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

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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

It is recommended that you consult your local school board and/or municipal regulations for proper disposal methods that may apply before proceeding.

Tips

- Prepare the agarose gel before class begins. It can be stored in the refrigerator under buffer for up to two weeks.
- Large weighing dishes (Flinn Catalogue No. AP1279) work well as staining trays for mini-gels.
- See the *Biotechnology* section of the *Flinn Scientific Canada Catalogue/Reference Manual* for tips on 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 Canada Inc.

Catalogue No.	Description
FB1260	Disposable Pipets, Needle-tip, pkg 400
AP1279	Weighing Dishes, 5¼ × 5 ¼ × ⅞
FB0314	M12 Complete™ Electrophoresis Package

Consult www.flinnsci.ca or your *Flinn Scientific Canada Catalogue/Reference Manual* for current prices.

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