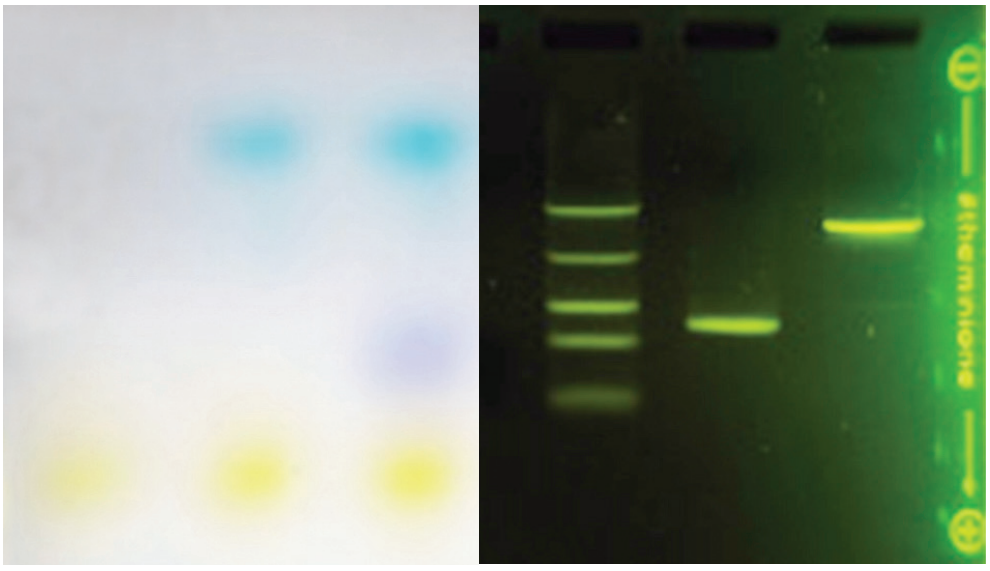
Electrophoresis 101 MiniLab

Student's Guide

Cat# M3001

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 analyze results and to calculate the sizes of unknown charged molecules from given information and experimental data.

Background

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 won't 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 added to the gel to enable visualization of the DNA, either before casting or after the run if DNA is going to be observed.

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.

Today, you are going to try this technique out to analyze various samples. You will be given a sample with a mixture of known DNA sizes and a sample of known dye sizes. Based on the migration results of the known samples, you can figure out the sizes of unknown mixtures by drawing a standard curve.

Part I - Electrophoresis

Materials

1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1%)
6 samples (3 DNA and 3 color dyes)
TBE running buffer (135 mL)
1 micropipette (2-20 µL) and 6 pipette tips

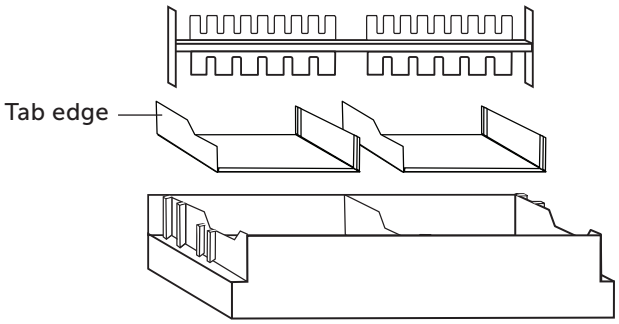
SAMPLE CHART:

Well	Sample Name	Loading Volume
1	Dye 1	10 µL
2	Dye 2	10 µL
3	Dye 3	10 µL
4	MiniOne Marker	10 µL
5	Unknown DNA 1	10 µL
6	Unknown DNA 2	10 µL

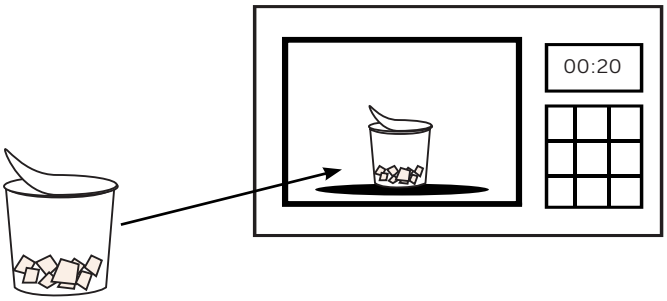
MiniOne Visual Instructions

How to Cast a Gel

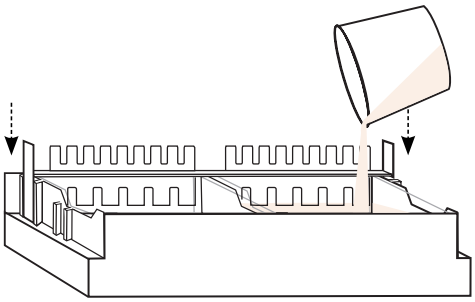
1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.



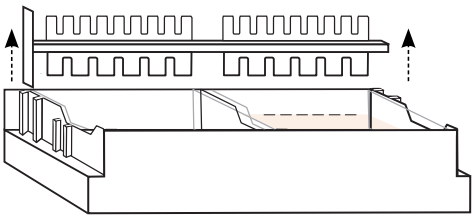
2. Partially peel the film off a GreenGel™ cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

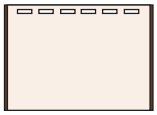


3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**



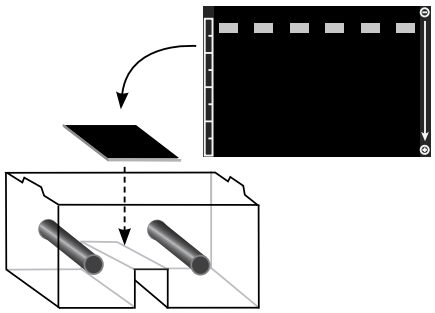
4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.



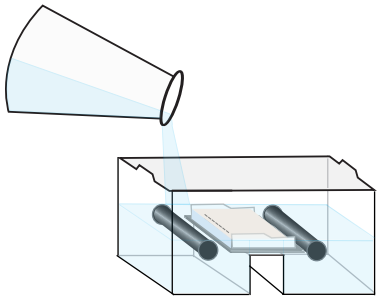


How to Load a Gel

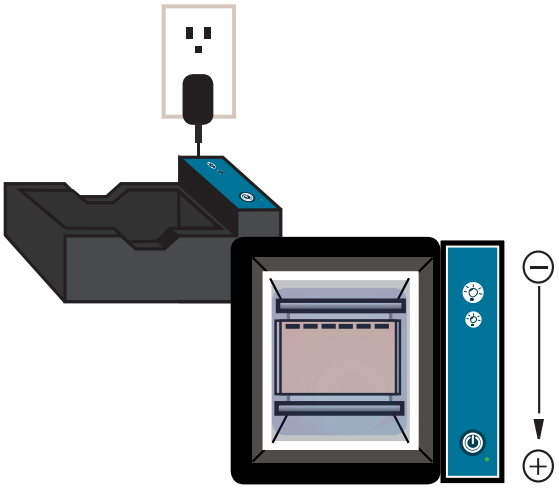
1. Ensure the black viewing platform is in the tank if it is not already installed and put the gel (along with the gel tray) into the tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



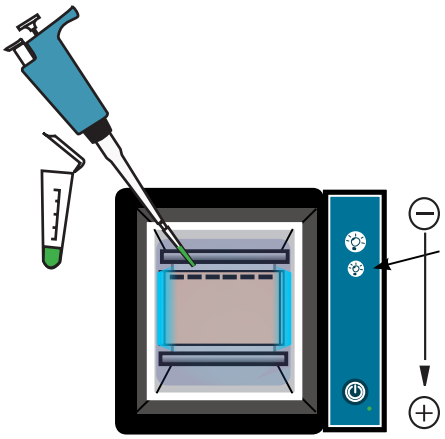
2. Measure 135 mL of TBE running buffer and pour into **one side** of the tank to push out the air, creating a nice even background without air bubbles or air trapped for imaging later.



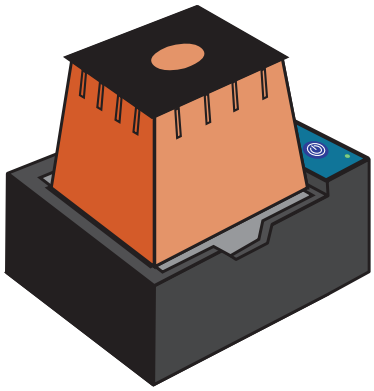
3. Plug the power supply into the wall. Place the tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.



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.**



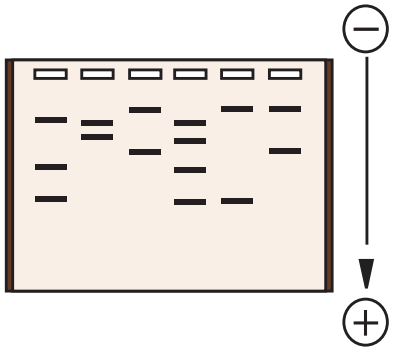
Run, Visualize and Capture Image



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.

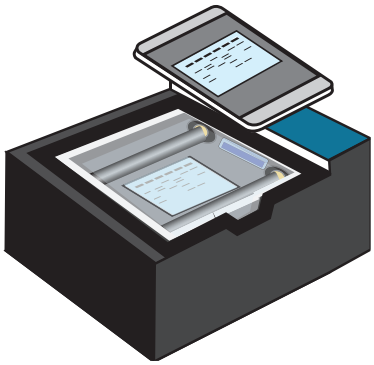
   The green power LED will not turn on if:

   - **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**



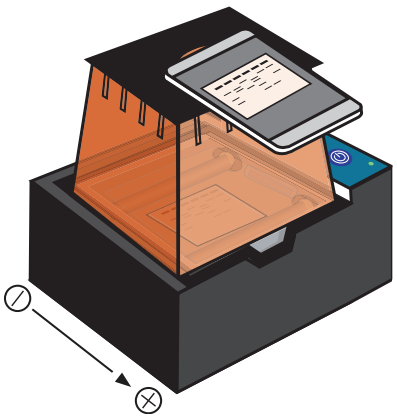
2. Allow the gel to run approximately **25 minutes** or until DNA separation is sufficient. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.

3. **Document your results.** At the end of the run, take photos following these steps:



   A. Remove the photo hood and **turn off** the blue LED light. Hold your cell phone or camera about three inches above the tank and take a picture of your gel.

   B. **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 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.

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Part II: Results

What does your gel look like? Record image(s) of the gel.

Part III: Analyze Your Data

1. According to your data, how many bands were resolved from each of your 6 samples? For the dye samples, note the color of each band you resolved.

2. What electrical charge did your samples carry? How do you know?

Part IV: Draw a Standard Curve

Looking at your gel, Dye 1 should have 1 band, and Dye 2 should have 2 bands. The orange bands are from a dye called 0range G, while the light blue band is from xylene cyanol. Dye 3 is an unknown mixture.

The DNA marker contains a mixture of known DNA sizes. A table is given with the sizes of known dyes and DNA. Measure and record the migration distance of these known bands. With the distance and the known sizes, you can then construct a standard curve. Based on the standard curve, you can approximate the size of the unknowns from their migration distances.

1. Using the images you took of the gel, measure the distance of the known bands relative to the positive edge of wells. Fill in the table.

Knowns

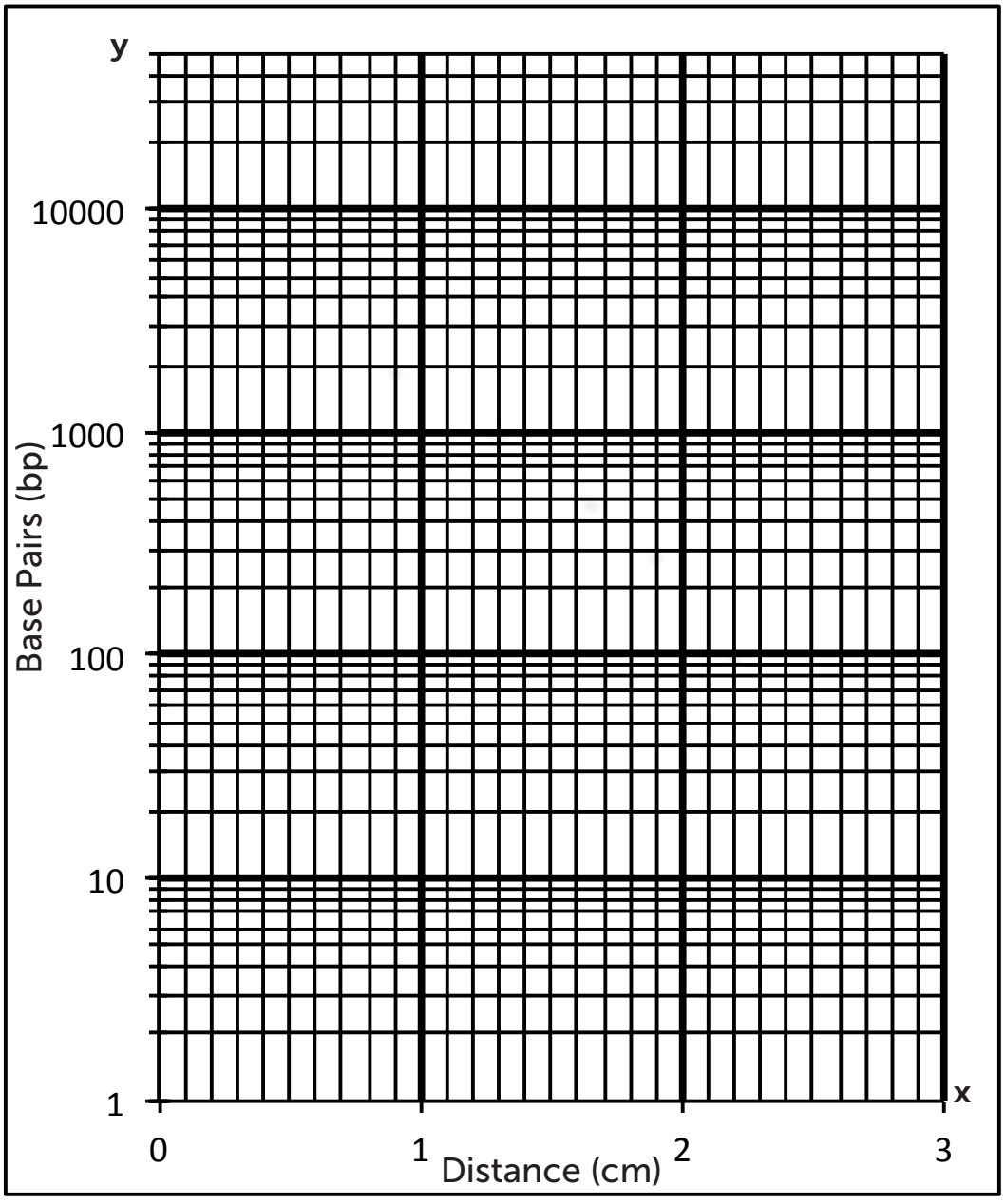
Dye/DNA	Color	Size (base pairs)	Migration Distance
Xylene Cyanol	Light blue	2800	
Orange G	Orange	70	
MiniOne Marker	Green Under blue light	2000	
		1000	
		500	
		300	
		100	

Unknowns

Dye/DNA	Color	Size (base pairs)	Migration Distance
Dye 3			
Unknown DNA 1			
Unknown DNA 2			

Part IV: Draw a Standard Curve (continued)

2. Use the filled in table to construct a standard curve on the semi log graph below. Distances are plotted linearly on the x-axis and base pairs are plotted on the log scale on the y-axis. Plot the graph for the knowns. Then draw a best-fit line based on your data.

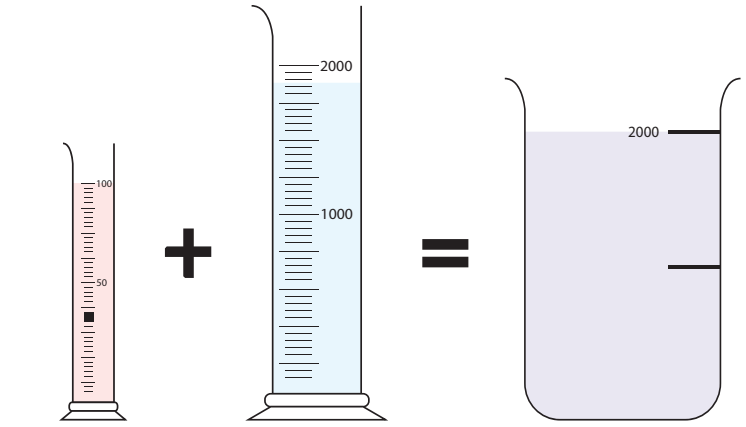


3. Measure the migration distances for the unknown bands. Use the standard curve to extrapolate the sizes of the unknowns. Record the results in the table (question 1).

Appendix A - 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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