STEM Electrophoresis Kit

Instruction Manual

Catalog Numbers
1665080EDU
1665090EDU

Warning! – The electrophoresis system that students will build in this laboratory exercise is designed to be used ONLY at low voltages (≤ 45 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as there is no isolation of the live electrical components from the users. NEVER connect this system to a power supply since serious or lethal shock could occur.

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BIO-RAD

Dear Educator,

As scientific discoveries advance there is a greater need, ability, and desire to understand systems as a whole, and this requires knowledge across several disciplines of study. Science is advancing faster than ever before because of advances in technology, and scientists can make use of new tools designed in various fields. Science and engineering have traditionally been taught as different thought processes—science as hypothesis-driven and engineering as an iterative process used to build devices. But as the pace of technology accelerates, there is increasing need for interdisciplinary curricula that thoroughly integrate the fields of science, technology, engineering, and math (STEM). Allowing students to apply these fields in real-world situations is key to helping them understand their principles and appreciate their interdependence.

With the STEM Electrophoresis kit, students can experience the relationships between the science of electrophoresis, the technology of separating molecules, the engineering of electrophoresis equipment, and the mathematical analysis of experiments. They can explore fields of inquiry ranging from which metal will oxidize the least in a saline environment to whether there is a better molecular sieve than agarose. They can relate experiments to their daily lives by using the electrophoresis instrumentation they build (and possibly improve upon) to determine what food dyes are present in familiar candies and drinks. This opens up a whole new discussion of why we dye foods, what the advantages and disadvantages of synthetic and natural dyes are, and whether dyes impact the flavor of foods or just our perceptions.

Students will be a part of the process of discovery and will learn to develop tools to answer questions about the world around them — and have fun doing it.

To quote one student tester, “I love being able to work with lab tools and getting to see something I helped make work!”

This curriculum was developed in collaboration with Dr. Kristi DeCourcy at the Fralin Biotechnology Center in Virginia. We would like to thank Dr. DeCourcy for her invaluable guidance and contribution to this curriculum.

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	STEM Practices	Activity Alignment
S	Ask Questions Observe Experiment	Chemistry of food dyes Electrophoresis Material properties Electricity Buffers, solubility and pH
T	Develop a Theory and Model Measure Collect Data Investigate	Separating molecules based on charge and size Pipetting/liquid handling Electrophoresis chamber Biotechnology
E	Evaluate Imagine Reason Calculate Predict	Circuit development Design parameters Safety Materials science Testing models
M	Develop Explanations and Solutions	Measuring volumes, distances, proportions, pH, temperature, Rf Calculating velocity, volume, area Analyzing results

Biotechnology and STEM go hand in hand. Science in the 21st century is driven by the integration of science, technology, engineering, and mathematics (STEM). Citizens and scientists alike are required to understand these fundamentals in order to make decisions about issues ranging from personal healthcare solutions to global energy challenges. Bio-Rad Explorer kits help you teach more efficiently by integrating STEM content into a single lab so that you can focus on what matters most — teaching.

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Kit Summary

Agarose gel electrophoresis is ubiquitous in molecular biology laboratories today. In order to perform agarose gel electrophoresis consistently, the science of separating molecules has to be combined with the engineering of equipment best suited to perform the separation. Early horizontal separation techniques used starch gels which did not give good molecular resolution. By the late 1970s, agarose had replaced starch as the gel of choice for horizontal gel separations and commercial instruments were designed and available to utilize this technology.

In this kit, students will analyze food dyes using a gel box prepared from simple materials. The principles demonstrated are the same, whether the apparatus is a commercial gel box with platinum electrodes or a plastic box with paper clip electrodes. The lessons can include chemistry, physics, biology, math, and more. Students will be introduced to the thought processes necessary for engineering a product. For example, they can determine the requirements for a good electrode material, and then determine what materials meet those needs.

The samples your students will analyze are food colors extracted from candies. Depending on the focus of your class, you may choose to have your students explore the food dyes that they consume, keeping a log of the dyes over the course of a week. Areas that can be explored include the pros and cons of artificial and natural food dyes and the pervasiveness of dyes in the foods we consume.

Timeline 1: Instructor prepares agarose gels in STEM boxes.

Pre-lab activities	Discussion of electrophoresis Discussion of food dyes and their uses	1–2 class periods
Teacher's advance preparation	Prepare STEM boxes and pour agarose gels	1.5 hr
Activity 1	Extraction of dyes from candy	15 min in lab
Activity 2	Set up STEM box and electrophoresis of reference dyes and student samples	45 min in lab
Post-lab activity	Discussion of results	1–2 class periods

Timeline 2: Students prepare agarose gels in STEM boxes.

Pre-lab activities	Discussion of electrophoresis Discussion of food dyes and their uses	1–2 class periods
Activity 1	Prepare a gel box and electrodes and pour the agarose gel	45 min
Activity 2	Extraction of dyes from candy	15 min
Activity 3	Set up STEM box and electrophoresis of reference dyes and student samples	45 min
Post-lab activity	Discussion of results	1–2 class periods

Note: Extraction of dyes (Activity 2) can be completed in the same class period while the agarose gel is solidifying (Activity 1).

Ideas for Further Inquiry

- pH change in electrophoresis buffer during electrophoresis
- Effect of electrode thickness and material choice on electrophoresis
- Effect of gel matrix
- Effect of TAE concentration and volume
- Putting it all together

Storage Instructions

All stock reagents are stable at room temperature.

Kit Inventory Checklist

This section lists the components provided in the STEM electrophoresis kit (classroom or demo kits). It also lists the required accessories. The classroom set contains sufficient materials for 8 student workstations, 4 students per workstation. The demo set contains sufficient materials to set up 2 workstations. As soon as your kit arrives, open it and check off the listed components to familiarize yourself with the kit.

Kit Components	Classroom Set 1665090EDU Quantity	Demo Set 1665080EDU Quantity	(✓)
Store at room temperature			
Dye extraction solution, 25 ml	1 bottle	1 bottle	☐
Blue 1 reference dye, 150 µl	1 vial	1 vial	☐
Yellow 5 reference dye, 150 µl	1 vial	1 vial	☐
Yellow 6 reference dye, 150 µl	1 vial	1 vial	☐
Red 40 reference dye, 150 µl	1 vial	1 vial	☐
Electrophoresis buffer, 50x TAE, 100 ml	1 bottle	1 bottle	☐
Molecular biology grade agarose, 5 g	1 bottle	1 bottle	☐
2 ml microcentrifuge tubes	72 tubes	72 tubes	☐
Hinged plastic boxes	4 boxes	1 box	☐
Paper clips	16	4	☐
Black alligator clip leads	8	2	☐
Red alligator clips leads	8	2	☐
8-well combs	8	2	☐

Required Accessories (Not Included)	Classroom Set 1665090EDU Quantity	Demo Set 1665080EDU Quantity	(✓)
9 V batteries (3-5 per workstation)	24–40	6–10	☐
Plastic rulers or plastic card to cut gels	8	2	☐
2–20 µl adjustable-volume micropipet (catalog #1660551EDU or 1660506EDU) or 10 µl fixed-volume micropipet (catalog #1660512EDU)	8	2	☐
2–200 µl pipet tips, 1,000/bag (catalog #2239035EDU)	1 bag	1 bag	☐
Eyedroppers or 100–1,000 µl adjustable-volume micropipet (catalog #1660553EDU or 1660508EDU)	8	2	☐
or disposable plastic transfer pipettes (DPTPs) (catalog #1660480EDU)	1 box	1 box	☐

Required Accessories (Not Included) cont.	Classroom Set 1665090EDU Quantity	Demo Set 1665080EDU Quantity	(✓)
100–1,000 µl pipet tips* (catalog #2239040EDU)	1 bag	1 bag	☐
Marking pen	8	2	☐
Plastic cups or small beakers	32	8	☐
Microwave oven or hot plate	1	1	☐
Balance	1	1	☐
Distilled water	3 liters	1 liter	☐
500 ml Erlenmeyer flask for microwaving agarose	1	1	☐
Candies with a variety of color coatings	variable	variable	☐

* Not needed if using DPTPs or eyedroppers instead of 100–1,000 µl adjustable-volume micropipets

Optional Accessories	Quantity per Kit	(✓)
Microcentrifuge (catalog #1660602EDU) or mini centrifuge (catalog #1660603EDU)	1	☐
Digital camera for imaging gels	1	☐
Microcentrtifuge tube racks (catalog #1660481EDU)	8	☐

Refills Available Separately

IDEA Kit Reagent Refill Pack, catalog #1665076EDU (includes Blue 1 reference dye, Yellow 5 reference dye, Yellow 6 reference dye, Red 40 reference dye, dye extraction solution, and 72 microcentrifuge tubes)

IDEA Kit Starter Pack, catalog #1665077EDU (includes Blue 1 reference dye, Yellow 5 reference dye, Yellow 6 reference dye, Red 40 reference dye, dye extraction solution, 72 microcentrifuge tubes, agarose, TAE, eight 10 µl fixed volume pipets, 1 bag of 20-200 µl pipet tips (qty 1,000), and IDEA instruction manual)

Molecular biology agarose, 5 g, catalog #1613116EDU

Molecular biology agarose, 25 g, catalog #1613100EDU

Electrophoresis buffer, 50x TAE, 100 ml, catalog #1660742EDU

Electrophoresis buffer, 50x TAE, 1 L, catalog #1660743EDU

STEM Electrophoresis Engineering Module, catalog #1665085EDU (2 workstations; includes one hinged plastic box, 2 pairs of leads, 4 paper clips, and 2 combs)

STEM Electrophoresis Demo Kit, catalog #1665080EDU (includes Blue 1 reference dye, Yellow 5 reference dye, Yellow 6 reference dye, Red 40 reference dye, dye extraction solution, 72 microcentrifuge tubes, agarose, TAE, one plastic hinged box, 2 pairs of leads, 4 paper clips, 2 combs, and STEM instruction manual)

STEM Electrophoresis Classroom Kit, catalog #1665090EDU (includes Blue 1 reference dye, Yellow 5 reference dye, Yellow 6 reference dye, Red 40 reference dye, dye extraction solution, 72 microcentrifuge tubes, agarose, TAE, four plastic hinged boxes, 8 pairs of leads, 16 paper clips, 8 combs, and STEM instruction manual)

STEM Electrophoresis Starter Pack, catalog #1665095EDU (includes Blue 1 reference dye, Yellow 5 reference dye, Yellow 6 reference dye, Red 40 reference dye, dye extraction solution, 72 microcentrifuge tubes, agarose, TAE, eight 10 µl fixed volume pipets, 1 bag of 20-200 µl pipet tips (qty 1,000), four plastic hinged boxes, 8 pairs of leads, 16 paper clips, 8 combs, and STEM instruction manual)

Background for Instructors

Separation Technologies

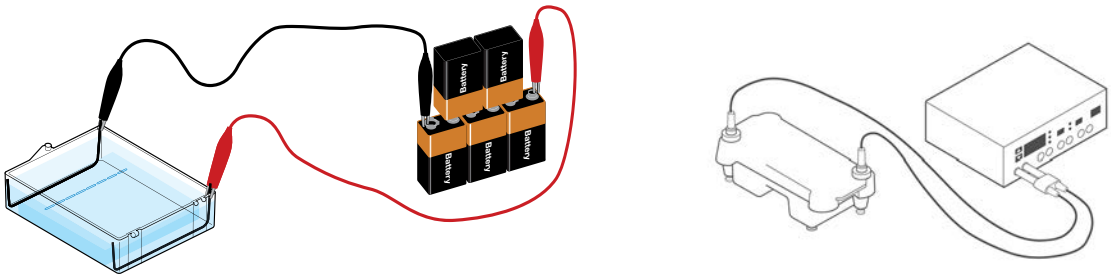
The first thing that comes to mind when technology is mentioned is computer technology. However, technology is much more than just automated processing of data. Technologies exist to improve the way we perform many tasks. For example, separation technology (separating a mixture into its components) is commonplace in science and industry. Numerous processes have been developed to separate mixtures, depending on the properties of the materials to be separated. An early example of separation technology is winnowing, the process of separating grain from chaff (the outer coating of seeds) that takes advantage of the differences in density between the grains and the chaff. When harvested, grain is tossed into the air, wind blows the lighter chaff away, and the grains drop to the ground. Distillation of alcohol is another separation technology that has been around for thousands of years. Ethanol boils at a much lower temperature than water, so heating the products of yeast fermentation of sugar will cause the ethanol to boil off and then it can be condensed and isolated. Some more examples of separation technologies are in Table 1.

Table 1: Common separation methods.

Materials to Be Separated	Property Used to Separate Components	Explanation
Iron filings from other metals	Magnetism	Iron filings will be attracted to a magnet while the other metals will not.
Salt from sand	Solubility	Salt will dissolve in water and can be separated from the sand.
Caffeine from coffee beans	Solubility	A solvent that selectively dissolves the caffeine (such as dichloromethane or ethyl acetate) but not most of the remaining components of the coffee oils is used.
Ethanol from fermented grain	Boiling point	Ethanol will boil off at a lower temperature than the other components of the fermented grain.
Wheat from chaff	Density	The wheat kernels are more dense and will fall to the ground while the chaff is less dense and can be blown away by the wind.
Tea from tea leaves	Size	Filtration can be used to separate the liquid tea extract from the large tea leaves.

Electrophoresis

Molecules can be separated by size and charge using a process known as **gel electrophoresis**. The term electrophoresis means *to carry with electricity.* Samples are placed in a gel matrix, which is put into a chamber filled with a conductive buffer solution (Figure 1). A direct current is passed between wire electrodes at each end of the chamber. Since all charged molecules will migrate in an electric field, negatively charged molecules will migrate toward the positive pole, or anode, and positively charged molecules will migrate towards the negative pole, or cathode. The separation matrix acts as a molecular sieve through which smaller molecules can move more readily than larger ones. Therefore, the rate at which a molecule migrates through the gel is inversely proportional to its size. Over a period of time, smaller molecules will travel farther than larger ones. Molecules of the same size stay together and migrate as a single band. In horizontal gel electrophoresis, pictured in Figure 1, the most commonly used separation matrix is agarose.



(A) (B)

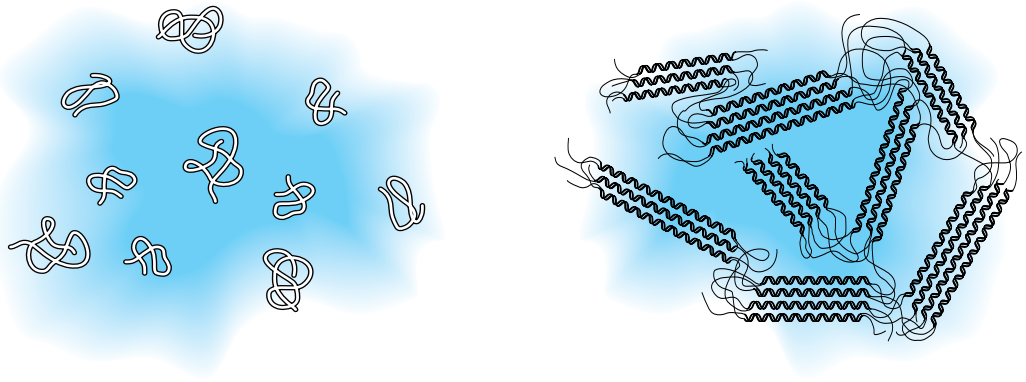
Fig. 1. Schematic drawing of two different gel electrophoresis systems. The samples are loaded into wells in the separation matrix. The electrodes are in a conductive buffer solution. When current is applied, negatively charged molecules will migrate toward the positive electrode and separate by size. **A,** a 9 V battery-powered electrophoresis system; **B,** a commercially available electrophoresis system with an electrical power supply.

Engineering an Electrophoresis Apparatus

What exactly is engineering? Engineering involves applying scientific and mathematical principles to create a machine or process to perform a specific task. While science tends to be hypothesis driven (experiments are designed to answer questions) and used to discover new things, engineering is driven by design parameters. In engineering, processes or devices are made to perform in certain controlled ways with predefined outputs. Engineers have to understand the science to design and build the tools they need. A chemist might need to understand the molecular details of how agarose polymers behave in solution and what happens to them when they come out of solution. To build an electrophoresis apparatus, an engineer would make use of the properties of agarose in solution and in a gel to create a molecular sieve that can separate molecules by size. In order to design such an apparatus, all of the physical components and their properties need to be defined, as does the process that occurs during electrophoresis to separate molecules.

Chemical Components in Electrophoresis

Agarose, the most commonly used matrix for separating molecules in horizontal gel electrophoresis, is derived from red algae. Agarose is purchased as a powder that is placed in buffer solution and heated until it dissolves. When agarose is in solution, the molecules are in random coils. The solution containing the dissolved agarose is poured into a mold to form a slab gel, and as the agarose cools, it forms regions of double helices linked by random coils (Figure 2). The structure is held together by hydrogen bonds and hydrophobic interactions.



(A) (B)

Fig. 2. Agarose gel structure. **A**, agarose molecules in solution are random chains; **B**, as agarose cools, the molecules form double helix areas connected by random chains.

When the agarose cools, it forms a mesh with pores through which the sample molecules will pass. The size of the pores is determined by the amount of agarose in the gel. For example, a gel with a lower percentage of agarose will have larger pores than a gel with more agarose. Researchers select the percentage of agarose in their gels based on the sizes of the molecules that they want to separate on the gel.

Samples in Electrophoresis

There are a number of factors that affect the migration of molecules in electrophoresis. The most obvious is charge, as the charge of the molecule will determine the direction of migration (toward the positive or the negative electrode). Food dyes and colors may be positively charged, negatively charged, or neutral. However, all of the food dyes certified by the Federal Food, Drug, and Cosmetic Act (**FD&C**) and most other commonly used food dyes carry a negative charge. DNA molecules, which are typically separated using agarose gel electrophoresis, have a negative charge, so they will always migrate toward the positive electrode.

The shape and size of molecules affect their rate of migration in gel electrophoresis as well. The food dyes used in this kit are relatively simple molecules and their shapes do not have measurable effects on their migration in the gel. Smaller molecules will migrate more quickly through the matrix than larger molecules. Selecting the correct percentage of agarose (and hence the correct pore size) for the size of the molecules that you wish to separate is an important consideration.

Electrophoresis Buffer

Different buffers can be used to perform agarose gel electrophoresis. The most common agarose gel electrophoresis buffers are TAE (Tris-acetic acid-EDTA) and TBE (Tris-borate-EDTA). The electrophoresis buffer must have several properties that will allow it to perform well. The buffer must carry a charge. This is why the buffer is not only used to overlay the gel but also used to prepare the gel. Since the buffer is used to prepare the gel, it must also be able to solubilize the gel matrix, though typically heat must also be applied to get the matrix into solution. The buffer also must be capable of absorbing heat. As a current flows through the gel and agarose, they heat up. If they heat up too much, then the agarose gel will melt.

The concentration of the electrophoresis buffer is also important. The ionic strength must be high enough to carry the current, but not too high. When the ion concentration is high, there is more electrical conductance and more heat is generated. Also, since more of the current is being carried by the ions of the buffer when the ionic strength is high, the sample molecules will move more slowly than they would in a buffer with lower ionic strength. Ionic concentrations of electrophoresis buffers are usually less than 100 mM and have a pH of around 8.

Physical Components of the Electrophoresis Apparatus

Gel Box

Electrophoresis takes place in a gel box. The box holds the gel and electrophoresis buffer, and must have electrodes at either end. When choosing materials to construct a gel box, keep in mind that the material should: 1) not conduct electricity, 2) be transparent (or at least translucent), and 3) be stable at temperatures up to 50˚C. The non-conductivity is important — the electric current should not run anywhere other than within the box. The transparency allows monitoring electrophoretic progress. Finally, heat is generated during electrophoresis, and the gel box material should not be deformed by the heat. Most gel boxes are made of transparent plastics.

Electrodes

An electrode is the material through which an electric current passes, so the first requirement for electrode material is that it conducts electricity. Most metal wires meet this criterion. Examples are copper, iron, gold, nickel, and platinum wire. Electrode material should also be relatively inert, or non-reactive. Metals that are easily corroded or oxidized do not make good electrodes, as electrodes are exposed to salts as well as to electric current. Most commercial gel boxes have platinum wire electrodes, because platinum is the most non-reactive of the metals. Platinum does not corrode and reacts only with very strong oxidizing acids. However, it is also an expensive metal, which is another consideration when designing a gel box.

During electrophoresis, the charged molecules in the buffer and the samples move towards a specific electrode depending on their charges. This is summarized in Table 2.

Table 2: Ions and electrodes.

Charged Molecule	Electrode	Migration Direction
Anion Negative charge (-)	• Anode – positive charge (+) • Typically red colored	• Anions move away from the anode • Anions move toward the cathode
Cation Positive charge (+)	• Cathode – negative charge (-) • Typically black colored	• Cations move away from the cathode • Cations move toward the anode

Gel Casting Chamber

An agarose gel is prepared by pouring molten agarose into a rectangular gel casting chamber. When the solution cools and sets, it forms the gel. In some systems, the gel is cast in a mold that is not part of the electrophoresis chamber and then once cooled, is placed in the electrophoresis apparatus. In other cases, the gel can be cast directly in the electrophoresis apparatus. In this kit, the casting chamber is a plastic box. A slab of gel at either end of the box is removed to form the buffer chambers.

A well former or comb is placed into the gel casting chamber (near one end of the mold) before adding the molten agarose. After the agarose has set, the comb is removed from the gel to form the sample wells. The size of the wells determines the volume of sample that can be loaded on the gel. Different sizes of combs are available, for loading volumes from 5–30 µl. Combs that form different numbers of wells are also available.

Power Source

Gel electrophoresis requires a power source that supplies direct current (DC). Agarose gels are typically run under constant voltage. Commercially available power supplies can run 2–4 gels at once. Simple power supplies may have predetermined settings, such as 50 or 100 volts. Other power supplies allow the user to set the voltage or current to the desired level. Batteries, such as those used in this kit, deliver constant voltage. When they are connected in a series, the voltage is additive. In other words, five 9 V batteries in a series will deliver 45 volts.

Warning! – The electrophoresis system that students will build in this laboratory exercise is designed to be used ONLY at low voltages (≤45 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as there is no isolation of the live electrical components from the users. NEVER connect this system to a power supply since serious or lethal shock could occur.

Samples to Be Separated – Food Dyes

Food Dyes

How often do you look at nutrition labels for the food and drinks that you consume? The next time you open a bottle of soda or a pack of candy, look at the list of ingredients and do not be surprised if you see “FD&C” followed by a color and a number. This means that what you are about to eat contains one or more of the color additives approved by the U.S. Federal Food, Drug, and Cosmetic Act (FD&C) for use in food.

Why Do We Dye Our Food?

How do consumers choose food? There are a variety of factors to consider when buying food, including price, nutritional data, sell-by date, and ingredients, but one of the first considerations is appearance. For many foods, it’s all about packaging, but for others it is the appearance of the actual food that is important. Will you choose a head of lettuce if the leaf edges are brown or a loaf of bread with green mold on it? In these examples, the quality of the food is evaluated by its color.

In other cases, our expectation of food is determined by its color. In the 1990s, consumers, encouraged by marketing and advertisements, began equating the clarity of products with their purity, and companies began making clear versions of existing products such as soft drinks. The products did not sell well — the public wanted caramel colored Coke and Pepsi. The lack of coloring affected consumer perception of the taste of the food.

The primary reasons foods are artificially colored are perception and expectation of the consumer. When you eat a purple-colored candy, you don't expect lemon flavor, you expect to taste grape. Our previous experiences lead us to expect margarine to be yellow, pickles to be green, and maraschino cherries to be red, but those colors are usually provided or enhanced by coloring agents (ß-carotene, FD&C Yellow 5, and FD&C Red 40 for margarine, pickles, and cherries, respectively).

History

Food dye agents have been around for a long time. References to adding coloring agents to food can be found in literature dating back thousands of years. Many colors were available from natural sources, such as yellow from saffron or egg yolks, red from huckleberries or beet juice, and green from spinach juice or parsley. Other colors were obtained by adding inorganic salts to foods, such as copper sulfate (blue), lead chromate (yellow), and mercury sulfide (red). Early consumers of purchased food had no knowledge of how the food had been prepared or what it contained, whether they were buying bread from the neighborhood baker or tea imported by the merchant across town.

In 1820, Frederick Accum published *A Treatise on Adulterations of Food, and Culinary Poisons*. In the preface he stated, "There is death in the pot." The treatise listed foods and beverages and the common additives for each. For example, he found cheese and cayenne pepper colored with red lead (lead tetroxide), pickles with copper sulfate, and green tea with copper carbonate. Despite the publicity garnered by Accum's work, food additives were largely unregulated until the 20th century.

In the U.S., the Pure Food and Drug Act of 1906 (also known as the Wiley Act, because of the work done by U.S. Department of Agriculture chemist Harvey Wiley to improve food safety) restricted the use of food additives and named seven dyes that were safe to be used in foods. Interestingly, two of these dyes are still on the approved list today.

Modern Food Dyes

In 1856, William Perkins, an English chemist, synthesized the first organic dye from coal tar, mauveine, which produced a purple color. This was the first of many organic dyes discovered. The synthetic food dyes used today are also coal tar or petroleum derivatives. Currently, there are seven synthetic dyes approved for use in the U.S. (the FD&C colors; see Table 3), although other countries permit the use of more dyes. For example, the UK, France, and Spain allow the use of 15 dyes: six of the FD&C dyes plus nine more.

Some synthetic dyes have been removed from approved lists when they were found to be harmful, but controversy is not limited to synthetic dyes. Such natural food colors as carminic acid (red), which is used in popsicles, candies, yogurt, and other foods, are derived from the bodies of crushed insects. People who follow vegan or vegetarian diets have pushed for the presence of this dye to be indicated on the labels of products where it is used. Starbucks removed carminic acid from its Strawberry Frappucinos upon pressure from customers, and is replacing it with a dye extracted from tomatoes. Another concern about some natural dyes is the level of purity obtained when separating the dye compound from all the other compounds that make up the source. It can be easier to separate a synthetic dye to very high purity from the five to ten different molecules used to make the dye. It can be much more difficult to separate a natural dye from all the other molecules that make up, for example, the abdomen of a beetle from which carminic acid is extracted. It also can be quite expensive to extract enough dye to color items from natural sources if a product sells worldwide in large amounts. For example, for a red candy, a large number of beets would need to be grown and processed in order to isolate as much of the dye molecule as would be obtained with a smaller amount of Red 40 dye synthesized and purified in a lab

Synthetic food dyes come in two forms, dyes and lakes. Dyes are water-soluble. Lakes are dyes that have been combined with salts to make them insoluble. Lakes can be used to color foods containing fats. The water-soluble FD&C dyes are used in the "food colors" found at the grocery store. Some "food colors" contain single dyes and others use combinations of dyes.

Table 3. Food dyes approved by the FDA for use in the U.S.

Dye Name	Also Called	CAS Number[1]	EEC Number[2]	Color in Solution	Used in	Maximum Absorbance[3]	Molecular Weight	Net Charge at pH 8
Blue 1	Brilliant Blue FCF, Acid Blue 9, Food Blue 2	3844-45-9	E133	Bright blue	Soft drinks, ice cream, canned and baked products, dairy products, candy	630 nm	792.86	–2
Blue 2	Indigotine, Indigo Carmine, Food Blue 1	860-22-0	E132	Deep blue	Pet food, candy, baked goods	610 nm	466.36	–2
Green 3	Fast Green FCF, Food Green 3	2353-45-9	Not approved for use in the EEC	Bright bluish-green	Soft drinks, canned vegetables, fish products, baked products, candy	625 nm	808.86	–2
Red 3	Erythrosine, Food Red 14	16423-68-0	E127	Bright bluish-red	Cocktail cherries, popsicles, candy, pistachio shells	526 nm	879.86	–1
Yellow 5	Tartrazine, Food Yellow 4	1834-21-0	E102	Bright lemon yellow	Canned peas, soft drinks, jelly, ice cream, pickles, popcorn, sports drinks, corn chips, candy	426 nm	534.37	–3
Yellow 6	Sunset Yellow FCF, Food Yellow 3	2783-94-0	E110	Bright yellow to orange	Soft drinks, ice cream, jams, fruit products, wine, cheese sauce, instant noodles, candy	485 nm	452.37	–2
Red 40	Allura Red, Food Red 17	25956-17-6	E129	Bright red	Soft drinks, ice cream, meat and fish products, desserts, candy	504 nm	496.43	–2

[1]CAS Registry numbers are unique numbers assigned to every chemical described in the scientific literature.

[2]EEC numbers are assigned to the dyes approved for use in food by the European Economic Community.

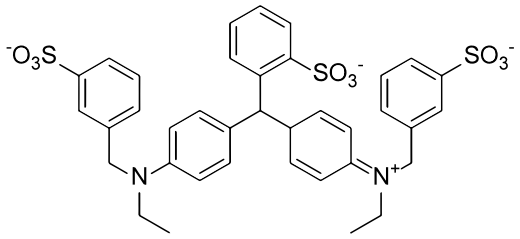
[3]Absorbance at pH 7 when dissolved in water.

Experimental Design

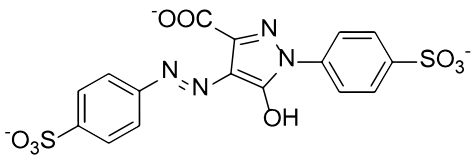
In this experiment, students will extract the food colors from candies and analyze the dyes using an electrophoresis apparatus they will build. The kit contains the four food dyes most commonly used in the U.S. These four reference dyes will be analyzed on the gels along with the student samples. The reference dyes are:

- Blue 1
- Yellow 5
- Yellow 6
- Red 40

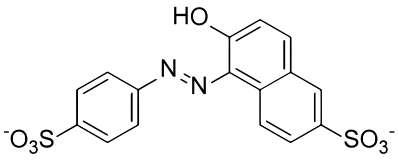
Structurally, these dyes are similar, all negatively charged at pH 8, with aromatic rings and sulfate groups.



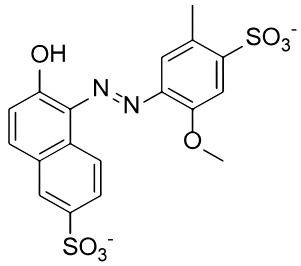
Blue 1



Yellow 5



Yellow 6

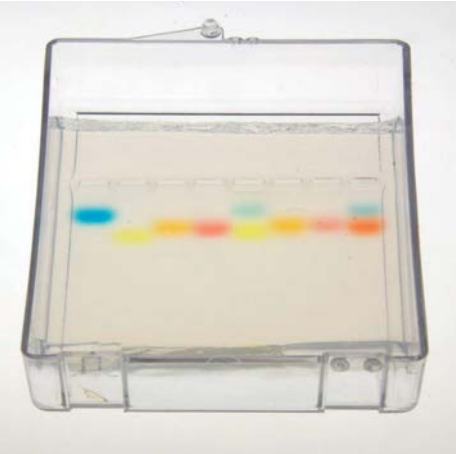


Red 40

Fig. 3. Structures of the reference dyes, the four most commonly used food dyes.

Typical Classroom Results

The data below represent typical classroom results for this experiment when the gel is run for 20 minutes using five 9 V batteries, a 1% agarose gel in a STEM electrophoresis engineering module box, and 1x TAE buffer.



Lane	Sample
1	10 µl blue 1 reference dye
2	10 µl yellow 5 reference dye
3	10 µl yellow 6 reference dye
4	10 µl red 40 reference dye
5	10 µl green Skittle
6	10 µl orange M&M
7	10 µl Hot Tamale
8	10 µl brown M&M

Fig. 4. Electrophoretic separation of standard dyes and color coatings extracted from standard U.S.-manufactured candies.

Recommended Candies

The candies that worked best in testing were candies with hard-shell color coatings such as M&M'S and Skittles. The brighter the color, the better the results. Candies with light pastel colors such as some jelly beans, do not work as well. In order to get strong bands, more than one piece of candy can be used. For candies such as plain M&M'S and Skittles, extracting the dye from two candy pieces of the same color is enough to produce bright bands. For smaller candies such as Red Hots, four or five candy pieces might be needed. Some recommended candies are listed in Table 4. Alternatively, students can experiment using candies that have not been tested.

Table 4. Recommended candies and products.

Best Results	Acceptable Results	Too Faint to See
M&M'S (regular or peanut)	Mike and Ikes	Gummy bears
Skittles	Jelly beans	Jujyfruits
Kool-Aid drink mixes	Gum balls	Peeps
Jawbreakers		Jolly Ranchers
Red Hots		
Hot Tamales		
Runts		
Reese's Pieces		

Frequently Asked Questions

1. **During electrophoresis, my students saw a brown color and white powder on and around the paper clip attached to the positive terminal of the battery. What is causing this discoloration?**

   The paper clips are made of galvanized steel, meaning that the steel is coated with zinc to prevent rusting. However, the combination of bending the paper clip, putting it into a salty environment and running electricity through it will expose some of the steel. Both the steel and the zinc may experience some corrosion during the gel run, producing iron oxide (brown/red rust) and zinc oxide (white powder). Commercially–made gel boxes use platinum wire for electrodes, as platinum is an inert metal and will not rust. Researching why the rust is seen at the anode and not at the cathode is a good extension activity for chemistry students.

2. **My students saw color bands that do not match any of the four standards. What might those be?**

   In the United States, the FDA currently approves seven dyes as food color additives. We have included the four most commonly used dyes as references in this kit. The other three FD&C dyes used are Blue 2, Green 3 and Red 3. Check the ingredients list from the sample to see if these dyes are present. Alternately, a natural food color could be on the list, such as ß-carotene or carmine. Researching natural food colors is a good extension activity for this experiment.

3. **My students used a sample that contained both FD&C Red 40 and Yellow 6, but they saw only a single band on their gel. Why didn't the colors separate?**

   Red 40 and Yellow 6 cannot be separated using 1% agarose gel electrophoresis because they are too alike in charge and molecular weight. The mass-to-charge ratio of molecules determines their rate of migration in electrophoresis. Both Red 40 and Yellow 6 have a charge of –2 (see Table 3) and they are similar in molecular weight, so their mass-to-charge ratio is too close for them to separate on the gel. The same is true for Red 40 and Blue 2 (purple Skittles).

4. **My students used a sample that contained a dye that is not on the FD&C list, and the dye migrated toward the negative electrode. Why did this happen?**

   Many candies manufactured outside of the U.S. do not contain any of the FD&C dyes. Instead, they contain synthetic and natural dyes approved for use in their country of origin. Also, some U.S.-made candies use natural dyes. These other dyes may have either a positive or a negative charge, or they may be uncharged (neutral). Hence, they could migrate toward either the cathode or anode, or, if the dye is neutral, not migrate at all. Determining the charge of these dyes is an excellent inquiry experiment for students.

5. **The list of ingredients has both Blue 1 and Blue 1 Lake. What is the difference?**

   The FD&C dyes are prepared in two forms: water-soluble and insoluble. When the water-soluble forms of the dyes are reacted with aluminum hydroxide, the reaction products are insoluble and are known as lakes or lake dyes or pigments. As food ingredients, they may be listed either as lakes or as aluminum lakes. Since lakes don't dissolve in 1x TAE buffer or dye extraction solution, they will not migrate during electrophoresis.

6. **Are there extension activities I can do with this laboratory?**

   Potential extensions to this laboratory include studying electrochemistry at the cathode and anode via pH changes, determining what makes a good electrode, studying gel-forming properties of different polymers such as gelatin and pectin, and determining the impacts of gel percentage, voltage, and TAE concentration on dye separation. See Appendix A for more information.

7. **How long will the bands be visible after running?**

The molecules are fairly small and difuse quickly so images should be taken within 30 minutes of completion of the run.

8. **Can I use this STEM box system for separating DNA?**

No, the STEM box system cannot be used to separate DNA. DNA is a much larger molecule (on the order of thousands of daltons or more in molecular weight) and DNA separation requires a much higher voltage (100 V or higher) and more time (30 minutes at 100 V).

The STEM box system should NEVER be used with more than five 9 V batteries or attached to a power supply because this system is not grounded and higher voltage can produce an electrical shock. Commercial electrophoresis systems are designed to cut the power and current flowing through the system when the lid is opened, preventing electrical shock that could be caused by the higher voltages. Commercial electrophoresis systems are designed to separate molecules such as DNA in a timely fashion when used with a commercial power supply. Asking students to consider the design parameters necessary to produce an electrophoresis system capable of separating DNA molecules is an excellent capstone question for this kit.

Instructor's Advance Preparation

This section outlines the recommended schedule for advanced preparation on the part of the instructor. Advance preparation details follow on pages 17–20.

Activity	When	Time required
Read manual	Immediately	1 hour
Prepare molten agarose	Prior to Activity 1	15 min
Prepare the gel boxes and electrodes and pour the agarose gels*	Prior to or during Activity 3	15–45 min
Aliquot dye extraction solution	Prior to Activity 2	15 minutes
Aliquot reference dyes	Prior to Activity 3	30 minutes
Set up workstations	The day of student labs	10 minutes/day

*These steps can be performed ahead of time by the educator or during a laboratory period by the students.

Workstation Checklist

Student Workstations. Materials and supplies that should be present at each student workstation prior to beginning each laboratory experiment are listed below. The components provided in this kit are sufficient for eight student workstations (we recommend two to four students per workstation).

Teacher's (Common) Workstation. A list of materials, supplies, and equipment that should be present at a common location, which can be accessed by all student groups, is also listed below. It is up to the discretion of the teacher whether students should access common buffer solutions and equipment, or whether the teacher should aliquot solutions and operate equipment.

Activity 1 Prepare the gel boxes and electrodes, and pour the agarose gels

Student Workstation

Material	Quantity	(✓)
Plastic chamber	1	☐
8-well comb	1	☐
Ruler	1	☐
Molten agarose	50 ml	☐
Marking pen	1	☐

Activity 2 Dye Extraction From Candies

Student Workstation

Material	Quantity	(✓)
Dye extraction solution	2 ml	☐
2 ml microcentrifuge tubes	4	☐
Microcentrifuge tube racks	1	☐
Marking pen	1	☐
Plastic cups or small beakers	4	☐
Eyedropper	1	☐
Colored candies*	4 varieties	☐

*Candy example: 3 green Skittles, 3 orange jelly beans, 4 Red Hots, 1 brown gumball.

Activity 3 Agarose Gel Electrophoresis

Student Workstation

Material	Quantity	(✓)
Agarose gel poured into plastic chamber	1	☐
Plastic ruler	1	☐
Paper clips	2	☐
Black lead with alligator clips	1	☐
Red lead with alligator clips	1	☐
9 volt batteries	3–5	☐
Blue 1 reference dye	15 µl	☐
Yellow 5 reference dye	15 µl	☐
Yellow 6 reference dye	15 µl	☐
Red 40 reference dye	15 µl	☐
1x TAE buffer	55 ml	☐
Dyes extracted from candies from **Activity 2**	4 samples	☐
20–200 µl adjustable-volume micropipet or 10 µl fixed-volume pipet and 8 tips	1	☐
Marking pen		

Common Workstation

Material	Quantity	(✓)
Gel documentation system (optional)	1	☐
Microcentrifuge or mini centrifuge (optional)	1	☐

Protective eye goggles should be worn in the laboratory at all times.

Proper safety precautions, such as no eating or drinking, should always be practiced.

Activity 1 Prepare the gel boxes and electrodes, and pour the agarose gels

Advance Preparation

Objectives: Preliminary preparation of STEM boxes
Pour agarose gels to prepare for activity 3
If you prefer to have your students pour their own gels during the lab, prepare the molten agarose ahead of time. If prepared in advance, molten agarose should be kept in a water bath set at 50–55°C until gels are poured
Set up student and instructor workstations

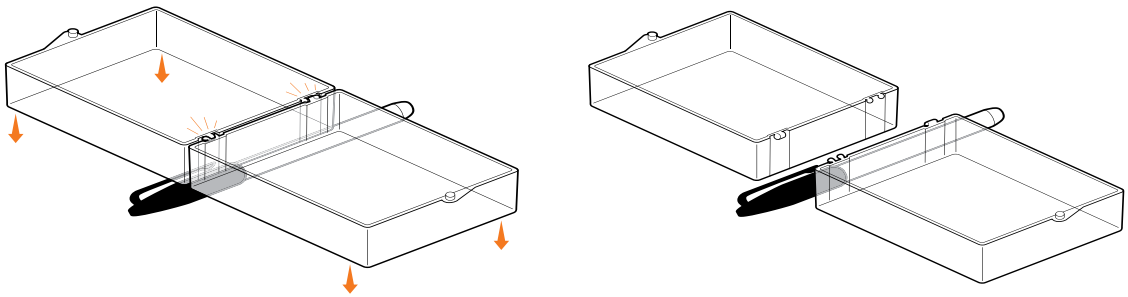
Note: These steps can be performed ahead of time by the educator or during a laboratory period by the students.

Procedure (estimated time for preparation of 8 STEM boxes and gels by educator: 1.5 hr)

Material Needed for Advance Preparation	Quantity
Electrophoresis buffer, 50x TAE	25 ml
Molecular biology grade agarose	5 g
Marking pen	1
Balance	1
Erlenmeyer flask	1
Hinged plastic boxes	4
Paper clips	16
Microwave oven or hot plate	1

1. **Prepare the gel boxes**

   The STEM gel boxes are hinged plastic boxes. Both the top and the bottom pieces of the boxes will be used as gel boxes. Gently separate the two halves by pulling them apart at the hinges. Be careful not to crack either of the boxes. If the hinge pieces crack off, that is okay.



2. **Prepare agarose**

   The recommended agarose concentration for gels in this classroom application is 1% agarose. This concentration of agarose provides good resolution and minimizes run time required for electrophoretic separation of the dyes. The volume required for each gel is 50 ml. This volume of agarose ensures that the wells are deep enough to easily pipet 10 µl of sample into each well and that there is space above the gel to overlay it with running buffer. **Be sure to use 1x TAE electrophoresis buffer, not water, to prepare agarose gels.**

A. 1x TAE electrophoresis buffer preparation

TAE (Tris-acetate-EDTA) electrophoresis buffer is provided as a 50x concentrated solution. In addition to the 1x TAE buffer needed to prepare the agarose gels, 55 ml of buffer is also required for each electrophoresis chamber. One liter of 1x TAE buffer will be sufficient to prepare and run 8 agarose gels. To make 1 L of 1x TAE buffer from 50x TAE concentrate, add 20 ml of 50x concentrate to 980 ml of distilled water and mix.

B. Agarose preparation

These procedures may be carried out 1 to 2 days ahead of time by the teacher or done during class by the individual student teams. The instructions below are for the preparation of the molten agarose assuming the students will prepare their own gel boxes and pour their own gels. **If STEM box preparation and pouring the agarose gels is to be done by the teacher, the protocols in the student section Pouring the Agarose Gel (see page 24) can be followed for the actual pouring of the gels and may be carried out up to a week ahead of time.**

If you are preparing the agarose gels ahead of time for your students, store the gels in the STEM box in a sealable plastic bag with the gel covered with 25 ml of 1x TAE buffer at room temperature for 1 day, or store in the refrigerator (4°C) for up to 1 week before using them.

i) To make a 1% agarose solution, use 1 g of agarose for 100 ml of 1x TAE electrophoresis buffer. Be sure to use electrophoresis buffer, not water.

Use this guide for gel volume requirements when casting single or multiple gels.

Number of gels	Volume of 1% agarose
1	50 ml
2	100 ml
4	200 ml
8	400 ml

ii) Add the agarose powder to a suitable container (for example, use a 500 ml Erlenmeyer flask for 200 ml or less). Add the appropriate amount of 1x TAE electrophoresis buffer. If clumps of agarose are visible, swirl the flask to mix.

iii) While heating the solution, some of the volume will be lost to evaporation. Prior to heating, mark the volume level on the flask or weigh the flask and contents and record the weight.

iv) Dissolve the agarose by heating in a microwave oven. If a microwave is not available, the solution can be heated on a magnetic hot plate.

Caution: Always wear protective gloves, goggles, and lab coat while preparing and casting agarose gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.

a. Microwave oven method

This technique is the fastest and safest way to dissolve agarose. Place the gel solution in an appropriate bottle or flask into the microwave. If you are using a bottle, **be sure to loosen** the cap before heating. The ideal microwave setting will depend on the volume of agarose solution that you are preparing and on the power of the microwave oven. For small volumes, microwave solution for 1 min, swirl the solution, then microwave in 20–30 sec intervals, swirling after each, until **all** of the small transparent agarose particles are dissolved. For larger volumes (e.g. 400 ml), heat initially for 2–3 min, swirl the solution, then microwave in 30–45 sec intervals, swirling after each, until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it back to the original volume and swirl to mix completely. Cool agarose solution to 55–60°C before pouring gels. If students are going to pour their own gels, keep the agarose at 55–60°C until it is ready to be poured by either stirring on a hotplate or in a waterbath. If the agarose is kept warm in a waterbath, make sure to swirl it thoroughly to mix the solution immediately before the gels are poured.

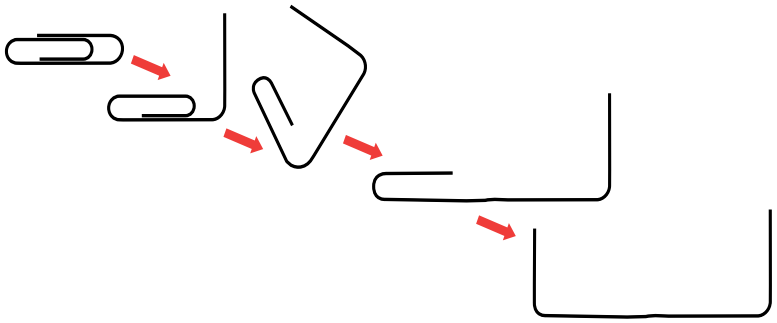
b. Magnetic hot plate method

Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Boil the solution until **all** of the small transparent agarose particles are dissolved.

Add water to the agarose solution to bring it back to the original volume and swirl to mix completely. Cool agarose solution to 55–60°C before pouring gels. If students are going to pour their own gels, keep the agarose at 55–60°C until it is ready to be poured by stirring on a hotplate.

3. Prepare paper clip electrodes

This step can be done ahead of time, or the students can prepare the electrodes in class. To get the best results, the paper clips should be unfolded so that the final product is flat. The steps are pictured below.



Activity 2 Dye Extraction From Candies

Advance Preparation

Objectives: Aliquot dye extraction solution
Set up student and instructor workstations

Material Needed for Advance Preparation	Quantity
Dye extraction solution	25 ml
2 ml microcentrifuge tubes	8
Marking pen	1
100–1,000 adjustable-volume micropipet and tips or DPTPs	1
Candies*	

*The candies can either be provided by the educator or by the students.

Procedure (estimated time: 15 min)

- **Aliquot dye extraction solution**
  Label eight clean 2 ml microcentrifuge tubes **Extraction** and aliquot 2 ml of dye extraction solution into each tube.

Activity 3 Agarose Gel Electrophoresis

Advance Preparation

Objectives: Aliquot reference dyes
Set up student and instructor workstations

Material Needed for Advance Preparation	Quantity
Blue 1 reference dye	150 µl
Yellow 5 reference dye	150 µl
Yellow 6 reference dye	150 µl
Red 40 reference dye	150 µl
2 ml microcentrifuge tubes	32
2–20 µl adjustable-volume micropipet and tips	1
Marking pen	1

Procedure (estimated time: 30 min)

1. If you have a centrifuge, pulse spin the reference dyes to pool the solutions at the bottom of the tubes.

2. Aliquot Blue 1 reference dye
Label eight clean 2 ml microcentrifuge tubes **Blue 1** and aliquot 15 µl of Blue 1 reference dye into each tube.

3. Aliquot Yellow 5 reference dye
Label eight clean 2 ml microcentrifuge tubes **Yellow 5** and aliquot 15 µl of Yellow 5 reference dye into each tube.

4. Aliquot Yellow 6 reference dye
Label eight 2ml clean microcentrifuge tubes **Yellow 6** and aliquot 15 µl of Yellow 6 reference dye into each tube.

5. Aliquot Red 40 reference dye
Label eight clean 2 ml microcentrifuge tubes **Red 40** and aliquot 15 µl of Red 40 reference dye into each tube.

Tips and Helpful Hints

- If you or your students are not running the agarose gels on the day they are prepared, you will need more than 1 L of 1x TAE buffer. The agarose gels should be stored under 25 ml of 1x TAE buffer until they are ready to be used. Before the electrophoresis laboratory class, prepare 500 ml of 1x TAE buffer by combining 10 ml of 50x TAE with 490 ml of deionized water and mix thoroughly
- When your students are cutting the slabs off the ends of the gel, they should cut straight down through the gel with something that is flat and thin; the end of a ruler will work, as will as a laminated ID card. Press straight down — do not slice across or the gel may tear. Slide the cutting device between the plastic box and the gel before removing the slab
- Electrodes should be as flat as possible and placed as close to the box edges as possible (as far away from the gel as possible) to prevent the gel from melting
- The alligator clips can be clipped both to the box edge and the wire of the paper clip to prevent the paper clip from moving. Students should try to keep the alligator clip from touching the buffer solution
- Three to five 9 V batteries can be used to run each electrophoresis setup. With new batteries, runs can be completed in as little as fifteen min when using five 9 V batteries. With three new 9 V batteries, separation is evident after twenty minutes. Batteries can be used for multiple separations/lab periods; 10-20 separations have been achieved from a single set of batteries. It should be noted that as the batteries are used, the separations slow down, so more time might be needed in later runs to achieve optimum separations
- It is important that all batteries in the battery tower work. If the batteries are not new, or relatively new, test that current is flowing (bubbles should form at the paper clip electrodes) before giving the batteries to students

Student Manual

Background

What exactly is engineering? Engineering involves applying scientific and mathematical principles to create a machine or process to perform a specific task. While science tends to be hypothesis driven (experiments are designed to answer questions), engineering is driven by design parameters. In engineering, processes or devices are made to perform in a certain controlled way to give a defined output. Engineers have to know a lot of science to design and build the tools they need.

Electrophoresis is a method of separating molecules by size and charge. Samples are placed in wells in a gel matrix, and the gel is put into a chamber filled with a conductive buffer solution. A direct current is passed between wire electrodes at each end of the chamber. Since all charged molecules will migrate in an electric field, negatively charged molecules will migrate toward the positive pole. The separation matrix acts as a molecular sieve through which smaller molecules can move more easily than larger ones.

So, if you put on your engineering cap to design an apparatus for electrophoresis, what do you need? Let's look at the description of electrophoresis and break down some of the components:

Component	Function	Desirable Characteristics
Chamber	Holds gel matrix and buffer	• Watertight • Desired dimensions • Transparent or translucent • Melting point > 60°C • Non-conductive • Easy and inexpensive to fabricate
Power Source	Source of electrical current	• Provide direct current • Provide the correct voltage
Electrodes	Material that carries electricity	• Can carry current • Easy and inexpensive to fabricate • Low reactivity (minimal rust, oxidation, corrosion, etc.) in an salty, aqueous environment
Comb	Forms wells in gel	• Easy and inexpensive to fabricate • Forms the correct volume wells and correct number of wells • Melting point > 60°C • Does not stick to solid agarose
Gel matrix	Forms a molecular sieve	• Easy to prepare • Inexpensive • Non-toxic • Separates molecules in size range of interest
Buffer	Conducts current and used to prepare gel matrix	• Conducts current • Absorbs heat generated by current • Dissolves gel matrix • Inexpensive • Easy to prepare

Choosing the “best” material from an engineering standpoint, however, is not the only consideration when designing a product or process. Cost and safety are also factors. For example, rare metals such as platinum and gold make excellent electrode materials, but they are very expensive. Glass might make an excellent container for the gel system from an engineering viewpoint, but glass is easily broken and hard to work with. Further, a gel material that would make an excellent molecular sieve but is toxic when handled would not be a good choice.

In this experiment, you will extract food dyes from various candies and construct an apparatus for gel electrophoresis. With the gel box you have prepared, you will use agarose gel electrophoresis to analyze the food dyes and compare them to reference dyes.

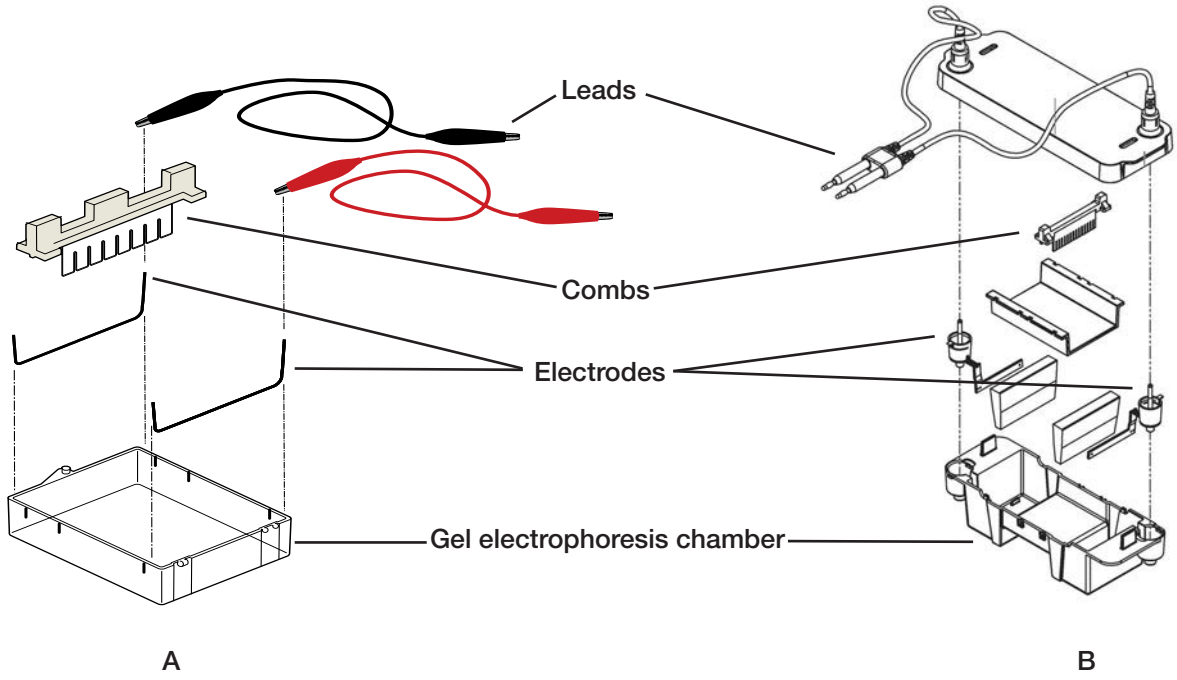


Fig. 5. Comparison of the components of two electrophoresis systems. A, a hand-built electrophoresis apparatus; **B**, a commercial electrophoresis apparatus.

Preparing the Gel Box and Pouring the Agarose Gel

Student Workstation	Quantity
Plastic chamber	1
8-well comb	1
Ruler	1
Molten agarose	50 ml
Marking pen	1

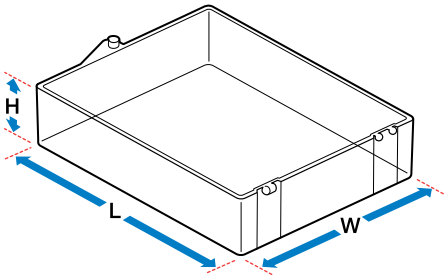
Protocol

1. Using a ruler, measure the length, width and height of the plastic box and record here.

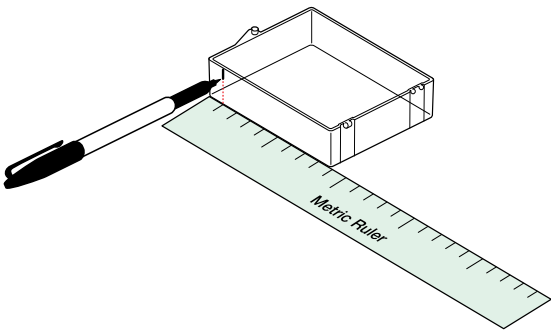
   Length L=_____ cm

   Height H=_____ cm

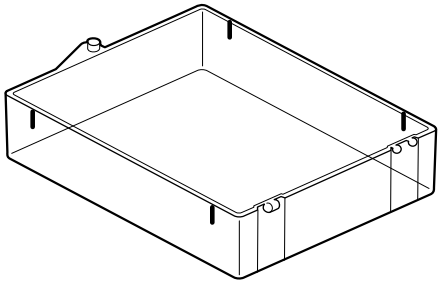
   Width W= _____ cm



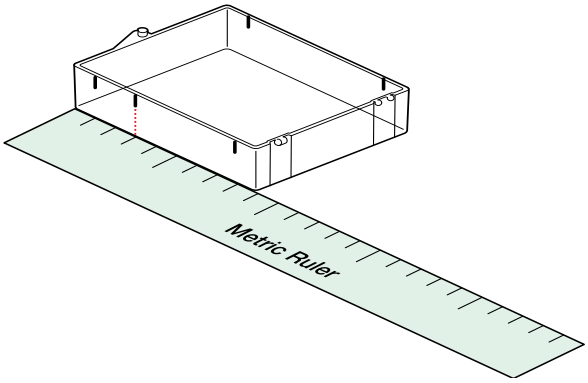
2. Measure a distance 1 cm from the end of the box on the longest side, and with a marking pen, make a dash on the outside of the box.



3. Repeat step 2 so that you have a mark 1 cm from the end of each of the longest sides of the box.

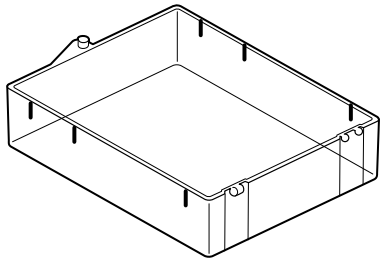


4. Measure a distance 3 cm from the end of the box on the longest side, and with a marking pen, make a dash on the outside of the box.

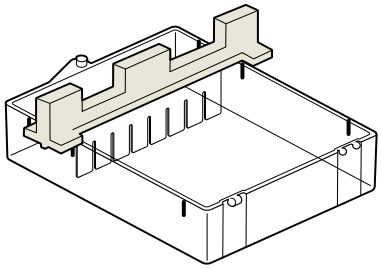


Protocol (cont.)

5. Repeat step 4 on the opposite side of the box. When you have finished, each side of the box should have three marks.

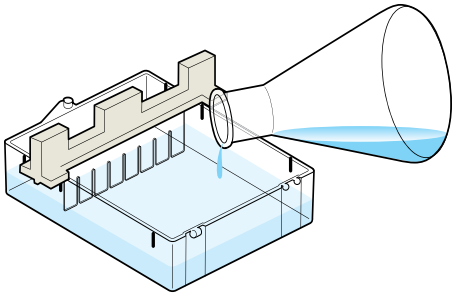


6. Place your 8-well comb on the marks that are 3 cm from the end. Make sure that the comb is centered so that none of the clear plastic well-formers touch the plastic box and that the comb is straight across the box.

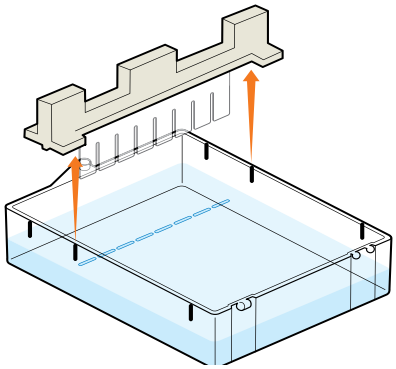


7. Carefully pour 50 ml of molten agarose into the box and allow the gel to solidify for 10–20 min. The gel will appear cloudy, or opaque, when ready to use.

   Caution: Always were protective gloves, goggles, and lab coat while preparing and casting agarose gels. Molten agarose or the flasks containing hot agarose can cause severe burns if allowed to contact skin.



8. Carefully remove the comb from the solidified gel by pulling gently in an upward direction.



9. If you do not have sufficient time to proceed to Agarose gel electrophoresis, store the gel in the box, covered with 25 ml of 1x TAE buffer in a sealable plastic bag at room temperature for 1 day, or in the refrigerator (4°C) for up to 1 week before using them. Be sure to label your plastic bag.

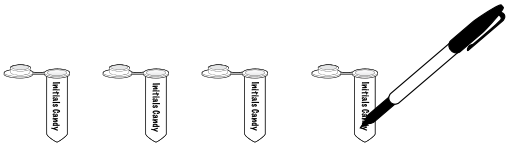
Dye Extraction From Candies

Student Workstation	Quantity
Dye extraction solution	2 ml
2 ml microcentrifuge tubes	4
Microcentrifuge tube rack	1
Marking pen	1
Plastic cups or small beakers	4
Eyedropper	1
Colored candies	4 varieties, 1–4 candies per variety*

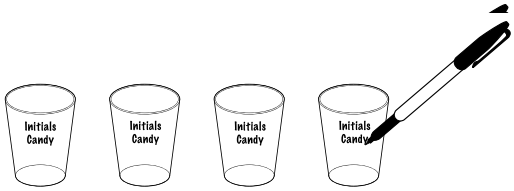
*Candy example: 3 green Skittles, 3 orange jelly beans, 4 Red Hots, 1 brown gumball

Protocol

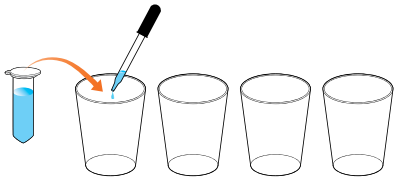
1. Label the four microcentrifuge tubes with your initials and the names and colors of the candies you are using.



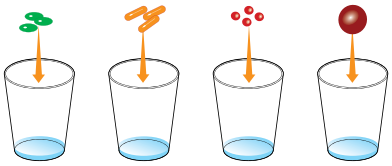
2. Label four cups with your initials and the names and colors of the candies you are using.



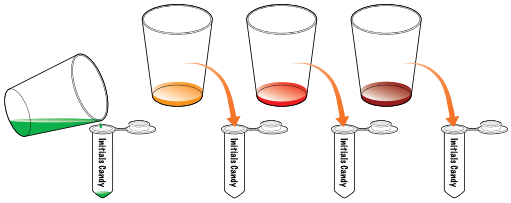
3. Using an eyedropper or pipet add 0.5 ml of dye extraction solution to each cup. Use the volume marks on the 2 ml microcentrifuge tube to measure the correct volume.



4. Place your candy into the appropriately labeled cup and swirl the candy in the dye extraction solution. If using a candy such as M&M'S or Skittles, just dissolve the color coating off until you get to the white layer of the candy. For all other candies, try to get as dark a solution of dye as possible.



5. Remove your candy from the cup. Pour the solution containing the dissolved colored candy coating into the appropriately labeled microcentrifuge tube.



Agarose Gel Electrophoresis

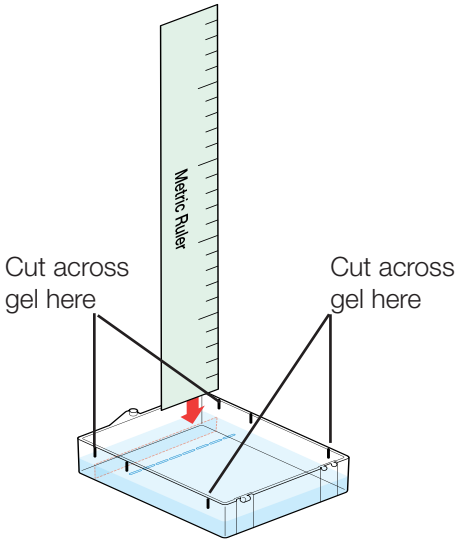
Student Workstation	Quantity
Agarose gel poured into plastic chamber	1
Plastic ruler	1
Paper clips	2
Black lead with alligator clips	1
Red lead with alligator clips	1
9 volt batteries	3-5
Blue 1 reference dye	15 µl
Yellow 5 reference dye	15 µl
Yellow 6 reference dye	15 µl
Red 40 reference dye	15 µl
1x TAE buffer	55 ml
Dyes extracted from candies from **Dye extraction from candies** activity	4 samples
2–20 µl adjustable-volume micropipet or 10 µl fixed-volume micropipet and 8 tips	1
Marking pen	1

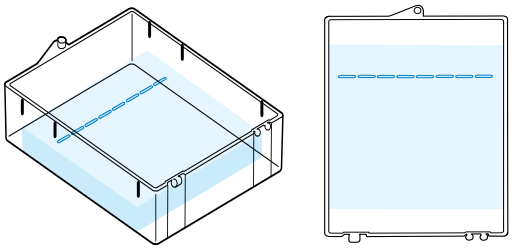
Protocol

1. Obtain your agarose gel in the plastic chamber. If you stored your gel after preparing it, pour off the 25 ml of 1x TAE buffer.

2. Using your ruler and following the marks you made one centimeter from the end of the box, cut a slab off the end of the gel using the end of a ruler. Press straight down through the gel to the box — do not slice across the gel. Loosen the slab by sliding the ruler between the end of the gel and the box end, then lift out the slab and discard.

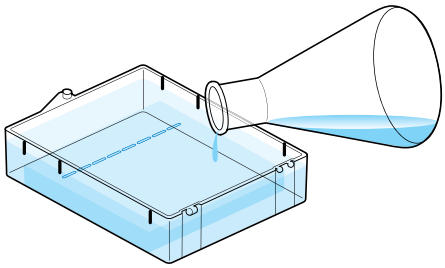
3. Repeat at the other end of the gel.



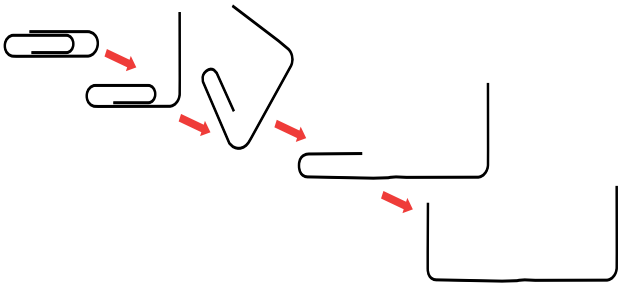

(Top view)

Protocol (cont.)

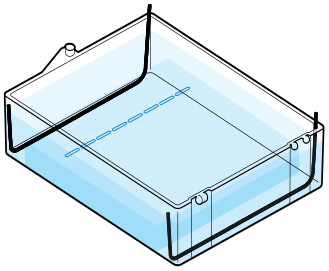
4. Add 55 ml of 1x TAE buffer to the box.



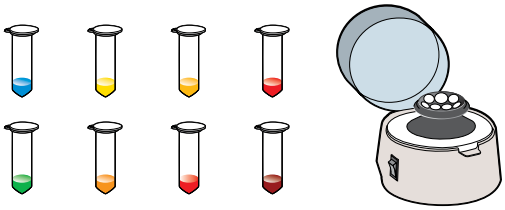
5. Construct your electrodes from two paper clips. Carefully straighten the paper clip and bend the two ends so they are perpendicular to the rest of the paper clip. Place your completed electrode on a flat surface. If it does not lie flat (in other words, if one of the angled pieces is not in the same plane as the rest of the electrode), hold the two ends and twist gently until the electrode will lie flat. The longer end will stick up above the gel box — this is where you will attach the alligator clip.



6. Place the electrodes into the gel box with the long ends on the same side. The electrodes should be as close to the end of the box as possible (as far away from the gel as possible).

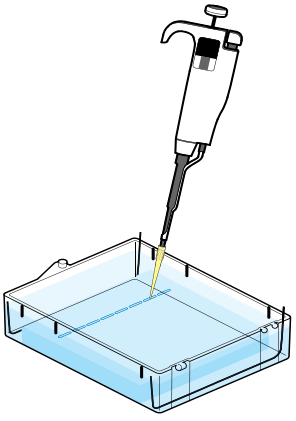


7. Prepare your extracted candy dye samples. If a centrifuge is available, pulse spin the microcentrifuge tubes in the centrifuge to bring all the liquid to the bottom of the tube and to settle any insoluble particles. Spin down your dye standard samples as well, if needed.



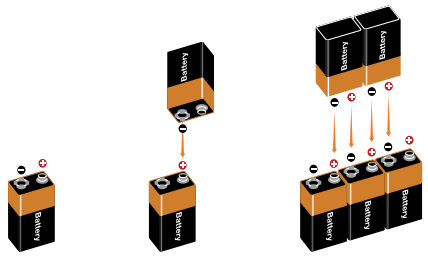
8. Using a separate tip for each sample, load 10 µl of each sample into 8 wells of the gel in the following order:

   Lane 1: Blue 1 reference dye
   Lane 2: Yellow 5 reference dye
   Lane 3: Yellow 6 reference dye
   Lane 4: Red 40 reference dye
   Lane 5: Candy 1 dye extract
   Lane 6: Candy 2 dye extract
   Lane 7: Candy 3 dye extract
   Lane 8: Candy 4 dye extract

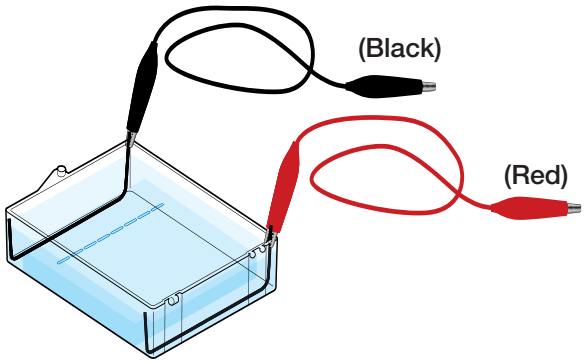


Protocol (cont.)

9. Assemble your battery tower by connecting negative nodes to positive nodes.

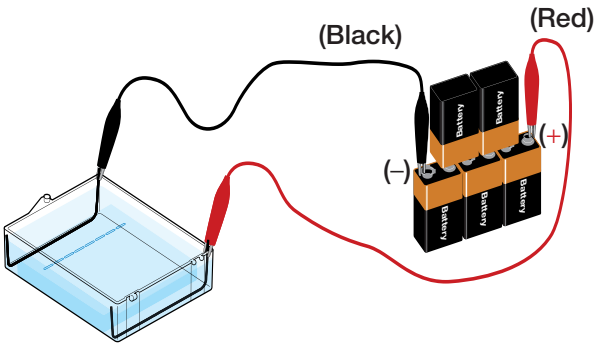


10. Attach the black alligator clip to the long end of the paper clip and box at the end of the box closest to the sample wells. Make sure the paper clip still remains on the bottom of the gel box under the buffer. Repeat the process for the red alligator clip and the electrode at the other end of the box.

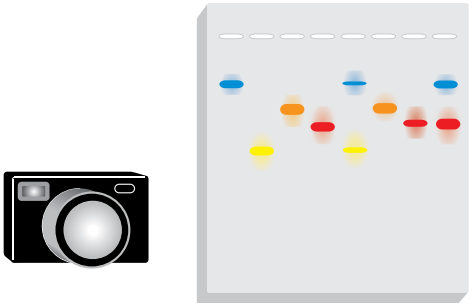


11. When you are ready to begin your electrophoresis run, attach the free black alligator clip on your lead to the (–) terminal of your battery tower and the free red alligator clip on your lead to the (+) of your battery tower. You should notice bubbles coming off of the paper clip electrodes if the circuit is complete.

12. Allow your gel to run for 20 min. Disconnect the red and black alligator clips from the battery tower.



13. Take a photograph of the gel for your records.



Focus Questions

1. When you analyzed the results of your gel, did any of your experimental samples contain dyes that did not match the four reference dyes? For example, did any of your samples produce:

   a. Dyes that are a different size than any of the standard bands?
   b. Dyes that are a different color than any of the standard bands?
   c. More than one color band?
   d. Dyes that you observed moving in the "wrong" direction (toward the cathode)?

   What might these dyes be?

2. Many popular dry dog foods and dog treats have FD&C dyes among their ingredients. For example, Beneful dry food contains Yellow 5, Red 40, Yellow 6, and Blue 2, and Snausages Breakfast Bites contain Red 40 Lake, Yellow 6 Lake, and Yellow 5 Lake. (Lake dyes are the insoluble forms of the FD&C dyes.) Why do dog food manufacturers put artificial food colors in dog food?

3. Keep a log of the coloring agents in the foods you consume for a 1-week period. What are the most common dyes on your list? Are there any natural dyes on your list?

   List the artificial dyes in a table, and look up natural food dyes that would produce the same color.

FD&C Dye	Color	Natural Alternative	Source of Natural Dye

   Are there any reasons why artificial food colors might be preferable to natural food colors?

4. You powered your electrophoresis with 9 V batteries connected in a series (multiple batteries with negative electrodes connected to positive electrodes to form a chain). For batteries connected in series, the total voltage is equal to the sum of the voltages of the individual batteries.

   a) How many batteries did you use? _____

   b) What would be the voltage if you used three batteries in series? _____ volts

   c) What if you used five batteries? _____ volts

   d) Would you expect your samples to migrate faster through the gel if you used three batteries or five batteries? Why?

5. Scientists often want results as fast as possible. What might be some disadvantages of using a higher voltage for the power supply?

6. For these experiments, you used a 1% agarose gel to separate the dyes. How do you think your results would differ if you used a 3% agarose gel? A 0.8% agarose gel?

7. Material engineers need to consider the uses of the products they design when they pick a material from which to make their product. What are some properties that need to be considered when picking a material to construct an electrophoresis box?

8. In this kit, you used zinc-coated steel paper clips to construct electrodes. Commercially-made gel boxes use platinum wire for electrodes. What are the characteristics needed for a good electrode? What other metals might make good electrodes?

9. Which direction would you expect a dye to run if it has no charge at pH 8?

10. The 1x TAE buffer serves several purposes in the electrophoresis setup. One is to provide ions to carry charge. A second purpose is to serve as a heat sink to absorb the heat generated by the current running from the batteries through the buffer and gel. If the buffer and gel get too warm, the gel will melt. A commercial electrophoresis apparatus typically uses about 275 ml of 1x TAE buffer for a 50 ml (cm^3) gel.

    a) Knowing the dimensions of the gel box, measuring the height of the gel, and calculating the volume of the gel pieces you sliced off, determine the volume of the gel in your STEM box.

    b) Calculate the ratio of the amount of 1x TAE in your STEM box to the amount of gel.

    c) Is this ratio larger or smaller than the same ratio for a commercial setup?

    d) Do you think the TAE in your STEM box will get warmer than the TAE in a commercial box if they are run at the same voltage for the same amount of time? Explain.

11. Calculate the rate that each dye moves in the gel.

    a) Using the photograph of your gel, measure the distance each reference dye moved from the wells in cm

    b) Divide the distance traveled by each reference dye by the time that you ran your gel (in minutes)

    c) Which dye has the fastest rate of movement in cm/min?

Appendix A
Independent Inquiry Activities

pH Changes in Electrophoresis Buffer During Electrophoresis

Water is hydrolyzed during electrophoresis. Water is reduced at the cathode and oxidized at the anode. The reaction is: $2\ H_2O \rightarrow O_2(g) + 2\ H_2(g)$

The hydrolytic reactions occurring at the electrodes are:

Reaction at the anode: $2\ H_2O \rightarrow O_2(g) + 4\ H^+ + 4\ e^-$

Reaction at the cathode: $2\ H_2O + 2\ e^- \rightarrow 2\ H_2(g) + 2\ OH^-$

Have your students prepare 1% agarose gels as described in the student activity. While they are running the experiment to separate their dyes, have students measure the pH at both the cathode and the anode using a pH meter every five minutes and record their results. If pH meters are not available, students can remove a small sample of buffer from the cathode and from the anode and measure the pH of each using pH paper.

Discussion of results can focus on why the chemical reactions are not the same at both electrodes.

Effect of Electrode Thickness and Material Choice on Electrophoresis

Electrodes are normally thin metal wires. What effect does the thickness of the wire have on electrophoresis? Use wires of different thickness to answer this question.

Since platinum is as costly as gold, it is not a good material for this experiment. Copper wire, however, is available in a number of thicknesses in electrical supply houses. The thickness of copper wire is given as the wire's gauge, a standardized measure based on the diameter of the wire. Thinner wire has a smaller gauge than thicker wire. Choose three to four thicknesses of copper wire to test. Make sure that one of the samples is the thinnest available.

Determine how much wire you will need to form an electrode. Cut the wires all to the same length and bend them into the shape of your paper clip electrodes. Prepare gels as you did in the activity, and run the dye standards on the gels, using electrode wires of different thicknesses. Pay particular attention to the electrode placement, ensuring that the electrodes are at the ends of the boxes, as far as possible away from the agarose.

As the gels run, compare the speed with which the samples migrate through the gels. Is there any difference? What about the gels themselves? Do you see any melting at either end? Do you see corrosion or discoloration around the electrodes, and, if so, does the thickness of the copper wire make a difference in the amount? Do you note any other differences between the gels run with thin wires compared to those with thicker wires?

As a further activity, compare other types of metal wire to copper wire to see if any other types make good electrodes. Choose wires of the same gauge, if possible.

Effect of Gel Matrix

For this laboratory experiment, 1% agarose gels were used and 50 ml of agarose was poured to create the gels. Have students experiment with the effects of pouring higher percentage and lower percentage gels, as well as thicker or thinner gels. What impact does this have on the amount of sample that can be loaded? On the separation of the dyes? On the ease of handling the gel?

Agarose is a polysaccharide or long chain of sugar groups. Have students research more into how agarose forms gels with pores. Other compounds such as gelatin also form gels. Gelatin is made from long protein chains, rather than polysaccharides. Have students design and run experiments to determine if gelatin forms gels at pH 8 in 1x TAE buffer. Will gelatin form a molecular sieve and separate dye molecules? What is the melting temperature of gelatin gels? If gelatin forms gels, what percentage gels are needed to separate the colored dyes? Is it the same as agarose?

Effect of TAE Concentration and Volume

The TAE buffer serves as a reservoir of ions to help move the samples via charge through the gel. Experiment with increasing and decreasing the TAE concentration in the buffer. What impact does this have on dye sample separation? On the temperature of the buffer? On the corrosion of the paper clip electrodes? What impact would keeping the TAE concentration the same but changing the volume of buffer use have on the separation? On the temperature of the buffer?

Putting it All Together

Challenge your students to design an electrophoresis box that separates the dyes in the most efficient manner taking into account time of separation, degree of separation, safety, and cost of the setup. Have them compare their design parameters to that of commercial electrophoresis equipment.

Appendix B
Glossary of Terms

Agarose — a polysaccharide derived from red algae. It has a neutral charge and is the most commonly used matrix for separating DNA by electrophoresis.

Aliquot — the division of a quantity of material into smaller, equal parts.

Anode — a positive electrode in an electrical device. The anode is the positively charged (red or +) electrode toward which the negative ions (anions) flow.

Buffer — an aqueous solution that contains acid/base pairs (either a weak acid with its conjugate base or a weak base with its conjugate acid) that serve to keep the pH of the solution constant.

CAS number — the number assigned to each chemical described in the scientific literature by the Chemical Abstracts Service. Also called the CAS Registry number.

Cathode — a negative electrode in an electrical device. The cathode is the negatively charged (black or –) electrode toward which the positive ions (cations) flow.

Electrophoresis — a technique that uses electricity to separate molecules by their physical characteristics, usually size and charge. In DNA agarose gel electrophoresis, DNA molecules are separated by size in an agarose matrix.

EEC number — the designation given by the European Union to food dyes approved for food use in the European Economic Community. Also called the E number.

FD&C Dye — dyes approved for use as food coloring agents by the US Food and Drug Administration. FD&C stands for the Federal Food, Drug, and Cosmetic Act. There are currently seven FD&C dyes approved for use in the U.S.

Food coloring — solutions of soluble dyes that can be purchased at the grocery store and are used to color food. These products may be made from a single FD&C dye or from a combination of dyes.

Lake dye — the insoluble forms of the FD&C food dyes. The soluble forms are reacted with aluminum hydroxide to form lake dyes, which are insoluble in aqueous solutions and will not migrate during electrophoresis. Lake dyes color foods by dispersing dye particles throughout the food. Some uses for lake dyes include coloring pet foods, pill coatings, cake mixes, and some candies.

Appendix C
Instructor's Answer Guide

Focus Questions

1. The four reference dyes included in the IDEA kit are the most commonly used artificial dyes in the US. There are a number of other dyes that students might encounter. Have your students check the ingredient lists from their candies to see what other dyes are present. Possible explanations for seeing bands of different sizes and colors from the control dyes include:
   a. There are three other FD&C dyes (see Table 3). All are negatively charged and of similar size to the four control dyes.
   b. Student samples may contain natural dyes, such as carmine or beetroot red. (Discussion of the sources and pros and cons of natural dyes is a good extension activity. See Focus Question 4.)
   c. Several dyes are too close in size and charge to separate on a 1% agarose gel, so students may see a single band for the two colors. For example, Yellow 6 and Red 40 will not separate, so students will see a single orange band if those two dyes are present in their sample.
   d. If the candy is from outside the US, it may contain dyes other than the FD&C dyes.
   e. Although they are not common, some food dyes may be positively charged and will migrate toward the negative (cathode or –) electrode.
   f. Many food colors are obtained by combining multiple food dyes, so some samples may produce more than a single dye band. For example, brown color is frequently a combination of Blue 1 and Red 40.

2. Dogs don't buy dog food and dog treats — their owners buy them! As with the other foods that we buy, appearance is very important in our decision-making, and the marketing departments of dog food companies know that. Colorful food is more appetizing to us, so we are more likely to buy it for our pets. Note how many dog foods have kibbles the colors (and shapes) of vegetables, so consumers are likely to associate the food with healthy eating.

   Even if dogs did take the time to note their colorful kibbles, remember that their vision is not like ours. Dogs have only 2 types of color receptors, so their vision is similar to that of humans with red-green color blindness.

3. Students will likely have four to five of the FD&C food dyes on their lists. They may also have some natural colors. For instance, cola drinkers will have caramel on their list. They may also encounter carmine in candy or yogurt. If they eat many foods from natural food stores, they may encounter other natural dyes, such as those made from beets and carrots.

   Internet searches will produce lists of natural food colors that can be prepared from plant materials, such as red from beets or cranberries, yellow from onion skins or daffodil blooms, purple from blackberries, blue from red cabbage, and green from spinach leaves.

   If none of your students has carmine or cochineal extract on his or her list, assigning those dyes to students to research for extra credit will result in interesting discussion points, as both of those dyes are considered "natural" and both are derived from the powdered bodies of insects. In the U.S., food manufacturers were not required to identify these dyes except as "natural colors" until January 2011. Since then, the dyes must be listed by name (but not by origin) on food labels.

   Reasons that artificial dyes might be preferable to natural dyes include that the chemistry of the FD&C dyes is well known, they are manufactured to food grade standards, and they are very strong dyes, so very little is needed to color food.

There is a demand for all things natural, so some natural food dyes are being produced commercially to food grade standards. Some natural dyes being manufactured include caramel from caramelized sugar, annatto from the seeds of the achiote tree, cochineal from *Dactylopius coccus* insects, and betanin from beets. In general, it takes a larger quantity of natural dyes to get strong colors and some of these natural dyes can impart flavor to the item being colored.

4. You powered your electrophoresis with 9-volt batteries connected in a series.
   a. Answers will vary
   b. 27 volts
   c. 45 volts
   d. The samples would migrate faster through the gel if five batteries are used than they would if three batteries were used. If there is more voltage, there is more current (V=IR). Current is the rate that charged molecules flow. If they can flow faster, then for the same amount of time, they will move farther.

5. The most important disadvantage is that higher voltages can produce higher currents. The higher current produced by the higher voltage will meet resistance to flow from the agarose. As the current hits resistance, it causes the buffer and the agarose to warm up. If the buffer and agarose get too warm, the agarose will melt.

6. A 3% agarose gel would slow down the larger molecules more than in a 1% or 0.8% gel. Within the same time frame, all of the dyes would run farther along the 0.8% gel than in a 1% or the 3% gel.

7. Answers will vary but may include: The material needs to be moldable. It needs to have a melting point above 60°C. It needs to be transparent or translucent. It needs to be able to withstand heating and cooling. It needs to be able to hold salt solutions without corroding. It needs to be non-conductive.

8. Answers will vary.

   A good electrode material must conduct electricity. All metals conduct electricity, but not all metals conduct electricity well. Poor conductors include tungsten and manganese. Good conductors include gold, silver, copper, and platinum.

   Electrode materials must be solid at working temperatures. For example, mercury conducts electricity, but it would be very difficult to make a mercury electrode for a gel box.

   Finally, the material should be as inert or non-reactive as possible. Electrodes are exposed to salt solutions and electricity. Materials that corrode easily, such as iron, make poor electrodes. Reactivity series — lists of materials and their reactivity — can be found on the internet. There are a number of metals that are reactive only with strongly oxidizing acids and would make good electrode materials, such as gold and platinum. Fortunately, they also meet the first two criteria.

   Most electrodes are metal (though carbon fiber conducts electricity and has been used as electrode material). Students should be able to find elements listed by their electrical conductivity and by their reactivity on the internet.

9. If it has no charge, the dye will not move out of the well and therefore will not migrate towards the anode or the cathode.

10. To determine the height of the uncut gel, students can either measure the height of the gel or they can calculate the height of the gel by knowing that they poured a gel of 50 ml = 50 cm^3.

    a. For example:

    Volume = 50 ml = 50 cm^3 = L x W x H where H = the height of the gel

    9 cm x 6.5 cm x H so H = 0.85 cm

    From each end of the gel, 1 cm of gel is cut off. To calculate the volume of gel removed
    Volume = L of gel removed x W x height of gel = 1 cm x 6.5 cm x 0.85 cm = 5.5 cm^3
    Since this volume of gel is removed from both ends, the total volume removed is
    Volume of gel removed = 2 x 5.5 cm = 11 cm^3

    So the final volume of gel present is 50 cm^3 – 11 cm^3 = 39 cm^3 = 39 ml

    b. So for the STEM box, the ratio of buffer to gel is 55 ml/39 ml = 1.4

    c. For a commercial gel box system, the ratio of buffer to gel is 275 ml/50 ml = 5.5

    d. The commercial gel box has a lot more buffer present per amount of gel present relative to the STEM box. Therefore the commercial gel box has more buffer present to absorb the heat generated at the electrodes, hence the gel in the commercial box would heat up less than in the STEM box.

11. The rate of movement of each dye can be calculated as follows:

    a. The distance each reference dye migrated in the gel will vary with the amount of time the gel was run and the number of batteries used.

    b. The time the gel was run should be 20 minutes.

    c. The reference dye that has the fastest rate of movement in the gel is Yellow 5.

Appendix D
References and Additional Resources

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