



Colorful Dye Electrophoresis MiniLab Student's Guide

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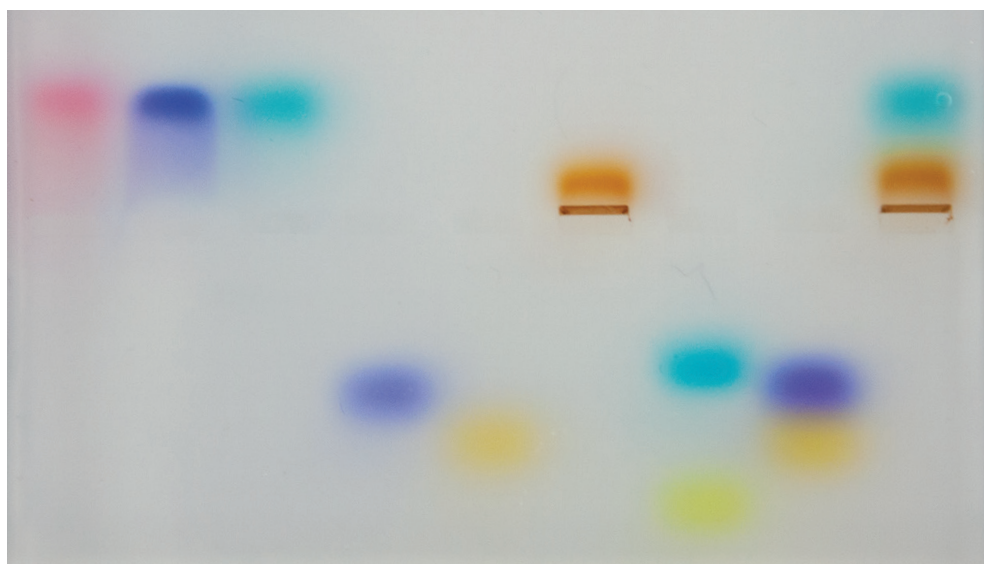


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Laboratory Safety

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.

Technical Objectives

- Correctly use an adjustable volume micropipette
- Prepare, load, and run an agarose gel
- Determine the composition of unknown dye mixtures

Intellectual Objectives

- Appreciate the importance of coloring agents in the world economy
- Understand the principles of gel electrophoresis and its importance in biology and chemistry
- Predict how molecular size and electrical charge affect a molecule's migration through separation matrix, such as agarose gel, in an applied electric field

Background

Colors in Our Lives

Coloring agents have been used by humans for art and commerce since prehistoric times. Water soluble dyes and water-insoluble pigments have become a ubiquitous feature of our lives in the industrial world. As companies compete to make the most novel, eye-catching products, vivid dyes and pigments have found their way into the clothes we wear, the food we eat, and the plastics that surround us every day. Food colorants alone are a billion dollar worldwide industry that employs chemists, food scientists, technicians, and product development experts.

For most of human history the only colorants available were natural extracts from plant, animal, and mineral sources, some familiar and others quite strange. Many traditional plant-based dyes are still used today: Brown extract of the henna plant has been used for millennia in hair dye and body art. Saffron dye, which gives Buddhist monk's robes their bright yellow color, is extracted from stigmas painstakingly plucked from crocus flowers. Indigo, which provides the deep blue of denim jeans, is traditionally extracted from *Indigofera* plants, but can also be produced synthetically. Some would be surprised to learn that the dye that gives red velvet cake and strawberry beverages their signature color is extracted from bodies of the cochineal insect, a 0.5 cm long scale insect that feeds on cactus pads. As a protection from predators, this insect produces carminic acid, as much as 10% of its body weight. Extracts containing carminic acid can be used directly as a colorant or treated with an aluminum salt to produce red carmine dye. A pigment called mummy brown was commonly used by painters in the 16th century and was actually made from ground up Egyptian mummies, but this practice declined as the supply of mummies ran out.

Science, economics, and safety concerns have driven the development of dyes and pigments. Metal ions complexed by organic compounds produce vivid colors, making them attractive coloring agents. During the 1700s and 1800s, toxic heavy metals (including mercury, lead, copper, and arsenic) were common in processed foods, especially colorful candies sold to children. These and other 'adulterants' were banned by the Pure Food and Drug Act of 1906. The first synthetic dye, a bright purple called mauveine, was produced accidentally in 1856 by a chemist attempting to synthesize the antimalarial drug quinine.

Synthetic dyes, which are often cheaper and more stable than natural dyes, have since become a staple of the industrial economy. A range of synthetic dyes, such as the Blue No. 2, Yellow No. 6, and others familiar from snack food labels, have been approved by the Food and Drug Administration for human consumption. However, concerns about safety have driven a renewed interest in natural dyes for food coloring.

Background (continued)

To understand how dyes and pigments give color to objects we must understand how their molecules interact with light. White light is a mixture of all colors within the visible spectrum. The colored appearance of a dye is a result of molecules absorbing all colors of light except the dye's characteristic color, which is reflected and perceived by the eye.

Blue and yellow are combined to produce green while red and blue are combined to produce purple. Many of the vivid colors we see in food and clothing are two or more colorants mixed together to achieve a desired hue.

In this lab you will:

- Explore the chemical properties of common dyes and dye mixtures.
- Use gel electrophoresis to determine the electrical charge of dye molecules.
- Determine whether samples contain a single type of dye molecule or a mixture.
- Use your knowledge to determine the composition of unknown samples.

Agarose 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. A comb is placed while the agarose is molten and 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 (\oplus anode) and negative (\ominus 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 \ominus cathode and negatively charged molecules migrate toward the \oplus 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 of molecule are present in the sample, they will separate from each other and each will form a distinct band.

In this lab you will run dye samples on an agarose gel and use the resulting pattern of colored bands to determine the charge of the dye molecules. You will also use the banding pattern from known samples to infer the composition of unknown samples.

Pre-Lab Preparation

Prepare Running Buffer (TBE)

You will need 135 mL of diluted running buffer to run one gel. If your instructor has already diluted your buffer for you, skip these dilution instructions.

1. Obtain TBE Buffer Concentrate from your instructor.
2. Dilute 1 volume of the TBE Concentrate with 19 volumes of DI water for running buffer.

Example calculation:

Final volume: 2000 mL

TBE Concentrate needed: $2000\text{mL}/(20)=100\text{mL}$

DI water needed: $2000\text{ mL} - 100\text{ mL} = 1900\text{ mL}$

Following the example calculation, calculate the volume of buffer you will need for a final volume of 135 mL.

Materials

1 MiniOne® Electrophoresis Unit	1 Micropipette and pipette tips
1 MiniOne® Casting System with gel tray and comb	1 Photo hood
1 agarose gel cup (1%), TBE buffer	9 x 12 µL Dye Sample Aliquot
135 mL running buffer	1 Table 1: Dye Chemical Structures (see page 13)

Dye Samples

Sample ID	Dye Sample (15 µL each)
SO	Safranin O
TB	Toluidine Blue
BG	Brilliant Green
BB	Bromophenol Blue
OG	Orange G
NR	Neutral Red
GFC	Green Food Coloring
Unk1	Unknown 1
Unk2	Unknown 2

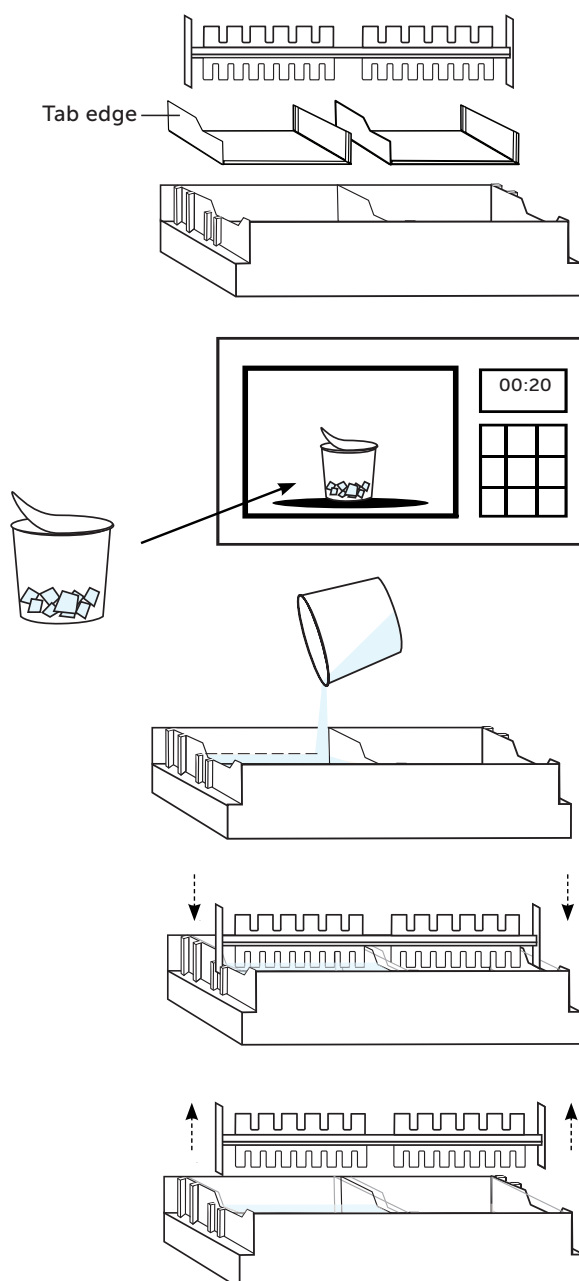
Pre-Lab Exercise

1. Predict which of the samples are mixtures of two or more dyes. Why do you think so?
2. Based on the information provided, predict which dye will migrate farthest in the gel.
3. Examine the MiniOne[®] Electrophoresis chamber. Locate the anode and cathode (positive and negative electrodes).
4. Use the Analysis Table to record which sample you will load in each well.


MiniOne Visual Instructions

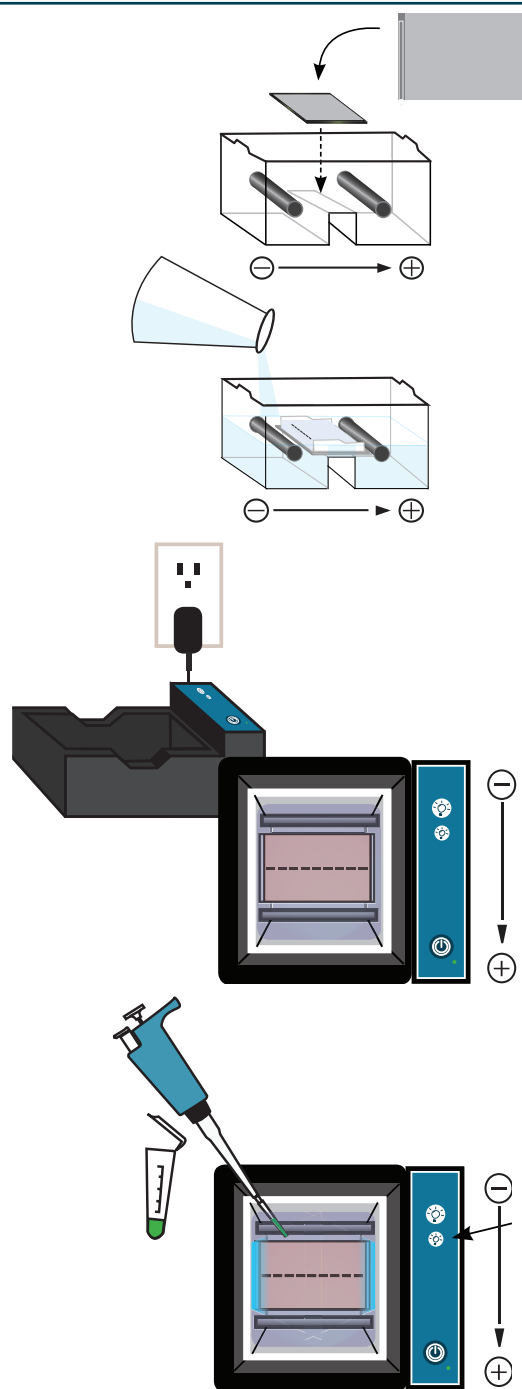
How to Cast a Gel

1. Place the MiniOne[®] Casting Stand on a level surface and place gel trays in the two cavities. For proper ray orientation place the tab edge of the tray on the left side.
2. Partially peel the film off a gel 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. Press down firmly on the gel tray. Make sure there are no air bubbles in the agarose solution.
4. Insert the comb into the slots at **the center** of the casting stand with the 9-well side facing down. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**
5. 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 grey viewing platform is in the tank if it is not already installed and put the gel (still on the gel tray) into the tank.
2. Measure approximately 135 mL of TBE running buffer and pour into **one side** of the tank to push out the air and create an even background.
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. Record the ID of each sample in the provided table (results section), corresponding to the correct well.

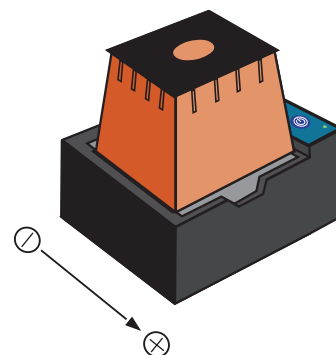



Run, Visualize, and Capture Image

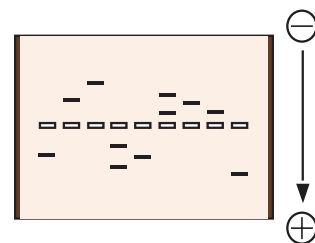
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the  button. The green LED next to the button will turn on.

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

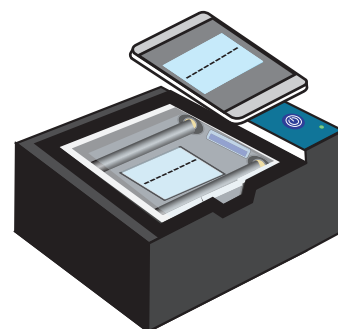


2. Allow the gel to run approximately **10 minutes** or until dye separation is sufficient. After your run is complete, turn off the power by pressing the  button.

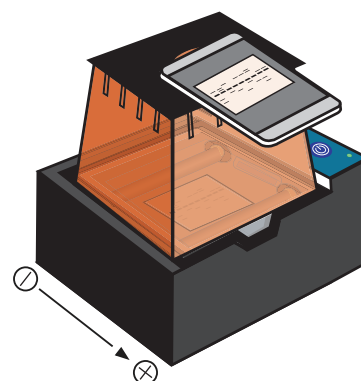


3. **Document your results.** At the end of the 10 minute run, take photos to document results.

- 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 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.

Results

1. Use the Analysis Table to record sample IDs, names, original and final colors, molecular weight (M. W.), their charges, and any other relevant information.

Well #	1	2	3	4	5	6	7	8	9
Sample ID									
Dye									
Original Color									
Final Color									
M.W.									
Charge									

2. Record your gel images here and label the colors with the Sample ID/Dye name.

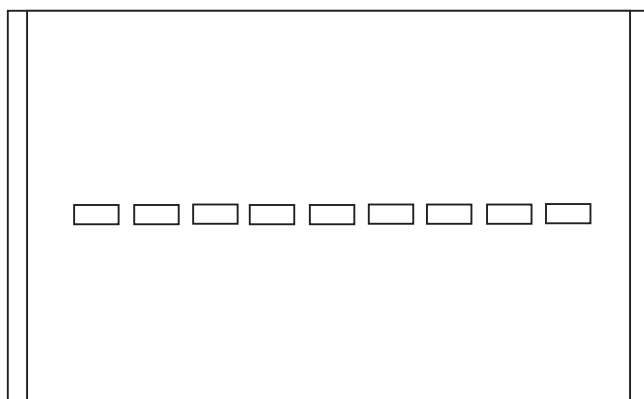


Image A: Blue LEDs OFF, without orange photo hood

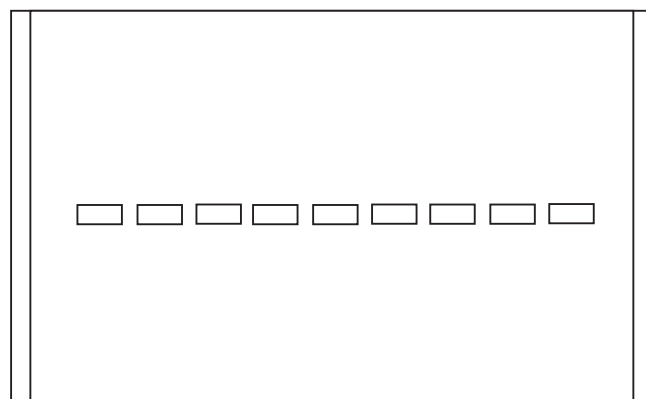


Image B: Blue LEDs ON, with orange photo hood

Analysis Questions

1. Which of the samples are mixtures of dyes? What colors do these mixtures contain? Can you identify the specific dyes in these mixtures by comparing to other lanes?
2. Which dye traveled the farthest? Provide an explanation for your observation.
3. Did the dyes migrate in the way you predicted above? If not, what do you think accounts for the difference between predicted and observed migration pattern?
4. Why is the comb placed in the middle of the gel for this experiment?
5. Which dyes are positively charged? How do you know?
6. Which dyes are negatively charged? How do you know?
7. View the gel first without the orange photo hood or the blue light, then with the photo hood on and the blue light on. What differences do you observe? How do you think the orange photo hood changes the appearance of the dyes?
8. Gel electrophoresis is used to separate macromolecules like DNA. The size of DNA is measured in base pairs (bp).
 - a. Look up the charge of DNA, which electrode will it migrate toward?
 - b. Based on the charge of DNA, which electrode will it migrate toward?
 - c. Where should you place the comb if you are going to run DNA and would like to maximize the use of the gel?
9. Assume that the charge to mass ratio of DNA is constant regardless of size. If you have three DNA fragments with sizes 2000 bp, 1000 bp, and 500 bp mixed together in one sample:
 - a. Which fragment will move the fastest?
 - b. Which fragment will move the slowest?

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 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 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.

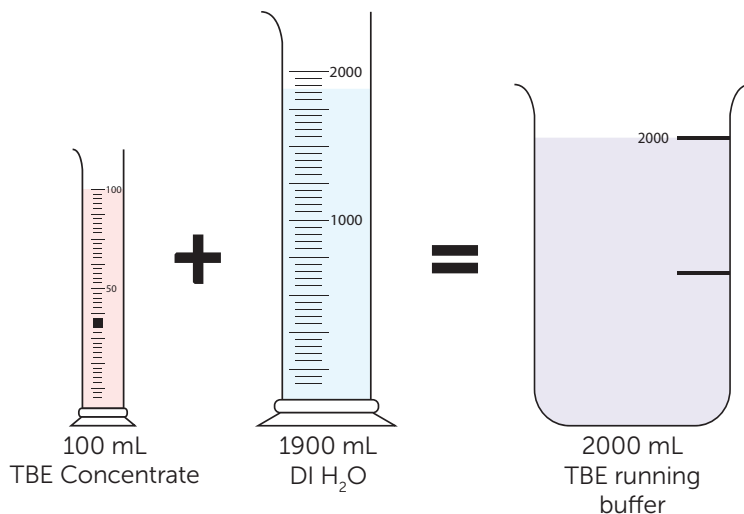
Sometimes a particle with a bigger size migrates faster than a smaller particle. This can happen if the strength of the charge of the larger particle is significantly stronger by comparison to the charge on the smaller particle. An example of this phenomenon is the loading dye Orange G. This dye often 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

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



TBE running buffer is stable stored at room temperature.

- 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

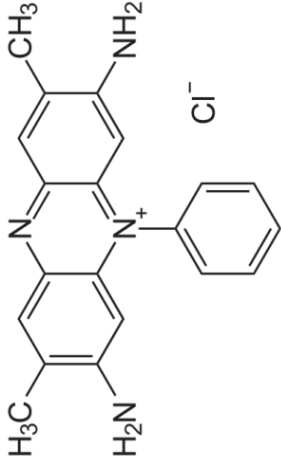
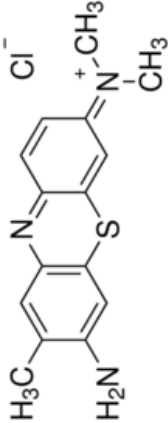
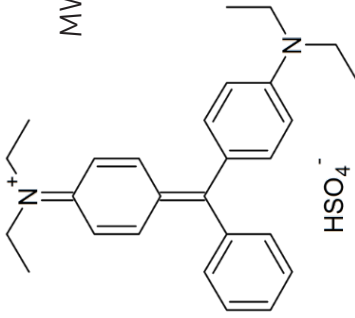
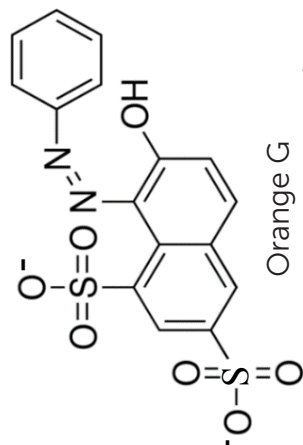
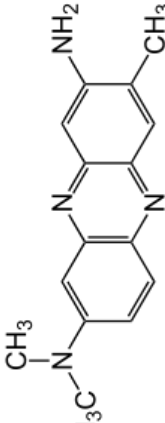
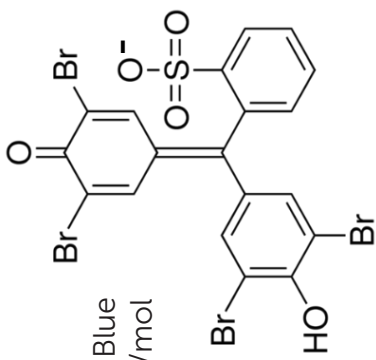
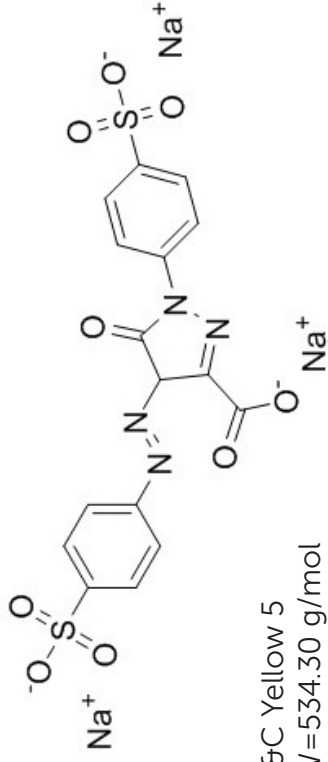
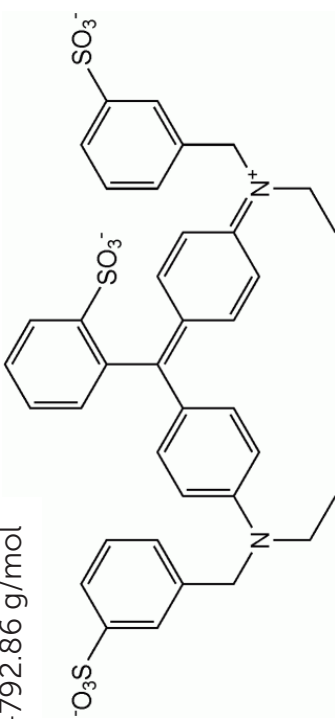
C_2 = Final concentration

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.

Table 1: Dye Chemical Structures

 <p>Safranin O MW = 350.84 g/mol</p>	 <p>Toluidine Blue MW = 305.83 g/mol</p>	 <p>Brilliant Green MW = 482.63 g/mol</p>
 <p>Orange G MW = 452.40 g/mol</p>	 <p>Neutral Red MW = 288.78 g/mol</p>	 <p>Bromophenol Blue MW = 691.94 g/mol</p>
 <p>FD&C Yellow 5 MW = 534.30 g/mol</p>	 <p>FD&C Blue 1 MW = 792.86 g/mol</p>	



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