



Biotechnology in the Classroom

PARTNERSHIP FOR BIOTECHNOLOGY & GENOMICS EDUCATION
UNIVERSITY OF CALIFORNIA DAVIS

Basic Biotechnology Kit GEL ELECTROPHORESIS OF DYES

Partnership for Biotechnology and Genomics Education

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GEL ELECTROPHORESIS

Dye Samples



Introduction

In this experiment, students will be using agarose gel electrophoresis to separate several dyes—a precursor to the DNA separations we will perform in later labs.

This lab will help students gain familiarity with equipment and techniques essential to biotechnology. They will learn to cast and load an agarose gel as well as correctly utilize gel electrophoresis equipment. Attached readings and activities will better illustrate this important technique.

Note: Your students should have completed the **Micropipet Technique: Dye Samples** lab prior to running this experiment.

Objectives

1. Understand the principles behind gel electrophoresis.
2. Become familiar with preparing, loading and running a gel.
3. Determine the effect of a molecule's electric charge and size on its movement through an agarose gel.
4. Determine the components of an unknown dye mixture

Materials

For Each Lab Group

- Samples of the following dyes:
 - Bromophenol blue
 - Orange G
 - Pyronin Y
 - Safranin O
 - Xylene Cyanol
 - Unknown mixture
- microtube rack

- electrophoresis gel box
- 1 gel tray with 6 tooth comb
- 50 ml beaker or graduated cylinder *
- disposable plastic pipette
- 250 ml beaker *
- 20 μ l micropipet with tips
- gloves
- safety glasses *
- paper to cover lab bench *

Common Materials

- power supplies
- 60°C water bath
- 0.7% agarose solution (enough for 25 ml per team)
- plastic carboy with TBE buffer (1X)
- gel disposal bags
- distilled water *

* **Materials provided by instructor.**

Advance Preparation

1. Dilute the concentrated TBE (10X) solution to a final concentration of (1X) and store in a plastic carboy for easy access by students. After the lab, the solution may be poured back into the carboy and reused for the **Restriction Digest Laboratory**.

Figure on about 300 ml per group—3,600 ml if your class has twelve groups.

$$M_1V_1=M_2V_2$$
$$(\text{molarity of buffer})(\text{vol. buffer})=$$
$$(1X \text{ buffer})(\text{total vol. of rxn.})$$
$$(10)(x) = (1)(3,600 \text{ ml})$$
$$x = 360 \text{ ml}$$

Use 360 ml of (10X) buffer and add **distilled** water to bring the final concentration to 3,600 ml.



2. Loosen the lid from the bottle of 0.7% agarose. Microwave in a food safe oven until the gel is melted – watch carefully! Then, immerse the bottle in a **distilled** hot water bath to keep liquid until needed by the students. Students will need to handle the bottles so have potholders available.
3. Aliquot 25 μ l samples of the 6 dyes in microtubes. Two groups may share one set of dyes.

Teacher Notes

USE OF MICROPIPETS:

See notes in *Micropipet Technique* laboratory

BUFFER SOLUTION:

Tris/Borate/EDTA (TBE) buffer is commonly used in electrophoresis systems. This salt solution both conducts the electric current and controls the pH of the solution during separation of DNA fragments, or in this case, dye molecules.

DISTILLED WATER:

Minerals in regular tap water will quickly stain equipment. Please have your students rinse and dry both the gel trays and gel boxes in distilled water. Be careful not to dislodge the wiring at the base of the gel box during the drying process.

GEL DISPOSAL

These gels may be disposed of in the regular trash.

USE OF POWER SUPPLIES:

When two gel boxes are running off of one power supply, expect a lot of condensation on the box lids.

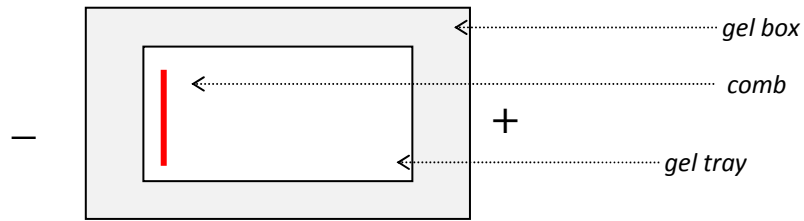
The power supply produces a voltage that is high enough to cause severe electrical shock if handled improperly.

- **DO NOT plug power supply into wall receptacle until the safety cover is positioned on the cell and all other electrical connections are properly made.**
- **This unit uses a 3-wire grounded plug. For safety reasons, it should NOT be used with 2-wire receptacle with a conversion plug.**
- **DO NOT operate in a damp or humid environment—any condensed moisture may short out electrical components. Make sure that all electrical equipment is dry.**
- **Inspect all power cords, patch cords, banana jacks and plugs for any defects, such as cracked and dried-out insulation and loose or wobbly banana jacks or plugs.**
- **DO NOT come in personal contact with or allow metal or any conductive material to come in contact with reservoir buffer or the electrophoretic cell while power supply is on.**
- **The power supply may continue to produce some voltage even when the power has been turned off. To eliminate any risk associated with this event, follow all required steps given in the procedure. When disconnecting the gel box be sure that the leads do not touch each other, come in contact with the buffer solution, or otherwise create any hazardous electrical condition.**



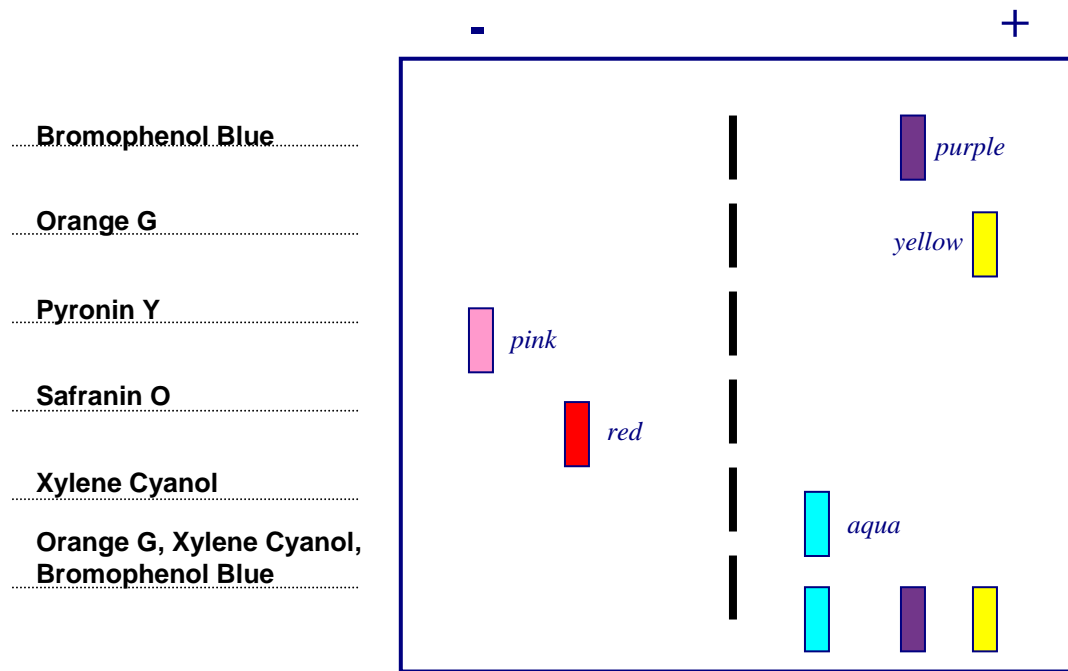
Answers to Student Activity

1.



2. The dyes have both positive and negative charges and will migrate in different directions.

3.



4. Bromophenol Blue, Orange G, and Xylene Cyanol.

5. Orange G

6. Bromophenol Blue, Orange G, Xylene Cyanol.

7. **Gel electrophoresis:** a technique used to separate charged molecular fragments in a matrix such as agarose, by applying an electric current. Fragments can be separated by size, because small fragments migrate quicker than large fragments.

GEL ELECTROPHORESIS

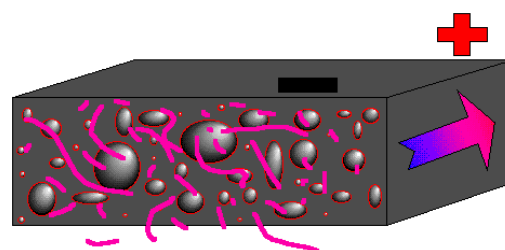
Dye Samples



Background

One of the most basic and frequently used tools of the molecular biologist is electrophoresis. In fact, DNA fingerprinting is based on this process. Electrophoresis can be used to separate molecules of different sizes and electrical charges, most often fragments of protein or DNA. In this lab, however, we will be using colored dyes. To perform gel electrophoresis three things are needed - a medium in which to separate the molecules, an electrical current, and a buffer solution.

During **agarose** electrophoresis, molecular fragments migrate through a gel when exposed to electricity. **Agarose** is a Jell-O-like substance derived from seaweed. The gel has a relatively large pore size, making it useful for size-separation of molecules. In the gel are several small wells, made by the teeth of a comb placed in the gel before it set. Samples to be tested are placed in the wells with a micropipet. The rate of movement of each sample depends upon molecule size and shape. In the presence of an electrical current, small molecules move quickly through the porous gel. Large molecules find it more difficult to move, so they travel more slowly. The direction of movement depends on the electric charge (+ or -) of the molecules, and the density of the gel through which the DNA moves. Since DNA molecules all have the same charge, they all move in the same direction – towards positive. Today, however, you will be separating different color dyes; some have a positive charge and others a negative charge.



A sponge makes a good comparison to a gel. Large and small particles would pass through a sponge at different rates according to particle size versus the size of the holes in the sponge.

When the gel is placed in a gel box, a buffer solution is poured over the gel, so that it fills the wells and makes contact with the electrodes at each end of the gel box. Salts dissolved in the buffer solution conduct electricity across the gel. The buffer also stops the gel from drying out. Prior to loading the samples in the wells of the gel, the molecules to be separated are mixed with a dense sugar solution, so that when the mixture is added to the wells, it sinks to the bottom, taking the test molecules (in this case, dye) with it. Without the sugar, the test molecules would simply float away in the buffer.

After the gel has been exposed to the electrical current – in this particular experiment, about 20 minutes - the dyes can be seen on the gel. However, remember that when using invisible DNA or protein fragments, the gel must be stained or specially-treated to reveal the familiar "bar code" patterns.

Attached readings and activities in the lab manual will help to clarify the process of electrophoresis.

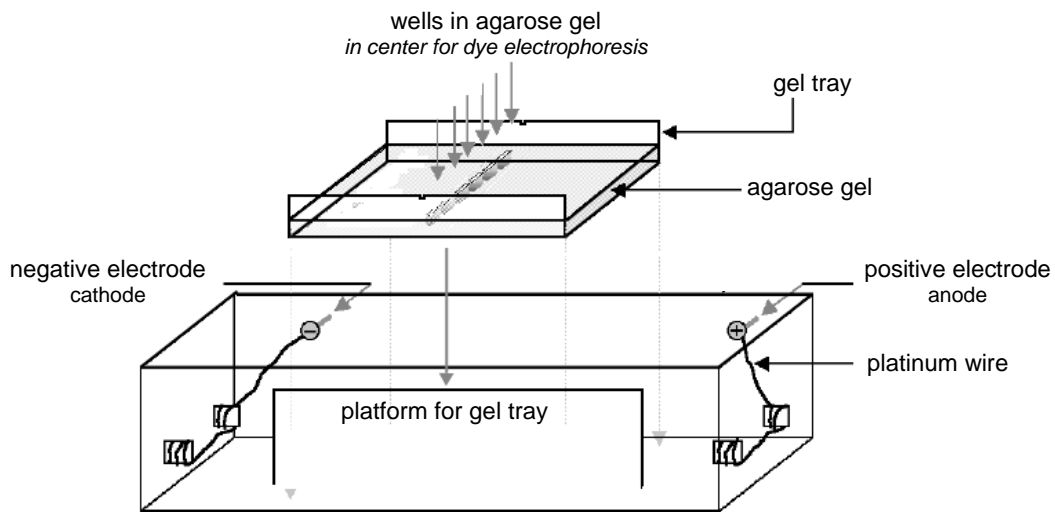


Diagram of Gel Box and Tray

Objectives

1. Understand the principles behind gel electrophoresis.
2. Become familiar with preparing, loading and running a gel.
3. Determine the effect of a molecule's electric charge and size on its movement through an agarose gel.
4. Determine the components of an unknown dye mixture

Materials

For Each Lab Group

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- microtube rack
- electrophoresis gel box
- 1 gel tray with 6 tooth comb
- 50 ml beaker or graduated cylinder *
- disposable plastic pipette
- 250 ml beaker *
- 20 μ l micropipet with tips
- gloves
- paper to cover lab bench *
- safety glasses *

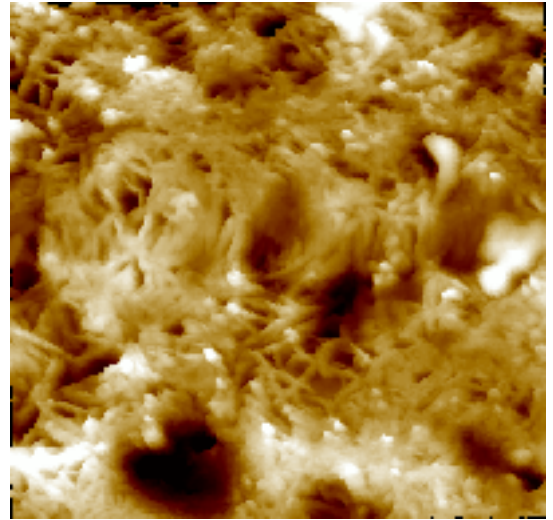
Common Materials

- power supplies
- 60°C water bath
- 0.7% agarose solution (enough for 25 ml per team)
- plastic carboy with TBE buffer (1X)
- gel disposal bags
- distilled water *
- *Materials provided by instructor.*



Precautions

- Follow directions in *Micropipet Technique* laboratory regarding micropipet handling.
- The power supply produces a voltage that is high enough to cause severe electrical shock if handled improperly. Assure that you are thoroughly drilled in the procedures regarding the use of this unit, making electrical connections, and that you are directly supervised by the teacher.
- DO NOT plug power supply into wall receptacle until the safety cover is positioned on the cell and all other electrical connections are properly made.
- Make sure that all electrical equipment is dry.
- DO NOT come in personal contact with or allow metal or any conductive material to come in contact with reservoir buffer or the electrophoretic cell while power supply is on.
- The power supply may continue to produce some voltage even when the power has been turned off. When disconnecting the gel box be sure that the leads do not touch each other, come in contact with the buffer solution, or otherwise create any hazardous electrical condition.



Polymerized agarose gel 1.5 μ m scan size.
Digital Instruments



Procedure

1. Gather all necessary materials. Put on gloves and safety glasses. *You will be sharing dye samples and a power supply with another lab group.*
2. Make sure the sides are up on your gel tray. If necessary, use tape to seal the ends. **Place the plastic comb in the middle of the tray.** Cover your lab space with paper prior to pouring the gel.
3. Go to the hot water bath and fill a 50 ml beaker with 25-30 ml of melted agarose.
4. Using a disposable plastic pipette, run a thin line of agarose around the inner edge of your gel tray.
5. Pour the remaining agarose into the gel tray. **Liquid should be approximately 1/3 of the way up the plastic teeth of the gel comb.** Remove any bubbles in the solution with a disposable pipette.
6. Let the gel harden on your desk for at least 15 minutes.
7. Once solidified, make sure that the gates are down or that the tape is removed from the ends of the tray. Carefully remove the comb by pulling straight out of the solidified gel. Place your gel tray on the center island of the gel box.
8. Obtain approximately 250 ml of TBE buffer from the plastic carboy. Pour the buffer into the side compartments of the gel box to completely fill the box and to cover the top gel surface with about 2 mm of solution. **Do not pour buffer directly onto the well area of the gel!** After filling gel box, pour any remaining TBE buffer back into the carboy.

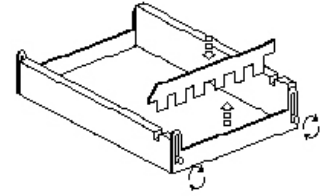
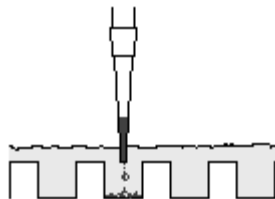


Diagram of gel tray – use screws on side of tray to raise and lower gates. If gates are not present, use lab tape to seal ends.

⌚ **STOP POINT:** If there is not enough time to run the gel in this lab period, gel boxes can be closed and left until the next class meeting.

9. On the gel, load 10 μ l of each dye into a corresponding lane. Keep track of which dye goes into which lane on the accompanying activity sheet. **Use a new tip for each dye and be careful not to puncture the bottom of the well.** Your teacher will demonstrate.

| | | | |
|---------|------------------|---------|---------------------|
| Lane #1 | Bromophenol Blue | Lane #4 | Safranin O |
| Lane #2 | Orange G | Lane #5 | Xylene Cyanol |
| Lane #3 | Pyronin Y | Lane #6 | Unknown dye mixture |



Insert tip of micropipet just over the top of the well and eject dye. The dyes are loaded with a sucrose solution and will sink. If you push the tip down too far, you may puncture the well.



10. Clean up any spilled buffer or any other liquid surrounding the gel box thoroughly.
11. **Make sure that the power supply is unplugged and switched off before proceeding.**
12. Carefully place the lid on the gel box (first making sure it is clean and dry).
13. Connect the red (positive) patch cord to the red terminal on the power supply. Similarly, connect the black cord to the black terminal. Each power supply will run two gel boxes. Notice what channel you have plugged your box into.
14. Wait until the group that is sharing your power supply has completed steps 1-12 before proceeding.
15. **IMPORTANT!** Have your teacher look over all of your connections before plugging in the power supply.
16. Plug in the power supply and turn on the machine on. The run light will illuminate, signifying that power is running to the cell.
17. Turn the display select switch to the appropriate channel for your group. Turn the voltage indicator knob to 100V. **Make sure both groups set their voltage.** *Observe the bubbles that form along the platinum electrodes.*
18. Let your gel run undisturbed for 20 - 30 minutes. Keep checking your gel to assure that the dyes do not run off the end of the gel into the buffer compartment. During this time complete the attached activity sheet.
19. When the dyes reach approximately 2 cm from the ends of the gel, turn off the power supply. Then disconnect the patch cords from the power supply and unplug the unit.
20. Make sure your gloves are on. Remove your gel tray from the gel box carefully. Tip tray over gel box to discard excess buffer solution. Record your observations on the activity sheet.
21. Follow your teacher's instructions for clean-up. Micropipet tips and gels may be discarded in trash. Gel boxes and trays must be rinsed in **distilled water** and air dried. Do not use paper towel to dry inside the boxes! Please be careful with the equipment.
22. Wash hands thoroughly and clean lab station before leaving the lab.



GEL ELECTROPHORESIS

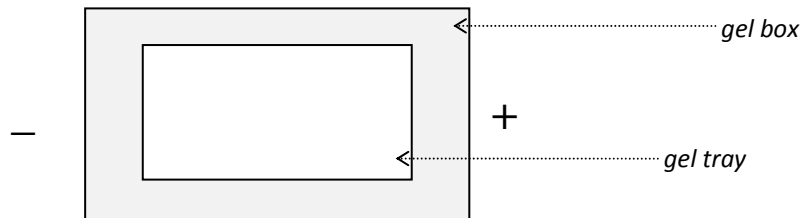
Dye Samples



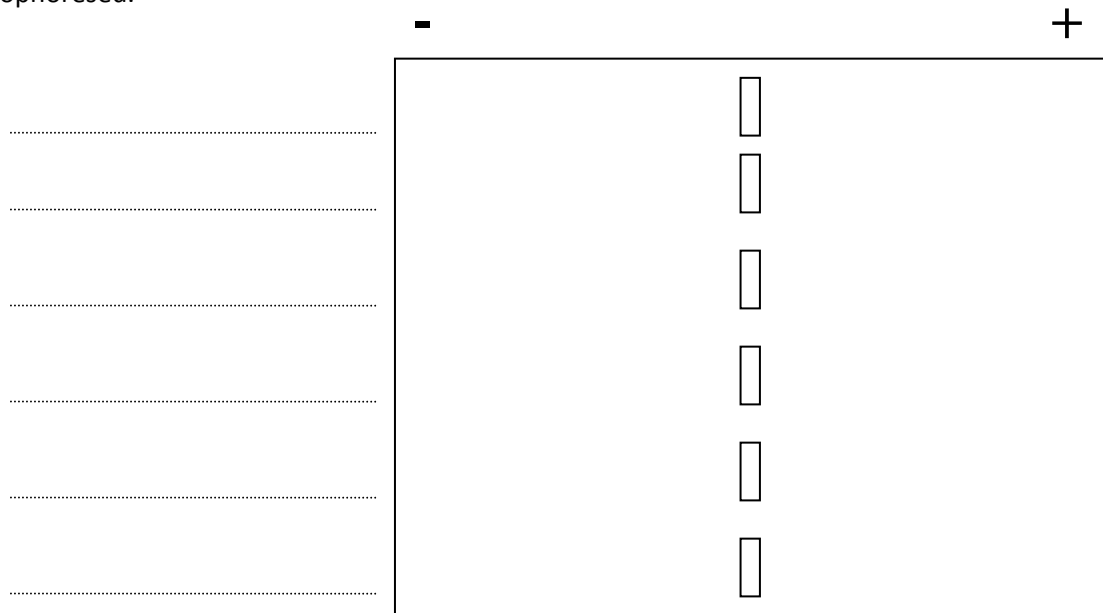
Name: _____

The sides of the DNA ladder are made up of alternating sugar and phosphate groups. These phosphate groups (PO_4) give DNA an overall negative charge.

1. If you were pouring a gel for DNA agarose gel electrophoresis, where would you place the comb? Draw a picture below.



2. Why is the comb placed in the center for this dye electrophoresis experiment?
3. The diagram below represents your gel. Using colored pencils or markers, draw in the results of your dye separation. Be sure to label each lane with the name of the dye that was electrophoresed.





4. What dyes were in the unknown mixture?
5. Which negatively charged dye seems to be the smallest molecule?
6. List the dyes that have a negative charge.
7. In your own words, explain how agarose gel electrophoresis works.