RAINFOUR GELS: AN INTRODUCTION TO ELECTROPHORESIS

STANDARDS

- 3.1.7, 3.1.10, 3.1.12
- 3.3.7, 3.3.10, 3.3.12

INTRODUCTION

This laboratory will demonstrate the basics of electrophoresis and the theory behind the separation of molecules on an agarose gel matrix. Electrophoresis means "carrying with electricity" and 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.

Size determines how fast a molecule can move through a gel matrix. Smaller molecules will travel faster through an agarose gel, whereas larger molecules will migrate more slowly. The electrical current that runs through an electrophoresis chamber acts like a magnet for the molecules; opposite charges attract, while like charges repulse each other. This means that molecules that have a negative (-) charge, like the nucleic acids DNA and RNA, will migrate from the negative pole (anode, black) toward the positive pole (cathode, red) (Fig. 1). Positively charged molecules, like some proteins, will migrate toward the negative pole. The shape of biological molecules may vary from a linear fragment of DNA to a globular protein; this too will affect how a molecule moves through a gel matrix.

Agarose gel electrophoresis is a procedure by which molecules, usually DNA or RNA, are separated on the basis of size. Agarose is a material derived from red seaweed (Phylum Rhodophyta). When agarose is melted and then cooled, it contains pores that act like a sieve. The size of the pores is determined by the concentration of the agarose in the gel. Increasing the agarose concentration decreases the pore size and limits the size of the DNA molecules that can fit through the pores. The agarose concentration therefore determines the range of DNA fragment sizes that can be effectively separated on a gel. On a standard 1% agarose gel, DNA fragments from approximately 500 – 10,000 base pairs (bp) can be effectively separated. Small molecules will travel more quickly through the agarose matrix, thus migrate the furthest from the gel well. Larger fragments will take longer to move through the gel matrix, therefore they will migrate more slowly and will be closer to the gel well.

The gel separation must be run while submerged in an electrophoresis buffer. This buffer contains salts for conducting the electrical current from one electrode to the other. In addition, the electrophoresis buffer helps maintain the pH during electrophoresis. If the buffer pH alters the charge of the molecules, their separation during electrophoresis may be affected. This is especially true for proteins. As the electrical current carries the
molecules, the type of gel matrix and the buffer being used will determine whether the molecules are separated by size, conformation or both.

These properties of electrophoresis can be demonstrated using standard biological dyes. When mixed with glycerin or sucrose, these 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.

**GUIDING QUESTIONS**

- What is electrophoresis?
- What is agarose and how is it used to separate biological molecules?
- Which properties of a molecule determine how it is affected by electrophoresis?
- Why is the electrophoresis buffer important?

**MATERIALS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
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<tbody>
<tr>
<td>1% TAE agarose gel</td>
<td>Loading dye samples</td>
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<tr>
<td>2-20 µL micropipettor</td>
<td>2-20 µL pipet tips</td>
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<tr>
<td>Electrophoresis chamber</td>
<td>Power supply</td>
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<tr>
<td>1x TAE electrophoresis buffer</td>
<td>Colored pencils</td>
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<tr>
<td>Plastic wrap</td>
<td>Practice gels (optional)</td>
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**SAFETY**

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.
PROCEDURE
1. Carefully remove the plastic cover from the gel tray and place the gel (plus the tray) into the electrophoresis chamber. Orient the gel so that the wells are closer to the negative (black) terminal (see Fig. 1).

2. Fill the electrophoresis chamber with 1x TAE buffer. Be sure that there is enough buffer to completely submerge the agarose gel (see Fig.1).

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 dye 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, every student should have a turn loading a well.

5. Load all the dye samples (A-E, and Mix). Record the order in which the samples are loaded on the diagram provided on your data sheet.

6. Gently place the plastic electrophoresis lid on the top of the chamber. Be sure to match the red and black electrodes! Plug the electric leads coming from the lid into the power supply. Again, match red to red, black to black.

7. Make sure that the area around the electrophoresis chamber and power supply is dry. There is an ON button (rocker switch) 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.

8. 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. More than 125 V is not recommended, as the agarose gel can melt.

9. Check to be sure that the leads from the gel are firmly 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.

10. Check the gel every 3-5 minutes and make observations in the table provided of how each dye is separating. Allow the gel to run for a total of 20 minutes.
11. Turn off the power and remove the lid from the gel box. Carefully lift the gel and its tray out of the electrophoresis chamber. 

**Note:** The gels will be very, very slippery! Keep your fingers over the open ends of the gel tray so that the gel does not slide out.

12. Gently slide the gel out of the tray onto some plastic wrap that is over white paper or a light box. Use a metric ruler to measure the distance from the loading well to each different colored pigment band. Record this information by sketching the different band patterns on the gel graphic provided in the Data Analysis section of the lab. Colored pencils are useful for this task.

**REFERENCES**
Adapted from “Rainbow Electrophoresis: An Introduction to Gel Electrophoresis”, May 2002. 
http://eagle.clarion.edu/~faculty/biscuts/Rainbow.html

**CREDITS**
This lab was revised and adapted from the above reference by Dr. Stephanie Corrette-Bennett.
OBSERVATIONS DURING ELECTROPHORESIS

Use the table below to record your observations of each dye sample. You will want to make predictions about:
- Composition of the dyes (do the dyes have a single or multiple pigments)
- Size of the molecules in the dyes (how fast are the different colors separating?)
- Charge (positive or negative)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
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<td>E</td>
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<tr>
<td>Mix</td>
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DATA ANALYSIS

Sketch a picture of your final gel results; colored pencils will make this easier. Use a ruler to help you measure the distance of the dye pigments from the gel wells. This will help you answer the questions.

QUESTIONS

1. Were any of the dyes composed of just one pigment? Did the different dye pigments migrate different distances?
2. What are the main factors which help separate the dye pigments (list 3)?

3. What charge is carried by the dye pigments? How did you determine this?

4. Which dye had the color molecule that is the smallest? Which dye had the largest? How were you able to determine this?

5. Compare the dye pigments in the mystery mix to the pigments from the other five colors (A-E). How can you use the results from the five dyes to determine what is in the mixed sample? Which dyes do you think are in the mixed sample?