blueGel™ Electrophoresis Rainbow Lab

Student Guide Contents

1. Synopsis

In this miniPCR Learning Lab, you will identify the dyes found in a mixture using two molecular analysis techniques, gel electrophoresis and paper chromatography. You will learn the key principles of gel electrophoresis and chromatography, and compare the two techniques. This lab is an introduction to key concepts about molecules and molecular properties that are relevant to important biological macromolecules such as DNA and proteins. You will explore how the size and charge of a molecule influence its migration through an agarose gel or in a paper chromatography separation.

- ***Techniques utilized***: Gel electrophoresis, paper chromatography, micropipetting
- ***Time required****:* One 45-minute period
- ***Reagents needed:*** 'blueGel™ Electrophoresis Rainbow Lab' reagents kit, gel electrophoresis reagents
- ***Suggested skill level****:* Familiarity with gel electrophoresis principles, basic familiarity with micropipetting techniques

2. Background

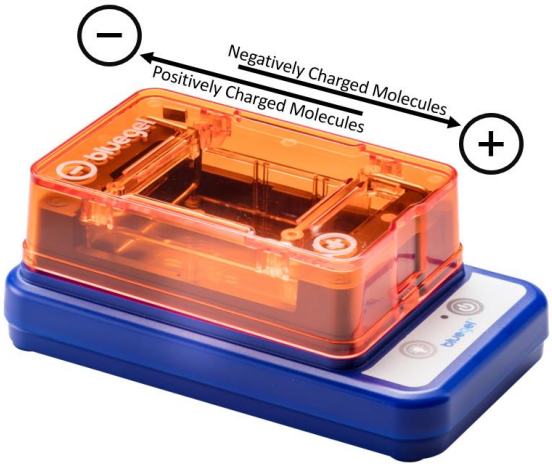
Gel electrophoresis

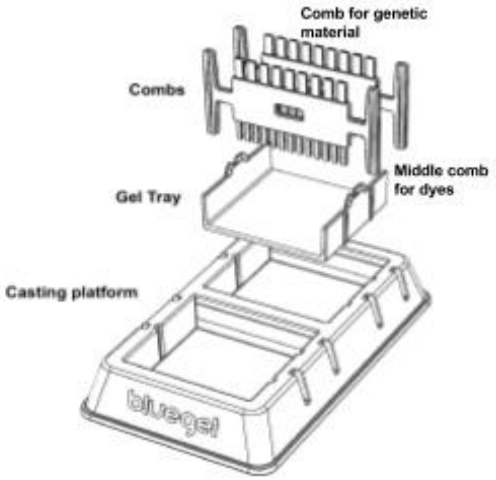
Gel electrophoresis is a technique used to separate samples of molecules by size and charge. The technique is most often used to analyze DNA, RNA, and proteins, but can be used for other molecules as well. During gel electrophoresis, an electric field causes the movement of molecules through a gel that contains small pores. A gel electrophoresis system consists of a platform to hold the gel, a chamber for buffer, electrodes positioned on opposite sides of the chamber, and a power supply that connects to the negative and positive electrodes and applies an electric charge to the electrodes.

In a fully assembled gel electrophoresis system, the gel is positioned on the platform within the chamber and submerged in buffer. The buffer conducts the electric current through the gel, driven by an electric field that causes molecules to migrate towards the oppositely charged electrode. Small molecules move more easily through the pores of the gel, and therefore travel further than larger molecules of the same charge.

When separating nucleic acids, the wells of the gel are placed closer to the negative electrode. This is because DNA and RNA have negative charges. When an electric field is applied, the negatively charged nucleic acids will be attracted to the positive pole. In this way, all the DNA or RNA is pulled through the gel in the same direction towards the more distant, positive electrode.

Some of the dyes that will be used in this experiment may be negatively charged and these dyes will move towards the positive electrode, like DNA and RNA. However, other dyes may be positively charged and will move in the opposite direction. For this reason, it is important to cast the gel with the wells **near the center** so molecules will be able to travel in both directions in your gel.





Why are molecules charged?

All molecules, including the dye molecules in this lab, are clusters of atoms held together by molecular bonds. Molecules can range in size from just a few atoms, as in H_2O, to the billions of atoms found in a long DNA molecule. Different atoms have different properties and some atoms are highly electronegative. Electronegativity is the ability of an atom to pull electrons towards itself. Some atoms, such as oxygen, pull on electrons more strongly than others. When an atom such as oxygen forms a covalent bond with a less electronegative atom, like hydrogen, the electrons forming that bond associate much more strongly with the oxygen nucleus. This allows the hydrogen nucleus (H^+) to leave the molecule and associate with a different molecule. The molecule that lost the H^+ now has a negative charge. The most common electronegative atoms found in biological molecules such as DNA and proteins are oxygen and nitrogen, which explains why these are often negatively charged.

More about agarose gels

A gel is made of a polysaccharide extracted from seaweed called agarose. Agarose gels are formed by adding dry powdered agarose to a buffer and melting it. The melted agarose is poured into a tray and allowed to solidify. To make a space that will hold the molecules you are studying, a comb is inserted into the agarose before it cools. After the agarose is solidified, the comb is carefully removed and where the teeth of the comb were, small holes, called wells, remain in the gel. Once solidified, the agarose gel forms a microscopic mesh, or web-like matrix, through which the molecules travel. When the solidified gel is placed into the platform in the electrophoresis machine and buffer is added to the chamber, the gel is submerged in the buffer. The buffer contains ions that make it a good conductor of electric current.

To perform gel electrophoresis, a small amount of liquid containing the molecules you want to study is added to the wells in the gel. When the power to the electrophoresis machine is turned on, the electric field facilitated by the ions in the gel and the buffer pushes the molecules through the gel. Positively charged molecules, also known as cations, are attracted by and move towards the negative electrode (the cathode) and negatively charged molecules, also known as anions, are attracted by and move towards the positive electrode (the anode).

How molecular size and charge affect migration

The speed at which a molecule moves through the gel depends upon its size, charge, and shape. The mesh of the agarose gel acts like an obstacle course that the molecules must travel through, so smaller molecules have an easier time moving through the gel than do larger molecules. If two molecules are similarly charged, the smaller molecule will move further through the gel. Sometimes molecules vary in both size and charge, and when this is the case, movement through the gel is dictated by the electric charge of the molecule relative to its size (the charge-to-mass ratio). For instance, a large molecule that is highly charged may move more quickly than a smaller, but lesser charged molecule. Finally, shape also influences movement of molecules in the gel. A compact molecule moves more easily though the mesh obstacle course than does a long linear molecule.

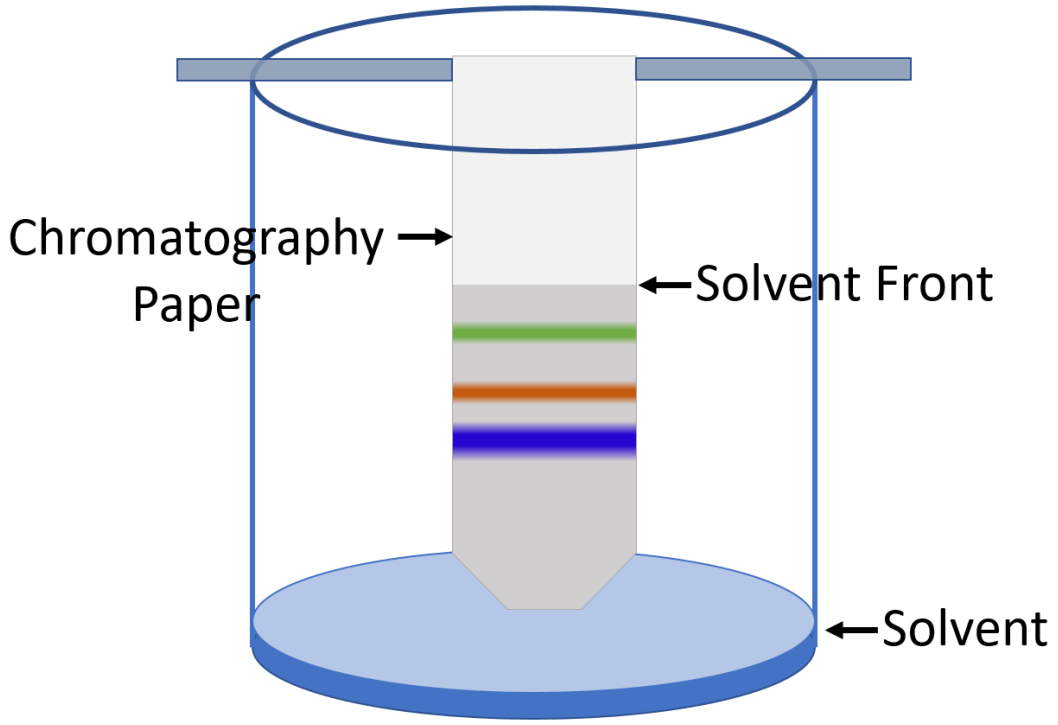
In this gel electrophoresis lab with **dyes**, *charge* determines which direction from the wells the dye will travel, and *size* plays the largest factor in distance traveled. That said, your will discover in this lab that size is not the only determinant, which can lead you to think about how charge-to-mass ratios and shape of the molecules influence their migration.

Please note that the most common use of agarose gel electrophoresis is to separate different sized DNA molecules. Because the movement of linear DNA molecules through a gel is based almost entirely on size, electrophoresis can be used to determine the size of DNA molecules with great accuracy. Why is size the predominant factor for electrophoresis of DNA? DNA is negatively charged due to the phosphate groups in the DNA backbone. Every nucleotide of a DNA molecule has one phosphate group, so as DNA molecules increase in size, their total charge increases at the same rate. The resulting charge-to-mass ratio of DNA therefore remains constant, and the movement of linear DNA molecules through a gel during electrophoresis is based almost entirely on size.

Paper chromatography

The term chromatography refers to many procedures, all of which separate parts of a mixture by passing a liquid solution through a non-liquid "stationary layer". The different components in a solution can be separated from each other based upon whether the substance more readily sticks to the stationary layer (in our lab, the paper) or dissolves in, and travels with, a solvent as it moves through the stationary layer.

In paper chromatography, the mixture to be separated is spotted on a piece of filter paper and the edge of the paper below the spot is submerged in a solvent, usually rubbing alcohol or water. As the solvent moves up the paper by capillary action, the components in the mixture (in this case the dyes) dissolve in the solvent and are carried along. The distance the dye moves on the strip depends on how soluble it is in the solvent. If both the solvent and the molecules of the dye are very polar, then the chemical will be very soluble in the solvent; if the solvent is polar and the chemical is non-polar, (or the other way around) then the chemical will not dissolve well in the solvent and instead will stick to the paper. The better the sample is dissolved, the more easily and faster the chemical will move up the paper with the solvent. If the sample placed on the chromatographic strip is a mixture of chemicals, the chemicals will separate based on their polarity, in other words, how well they dissolve in the solvent.



Today's lab

Today, you will analyze dyes of different colors, molecular weight, and charge using gel electrophoresis. You will see that the molecules that move more slowly through the gel tend to be larger than the molecules that move more quickly. You will also observe that the negatively charged dyes move towards the positive electrode and the positively charged dyes move towards the negative electrode. Below is a table detailing relevant information on the dyes used in this lab.

Table 1: Dyes included in this lab:

Dye	Color	MW	Charge	Concentration
Allura Red AC	Red	496.42 g/mol	-	1% v/v
Orange G	Yellow	452.38 g/mol	-	0.04% w/v
Methylene Blue	Blue	373.90 g/mol	+	0.04% w/v
Bromophenol Blue	Purple	669.96 g/mol	-	0.04% w/v
Bromocresol Purple*	Purple	540.22 g/mol	-	0.08% w/v

**Bromocresol Purple may appear yellow if the pH of the solution falls below 6.8. If the Bromocresol purple appears yellow, it should change back to purple when added to the buffered solution in the electrophoresis gel.*

Important note:
Along with the five dyes listed above, your teacher will give you two "unknowns". These unknowns will be different mixtures of the above dyes that you must separate and identify using electrophoresis and chromatography techniques.

Paper chromatography is also included in this lab to serve as a comparison to gel electrophoresis. The two processes are similar in that both separate samples that are a mixture of two dyes, but the mechanism of separation is different. Gel electrophoresis separates molecular mixtures due to differences in size and charge in response to an electric field. In chromatography, the dyes separate based upon a difference in affinity towards a solvent. In this way, chromatography is measuring a sample's polarity. Unlike gel electrophoresis, in which the dye sample can move either way on the gel depending on charge, the dyes in paper chromatography will only travel up the paper strip, never down.

3. Student laboratory guide

Experiment Overview

A **Load Gels and Run Gel Electrophoresis**
- 30 min

B **Paper Chromatography**
- 10 min – to be started while gel electrophoresis runs

C **Interpretation**
- 10 min – observation and discussion

READ ALL INSTRUCTIONS BEFORE PERFORMING THE LAB

Important note:

If your teacher is providing you with a gel, you may skip to page 9 - "***Gel electrophoresis – Running the gel***"

A. ***Gel electrophoresis – Pouring agarose gels (can be done ahead of time)***

1. Prepare a clean and dry agarose gel casting tray.
   - Seal off the ends of the tray as indicated for your apparatus (not needed for blueGel™ users).
   - Place a well-forming comb at the **middle of the gel** (7 lanes needed per group).

2. Prepare a 0.8% agarose gel using 1X TBE buffer.
   - Adjust volumes and weights according to the size of your gel tray.
     - For example, add 0.4 g of agarose to 50 ml of electrophoresis buffer.
     - **blueGel™ users:** add 0.2 g of agarose to 25 ml of 1X TBE buffer.
   - Mix reagents in glass flask or beaker and swirl to mix.
   - If pouring more than one gel, you can dissolve all the agarose at once (e.g. 1.6 g in 200 ml for blueGel™ for 8 gels).

3. Heat the mixture using a microwave or hot plate.
   - Until agarose powder is dissolved and the solution becomes clear.
   - 40 seconds is usually sufficient for heating 50 ml in a microwave.
   - Use caution, as the mix tends to bubble over the top and is very hot.

4. Cool the agarose solution for about 2-3 min at room temperature.
   - Swirl the flask intermittently to cool evenly.

5. Slowly pour the cooled agarose solution into the gel-casting tray with comb (**comb should be in the middle of the gel**).

6. Allow gel to completely solidify (until firm to the touch) and remove the comb.
   - Typically, 20-25 minutes
   - At this point, gels may be stored for future use. If storing gels, place in a sealed container or plastic bag and place in the refrigerator. Stored gels should be used within one week of pouring.

7. Once the gel is completely cool, remove the comb.

Gel electrophoresis – Running the gel

**Gloves are not needed for this experiment because all dyes are nontoxic, but they are highly recommended as dyes will stain skin. Dyes will come off skin by washing point of contact with soap and water. If using gloves, put them on at this point, before loading the dyes.*

1. Place your gel into the electrophoresis chamber.

2. Cover your gel with 1X TBE buffer. Make sure gel is completely submerged in electrophoresis buffer.
    - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).
    - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to *barely* cover the gel and wells.
    - blueGel™ users: Use no more than 25 ml of 1X TBE electrophoresis buffer.

3. Load 10 µl samples of each dye into the wells in the following sequence
    - Lane 1: Bromophenol Blue (BB)
    - Lane 2: Methylene Blue (MB)
    - Lane 3: Orange G (OG)
    - Lane 4: Bromocresol Purple (BP)
    - Lane 5: Allura Red AC (AR)
    - Lane 6: Unknown A
    - Lane 7: Unknown B
    - Lane 8: Unknown C

4. Place the cover on the gel electrophoresis box.

5. Insert the terminal leads into the power supply (not needed if using blueGel™.)

6. Start electrophoresis.
    - If using blueGel™, simply press the “Run” button.
    - If using other gel electrophoresis system, set the voltage to between 100 and 120 volts.
    - Check that small bubbles are forming near the terminals in the box.
    - Run for 15-20 minutes, or until the colored dyes have progressed to about half the length of the gel.

7. Electrophoresis can be considered complete when significant separation is seen between the dyes.
    - Longer electrophoresis times will result in better size resolution.

8. Once electrophoresis is completed, turn the power off.
    - Gels should be viewed soon after termination of the electrophoresis run as dyes will diffuse in the gel making resolution of the bands less clear.

B. ***Paper chromatography*** *- to be completed while your gel is running*

1. Obtain from your teacher 1 cm wide chromatography paper (one per lab group and included in kit), isopropyl alcohol, and 500 ml beakers (also could use an Erlenmeyer flask or a test tube).

2. Cut one end of paper to make a point.

3. Using a toothpick, dip the end in Unknown A. Make a spot about 2.5 cm above the point of the paper with dye on the end of the toothpick.

4. Pour a small amount of alcohol into the beaker – just enough to cover the bottom, and place a pencil or stirring rod horizontally across the top of the beaker.
    - NOTE: your teacher may have you use water instead of rubbing alcohol.
    - If using an Erlenmeyer flask or test tube, the pencil is not needed.

5. Make a fold in the non-pointed end of the paper so that it can hang on the pencil. The point on the paper should just barely be submerged in the alcohol. No more than a centimeter of the paper should be submerged.
    - If using an Erlenmeyer flask or test tube, hang the paper over the edge of the flask or tube with the fold in the paper.
    - The spot of dye drawn on the paper should **not** be in contact with the alcohol at the bottom of the beaker.

Schematic of chromatography set up is included in the "background" section of this lab guide. (page 5)

6. Observe the paper as the solvent (the alcohol) climbs up the paper with the pigment of the dye with it.
    - It should take about 5 minutes for the sample to separate into its two respective dyes.

C. Size and charge determination and interpretation

1. When your gel has completed running and the power is turned off, place 5x7 piece of white paper under the buffer chamber.
    - If using blueGel™, carefully lift the buffer chamber and place the paper on the blue base. Return the chamber to its original position.

    - If using other clear gel electrophoresis apparatuses, the paper can be under the entire chamber.

2. Observe location of dyes in the gel.
    - If needed, use a flashlight or other light source to better see the dyes.

3. If you do not see clear separation between dyes, increase running time.

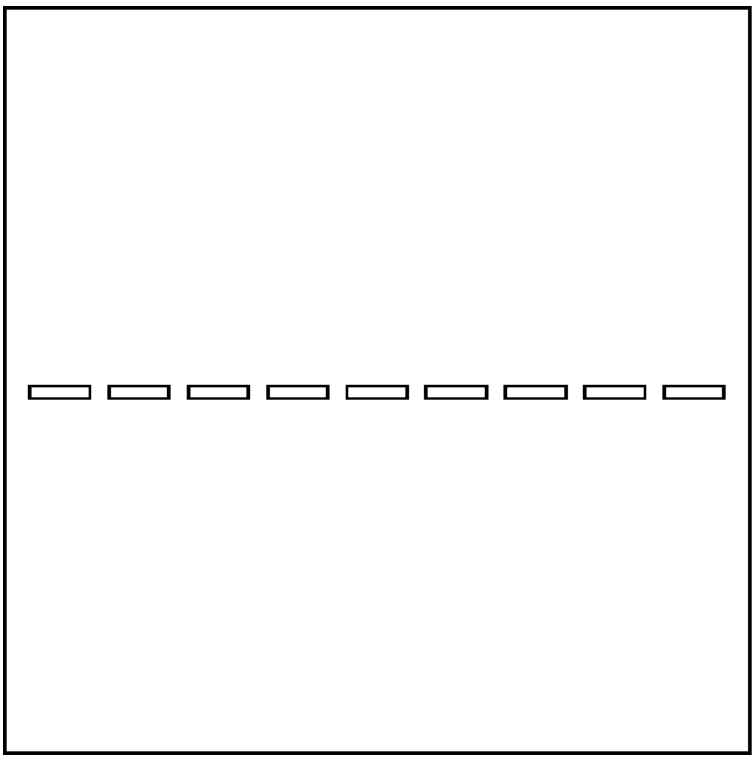
4. Document the size and charge of the dye chemicals.
    - Using a ruler measure the distance from the well to the approximate center of each band of dye. Record the distance in the *Student Data Table*.
    - Fill in data in Student Data Table.
    - If available, capture an image of your gel using a camera or other gel documentation system.

4. Student Data Table

Lane	Dye	Mol. weight g/mol	Direction run	Distance run	Color
1	Bromophenol Blue	669.96			
2	Methylene Blue	373.90			
3	Orange G	452.38			
4	Bromocresol Purple	540.22			
5	Allura Red AC	496.42			
6	Unknown A				
7	Unknown B				
8	Unknown C				

Gel Diagram

Use this gel diagram for questions 9 and 10 in the "Questions for after gel electrophoresis and visualization" section.



5. Study questions

Questions to be completed before experimental set-up

1) What is the force that causes the molecules to move in gel electrophoresis?

2) What is the purpose of the buffer solution that the gel is placed in?

3) Normally when doing DNA gel electrophoresis, the comb is placed at one end of the gel so that wells will be closer to the negative electrode. Why did you put the comb in the middle of the gel?

4) Imagine that you have two dyes that are the same size. After running the gel, the dyes travel different distances. Explain how this could happen.

5) How could the same dye molecule move different distances if run by two different gel electrophoresis experiments?

6) Based only on the information in Table 1, fill in the table below. List the dyes in order from the dye that you expect to travel the shortest distance to the dye that will travel the greatest distance.

Dye(s) that will move towards the positive electrode	Dye(s) that will move towards the negative electrode

Questions for after paper chromatography

1) How many dyes were in the unknown sample you put on your chromatography paper? Can you tell which dyes they are from their color?

2) What determines the rate at which the dyes move up the chromatography paper?

3) According to your results, which dye in the sample do you think is more soluble?

4) Both alcohol and water are polar solvents. Therefore, which dye in the sample is more polar?

Questions for after gel electrophoresis and visualization

1) Which dyes are positive and which are negative? How do you know this?

2) Using only the data collected from your gel, which dyes would you expect to be the smallest and which do you expect to be the largest molecules? How did you determine their relative sizes?

3) Based on the molecular weight of the molecules given in table 1, were you able to predict roughly how far on the gel each dye would run relative to each other? Which dyes followed your predictions; which ones did not? What explanations can you give for the dyes not following your predictions?

4) Which dye ran the farthest? What was its weight and charge?

5) Unknown A was run on both the chromatography paper and on the electrophoresis gel. How were the results of the chromatography and the gel for this mixture similar? How were they different? Can you explain these similarities and differences?

6) Why did Bromophenol Blue and Bromocresol Purple travel roughly the same distance even though Bromophenol Blue is almost 130g/mol heavier than Bromocresol Purple?

7) Propose an explanation for why Methylene Blue ran in a different direction on the gel than the other dyes used in this lab.

8) The two unknown samples should have separated into two bands, demonstrating that the samples were made from two dyes. What dyes are these two samples made of?

9) Fill in the *Gel Diagram* found below the *Student Data Table* with your observed results; use markers or colored pencils if they are available to you. Label each band with the correct name of the dye.

10) On the *Gel Diagram*, predict what you would see if you ran another mix of dyes in the final empty lane. The dyes in the final mixture are as follows:
    a) A green dye with the same weight and charge-to-mass ratio as Bromophenol Blue, but the charge is positive instead of negative.
    b) A brown dye that has the same charge-to-mass ratio as Methylene Blue but weighs half as much.
    c) A yellow dye that weighs twice as much as Allura Red and also has half the charge-to-mass ratio.

Calculating:

1. How many individual molecules are in the 10 µl aliquot of dye that you placed in the well?
    a) Use the molecular weight and the weight per volume concentration given in table one to calculate the approximate number of total molecules placed in the well for Orange G. You will need to use Avogadro's number, 6.022×10^{23} (the number of molecules in a mole) to find your answer.

        *You can calculate this number for all dyes except Allura Red AC. The concentration of Allura Red AC is given volume/volume and therefore the number of total molecules available cannot be calculated given the data available.

    b) There are roughly 7.5 billion people alive on Earth. How does the number of molecules in your 10 µl aliquot compare to the population of Earth?