Gel Electrophoresis of Dyes
An Introduction

Rationale
This lab provides a beginners' introduction to the function of a gel electrophoresis apparatus. It will train students how to make an agarose gel and set up the electrophoresis apparatus. After students complete this exercise, they can be held responsible for setting up any future labs that involve electrophoresis.

This lab may not be necessary for advanced students who have had previous lessons in gel electrophoresis in a biology course.

Objectives
Students will:
- Understand the principles behind gel electrophoresis.
- Become familiar with the procedure for running a gel.
- Determine the components of an unknown dye mixture.
Engage

- Pre-assess what students have already learned in previous biology courses by asking:
  - What property of DNA is the basis for sorting DNA fragments by gel electrophoresis?
  - Why don't all DNA fragments move through the gel at the same rate?
  - Why do scientists include a marker when they run a DNA sample?
  - View Virtual Electrophoresis as a class to clarify above questions.

Key Points

Activity

1. Students complete the Pre-Lab Activity. This activity allows for gel preparation and practice loading of gels. Assessment is skill based and can be easily recorded as teacher initial.
   a. Gels can be stored in a plastic bag with buffer for up to a week.

   Pre-Lab Set Up:
   - Gel preparation materials (agarose, buffer, casting trays, hot plate, microwave).
   - Make a 0.8% agarose gel for students to practice loading samples under buffer.
   - Dyes can be ordered from science supply company and/or ask the chemistry department on your campus. These are often used in chemistry courses. Dye recipe provided at end of lesson.

2. Lab Set-Up Tip: Have students set up their lab stations using a set-up key. If they have been trained to know the locations of all lab supplies, this should be an easy task for them. An example lab station set-up is below. A printable copy can be found at the end of the lesson.
3. Lab Grading Tip: Students sign up with their lab groups on the “Lab Station Sign Up” sheet. Display sheet on front board. Mark lab station for infractions/accomplishments during the lab (example: leaving a dirty station, correctly making a gel, setting up lab station properly, misconduct, open toe shoes, violation of safety rule). At the end of lab, record grade in grade book as lab grade. An example “Lab Station Sign-Up” is below. A printable copy can be found at the end of the lesson.

4. Students complete Lab Activity. Sample Lab Results and Key below:
5. Students complete “Post Lab” Handout.
6. Students peer-grade “Post Lab” using grading criteria provided.
7. Randomly select “Post Lab” to check for accurate grading.

Assessment
- Pre-Lab Skill Assessment
  o Calculations
  o Preparing agarose gel
  o Loading agarose gel under buffer
- "Lab Station Sign Up" Grade
- Post Lab Questions
- “Grading Criteria” Handout

Materials
- Internet access needed to view procedural videos in pre-lab
- Worksheet: "Pre-Lab Gel Electrophoresis: Dye Samples"
- Lab Activity: “Gel Electrophoresis: Dye Samples”
- Worksheet: “Post Lab Gel Electrophoresis: Dye Samples”
- Handout: “Preparation of Dyes for Electrophoresis”
- Handout: “Lab Station Set-Up: Gel Electrophoresis”
- Sheet: “Lab Station Sign Up”
Lab Materials
For Each Lab Group:
- Samples of the following dyes:
  - Crystal violet
  - Orange G
  - Methylene blue
  - Xylene cyanol
  - Safranin O
  - Bromophenol blue
  - Unknown sample
- 1 microtube rack
- 1 electrophoresis gel box
- 1 0.8% agarose gel that has been cooled and initialed by instructor
- 250 ml beaker
- 250 ml flask of TBE buffer (1X)
- 20 μl micropipette and tips

Common Materials
- power supplies
- distilled water
- biohazard can

Accommodations for Learning Differences
- Visit the Special Populations section of the CTE Career and Technical Education Website: http://cte.unt.edu/special-pops.

National and State Education Standards
Texas College and Career Readiness Standards
I. C1, C2, C3, E1, E2
II. A7
III. A1, B1, B2, B3, C1
Gel Electrophoresis: Dye Samples

1. View Instructional Video :Pouring an Agarose Gel and answer the questions below.

2. Show calculations to prepare 30ml of a 0.8% agarose gel.

   Instructor Initials: Calculation Correct

3. Why is it necessary to TARE the electric balance?

4. The purpose of the foil is

5. How long will you boil the solution?

6. The solution should be cooled below _______ because _______________________

7. Sketch and label the parts of a gel casting tray.

Instructor Initials: Gel Made Correctly

8. Generate an SOP using Microsoft Word for making an agarose gel. Print SOP out and turn in with your lab activity.

9. Prepare a 0.8% agarose gel using your SOP.

10. Once your gel has solidified, your instructor will verify that the gel was made correctly.
11. View Instructional Video: Preparation of the Agarose Gel

12. The gel should be placed in the electrophoresis chamber with the wells closest to the
   ________________ because ________________

13. Could distilled water be used in place of buffer? Explain.

14. Show what the window of a P-20 would look like if it were set to 10ul:

15. Predict the consequences of:
   a. Expelling the liquid too fast: ________________________________
   b. Inserting the tip too far into the well: __________________________
   c. Pressing beyond the first stop of the micropipettor: ______________
   d. Releasing the micropipettor while still under buffer: ______________

16. How will you be able to tell if the gel is running properly?

17. Practice loading a sample in the sample well at your lab bench. Have your instructor check your
technique.
Gel Electrophoresis: Dye Samples

Laboratory

Objectives
1. Understand the principles behind gel electrophoresis.
2. Become familiar with the procedure for running a gel.
3. Determine the components of an unknown dye mixture.

Materials
For Each Lab Group:
- Samples of the following dyes:
  1. Crystal violet
  2. Orange G
  3. Methylene blue
  4. Xylene cyanol
  5. Safranin O
  6. Bromophenol blue
  7. Unknown sample
- 1 microtube rack
- 1 electrophoresis gel box
- 1 0.8% agarose gel that has been cooled and initiated by instructor
- 250 ml beaker
- 250 ml flask of TBE buffer (1X)
- 20 μl micropipette and tips

Figure 1: DNA is made up of sugar and phosphate molecules which are held together by phosphodiester bonds. The phosphate molecules on the DNA backbone are negatively charged. These phosphate molecules confer a negative charge on the entire DNA molecule. Therefore, because opposites attract, when placed in an electrical field, the negatively charged DNA will migrate toward the positive pole.

Figure 2: Because all DNA is negatively charged, regardless of the length or source, the rate of DNA migration and separation through an agarose gel depends on the size of the DNA molecule. Agarose provides a gel matrix through which the DNA molecules migrate. Smaller molecules travel faster through the gel matrix than larger molecules.
Procedure

1. Gather all necessary materials. Put on safety glasses. You will be sharing a power supply with another lab group.

2. Once your gel has solidified, remove the dams from the side of the tray. Carefully remove the comb by pulling straight out of the solidified gel. Place the gel in the chamber.

3. Pour the TBE 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!

4. Using the P20 pipettor, load 10 µl 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. Dispose of used tips into the 1 L beaker. Your lab instructor will demonstrate loading.

   | Lane #1  | Lane #2  | Lane #3  | Lane #4  |
---|----------|----------|----------|----------|
Crystal violet (C) | Orange G (O) | Methylene blue (M) | Xylene cyanol (X) |
Safranin O (S) | Bromophenol blue (B) | nothing | |

5. Make sure that the power supply is unplugged and switched off before proceeding.

6. Carefully place the lid on the gel box (first making sure it is clean and dry).

7. 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.

8. Wait until the group that is sharing your power supply has completed steps 1 - 10 before proceeding.

9. Plug in the power supply and turn on the machine.

10. Turn the voltage indicator knob to 100V. Observe the bubbles that form along the platinum electrodes. If there are no bubbles your unit is not working. After 5 minutes check your gel to make sure your sample have begun to migrate out of the wells moving in the right direction.

11. Let your gel run undisturbed for 20 - 40 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.

12. 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.

13. Carefully remove your gel tray from the gel box. Tip tray over gel box to return excess buffer solution into the TBE container (it will be re-used). Record your observations (dye location, distance traveled) on the post-lab answer document.
Gel Electrophoresis: Dye Samples

The sides of the DNA ladder are made up of alternating sugar and phosphate groups. These phosphate groups (PO₄) 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.

   ![Diagram of gel box and gel tray]

2. Why is the comb placed in the center for this dye electrophoresis experiment?

3. For each known sample, add to this diagram a spot where the dye migrated.

   - Crystal violet
   - Orange G
   - Methylene Blue
   - Xylene cyanol
   - Safranin O
   - Bromophenol blue
4. Fill in the following table with your observations. When measuring distance dye migrated from well, measure the distance from the center of the well to the center of the dye spot.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Dye</th>
<th>Size</th>
<th>Distance migrated from well (mm)</th>
<th>Color of Spot</th>
<th>Moved toward which electrode (+ or -)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crystal violet</td>
<td>408</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Orange G</td>
<td>452</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Methylene blue</td>
<td>373</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Xylene cyanol</td>
<td>538</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Safranin O</td>
<td>351</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bromophenol blue</td>
<td>670</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Unknown #</td>
<td>??</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Create a standard curve comparing distance migrated (x-axis variable) to size of the dye (y-axis variable) using EXCEL. Add a trendline to the graph. Print your graph and attach it to this sheet.

a. What can you conclude about the relationship between distances each dye migrated compared to each dye’s size and how did you arrive at this conclusion?

6. Using your trendline data, identify which dyes are in the unknown sample?

7. List the dyes that have a net negative charge. Which electrode did they migrate towards?

8. List the dyes that have a net positive charge. Which electrode did they migrate towards?

9. In your own words, explain how agarose gel electrophoresis works.
## Gel Electrophoresis: Dye Samples

### Pre-Lab

<table>
<thead>
<tr>
<th></th>
<th>ALL (5 pts)</th>
<th>MOST (3 pts)</th>
<th>FEW if ANY (1pt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teacher Initials Obtained</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questions Answered with Thought</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Lab

<table>
<thead>
<tr>
<th></th>
<th>ALL THE TIME (5 pts)</th>
<th>MOST OF THE TIME (4pts)</th>
<th>RARELY (1pt)</th>
<th>NEVER (0 pt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On TASK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Followed Directions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proper Use of Equipment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaned Lab Table</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

### Post Lab Handout

<table>
<thead>
<tr>
<th></th>
<th>ALL (10 pts)</th>
<th>MOST (8 pts)</th>
<th>FEW if ANY (1pt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Questions Answered Correctly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data Table Complete</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graph Generated with Criteria</td>
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</table>

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Preparation of Dyes for Electrophoresis

This procedure requires making a 2% stock for each dye. The dye stocks are then diluted into a sugar-based solution for loading the agarose gel. The sugar, such as glycerol, increases the density of the sample so that it sinks to the bottom of the well.

2% Dye Stock
The dye stocks are used to prepare samples for agarose gel electrophoresis with dyes.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balance</td>
<td>Pyronin Y</td>
</tr>
<tr>
<td>6 tubes with caps (10-15 ml size) Methyl Orange</td>
<td>Xylene Cyanol</td>
</tr>
<tr>
<td>10 ml graduated cylinder or pipet Safranin O</td>
<td>Bromphenol Blue</td>
</tr>
<tr>
<td>Weighing paper</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Deionized or distilled water</td>
</tr>
</tbody>
</table>

1. Measure 0.2 g of one of the dye powders on a square piece of folded weighing paper.

2. Using the crease in the paper, pour the dye powder into a tube. The tube should be able to hold between 10 and 15 ml.

3. Add 10 ml deionized or distilled water to the dye and cap the tube. Shake the tube well to dissolve the dye in the water. For some dyes, not all the powder will go into solution.

4. Repeat steps 1-3 for each dye.

5. The dye stocks (2% concentration) can be stored indefinitely at room temperature.

Dye Sample
The dye samples for electrophoresis are prepared from 2% dye stocks. The colored samples allow students to observe the dye molecules migrate during electrophoresis. One of the samples is the "unknown," which contains a mixture of three of the other dyes.
<table>
<thead>
<tr>
<th>Equipment</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml pipet or graduated cylinder</td>
<td>Glycerol</td>
</tr>
<tr>
<td>25 ml graduated cylinder</td>
<td>2% dye stocks</td>
</tr>
<tr>
<td>Small container with cap or lid for each dye</td>
<td>Deionized or distilled water</td>
</tr>
<tr>
<td>Stirring rod</td>
<td></td>
</tr>
</tbody>
</table>

The dye stocks are Methyl orange, Bromophenol blue, Xylene cyanol, Pyronin Y, Safranin O, and Unknown.

1. Prepare a 50% glycerol solution. Pour 10 ml glycerol into a 25 ml graduated cylinder (note: glycerol is very viscous). Next, add 10 ml deionized or distilled water to the same cylinder. Use a stirring rod (a pipet would also work) to mix the glycerol and water. The glycerol solution can be stored in a small, closed container in the refrigerator.

2. Prepare a dilution of the dye stock for electrophoresis. Using a pipet, add 2 ml 50% glycerol solution into a 25 ml graduated cylinder.

3. In the same cylinder add 1 ml of a 2% dye solution.

4. Add 17 ml deionized or distilled water to make a final volume of 20 ml. Use a stirring rod to mix the ingredients together. This batch of dye sample is ready to use - we use 12 ul of the dye sample per lane on an agarose gel. You can store any unused dye sample in a small closed container in the refrigerator. Repeat steps 2 - 4 for each dye stock.

5. Prepare the unknown dye sample. Add 2 ml 50% glycerol solution to a 25 ml graduated cylinder. Next, add 1 ml each of 2% methyl orange, 2% bromophenol blue, and 2% xylene cyanol. In the same cylinder add 15 ml deionized or distilled water. Use a stirring rod to mix the ingredients together. The unknown dye sample is ready to use, and store any unused dye sample in a small closed container in the refrigerator.
Gel Electrophoresis Lab Station Set-up

- Microtube Rack
  - Collect 7 dye samples from instructor

- 250 ml beaker

- TBE Buffer

- Agarose gel made in Pre-Lab Activity

- HOT PLATE

- Ruler

- Common Lab Area Materials
  - Electrophoresis Box
  - Power Supply
  - Distilled Water
  - Electric Balance
  - Agarose
Lab Station Sign Up for Period _______
Lab Exercise:___________________ # of Points: ______

<table>
<thead>
<tr>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
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<tbody>
<tr>
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<td>Station 4</td>
<td>Station 5</td>
<td>Station 6</td>
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