Dyeing for Electrophoresis

Introduction

How can a mixture of molecules, too small to be seen with even a high-powered microscope, be separated from one another? Such was the dilemma facing scientists until the development of a process that is now standard in laboratories worldwide—gel electrophoresis. Laboratories rely heavily on this proven and reliable technique for separating a wide variety of samples, from DNA used in forensics and for mapping genes, to proteins useful in determining evolutionary relationships.

Concepts

- Biological molecules
- Electrophoresis

Materials

Agarose, 0.48 g
Biological dye solutions, 0.5 mL, 6
Glycerin, 3 mL
Tris-acetate electrophoresis buffer, 50X, 10 mL
Water, deionized or distilled (DI)
Balance, 0.01-g precision
Cotton, non-absorbent or foam plug
Electrophoresis chamber
Erlenmeyer flask, 500-mL
Erlenmeyer flask, borosilicate, 125-mL
Gel casting tray with well comb
Graduated cylinder, 25-mL
Graduated cylinder, 100-mL
Marker or wax pencil
Microwave or stirring hot plate
Overhead projector
Pipets, graduated, disposable, 7
Pipets, needle-tip, disposable or micropipets with tips, 6
Power supply
Spot plate or reaction plate
Stirring rod
Thermometer, 0–100 °C
Toothpicks, 6
Weighing dish, small

Safety Precautions

Be sure all connecting wires, terminals and work surfaces are dry before using the electrophoresis chamber and power supply. Treat these units like any other electrical source—very carefully! Do not try to open the lid of the chamber while the power is on. Use heat protective gloves and eye protection when handling hot liquids. Biological dyes and stains will stain clothes and skin—avoid all contact. Many biological dyes are also moderately toxic by ingestion. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Prepare the biological dyes for electrophoresis.
   a. Use a clean graduated pipet to measure 0.5 mL of the biological dye. Transfer this dye into well 1 of the spot plate.
   b. Use a clean graduated pipet to measure 0.5 mL of glycerin. Add the glycerin to well 1.
   c. Mix the glycerin and dye thoroughly with a toothpick.
   d. Repeat steps *a* through *c* using a clean well and a clean graduated pipet for each biological dye or stain provided (see *Discussion* section for a list of possibilities).
2. Prepare 500 mL of 1X tris-acetate electrophoresis buffer.
   a. Use a graduated cylinder to measure 10 mL of the 50X-concentrate tris-acetate electrophoresis buffer into a 500 mL Erlenmeyer flask.
   b. Dilute to 500 mL with DI water.

Publication No. 10901
111417

3. Prepare a 0.8% agarose gel.

   a. Weigh out 0.48 g of agarose.

   b. Use a graduated cylinder to transfer 60 mL of the 1X tris-acetate electrophoresis buffer into a clean 125-mL Erlenmeyer flask.

   c. Mark the level of the liquid on the side of the Erlenmeyer flask. (Stopper the flask with non-absorbent cotton or a foam plug.)

   d. Dissolve the agarose by heating the flask in a microwave, or on a stirring hot plate. Microwave for 30–40 seconds, stir, and repeat. *Caution:* Be careful not to superheat the solution because it will NOT boil until it is disturbed, whereupon it may spontaneously boil out. Use heat protective gloves when handling the hot flask.

   e. Heat until the solution is clear and agarose appears to be fully dissolved.

   f. Check the level of the solution. Add distilled or deionized water, if needed, to bring the volume back to the original height on the flask.

   g. To prevent damage to the gel casting trays, allow the agarose to cool to 55 °C before pouring into the gel casting trays.

   h. Ensure the gel casting tray is on a level surface and place the well-forming combs in the *center* position in the gel casting trays.

   i. Slowly pour the melted agarose into the gel casting tray, being careful not to create bubbles in the gel. Only add enough agarose to fill the tray 2–3 mm above the bottom of the well-forming comb.

   j. Allow the agarose to sit undisturbed in the casting tray for at least 20 minutes until the gel is firm to the touch. The set gel will appear opaque and somewhat white. Sixty minutes or more is optimal.

   k. Once the gel is thoroughly set, carefully remove the well-forming comb and the end dams of the gel casting tray. Be careful not to tear the gel. If the gel does tear, remelt and repour the gel.

4. Prepare to run the gel.

   a. Assemble the electrophoresis chamber according to the manufacturer's instructions.

   b. Place the electrophoresis unit on an overhead projector. Do not move the electrophoresis chamber after loading the samples.

   c. Place the agarose gel and gel casting tray, without end dams or end tape, into the electrophoresis chamber.

   d. Pour enough electrophoresis buffer into the chamber to submerge the entire gel surface to a depth of 2–5 mm.

   e. Withdraw 10 µL one of the prepared biological dye samples (see step 1 *a–d*) by filling only the needle tip of a needle-tip pipet.

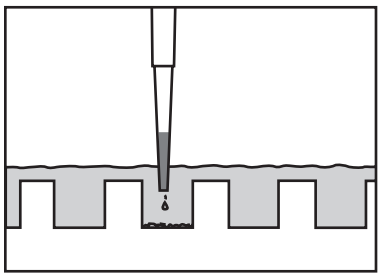

Figure 1.

   f. Dispense the dye sample into the well by holding the pipet tip just above the well. The sample will sink to the bottom of the well. Repeat, using a fresh pipet for each biologigal dye or stain (see Figure 1).

5. Run the gel.

   a. Place the lid on the electrophoresis chamber and connect the unit to the power supply according to the manufacturer's instructions.

   b. Run the gel at 125V until the biological dyes have separated sufficiently for students to see how size and charge affect how far and in which direction each dye migrates within the gel (about 20 minutes).

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. All solutions used in this lab may be disposed of down the drain with plenty of excess water according to Flinn Suggested Disposal Method #26b. Agarose gels may be disposed of in the regular trash according to Flinn Suggested Disposal Method #26a.

Tips

- Many biological dyes and stains may be used in this activity. As always, test each one first to see how well they work. Some dyes may need to be diluted prior to use. See the *Discussion* section for examples.
- The glycerin alters the density of the solutions, ensuring they sink into the agarose well.
- Gel loading solution contains bromphenol blue, xylene cyanol dyes and sucrose to increase the density of the solution. Gel loading solution may be loaded into a well without diluting or adding glycerin. Two different colored dye bands will separate during the electrophoresis.
- Mix two or more dyes that will not move at the same rate through the gel (for example, malachite green and methyl orange). Allow students to see how the dyes separate during electrophoresis.
- Extend the activity by having students research the molecular structure of the dyes and stains to determine why they migrated the particular direction and distance they did.
- Zwitterions, like Rhodamine B, will not migrate through the gel. Zwitterions, like amino acids, are dipolar ions with both positive and negative side chains on the same molecule. The positive and negative side chains on Rhodamine B create a net neutral charge at pH 7.8, the pH of the electrophoresis buffer.
- Flinn Scientific offers many gel electrophoresis kits. "A Process to Dye for: Gel Electrophoresis" (Flinn Catalog No. FB1736) and "Why Do People Look Different?" (Flinn Catalog No. FB1817) are both dye-based electrophoresis kits. "DNA Forensics" (Flinn Catalog No. FB1798), "DNA Paternity Testing" (Flinn Catalog No. FB1799), and "The Genetics of Cancer" (Flinn Catalog No. FB1800) are all DNA electrophoresis kits.
- The Flinn Scientific Electrophoresis Guide (Flinn Catalog No. FB1904) is a complete reference containing many electrophoresis tips.

Discussion

As it became more important for biological substances to be identified by their molecular structures rather than by direct observation, scientists were challenged with the task of separating and isolating molecules from one another. Gel electrophoresis is a laboratory technique used to separate segments of DNA, RNA or proteins according to the size of the segment and the relative electric charge of that segment.

In 1950, American scientist Oliver Smithies (born 1925) determined that a gel made of starch acts like a molecular filter or sieve for biological molecules when it is positioned between positive and negative electrodes. When molecules of different sizes and shapes or mixtures of molecules are placed within the gel and an electric current is applied, the molecules travel through the gel and separate from one another according to each molecule's charge, size, and shape. Molecules with a negative charge, such as DNA, migrate through a gel toward the positive electrode. Small globular molecules are able to travel through the gel faster than large linear molecules. When the current is turned off, all the molecules are stopped within the gel. Biological dyes and stains each have a different size, shape and charge making them ideal models for introducing the principles of electrophoresis to students.

Many biological dyes and stains are also acid–base indicators. The color of an acid–base indicator will change depending upon the pH of the solution. Each indicator is unique; please refer to the *Flinn Scientific Catalog/Reference Manual* for more information about each indicator. Table 1 lists some common biological dyes and stains, their formula weights and charges, the recommended concentrations, and the color of each dye in tris-acetate electrophoresis buffer.

Biological dye or stain	Formula Weight	Charge	Concentration (%)	Color in tris-acetate Buffer
Alizarin red	342	-	1	Pink
Bromcresol green	720	-	0.04	Blue
Bromcresol purple	562	-	0.04	Purple
Bromphenol blue	692	-	0.04	Purple
Bromthymol blue	646	-	0.1	Blue
Chlorphenol red	423	-	0.04	Hot pink
Congo red	697	-	0.1	Orange
m-Cresol purple	404	-	1	Brown
Crystal violet	408	+	0.04	Purple
Fuchsin basic	586	+	0.04	Red
Janus green B	511	+	1	Blue
Malachite green	464	+	0.04	Turquoise
Methyl orange	328	-	0.1	Yellow
Methyl red	291	-	0.02	Yellow
Methylene blue	374	+	0.04	Blue
Neutral red	289	+	0.5	Orange
Safranin O	351	+	0.04	Red
Thymol blue	489	-	0.04	Yellow
Toluidine blue O	306	+	0.04	Blue
Xylene cyanol	539	-	0.04	Blue

NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

Disciplinary Core Ideas: Middle School

MS-LS1 From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

MS-LS3 Heredity: Inheritance and Variation of Traits
- LS3.A: Inheritance of Traits
- LS3.B: Variation of Traits

Disciplinary Core Ideas: High School

HS-LS1 From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

HS-LS3 Heredity: Inheritance and Variation of Traits
- LS3.A: Inheritance of Traits

Science and Engineering Practices

- Developing and using models
- Analyzing and interpreting data
- Constructing explanations and designing solutions

Crosscutting Concepts

- Patterns
- Cause and effect
- Structure and function

Materials for *Dying for Electrophoresis* are available from Flinn Scientific, Inc.

Catalog No.	Description
A0209	Alizarin Red S, 5 g
B0039	Bromcresol green, 1 g
B0043	Bromphenol blue, 1 g
C0280	Crystal violet, 1 g
FB0324	Electrophoresis Reagent Package
FB1904	Flinn Scientific Electrophoresis Guide
G0019	Glycerin, 100 mL
M0072	Methylene blue, 25 g
M0129	Malachite green, 10 g
S0339	Safranin O, 10 g

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.