Molecular Rainbow
Dye Electrophoresis
Student Materials

Introduction........................................................................................................................................... 2

Pre-Lab Questions................................................................................................................................ 4

Lab Protocol......................................................................................................................................... 5

Data Collection Worksheet.................................................................................................................. 6
Molecular Rainbow

Introduction

Biology is the study of living things, but how are living things studied? Today you are going to explore one tool that scientists use to study things at the molecular level.

Molecules like DNA, RNA and proteins are not living, but understanding these molecules helps us to learn about living things. How can you study molecules of DNA, RNA or protein when they are too small to be seen, even with a microscope? A good first step may be to determine the size of a molecule.

A common way to measure molecular size is by **gel electrophoresis**, which is a big name for a simple concept. Here is an analogy to explain gel electrophoresis. Imagine a very dense forest of trees where each tree is only one foot away from every other tree. A boy and a mouse must each run through the forest from point A to point B. The boy and the mouse begin at the same time.

![Forest analogy](image)

**Figure 1.** Gel electrophoresis separate molecules by size. Like a race through a forest the small molecules will move more quickly that the bigger molecules.

◊ **Who will reach the other side first, the boy or the mouse? Write your ideas here:** __________

The gel in gel electrophoresis is like the forest except instead of trees, it is a mesh formed of polymers (see **Figure 2**). It looks and feels a lot like JELL-O, but instead of being made of gelatin, the gels in electrophoresis are made of **agarose**. Agarose is a sugar, purified from seaweed, while gelatin in JELL-O is made from animal products like collagen. Once set, the gel is covered in a tank with **electrophoresis buffer** (a salt solution) that controls the pH and conducts electricity. The molecules to be studied are injected into wells in the gel, and an electric field is applied (see **Figure 3A**).
Why is the gel covered in electrophoresis buffer?

What force makes the molecules move through the gel?

When it comes to electricity, opposites attract. Molecules with a positive surface charge will move through the gel towards the negatively charged electrode. Molecules with a negative surface charge will move through the gel towards the positively charged electrode. If the relative charges of the molecules are the same, then the molecules will move through the gel at different rates based on their size. Just like in the example with the boy and the mouse, the smaller molecules will move faster through the small holes in the gel. (see Figure 3B). Smaller (faster) molecules will move further from the wells than the larger (slower) molecules.

In what direction will molecules with a negative surface charge move? 

In what direction will molecules with a positive surface charge move? 

Which dye in Figure 3B is smaller the red dye or the yellow/orange dye?

Figure 3. Illustrations of gel electrophoresis. (A) Shows the set-up of the equipment. (B) Represents a gel where dyes have been separated based on their size and charge. Notice how the placement of the wells is different between the two gels shown.

In this activity, you will use agarose gel electrophoresis and dye standards to determine what dye(s) are found in three mystery samples.
Molecular Rainbow
Pre-Lab Questions

Directions: After reading the introduction and protocol for the Molecular Rainbow lab, answer the questions below.

1. How does agarose gel electrophoresis work to separate molecules from one another? What is the force that causes the molecules to move?

2. You observe the following while running gel electrophoresis on two molecules.

   ![Diagram of gel electrophoresis with Molecule A and Molecule B]

Even though the molecules are the same charge, you notice that molecule A barely moves from the well while molecule B moves 2 cm towards the positive electrode. Give one reason why these two molecules of the same charge run differently in your experiment.
**Molecular Rainbow**

**Lab Protocol**

**Procedure:** Check your workstations to make sure you have all the supplies before beginning the lab.

<table>
<thead>
<tr>
<th>Student Workstation:</th>
<th>Common Workstation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 tubes standard dye samples</td>
<td>1X electrophoresis buffer</td>
</tr>
<tr>
<td>3 tubes unknown dye samples (A, B, C)</td>
<td></td>
</tr>
<tr>
<td>1 agarose gel (0.8%) with wells in the center</td>
<td></td>
</tr>
<tr>
<td>1 p20 micropipette and tips</td>
<td></td>
</tr>
<tr>
<td>1 microcentrifuge tube rack</td>
<td></td>
</tr>
<tr>
<td>1 waste container</td>
<td></td>
</tr>
<tr>
<td>1 gel electrophoresis unit with power supply</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure:**

1. Make or get a 0.8% agarose gel and 1X electrophoresis buffer.

2. Put together the gel box
   - Place the gel tray into the box so that the wells are in the center.
   - Add just enough buffer to cover the gel.
   - Place it on the lab bench where it will run.

3. Using a p20 micropipette, load 12 µL of each known dye into the gel wells in the following order.
   - **Reminder:** Use a new tip for each sample.
   - Lane 1: Bromophenol Blue (BB)
   - Lane 2: Methylene Blue (MB)
   - Lane 3: Orange G (OG)
   - Lane 4: Bromocresol Purple (BP)
   - Lane 5: Allura Red AC (AR)

4. Skip Lane 6. Using a p20 micropipette, load 15 µL of each unknown sample into the gel wells in the following order.
   - Lane 6: empty
   - Lane 7: Unknown A
   - Lane 8: Unknown B
   - Lane 9: Unknown C

5. Place the cover on the electrophoresis box.

6. Turn on the power ⚡ and run your samples for **3 minutes** and write what you see in the lanes for unknowns A, B and C on the **Data Collection Worksheet**.

7. Continue to run your gel until the fastest moving dye is about two-thirds of the way down the gel – about **10-15 minutes**.

8. Turn off the power supply, remove the cover and observe your gel. 📷 Take a photograph of your gel and/or ✏️ draw a picture of your gel on the **Data Collection Worksheet**.
1. After you ran the gel for **three** minutes, what did you see in the lanes for unknown A, B and C? Write your observations below.

2. After you have stopped running your gel, draw your results on the gel picture below. Be as accurate as you can.

3. Label the positive and negative electrodes in the picture above.

4. Compare the bands seen in the lanes for A, Unknown B and Unknown C with the bands for the known dyes. Can you figure out the mixture of dyes in each unknown?

   - Unknown A = _________________________________
   - Unknown B = _________________________________
   - Unknown C = _________________________________

5. What dyes are positively charged?

6. What dyes are negatively charged?

7. Which dye molecule is larger Orange G or Allura Red? How do you know?