

Investigating Gel Electrophoresis

You may want to refer students to Section 13–2 in the textbook for a discussion of genetic engineering techniques.

Time required: 30 to 90 minutes for electrophoresis, 30-minute observation period

Introduction

Gel electrophoresis is a method of separating molecules such as DNA and RNA by charge, size, and shape. When an electric voltage is applied to the gel, negatively charged molecules move toward the positive electrode, and positively charged molecules move toward the negative electrode. The charge, size, and shape of a particular molecule all affect the rate at which it moves through the gel.

In this investigation, you will run a gel to compare the movement of several molecules. You also will design and conduct an experiment to determine the sizes of DNA fragments in an unknown mixture using a DNA ladder (a mixture of DNA fragments of known size).

Problem

How can gel electrophoresis be used to separate a mixture of different molecules? How could this technique be used to determine the sizes of unknown molecules?

Pre-Lab Discussion

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

1. For what purposes do scientists use gel electrophoresis?

Scientists use electrophoresis to separate charged molecules of DNA and RNA.

2. What properties of molecules affect how they migrate (or move) through the gel? Predict what types of molecules will move closer to the negative electrode and what types will move closer to the positive electrode.

The charge, size, and shape of molecules affect migration of molecules through the gel. Positively charged molecules will move closer to the negative electrode, and negatively charged molecules will move closer to the positive electrode. The larger a molecule is, the more slowly it will move.

3. The gel used in electrophoresis has microscopic pores that act like a sieve. Why would a small, compact molecule move farther through such a gel than a larger, less compact one?

Smaller, more compact molecules can move more easily through the pores of the gel. Larger, less compact molecules will move more slowly than smaller ones.

4. In your own words, summarize the procedure for gel electrophoresis.

Load the samples into the wells, close the cover of the apparatus, connect the power source, run the electrophoresis for the appropriate time, turn off the power source and disconnect the gel, carefully remove the gel and observe the DNA bands.

5. How could gel electrophoresis be used to determine the size of DNA fragments in an unknown mixture?

The distance that the molecules of known sizes migrate can be compared to the distance that those of unknown sizes migrate.

Suggested Materials (per group)

gel electrophoresis apparatus
direct current power source
transfer pipettes
DNA samples

Electrophoresis kits containing DNA samples are available from biological supply companies. (Introductory kits that use prepared dyes instead of DNA are also available.) Follow the directions provided in the kit. Some directions may tell you to store the kits in the refrigerator. To save time, you may decide to prepare the gel and buffer beforehand and have the gel apparatus set up for students at the beginning of the lab period. If so, be sure to explain to students how this was done.

Safety



Put on safety goggles. Put on plastic gloves and a laboratory apron. Observe proper laboratory procedures when using electrical equipment. Never touch or taste any chemical unless instructed to do so. Note all safety alert symbols next to the steps in Design Your Experiment and review the meanings of each symbol by referring to Safety Symbols on page 8.

Design Your Experiment

Part A. Electrophoresis Technique

1. Using a transfer pipette, carefully load each of the DNA samples into the wells in the middle of the gel in consecutive order. Load each well until it is full. **Note:** *Do not move the apparatus after samples have been loaded.* **CAUTION:** *Wear safety goggles, plastic gloves, and a laboratory apron.* Demonstrate how to load samples into the wells.
2. After loading the samples, carefully close the cover of the apparatus.
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.

Follow instructions accompanying kit to prepare the gel and buffer and to set up the gel apparatus. If there is time, it may be worthwhile for students to help with this process. It is recommended that students practice loading samples before beginning experiment; some kits provide samples for this purpose.

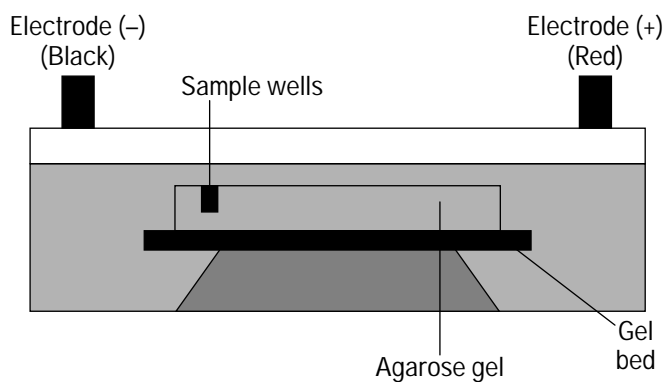
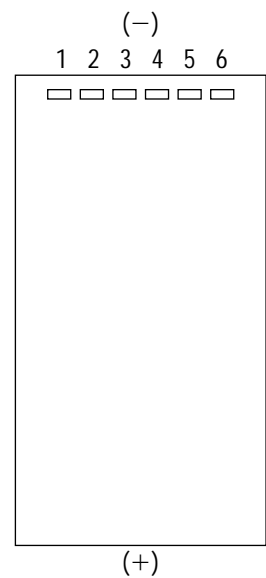


Figure 1

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. **CAUTION: Make sure that power is disconnected before removing the gel.**
8. In Figure 2, indicate the relative positions of the bands of DNA.



Part B. Your Own Experiment

1. In the spaces that follow, design an experiment to determine the size of DNA fragments in an unknown mixture using a DNA ladder (a mixture of DNA fragments of known size).
2. Submit a written experimental design to your teacher for approval using the space below. Once your teacher has approved your design, you may carry out your experiment.

Figure 2

See instructions included in kit for recommended voltage and running time.

Students should visually represent their results as accurately as possible in Figure 2.

(See kit instructions for position of bands.)

Provide students with a list of the sizes of the DNA fragments in the ladder mixture. The

procedure should be carefully examined and approved by the instructor before students begin. A

process similar to the one used in the first part of the lab should be followed.

Refer to the instructions provided with the DNA samples in the kit.

See kit instructions for more detailed instructions. Provide students with sizes of "known" molecules.

Hypothesis:

Sample: The relative position of the DNA samples on a gel can be used to determine the size of unknown molecules.

Manipulated variables:

Sizes of DNA molecules

Responding variables:

Positions of the bands on the gel

Procedure:

Procedure will be the same as the previous experiment; however, migration distances on the gel will be measured and compared to molecules of known size.

3. **Communicating Results** When you have finished running the gel, draw your results as accurately as possible on a separate sheet of paper. Number the lanes and show the relative positions of the bands. Measure and record the distance each band traveled on your drawing. Your teacher will provide you with the sizes of the DNA molecules in the ladder. Use this information to determine the sizes of the unknown DNA molecules.

Analysis and Conclusions

1. **Inferring** Why did some of the samples migrate greater distances than others?

The smaller the DNA molecules are, the farther they migrate.

2. **Drawing Conclusions** How did you determine the sizes of the unknown molecules?

The sizes of the "unknowns" were determined by comparing each migration distance to those of the ladder DNA fragments.

3. **Predicting** How would the concentration of the gel used affect the results of electrophoresis?

A more concentrated gel would be a denser sieve. DNA molecules would move more slowly through the gel, and it would take longer to run the gel at the same voltage.

Going Further

Research a genetic engineering technique that uses gel electrophoresis such as DNA fingerprinting (for determination of genetic diseases), recombinant DNA, or DNA sequencing. Write a short essay to explain the steps carried out in the technique and present your findings.