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Let's Move It! Gel Electrophoresis using Food Dyes Teacher Guide



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Unit Overview

Overview: This is the third lesson in the getting started series. In this unit, students will explore the process of electrophoresis with food-grade colored dyes. Using multiple dimensions of NGSS, the phenomenon will be introduced via a video. Students will predict the direction of the migration of dyes on paper and test their prediction using gel electrophoresis. Students will then extract food color from Skittles (predicting the color(s) they contain) then run the extracts on an agarose gel in a comparison with the known food dyes.

Suggested Background Learning Activities

- Students should have completed the “How to Use a Micropipette” lesson and be comfortable using a micropipette to deliver small volumes.
- Students should be familiar with basic concepts of electricity such as electrical current, positive and negative charges, and voltage.

Pre-Lab Activity

Includes: phenomenon video, jigsaw reading, dye cards, board model and electrophoresis loading video

Students will...

- be introduced to the phenomenon of colored dyes moving through a gel matrix
- use modeling to explain the phenomenon
- engage in small group learning with an elbow partner
- make a hypothesis about the outcome of their experiment based on their model

Lab Activities

Includes: Food Dye Gel Electrophoresis, Which Dyes are in Skittles?

Students will...

- learn the structure and function of the gel tray and gel box.
- load the gel with six different colored dyes and two unknowns.
- observe the electrophoresis process, while recording their observations and determining the names of each of the color dyes.
- deduce the unknown dyes by comparing with the known colored dyes they are running.
- extract the dye from Skittles, predict which dye each color contains and run on a gel to see if their prediction is correct.

Post-Lab Worksheet

Students will...

- go back to the predictions that they made in the pre-lab and compare their results with their predictions
- make conclusions based on evidence about the properties that affect the movement of molecules
- redo their model based on the conclusions from their experiment

NGSS Alignment

Phenomenon: Electrophoresis

- Molecules move based upon their charge and mass.
- Generally, the larger the molecule, the slower it moves.
- Opposite charges attract and like charges repel.
- Things that have the same mass-to-charge ratio move at the same rate.
- Molecules that are different move at different rates.

Disciplinary Core Ideas

HS-PS1-3 Matter and Its Interactions - Inferring strength of electrical forces between particles.

HS-PS2-6 Motion and Stability: Forces and Interactions – Communicate why molecular-level structure is important in functioning of designed materials

HS-ETS1-2 Engineering Design – Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering

Cross-cutting concepts

Patterns, Cause and Effect, Structure and Function

- Students explore how the different charges, mass, and shape of food dye molecules affects their migration through a gel matrix, examining the direction and distance each molecule travels.

Science & Engineering Practices

Asking Questions and Defining Problems

- Students question why some color dyes move differently, and develop predictions that it may have to do with the mass, charge or shape of these molecules

Developing and Using Models

- Students create a series of predictive models prior to running each step of the experiment. They then compare their results to this model.

Planning and Carrying Out Investigations

- Students extract food dye from Skittles and plan how to load their dye for comparison to known dyes.

Analyzing and Interpreting Data

- Students examine the direction and distance that each dye molecule has traveled. Students examine “unknown” samples to determine which dyes were used to make this sample. Not all food dyes used in Skittles are provided for the “known dye” portion of the experiment, forcing students to consider alternatives to making certain Skittles color shells.

Constructing Explanations and Designing Solutions

- Students examine their predictions and experimental evidence to develop an understanding of how electrophoresis moves particles.

Engaging in Argument from Evidence

- Students examine their predictions and experimental evidence to develop an understanding of how electrophoresis moves particles, and can compare their understanding with one another.

Obtaining and Evaluating Information

- Students examine written statements, dye cards and experimental evidence to develop a conclusion about how electrophoresis moves molecules.

Suggested Timeline

This Activity could be completed in one 75 minutes block or in two 50 minute periods. For two 50 minute periods, it is recommended you stop after the practice gels. To be most time efficient, have students extract their Skittle dye, and make predictions about these colors, while the Food Dye gel with known colors are running in the gel electrophoresis.

Possible Alternative structure: Have students extract dye from Skittles first and pipetting into tubes for later use. Engage them with a discussion of how they think foods like Skittles get their colors. Students could examine Skittles packaging labels for ideas. Then proceed through the rest of the lesson.

Inventory Sheet

Listed below are the reagents and consumables provided in the “Let’s Move It, Gel Electrophoresis using Food Dyes” from BABEC, as well as additional reagents and consumables. Make sure also to read the list of equipment needed for this laboratory activity. For this activity, a team is considered 2 students. The kit contains enough reagents for **40 students (20 teams)**. Teachers in the GeneConnection service area may find these materials supplied in their portable beginning Fall 2018.

Reagents provided in the BABEC reagent kit:

✓	Item	Storage	Amount Per Kit	Amount Per Team
	FD&C Blue #1	Room Temp (20–25C)	500 µL	15 µL
	FD&C Yellow #5	Room Temp (20–25C)	500 µL	15 µL
	FD&C Yellow #6	Room Temp (20–25C)	500 µL	15 µL
	FD&C Red #40	Room Temp (20–25C)	500 µL	15 µL
	Brilliant Green	Room Temp (20–25C)	500 µL	15 µL
	Janus Green	Room Temp (20–25C)	500 µL	15 µL
	For Extraction of Skittles			
	1X TAE	Room Temp (20–25C)	50mL	2mL

Reagents/Consumables NOT provided in the BABEC Reagent Kit

✓	Item	Comments
	Skittles®	2 bags; both “Classics” and “Brights” work well
	Microfuge tube rack	1 for every team or every 2 teams
	Microfuge tubes	For aliquoting and for Skittles
	Micropipette & tips	P-20s, for loading gels; 1 micropipette and tip box per team
	Markers	1 per team
	Dixie Cups	4 per team (for extracting Skittles)
	Tweezers	1 per team (for removing extracted Skittles)
	Transfer pipette or P1000	For transferring 500 µL to Dixie Cups for Skittles extractions; 1 per team
	Item (for gel electrophoresis)	Comments
	1X TAE buffer	Running agarose gel electrophoresis
	0.8% agarose	For making agarose gels; 2 gels per team; Gels can be re-used!*
	Item (general)	Comments
	Gloves / lab coat / goggles	Optional: For personal protection
	Waste containers	For disposal of the tips. 1 per station of 2-4 students
	Zip lock bags (optional)	To store the gels in the refrigerator (if gels are poured the day before)
	Equipment	Comments
	Gel boxes, casting trays and comb, power supply	The number of gel boxes depends on the number of student groups and the number of wells in each gel. Each student group needs to load 5 lanes plus
	Smartphone or digital camera	Optional: Taking pictures of their results

**To reuse gels, put gels into bottle/beaker, microwave until completely clear, swirling often. Re-cast when gel is cooled to touch.*

Suggested Lab Preparation Sequence

	Activity	When	Prep Time *
1	<input type="checkbox"/> Read this packet, create a lesson plan. <input type="checkbox"/> Make copies of the Student Guide, jigsaw readings and worksheets. <input type="checkbox"/> Cut out dye molecule cards.	Before starting lab prep	2 hours or more
2	<input type="checkbox"/> Aliquot dyes to microfuge tubes according to the number of groups you will have. <input type="checkbox"/> Prepare agarose gels, wrap in plastic and store in 4C until ready to use.	24 hr prior to student use	30 min or more
3	<input type="checkbox"/> Set-up student group stations by providing microfuge tubes and racks, Dixie cups, tweezers, etc. Note: In our experience, holding off on the Skittles until just prior to use is probably a good idea.	Night before or prior to start of lab	1 hour

** Time needed depends on number of group stations and class sections.*

Prelab Activity Summary

Goal of the Lesson:

In this activity, students will take an inquiry-based approach toward understanding a basic separation technique, electrophoresis. First, they will construct a written model based on the phenomena of dye moving in an agarose gel. Then they will read with an elbow partner, discuss, and revise their models. Next, they will make predictions based on the size and charge of the dye molecules for the outcome of their experiment.

The Phenomenon: Electrophoresis

For teacher only, do not tell the students the background of this phenomenon

- Molecules move based upon their charge and mass.
- Generally, the larger the molecule, the slower it moves.
- Opposite charges attract and like charges repel.
- Things that have the same mass-to-charge ratio move at the same rate.
- Molecules that are different move at different rates.

This activity involves:

1. Video of Dye Migration
 - a. Video from the BABEC website “Dye Electrophoresis time lapse video”. This video is the “phenomenon” around which students will frame their learning. The phenomenon is how molecules migrate at different rates through a matrix.
 - b. After watching the video, have students write down “I notice...I wonder...” Remember, you have not told students the information. Students will uncover new information and revise their understanding as they progress through the lesson and learn the concepts.
 - c. Group Discussion & Hypothesis: “Why are the colors going toward different directions?”
 - d. Draw a model of your hypothesis on the pre-lab activity sheet.
2. Electrophoresis Reading - Jigsaw
 - a. Break students into groups of 2-3, giving each student a different reading strip. See page T3 for reading.
 - b. Take 1-2 minutes for each student to read silently and independently their assigned reading. This activity provides them with some core content that may be used to revise their model and make predictions about the outcome of their experiment.
 - c. Jigsaw Group Discussion
 - i. 2-3 minutes to share what they read to their group.
 - ii. Have students come up with a list from the readings to answer the question “How does electrophoresis separate molecules?”
 - iii. Do their hypotheses match with the earlier one (#1e)? If not have students revise their hypothesis.
3. Lecture – electrophoresis. Slides are available on the BABEC website. BABEC.org→Curriculum→Teacher Resources
4. Tabletop prediction using dye cards:
 - a. See page T4 for card templates. Print out and cut this page into six individual cards. Each card contains information about each of the dyes that the students will electrophorese: molecular structure, charge, and molecular weight.
 - b. Students will use these cards to make predictions about the molecules and how they will run (towards anode or cathode) and how far.
 - c. Students will predict the migration on their tabletop. Make sure they indicate polarity and the well origin or starting point.
5. Videos – Also see the BABEC website for links.
 - Agarose Gel Electrophoresis (TXBiolab). This video shows how to set up and load a gel properly: <https://www.youtube.com/watch?v=9f2VSyVhsGI>
 - Improper Gel Loading (Fisher Science Ed): <https://www.youtube.com/watch?v=OF9fFdTdqFY>

Electrophoresis Jigsaw Reading Material

Prepare sets of three strips per group

- Separate students into groups of 2-3.
- A student of each group will get a different reading.
- Read silently for 1-2 minutes, independently, and make note of what they think it means.
- Students share what they have read answering the question: "How does electrophoresis separate molecules?"
- TIP: Sometimes allowing students to check understanding of their selection with someone else with the same selection before sharing back to their team builds confidence and understanding.

Reading #1

Electrophoresis is a versatile tool in molecular biology. There are many different types of electrophoresis but they all work on the same principle. Samples are placed on one end of a 'gel'. The gel is a substance that consists of a molecular matrix of evenly sized and spaced openings. An electrical current is passed through the gel. The current moves the molecules in the sample through the gel. The rate at which the molecules move depends on both their size and their charge. Large molecules will be slowed by the matrix while small molecules will move more quickly. Molecules with a charge will move rapidly while uncharged molecules will move slowly.

Reading #2

Gel Electrophoresis: The process in which molecules (such as proteins, DNA, or RNA fragments) can be separated according to size and electrical charge by applying an electric current to them while they are in a gel. The current forces the molecules through pores in a thin layer of gel, a firm jelly-like substance. The gel can be made so that its pores are just the right dimensions for separating molecules within a specific range of sizes and shapes. Smaller fragments usually travel further than larger ones.

Reading #3

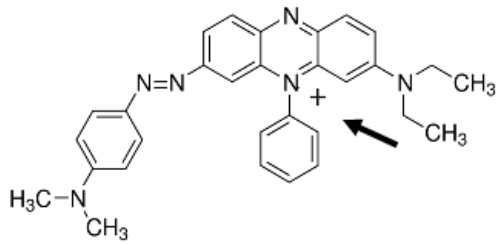
Gel electrophoresis is a laboratory method used to separate molecules according to size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. The molecules travel through the pores in the gel at a speed that is inversely related to their lengths. This means that a small DNA molecule will travel a greater distance through the gel than will a larger DNA molecule. After just a few minutes, the mixture will start to separate.

Sources

- Retrieved from: https://highered.mheducation.com/sites/9834092339/student_view0/chapter20/electrophoresis.html
- Retrieved from: <http://www.medicinenet.com/script/main/art.asp?articlekey=13534>
- Retrieved from: <https://www.nature.com/scitable/definition/gel-electrophoresis-286>

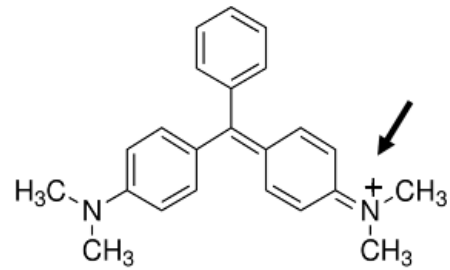
Dye Cards Materials – Make copies and cut out each molecule; make sets for each student group

Janus Green



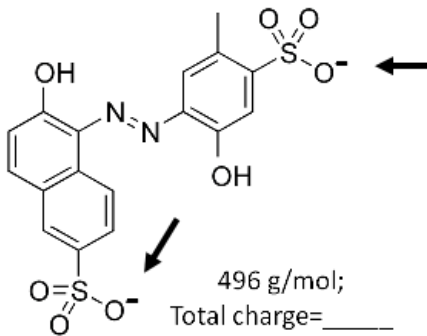
511 g/mol; Total charge= _____

Brilliant Green



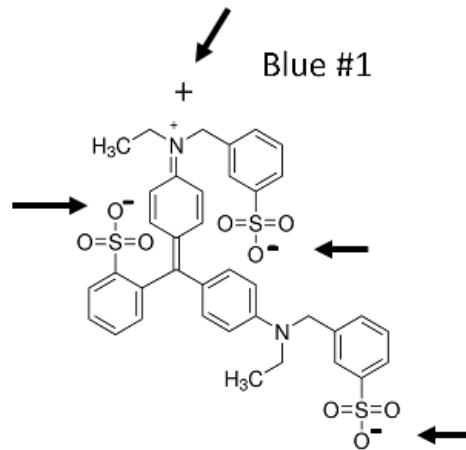
482 g/mol; Total charge= _____

Red #40



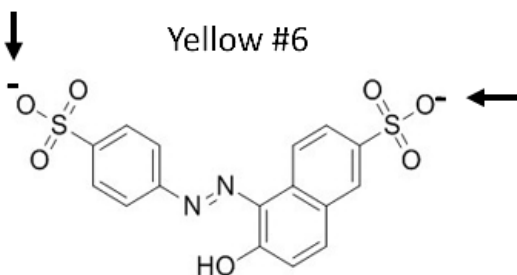
496 g/mol; Total charge= _____

Blue #1



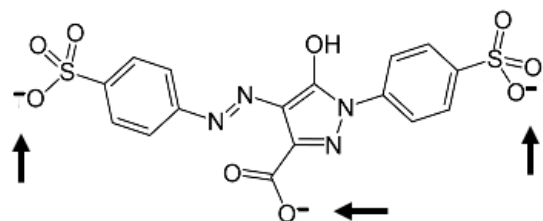
793 g/mol; Total charge= _____

Yellow #6



452 g/mol; Total charge= _____

Yellow #5



534 g/mol; Total charge= _____

Lab Activity Instructions

A. Practice loading into wells with loading dye.

Students practice correct methods of using the micropipette to load loading dye into the wells.

Emphasize:

1. When loading a well, place both elbows on the table for stability and hover over a well; do not poke into the well.
2. When expelling, stop at the first stop; otherwise, bubbles and air will push the color out of the well.
3. Keep the micropipette depressed at the first stop; take the micropipette completely out of the well/buffer before releasing the plunger.

B. Food Dye Gel Electrophoresis

In this activity, students will load eight samples and run the gel to separate the dyes by electrophoresis. They will determine which direction the dyes are moving as well as how far from the well each molecule will move. Students will use the results of six known colored dyes as a key to identify two unknowns as well as the outcome of the Skittles color experiment.

C. Which Dyes are in Skittles? (Skittles Food Dye Gel Electrophoresis)

In this activity, students will:

1. Extract the dye colors from the candy Skittles
2. Predict which colors correspond to the “known” colors, by predicting towards which electrode and how far the dye will run.
3. Run the dyes on 0.8% agarose gel electrophoresis.
4. Analyze and write the results for this experiment.
5. Determine if their prediction matched with the experimental results.

These activities require:

1. **Electrophoresis systems.** Ideally one system for each team of 2-3 students. The system includes a power supply, a gel box, and a gel casting tray
2. **P20 micropipettes**, with corresponding yellow tips.
3. **Agarose.** You will need to make an 0.8% solution; see instructions on page T9.
4. **TAE Buffer.** Used to run the gel during electrophoresis and to make the 0.8% agarose solution. Place the 1X TAE Buffer in the classroom.
5. **Dyes.** Concentrations of dyes are on page T6. Below is a table of the different dye properties that will influence the speed with which they migrate through an agarose gel during electrophoresis:
6. **1X TAE for Skittles food dye extraction**
7. **Small Dixie cups or 125ml Beakers**
8. **Tweezers for removing the Skittles from the cups.**

Tube #	Common Name	Molecular Weight	Chemical Formula	Charge
1	FD&C Blue #1	793 g/mol	$C_{37}H_{34}N_2Na_2O_9S_3$	- 2
2	FD&C Yellow #5	534 g/mol	$C_{16}H_9N_4Na_3O_9S_2$	- 3
3	FD&C Yellow #6	452 g/mol	$C_{16}H_{10}N_2Na_2O_7S_2$	- 2
4	FD&C Red #40	496 g/mol	$C_{18}H_{14}N_2Na_2O_8S_2$	- 2
5	Brilliant Green	482 g/mol	$C_{27}H_{33}N_2.HO_4S$	+ 1
6	Janus Green	511 g/mol	$C_{30}H_{31}ClN_6$	+ 1

Materials needed per team:

1. Electrophoresis System: power supply, gel box, gel tray with 8-well comb and 300 mL 1X TAE buffer
2. Individual 1.5mL microtubes of each of the 6 dyes and 2 unknowns labeled A and B
3. [0.8%] Agarose, melted and kept hot in a 65°C bath or incubator; or pre-made 0.8% agarose gel with 8-wells
4. P20 micropipette & tips
5. Skittles
6. Dixie Cups small beakers for extracting the color dyes from Skittles with 1X TAE
7. Container for waste

Reagent Preparation instructions

Preparing 0.8% agarose solution

You will need 0.8% agarose in 1X TAE buffer. Each bottle of 400 mL supplies 16–25 mL gels.

To make 400 mL of 0.8% agarose:

1. Mix 3.2 g of agarose power in 400 mL of 1X TAE buffer
2. Microwave for 2 minutes and check to see if all the agarose has dissolved by swirling. If not, microwave at 30 second increments until fully dissolved.
3. Store the melted agarose in a water bath set at 65°C
4. When ready, dispense 25 mL/gel in a small beaker for each team. Make sure that students cast gel immediately. Note: tell students not to pour gel if they see the agarose coagulating. Microwave again until fully dissolved and at safe temperature before continuing.
5. To save instructional time, you can pre-cast gels for your class into gel trays, or even into petri dishes as “practice” gels.

Setting up the electrophoresis system

- I. Set up the system according to the manufacturer’s instructions.
- II. Preparing the Gel Tray:
 - a. Make sure that there is a washer with each screw to prevent leakage.
 - b. Have students raise the gates on the gel tray and gently turn the screws until they are just barely tight. Caution them that overtightening the screws can break them. Use tape if gate is not present.
 - c. Students will insert the comb into the middle notches.
 - d. Have students place the trays on a paper towel, prior to pouring the hot agarose.

Colored Dyes (Provided by BABEC)

- Dyes have been dissolved in 50% glycerol, 10mM Tris, pH 8.
- Glycerol is used to increase the density, so that the sample sinks into the wells.

Unknown dyes

- There are 2 unknown dyes labeled A and B. The unknown dyes are a mix between any of the dyes you are running.

Skittles Dye extraction – Students will perform this extraction

1. You will need 4 small cups or beakers per group for the four different Skittles color (both original and bright color types work well).
2. Choose **four colors** of Skittles to work with; make piles, **four pieces per color**.
3. In each small cup, add 500 μ L of (Food Dye Extraction buffer/1X TAE Buffer).
4. Starting with only 1 Skittle color at a time per student, place one Skittle into the cup.
5. Rock the Skittle gently until the color begins to dissolve off the candy.
6. When you see the white of the candy, remove the used Skittle with tweezers and replace with another Skittle of the same color.
7. Repeat steps 5 and 6 until all the Skittles of each color is used.
8. Use a micropipette to transfer the dye colors into microfuge tube, labelling each tube with the color.

What to demonstrate/explain

1. Remind students that the level of the buffer should be 1 cm above the gel.
2. Demonstrate how to drop the gel tray gates: After allowing the agarose to solidify for 10 minutes, loosen the screws of the gel tray by turning them half-a-turn counterclockwise, drop the gates, then gently tighten the screws to prevent the gates from moving.
3. Demonstrate how to place the gel tray onto the platform in the gel box: Show students how to tilt the gel tray so that it enters the gel box filled with buffer solution.
4. Demonstrate how to remove the comb and place it on the power supply: Show students how to wiggle the comb, allowing the buffer solution to loosen the gel, then remove the comb from the gel. Always place the comb on the power supply to locate easily.
5. Demonstrate how to load the well with dye: Rest elbows on the table, while looking down on the wells, release dye directly above the well.

Troubleshooting

Dyes may move into adjacent wells. Check the pH if you suspect water was used to make the gel instead of 1X TAE buffer. A pH of 8 or 9 is expected if the gel solution is made with a correct buffer concentration. Gels made in greater than 1X TAE buffer will heat up more quickly and dyes will be smeary. Check the current for clues about buffer concentration.

If you are using agarose sent from previous schools, check for 2 possible problems:

1. Incorrect concentration of agarose:
 - a. <0.6%. Dyes will migrate more quickly
 - b. >1%. Separation will still occur, but dyes will move slowly
2. Incorrect buffer concentration: Gels made in water will solidify clearer than gels made with buffer.

When using electrophoresis systems, it is advised to keep the voltage under 100-125V, and amperage to 40 milliamps for one gel and 80 milliamps for two. Higher voltage / amperage may allow the system to run too hot, which can ultimately damage the gel tray or gel box, including melting in severe cases.

If a gel box is not producing bubbles, check to see that the lid is fitting properly and that there is no damage to the black or red leads, or to the thin platinum wire filament inside the box. To avoid breaking this filament, rinse gel boxes with distilled water when done, then allow to air dry. Using a towel can break the wire, and scratch the plastic. Also, never screw/unscrew the leads. They should only be pulled out or pushed in carefully.

For efficient use of time, you may want to have students make predictions about the Skittles colors and prepare their extractions while their first gel is running.

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

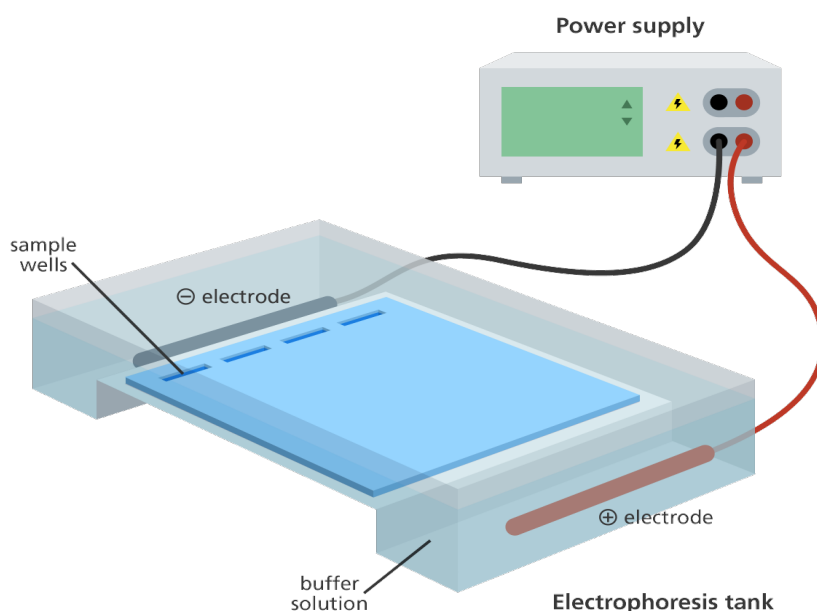
Gel Electrophoresis is a method used to separate molecules based on size and electrical charge. If we break the word electrophoresis into smaller parts, we can see that **electro-** refers to electricity, and **-phoresis** is Latin for “to carry.” So, the word **electrophoresis** means “to carry electricity.” 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

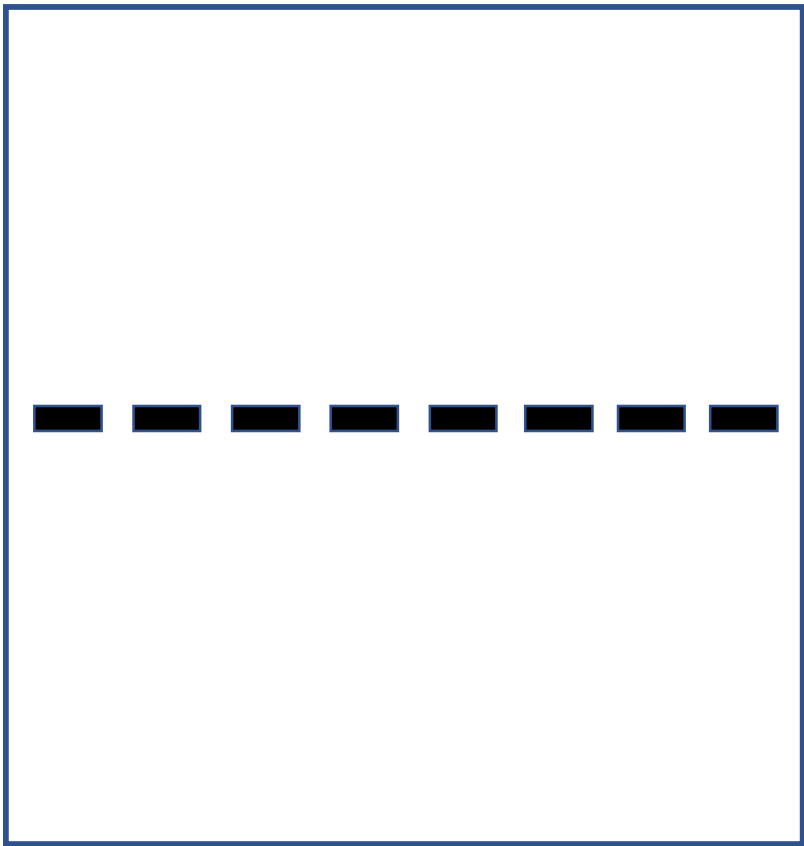
- Read your assigned selection silently for one minute. Each person in your group has a different reading.
 - Read one more time looking for information supporting this, “How does electrophoresis separate molecules?”
4. Answer the question: “How does electrophoresis separate molecules?”. Use evidence 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.

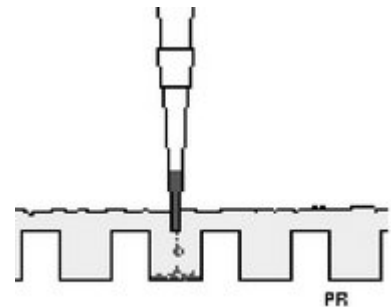
Electrophoresis is a technique for separating and analyzing mixtures of charged molecules. Samples of mixtures are "loaded" into the "wells" of an agarose gel. Again, agarose is a purified form of agar, which is made from seaweed.

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 μ L 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 μ L.
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 μ L 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:

- What happens when you push down to the 2nd stop?
- What do you think would happen if you poked the bottom of the well with your micropipette?
- What would happen if you loaded too much into a well?

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

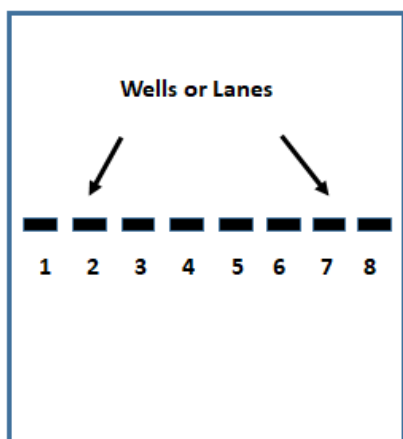
- You will be given eight dyes in tubes. Six are known dyes: Blue #1, Yellow #5, Yellow #6, Red #40, Brilliant Green and Janus Green. Two tubes are Unknowns: A and B.
- Write down the order in which you will load the dyes:

Well	1	2	3	4	5	6	7	8
Tube #								

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



Anode



Cathode

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 (electrophoresis) 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: <i>Predicted Color(s)</i>	Red #40				
Results: <i>Color(s) after electrophoresis</i>	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 μ L 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 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 μ L 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?