

Introduction to Electrophoresis

Running a DNA Sample on an Agarose Gel



Introduction

Deoxyribonucleic acid (DNA) is the genetic material found inside the nucleus of eukaryotic organisms. The information coded by DNA determines the characteristics of an organism, including its size, shape and other unique features. How can this genetic material be isolated and identified? This activity describes one of the most common techniques used to examine DNA—electrophoresis.

Concepts

- Electrophoresis
- Deoxyribonucleic acid (DNA)

Background

All eukaryotic organisms have DNA in their nuclei, including humans. The specific sequence of the nucleotide bases in DNA makes each individual of a particular species unique. Different sections of DNA code for specific genes. Most genes actually are blueprints for specific proteins used by the organism to do a multitude of different things. Slight changes in the DNA sequence in a specific gene may alter the protein that is produced by the cell. Variations in DNA sequences also account for phenotypic visible variance among individuals, such as blue eyes versus brown eyes.

Materials

Agarose, 0.48 g	Gel loading solution, 6X, 0.5 mL
Fish DNA, 1 g	Light box (optional)
Methylene blue, 0.02%, 40 mL	Micropipets, 3
Tris-acetate (TAE) buffer solution, 350 mL	Microwave, hot water bath or hot plate
Beakers	Mini-gel casting tray with end dams
Comb, 6-well	Rubber gaskets, 2
Electrophoresis chamber with power supply	Spot plate
Erlenmeyer flask, 250-mL	Staining tray

Safety Precautions

Do not operate the power source with wet hands or on a wet surface. Make sure the power supply is off before connecting the leads to the electrophoresis apparatus. Turn off the power supply before disconnecting the leads and removing the cover at the end of the demonstration. Never reach in the electrophoresis apparatus while the power supply is on. Methylene blue and gel loading solution will stain skin and clothing. Wear chemical splash goggles, chemical-resistant gloves, and chemical resistant apron. Please review current Safety Data Sheets for additional safety, handling and disposal information.

Procedure

Part A. Loading and Running the Gel

1. Place the rubber gaskets on the ends of the mini-gel casting tray and insert the 6-well comb into the end position of the tray.
2. Prepare a 0.8% agarose gel by combining 0.48 g of agarose with 60 mL of TAE buffer solution in an Erlenmeyer flask.
3. To dissolve the agarose, heat the buffer solution to about 80 °C, stirring frequently.
4. Cool the resulting agarose gel solution to about 50 °C, and then pour the solution into the mini-gel casting tray.
5. While the gel is solidifying, fill the electrophoresis chamber with about 250 mL of TAE buffer solution.

6. To prepare the DNA, add 1 g of fish DNA to 30 mL of TAE buffer. *Note:* The fish DNA tends to clump like starch so add slowly to buffer and stir vigorously.
7. Using a micropipet, transfer two drops of fish DNA to five wells on a spot plate. Add two drops of 6X gel loading solution to each of the six wells
8. Combine the loading solution and DNA by flushing with a clean pipet two or three times.
9. After the gel has solidified (about 20 minutes), carefully remove the comb and gaskets from the end of the tray and submerge the gel (on the tray) in the electrophoresis chamber filled with TAE buffer (step 5).
10. Using a micropipet, load each of the five samples into a different well on the agarose gel.
11. Run the sample for about 20 minutes at 125 V until the first gel loading dye marker (bromophenol blue) is about half way across the gel. *Note:* The fish DNA will run ahead of the first dye marker.
12. Turn off the electrophoresis apparatus.

Part B. Staining the Gel

1. Remove the gel and tray from the electrophoresis chamber.
2. Slide the gel off of the casting tray onto a staining tray. Gently pour 40 mL of 0.02% methylene blue staining solution into the staining tray. *Note:* Wear gloves—methylene blue will stain.
3. Allow the gel to stain for five minutes. Pour off the stain into the “used methylene blue” beaker. The stain may be reused.
4. To destain the gel, gently pour cold tap water into the staining tray. Do not exceed 37 °C—warmer water may soften the gel.
5. Occasionally agitate the water for two minutes. Repeat steps 1–3 until the DNA bands are clearly visible.
6. (*Optional*) Place the destained gel onto a light box for easier viewing of the DNA bands.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The gel may be disposed of in the trash according to Flinn disposal method #26a. The electrophoresis buffer solution and methylene blue stain may be reused several times before being disposed of down the drain with excess water according to Flinn Suggested Disposal Method #26b.

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

Disciplinary Core Ideas: High School

- HS-PS1 Matter and Its Interactions
 - PS1.A: Structure and Properties of Matter
- HS-PS2 Motion and Stability: Forces and Interactions
 - PS2.B: Types of Interactions
- HS-LS1 From Molecules to Organisms: Structures and Processes
- 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

Crosscutting Concepts

- Cause and effect
- Structure and function

Tips

- Prepare the agarose gel before class begins. It can be stored in the refrigerator under buffer for up to two weeks.
See *Biotechnology for Young Scientists* lab kit available from Flinn Scientific (Flinn Catalog No. FB1806) for an introductory level DNA isolation and electrophoresis experiment.
- Large weighing dishes (Flinn Catalog No. AP1279) work well as staining trays for mini-gels.
- See the *Biotechnology* section of the *Flinn Scientific Catalog/Reference Manual* for tips on preparing an agarose gel and how to safely operate an electrophoresis unit.
- Tris-acetate (TAE) buffer is available as a 50X concentrated buffer solution. To prepare 500 mL of working strength 50X buffer needed for electrophoresis, dilute 10 mL of the 50X concentrate to a final volume of 500 mL with distilled or deionized water.

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

Catalog No.	Description
D0025	Deoxyribonucleic Acid, 10 g
FB0324	Electrophoresis Reagent Package
FB1260	Disposable Pipets, Needle-tip, pkg 400
AP1279	Weighing Dishes, 5 1/20 × 5 1/20 × 7/80
FB0314	Mini-gel Electrophoresis Apparatus
FB0325	Tris Acetate Buffer, Concentrated

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