Name_____ Class_____ Date _____

Investigating Gel Electrophoresis

Introduction

Agarose gel electrophoresis is a commonly used method of separating molecules based on their charge, size, and shape. It is especially useful in separating charged molecules of DNA and RNA. When an electric current is applied to the gel, negatively charged molecules move toward the positive electrode (anode), and positively charged molecules move toward the negative electrode (cathode). The charge, size, and shape of a particular molecule all affect the rate at which a molecule moves through the gel.

In this investigation, you will compare the rate and direction of movement of several different dye samples in an agarose gel and draw conclusions about their charge and chemical composition based on your observations.

Problem

How can gel electrophoresis be used to separate different molecules?

Pre-Lab Discussion

Read the entire investigation. Then, work with a partner to answer the following questions.

1. Why should you use a clean transfer pipette for each sample?

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2. How does gel electrophoresis separate molecules?

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3. In which direction do negatively charged molecules move? Positively charged molecules?

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_____

4. How will you know if the current is flowing properly in your electrophoresis apparatus?

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5. Before you begin the investigation, your gel is transparent. What will it look like at the end of the investigation?

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_____

Materials *(per group)*

gel electrophoresis apparatus
direct current power source
transfer pipettes
numbered dye samples
plastic gloves

Safety

Put on a laboratory apron. Put on safety goggles and plastic gloves. Never taste anything used in this laboratory investigation. Always work with special caution when working with laboratory chemicals, as they may irritate the skin or stain skin or clothing. Observe proper laboratory procedures when using electrical equipment. Use extreme care when working with heated equipment or materials to avoid burns. Wash your hands thoroughly after carrying out this investigation. Note all safety alert symbols in the Procedure and review the meaning of each symbol by referring to Safety Symbols on page 8.

Procedure

1. **CAUTION:** *Put on a laboratory apron, safety goggles, and disposable gloves before beginning this investigation. Do not smell or taste any chemicals in this investigation. If a chemical gets on your clothes or skin, flush the area with water immediately and notify your teacher. When loading the samples into the wells, always point your pipette away from yourself and others, especially the face and eyes.* Using a transfer pipette, carefully load each of the dye samples into the wells in the middle of the gel in consecutive order. Follow your teacher's directions for the proper way to load the samples into the wells. Use a clean transfer pipette for each sample. Load each well until it is full.
2. After loading the samples, carefully close the cover of the apparatus onto the electrode terminals. Be sure to align the negative and positive marks on the cover with the corresponding marks on the apparatus chamber.
3. Insert the plug of the negative (black) wire into the negative (black) input of the power source. Insert the plug of the positive (red) wire into the positive (red) input of the power source. See Figure 1. **CAUTION:** *Do not use electrical equipment near water or with wet hands.*

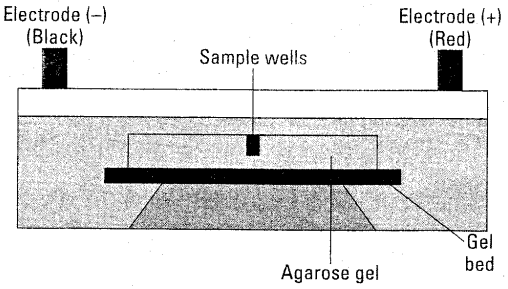

Figure 1

Name_____ Class_____ Date _____

4. Set the power source at the voltage determined by your teacher.
5. Run the electrophoresis for the appropriate length of time based on the voltage you are using as determined by your teacher. Look for bubbles forming on the electrodes to be sure that current is flowing properly.
6. When the electrophoresis is completed, turn off the power, unplug the power source, disconnect the wires, and remove the cover from the apparatus.
7. Carefully remove the gel on its bed, holding each end of the gel to prevent it from slipping off the bed.
8. In Figure 2, indicate the relative positions of the bands of dye.
9. Dispose of all gels, solutions, and equipment according to your teacher's instructions. Wash your hands with soap and warm water after completing this investigation.

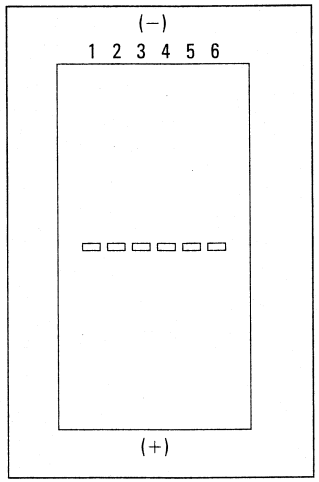

Figure 2: Results of electrophoresis

Analysis and Conclusions

1. **Inferring** Based on your results, which samples have a negative charge? A positive charge?

_____

_____

2. **Drawing Conclusions** What can you conclude about the samples in lanes 4 and 6?

_____

_____

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3. **Controlling Variables** What steps did you take during the procedure to avoid contaminating samples?

_____

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4. **Inferring** Why might it be important to avoid contaminating DNA samples when doing a DNA analysis?

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5. **Analyzing Data** Assume that samples 4 and 6 are each a combination of samples 1–3 or 5. Based on this assumption, can you identify the dyes in samples 4 and 6?

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_____

Going Further

In DNA sequencing, strands of DNA are selectively broken into smaller pieces that can then be separated by gel electrophoresis. Using reference materials available in your school or public library or on the Internet, research the process of DNA sequencing. What chemicals are used to break DNA strands into pieces? What are some applications of this process? Present your findings to the class.