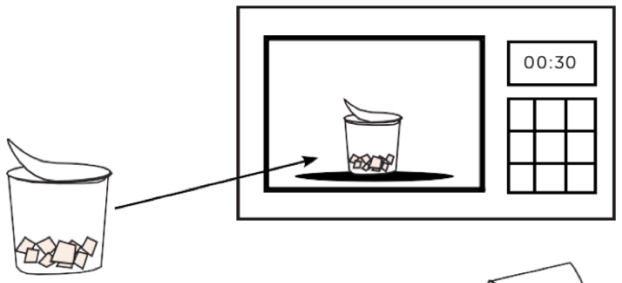
Lane #	Sample Name	Volume
1	Female L	10 µL
2	Female M	10 µL
3	Male A	10 µL
4	Male B	10 µL
5	Male C	10 µL
6	Empty	

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

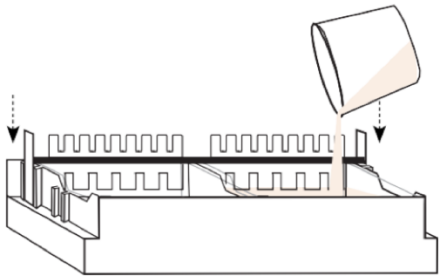


2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.

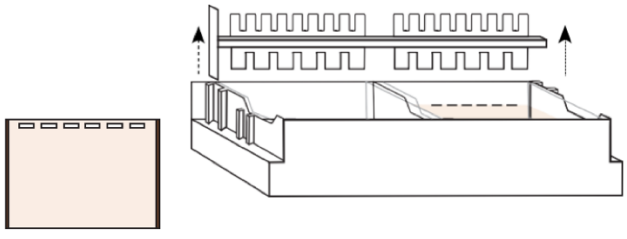


3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.

   DO NOT disturb the gel until time is up.

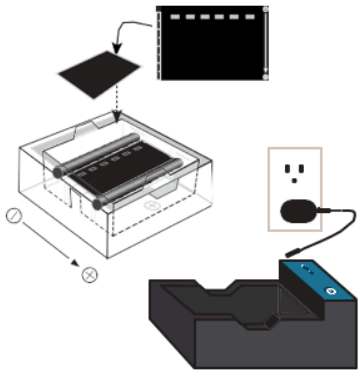


4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.



How to Load a Gel

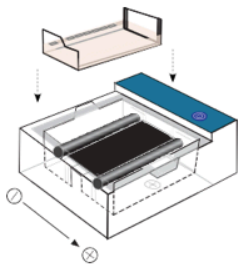
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

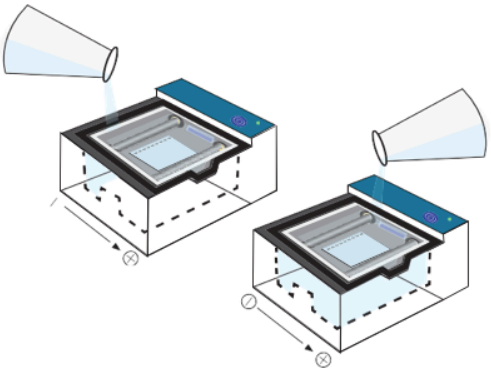
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



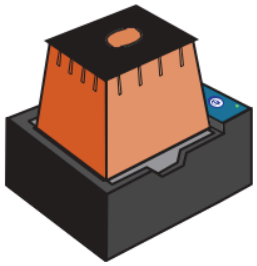
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**

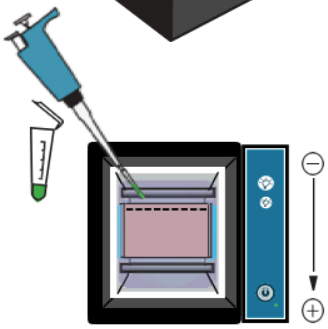


7. Place photo hood on the carriage.



8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



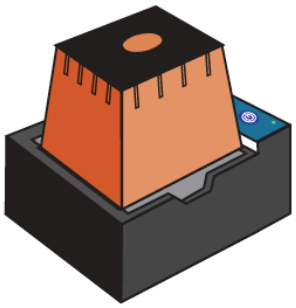
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

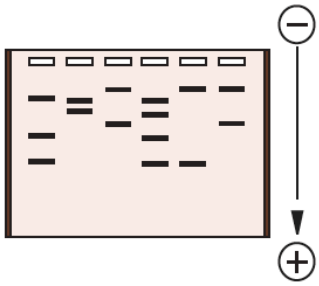
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage. There is no buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage. There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne Electrophoresis Instruction Manual**



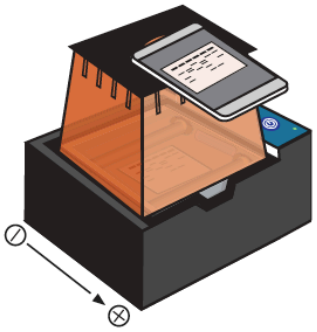
2. Have students periodically check the migration of the bands (~every five minutes).



3. Allow the gel to run **20 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.

4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.



5. Clean up. Follow teacher's instructions on disposal and clean up.

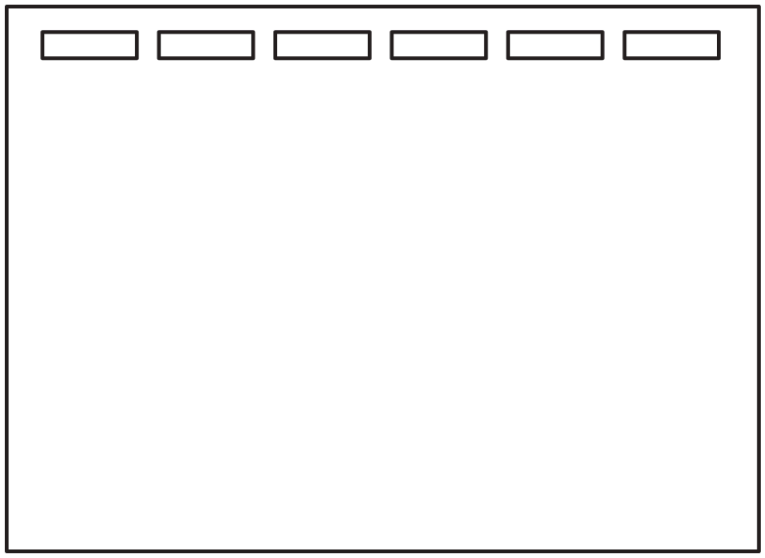
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____

Part III: Analyze Your Data

1. How many bands does Luna have in her fingerprint? What about her mom? How many bands do they have in common?

2. Compare Luna's fingerprint to each of the possible fathers. How many bands does Luna have in common with Male A? Male B? Male C? Based on your data, who is most likely to be Luna's father?

3. Using your gel image can you figure out which whale is Luna's father? Why?

Appendix A - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

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