**RAINBOW ELECTROPHORESIS**1

# An Introduction to Gel Electrophoresis

# STANDARDS ADDRESSED

# 3.1.9-12.P Ask questions to clarify relationships about the role of DNA and chromosomes in coding instructions for characteristic traits passed from parents to offspring

**INTRODUCTION**

This laboratory will demonstrate the basics of electrophoresis and the theory behind the separation of molecules on an agarose gel. Electrophoresis is commonly used to separate DNA fragments or proteins. How a molecule migrates through a gel is dependent upon its size, electrical charge and shape. Smaller molecules will travel faster through pores of an agarose gel, whereas larger molecules will migrate more slowly.

Negatively charged molecules will run toward the positive (red) pole of the gel, while positively charged molecules will migrate toward the negative (black) pole. Migration of a molecule may also affected by the density of the gel and the type of buffer used.

These properties of electrophoresis can be demonstrated using food coloring. When mixed with glycerin or sucrose, food dyes can be loaded into the wells of an agarose gel and separated into their constituent pigments. Based on the migration of the pigments during electrophoresis, students should be able to form hypotheses about the size and charge of each pigment.

# PURPOSE

The goal of this exercise is to familiarize students with the methods and theory of electrophoresis. This activity should demonstrate:

1. how to make an agarose gel for electrophoresis
2. how to load samples into a well
3. how to analyze the results of electrophoresis

# MATERIALS

* electrophoresis chamber & power supply
* dye samples mixed with glycerin or sucrose
* (4 colors plus 1-2 “mystery” mixes)
* micropipettor and tips
* plastic sandwich bag
* colored pencils
* paper towels

# SAFETY PROCEDURES

Exercise caution when using the power supply. The area around the supply and the electrophoresis chamber should be dry. Be sure all the connections are in place before turning on the power. Likewise, the power supply should be shut off before disconnecting any of the electrical leads.

1Adapted from “Rainbow Electrophoresis: An Introduction to Gel Electrophoresis” <http://eagle.clarion.edu/~faculty/biscuts/Rainbow.html> (8 May 2002)

# PROCEDURES

Electrophoresis

1. Carefully remove the tape from the ends of the casting tray and place the gel into the electrophoresis chamber. Remove the comb slowly by pulling straight up on it. You must do this carefully so that the bottoms of the wells do not rip. Orient the gel so that the wells are near the negative (black) terminal.
2. Fill the electrophoresis chamber with 1x TBE. Be sure that there is enough buffer to completely submerge the agarose gel.
3. The teacher will demonstrate the proper way to hold a micropipette, fill it with sample and dispense the sample into a well.
4. Fill the pipet with 10 µL of a food color sample. Place the tip over the top of one of the wells. The tip should be submerged in the buffer at this point. Holding the pipet steady, gently dispense the sample into the well. The glycerin in the sample will allow the sample to sink into the well. Do not place the pipet tip directly into the well or you will risk poking a hole in the side or bottom of the well, and your sample may leak out of the gel. A new pipet tip should be used for each sample. If possible, each student should have a turn loading a well.
5. Record the order in which the samples are loaded, either left to right, or top to bottom.
6. Make sure that the area around the electrophoresis chamber and power supply is dry. There is an ON button on the back of the power supply. This is on when a red “0” is displayed on the LED at the front of the machine. There are several buttons on the front of the power source. To set the supply to show volts, press the two “ ▀ “ buttons until a green light comes on next to the “V” (for volts). Set the power source to 100V by pushing the “▲” to the left of the LED display. Check to be sure that the leads from the gel are plugged into the power supply. When the leads are connected properly (red to red, black to black), press the “run” button (a picture of a man running). A green light should come on next to the run button to indicate the power supply is working. There should be bubbles forming on the electrodes in the electrophoresis chamber if the current is flowing properly. More than 125 V is not recommended, as the agarose gel can melt. Check the gel after approximately 5 min. to make sure the sample is migrating through the gel in the correct direction. Allow the gel to run for a total of 30-45 minutes.
7. Have the students predict what will happen to each sample during electrophoresis. Will all the dye pigments migrate at the same rate? Is each color pure or a mixture of other colours?

# DATA COLLECTION

1. Remove the gel from the electrophoresis chamber and place it on plastic wrap or in a sandwich bag.
2. For best viewing, the gel should be placed on a light source (a light box or overhead projector) or on a white background (a piece of unlined paper).
3. The gel results should be recorded within a couple of hours after running the gel. After this time period, the dye pigment bands will start to diffuse into the gel and will be difficult to visualize.

# RAINBOW ELECTROPHORESIS

Analysis of Results

## Name:

1. Draw a detailed sketch of the gel. Use colored pencils to show the colors of the samples as originally loaded. Draw the different color bands that resulted from electrophoresis of each dye. Special attention should be paid to the differences in dye migration and the relative positions of the bands in each lane.
2. How do the results compare to the predictions you made about each dye? Were any of the colours pure? Did the different dye pigments migrate different distances?
3. What are the main factors which help separate the dye pigments (list 3)?
4. What charge is carried by the dye pigments? How did you determine this?
5. Which colour molecule is most likely the smallest? How did you determine this?
6. Compare the dye pigments in the mystery mix to the pigments from the other four colours (red, yellow, blue and green). How can you use the results from the four basic colours to determine what is in the mixed sample. What two food colors are in the mystery mix?

# TEACHER NOTES

1. The actual experimentation portion of this lab is written for two 50 min class periods, but could be modified for one class period. This would require that the teacher prepare the gel, food color sample and the electrophoresis chamber prior to class. The gel would need to be loaded and electrophoresis (at 110-115 V) started as soon as class has begun. While the gel is running, the principles of electrophoresis could be taught. After about 30-35 min, electrophoresis could be stopped and the gel analyzed for separation of pigments. If time does not allow for analysis, the gel can be wrapped and stored in a refrigerator until the next class period.
2. If running more than one gel in a class, there are variations which would allow the students to compare the effect of external variables on the separation of the dye pigments.
	1. Pour gels of different densities. Select 3 different percentages of gel and run all three for the same amount of time at the same voltage. (0.8% agarose, 1.0% agarose, and 1.2% agarose are densities commonly used in research labs.) Then have the students compare the differences in pigment migration for each gel. Which percentage has the fastest rate of migration? Are only certain pigments affected by the change in agarose density?
	2. Use different electrophoresis buffers. In addition to TBE buffer, TAE (Tris- acetate, EDTA) and TPE (Tris-phosphate, EDTA) are also commonly used. By running 1% agarose gels at the same voltage, it is possible to compare the effects of the buffers on pigment separation. If TPE buffer is used, a lower voltage (60-70 V) will need to be used, as a higher current in this buffer tends to melt agarose gels more easily.

## Example of Possible Results

In general, the food colours should separate into the following dye pigments: green = dark blue and yellow bands

red = pink and red/orange bands

yellow = pink, orange and yellow bands

blue = light blue, dark blue and dark red bands

An example of the electrophoresis results is provided below. It is recommended that the students record and draw their experimental results in a similar fashion. This should make determination of the mystery mix somewhat easier.

Order of Samples

1 2 3 4 5

## Well # Food Dye Sample wells

M green

* + 1. red
		2. yellow
		3. blue
		4. mystery mix