miniOne®
MINILABS

DNA Fingerprinting MiniLab

Student's Guide

Cat# M3004

Version 020718

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Laboratory Safety

1. Exercise caution when heating or melting reagents.
2. Exercise caution when working with electrical equipment.
3. Gloves and eye protection should be used whenever needed as part of good laboratory practice.
4. Always wash hands thoroughly after handling biological materials or reagents.

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?

Part I - Electrophoresis

Materials

1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1%)
5 DNA sample aliquots
TBE running buffer (135 mL)
1 micropipette (2-20 µL) and 5 pipette tip

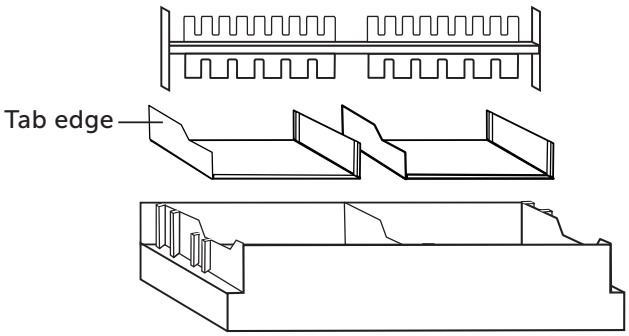
SAMPLE CHART:

Well	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

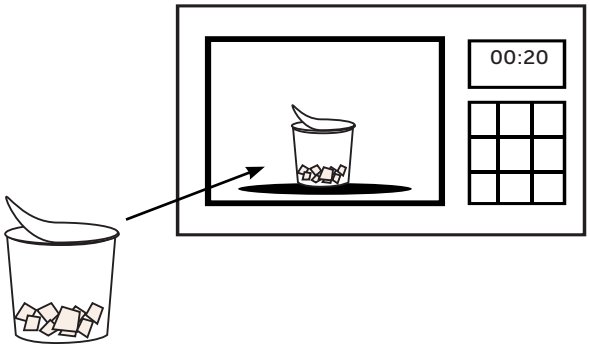
MiniOne Visual Instructions

How to Cast a Gel

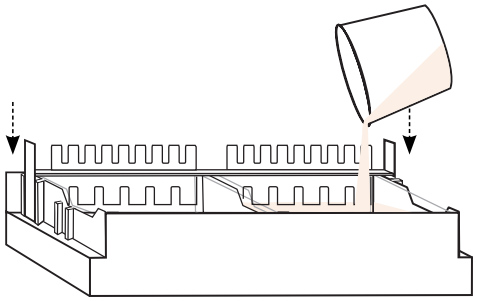
1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. The straight edge should be on the right side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.



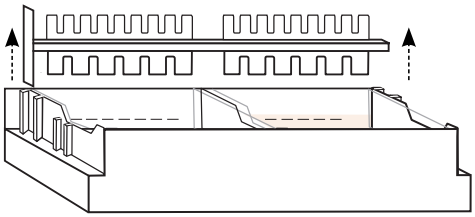
2. Partially peel the film of a GreenGel™ cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

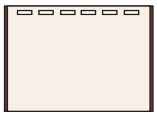


3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque**. DO NOT disturb the gel until time is up.**



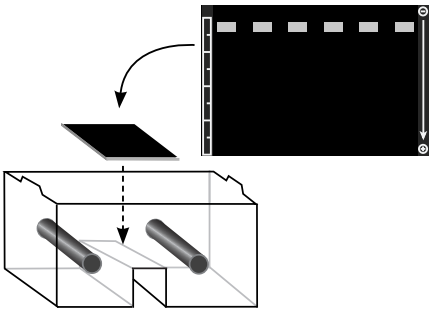
4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray



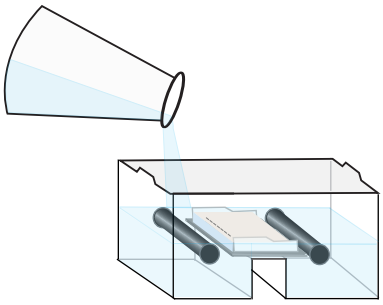


How to Load a Gel

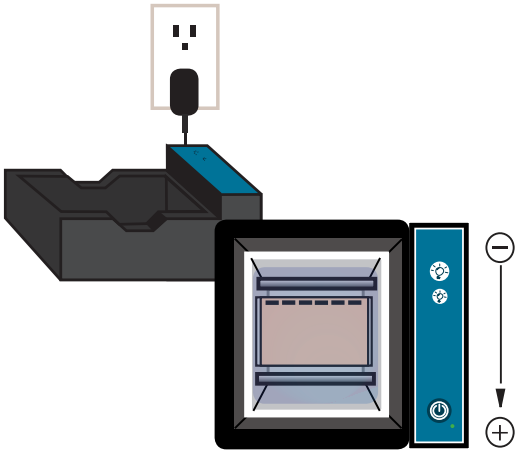
1. Ensure the black viewing platform is in the tank if it is not already installed and put the gel (along with the gel tray) into the tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



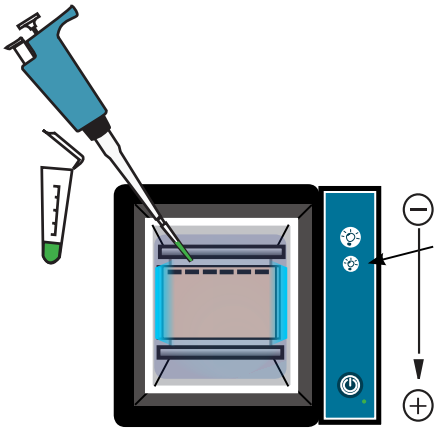
2. Measure 135 mL of TBE running buffer and pour into **one** side of the tank to push out the air, creating a nice even background without air bubbles or air trapped for imaging later.



3. Plug the power supply into the wall. Place the tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

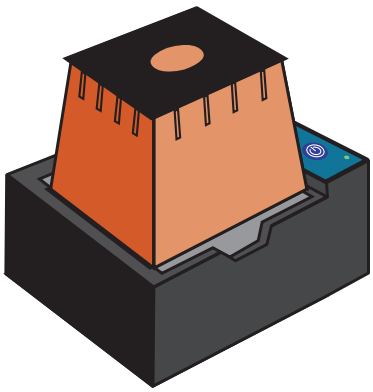


4. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**



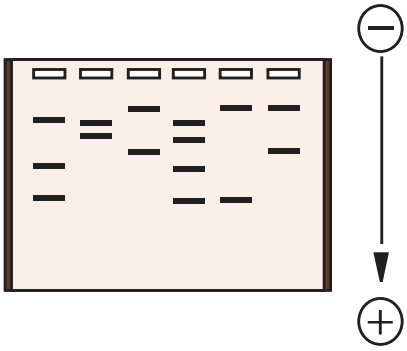
Run, Visualize, and Capture Image

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.



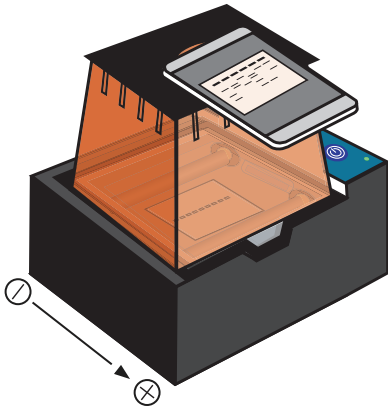
The green power LED will not turn on if:

- **The tank is not properly placed inside the carriage**
- **There is no buffer in the tank**
- **The buffer is too concentrated or too diluted**
- **The photo hood is not on the carriage**
- **There is too much or too little running buffer**
- **The power supply is not plugged in. Check by turning on the blue LEDs**



2. Allow the gel to run approximately **25 minutes** or until DNA separation is sufficient. 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.

3. **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.

Clean Up

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. 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 cleanup and storage.

Part II: Results

What does your gel look like? Record images of the gel.

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? Who is most likely to be Luna's father? 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?

Looking at a sample of green dye, how can you know if it is really green? Could it be a mixture of blue and yellow dyes? Electrophoresis is a technique used in many areas of science to analyze and separate samples by applying a constant electric field. Biologists or forensic scientists can use this technology to separate mixtures of DNA or dyes into each component based on size and electrical charge.

The gel in gel electrophoresis is essentially a matrix through which particles travel. Gels can be made from different substances depending on what is being separated (DNA, RNA, proteins, etc.), but it should be both conductive and have the ability to form a uniform matrix with appropriate pore sizes. The matrix is like a sieve or collander: if the holes are too big or too small it wont work very well. One of the most commonly used and effective reagents for DNA separation is agarose. Agarose gels are usually cast in a tray with molten (melted) agarose. A comb is placed while the agarose is molten and then removed after the gel solidifies to create wells in which to load samples. A DNA stain is used to enable visualization of the DNA.

As an electric field is applied to the agarose gel, the particles in the wells will begin to move. The direction that particles migrate depends on their charge. DNA has a negative charge, so it will be attracted to a positive electrode. Some dyes and other particles have a positive charge and will thus migrate toward a negative electrode. The relative speed of migration is determined mainly by the size of the particle but also by the strength of the particle's charge. Like an obstacle course, larger particles have more difficulty passing through the matrix with their bulk and do not travel very far, while shorter and smaller ones can maneuver much more easily and therefore travel faster and farther.

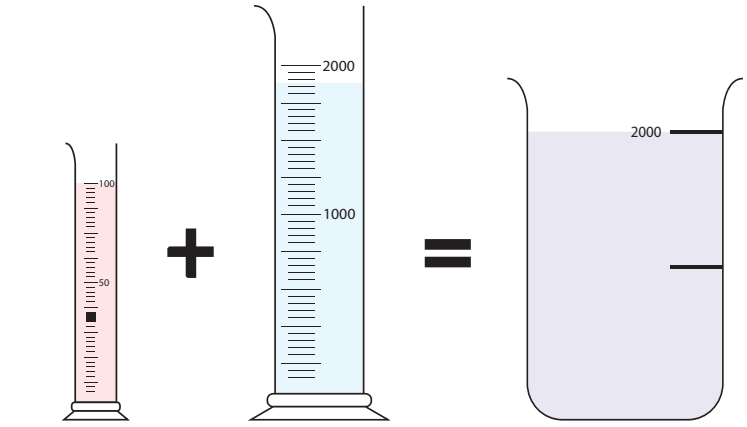
Sometimes a particle with a bigger size migrates faster than a smaller particle. This can happen if the strength of the charge of the larger particle is significantly stronger by comparison to the charge on the smaller particle. An example of this phenomenon is the loading dye Orange G. This dye often runs faster than the smaller DNA fragments and other relatively small particles because it is more negatively charged and has a stronger attraction to the electrode than the smaller particles.

Both particle size and electrical charge can affect the results of gel electrophoresis experiments. In general however, gel electrophoresis separates charged particles and fragments by size.

Appendix B - TBE Concentrate Dilution Instruction

Dilute **1** part TBE Concentrate with **19** parts deionized or distilled water

1. **To make 2000 mL TBE running buffer (1X),** mix 100 mL TBE Concentrate and 1900 mL deinonized or distilled water



TBE running buffer is stable stored at room temperature.

2. **To make various volumes of TBE running buffer use this formula:**

$$C_1 \times V_1 = C_2 \times V_2$$

Where:
- C_1 = Original TBE Concentrate
- V_1 = Volume of the Original TBE Concentrate needed
- C_2 = Final concentration
- V_2 = Total final volume desired

Once you have calculated the volume of TBE Concentrate needed, SUBTRACT that amount from the total volume of TBE running buffer desired to find the volume of water needed.

We recommend diluting buffer in batches for accuracy.

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theminione.com (858) 684-3190 info@theminione.com