Let's Move It!
Gel Electrophoresis Using Food Dye
Student Guide

Purpose

This lab explores the principle of electrophoresis, an important technique used in biochemistry and molecular biology. You will:

- Practice the steps required in loading an agarose gel.
- Observe the movement and separation of known colored dyes used in food.
- Observe the "known" food color dyes to determine which electrode and how far your color dye travels.
- Compare the "known" food color dyes with 2 unknowns and make a conclusion about the component(s) of the unknown color dye.
- Extract the color dye from Skittles candy, predict what color dyes are used and run the experiment with the known food dyes to see if your prediction is correct.

Using Gel Electrophoresis to Separate Molecules

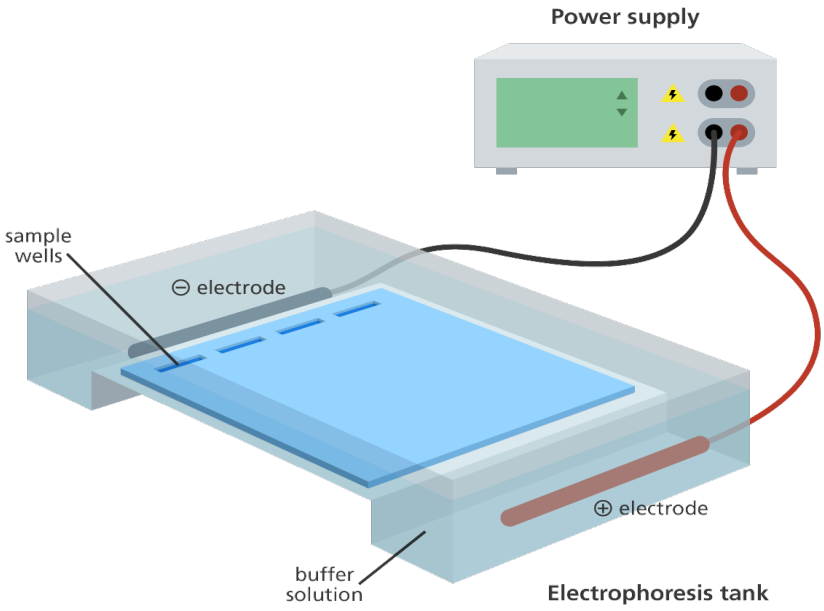
Gel Electrophoresis is a method used to separate molecules based on size and electrical charge. If we break the word electrophoresis into smaller parts, we can see that ***electro-*** refers to electricity, and ***-phoresis*** is Latin for "to carry." So, the word **electrophoresis** means "to carry electricity." In this activity, you will be using liquid dyes of various colors and separate out the different colored individual molecules using electrophoresis. For example, a purple dye may become blue and red, while a green dye may become blue and yellow.

We use a gel made from **agarose,** which comes from seaweed, that has the consistency of gelatin. It is used for electrophoresis because it is porous, which means it has lots of microscopic holes that molecules can travel through. Different concentrations of agarose produce different size pores allowing for varying migration speeds depending on the size of the molecules. It also allows electric currents to pass through, resulting in a positive charge on one end, and a negative charge on the other. In the presence of a solution that contains ions, the electrical current can carry the charged molecules in your dye through the gel.

About the Equipment

The system contains:

1. **Power Supply:** the source of electricity
2. **Electrophoresis Tank:** where the agarose gel sits and the reaction takes place
3. **Buffer Solution (TAE):** water and ions which fills the tank and conducts the electricity
4. **Electrodes:** where the electrical current enters (cathode, +) and exits (anode, -) the electrophoresis tank
5. **Agarose gel with sample wells:** the gel has indented areas for adding each of the different colored dyes



Pre-Lab Activity

Name _____

Date _____ Class Period _____

Part 1 - Make a Model

Your teacher will show you a short video of dye electrophoresis. After watching the video fill in the following:

1. I notice... _____

2. I wonder... _____

3. In the space below, draw a model to answer the questions: Why are colors moving in different directions? Why don't all colors move the same way? Write a brief caption explaining what you drew.

Part 2 – Jigsaw Reading

- Read your assigned selection silently for one minute. Each person in your group has a different reading.
- Read one more time looking for information supporting this, "How does electrophoresis separate molecules?"

4. Answer the question: "How does electrophoresis separate molecules?". Use evidence from readings to help.

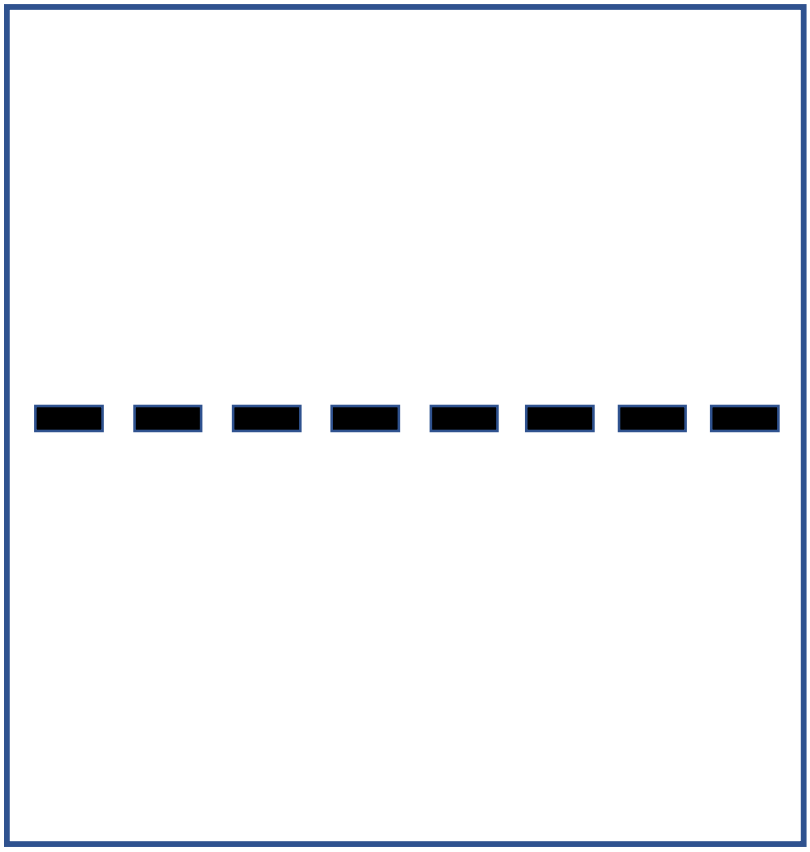
Part 3 – Revise your model

5. Revise or make changes to your model (in the box above) based on the readings.

Part 4 – Make Predictions with dye cards

- Your teacher will pass out a set of cards with pictures of each dye's molecular structure and mass (weight).
- Based upon the reading and video, predict how each the 4 dyes would move during gel electrophoresis.

6. Predict:
    a. Why way, (-) or (+), the molecules will travel? Up is (-) and down is (+)
    b. Which molecule will travel the farthest in each of the directions?
    c. Draw out where you think each dye will go. Then, write down your reason(s) on the line below:



Wells or lanes - Start of electrophoresis

I predict that…

_____

_____

Because…

_____

_____

Lab Activity – Practice Loading Dye
Lab Activity – Electrophoresis of Food Dye

Name _____

Date _____ Class Period _____

Background

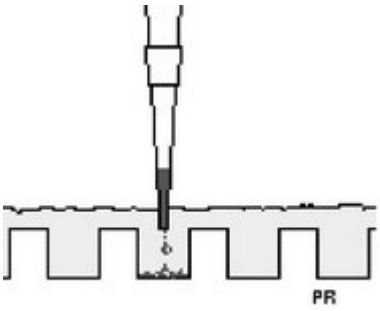
Recall that ions are atoms that have a positive or negative charge because they have lost or gained electrons. The migration of ions at different speeds is the basis of electrophoresis. During electrophoresis, the current splits the water into hydrogen ions (H+), which are acidic, and hydroxyl ions (OH-), which are basic.

Electrophoresis is a technique for separating and analyzing mixtures of charged molecules. Samples of mixtures are "loaded" into the "wells" of an agarose gel. Again, agarose is a purified form of agar, which is made from seaweed.

To prepare or "cast" an agarose gel, agarose powder is mixed with buffer, heated, and poured into a casting or gel tray containing a comb. After the gel has cooled down and solidified, lower the end gates and place the entire tray into the gel box. Fill the gel box with buffer, which allows the electricity to flow, and prevents changes in pH. Remove the comb after the buffer is added. When ready, use a p20 micropipette to load 15 μL of dye into each well.

Gel electrophoresis is commonly used to separate out DNA samples. To track where the invisible DNA runs on a gel, we add loading dye to the DNA samples. Usually, loading dye contains two dyes: one dye that runs slightly faster and farther than DNA, the second dye runs slower and not as far as the DNA. Different dyes move at different rates. Your instructor will put out a variety of dyes for you to test in the gel system. Your task is to predict distance and direction the dyes will travel, then observe and analyze the results.

I. Practice loading dye into practice gels.



1. Use a P20 micropipette to practice your loading technique into a well on a practice gel. Set micropipette to 15 μL.
2. **Depress** the plunger to the first stop and **keep it there before** lowering the tip into the dye. Slowly release the plunger to extract 15 μL of dye.
3. Put your elbows on the lab bench and steady the pipette over the well. Use your second hand to support your pipetting hand or arm.
4. Lower the tip of the pipette under the surface of the buffer directly over the well. **Avoid** puncturing the bottom of the gel.
5. Gently depress the plunger **to the first stop only** to slowly expel the loading dye into the well. If the tip of the micropipette is centered over the well, the dye will sink to the bottom of the well.
6. Keep the pipet plunger depressed until the pipet tip is out of the gel box. This prevents the dye from returning into the tip!

Questions:

a. What happens when you push down to the 2nd stop?

b. What do you think would happen if you poked the bottom of the well with your micropipette?

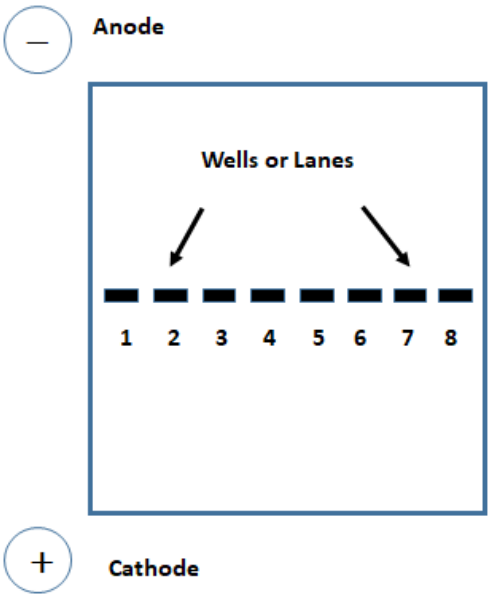
c. What would happen if you loaded too much into a well?

II. Loading the Gel of 6 Food Dyes and 2 Unknowns

1. You will be given eight dyes in tubes. Six are known dyes: Blue #1, Yellow #5, Yellow #6, Red #40, Brilliant Green and Janus Green. Two tubes are Unknowns: A and B.
2. Write down the order in which you will load the dyes:

Well	1	2	3	4	5	6	7	8
Tube #								

3. Load each well left to right with the corresponding tube. Load 15µL per well.



Important notes:

- When two teams are connecting their gel boxes to one power supply, be sure to communicate with each other whether the power supply is turned ON or OFF. The power supply must be OFF every time anyone needs to touch or open a gel box.
- Place your gel box so that you can reach it comfortably during the loading process. Also, close the box and confirm that the wire leads reach the power supply from this location. After loading your samples into the wells, any movement of the box may cause your samples to be swirled out of the wells and/or mix!!!!

III. Electrophoresis of the Dye

1. Close the top of the gel box and connect electrical leads positive to positive (red to red) and negative to negative (black to black). Both electrodes will be connected to one power supply channel.
2. Set the power supply to approximately 100 V, and turn it ON. To double-check this, switch the display from V to mA to look at the current. If one gel is running, it has about 40 milliamps; with two gels it reads about 80 milliamps.
3. Shortly after the current is running, you will see the dyes slowly moving through the gel.
4. Run the gel box (electrophorese) for 10-20 minutes. Record the final location of the dyes on your Activity Sheet, p2. Take a photo for your records.
5. Turn off the power and disconnect the leads.
6. Leave the buffer in the gel box for reuse by the next class. Your teacher may ask you to replace the buffer or dispose of it down the sink if yours is the last class of the day. Please allow the gel boxes to **air dry**. Drying your gel box with paper towels may tear the platinum wires in the box.
7. Return gels to teacher for reuse.
8. **Record your observations on the worksheet on the next page.**

Upon completion of this lab

1. Dispose of designated materials in the appropriate places
2. Leave equipment as you found it
3. Wash your hands

Lab Worksheet – Electrophoresis of Food Dye

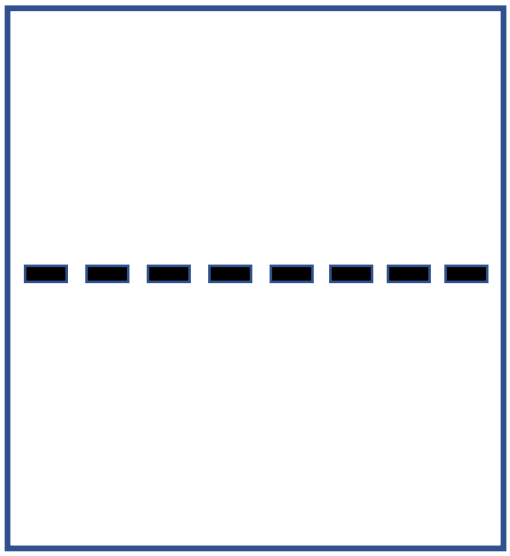
Name _____

Date _____ Class Period _____

1. Fill in the table below with your results. Refer to the dye cards for information about charge and weight.

Well #	Name of Dye	Charge	Molecular Weight	Go toward (+) or (-)?	How far from well? Closest? Farthest? In Between?
1					
2					
3					
4					
5					
6					
Unk A					
Unk B					

2. Label the positive and negative ends of the gel in figure below. Record the location of the 6 dyes and 2 unknowns in the diagram below.



3. Why did you want the wells to be in the center of the gel?

4. Look closely at the diagrams and results from the two yellow dyes and answer this question: Which, charge or mass (size), has more of an effect on the distance travelled during electrophoresis?

5. Compare your predictions to your observations. Did you correctly predict what happened? Explain what you may have overlooked in your predictions or what you have learned.

Lab Activity – Which dyes are in Skittles?

Name _____

Date _____ Class Period _____

Extraction of dyes from Skittles – Each group will work with 4 colors each.

1. Choose **four colors** of Skittles to work with; sort and pile **four pieces per color.**
2. **Predict what "known" dyes from the previous activity makes up the color in the chart below.**
3. **Extract** and **separate** the dyes as instructed on the following page.

Prediction Chart:

	Example Prediction	Skittle #1	Skittle #2	Skittle #3	Skittle #4
Color Skittle:	Red				
Prediction: *Predicted Color(s)*	Red #40				
Results: *Color(s) after electrophoresis*	Red #40 and Yellow #5				
Conclusions and New Questions:	I didn't think there were 2 colors in the red Skittles. I wonder why they needed the two colors?				

Skittles Extraction:

1. Get 4 Dixie cups or 125 mL beakers. Label the cups with the 4 chosen colors.
2. Into each Dixie cup or small beaker, transfer 500 μL of TAE buffer and one Skittle.
3. Rock each cup or beaker gently.
4. When enough dye is removed that the candy starts to look white, remove it and replace with another Skittle of the same color (into the same cup.)
5. Repeat steps 4 and 5 until all the Skittles of each color are used. Congratulations! You have made your dye extraction!
6. Now, make a make a map or legend of which colors you will load into which well. Be sure to include the known colors (Blue #1, Red #40, Yellow #5 and Yellow #6) and your newly extracted Skittles colors.
7. Using the correct micropipette, load 15 μL of each dye known and Skittles colors into the corresponding well on a fresh 0.8% agarose gel.
8. Finally, refer to the steps on page 6 to safely electrophorese (run) your gel.

Well	1	2	3	4	5	6	7	8
Tube #								

How well did your prediction match your results or findings by running the experiment?

Why do you think you ran the "known" colors with your Skittles extracts?

What do your results not tell you?

Let's Move It: Apply What You Know

Name _____

Date _____ Class Period _____

Having completed these activities on food dye electrophoresis, how do you think scientists might use the properties of electrophoresis to solve problems using DNA?

Write an answer that best reflects your understanding of how electrophoresis works, and how it might be applied to problems using DNA below.

Things to consider: Is DNA a molecule? If so, how can it differ between individuals? What kind of samples do scientists collect that might contain DNA? What kind of problems might separating DNA help scientists solve?