Overview

This lab uses gel electrophoresis and the properties of DNA to separate fragments of DNA, which in this case have been precut by restriction enzymes.

Learning Targets

1. To understand what **physical properties** allow **fragments of DNA** to separate by gel electrophoresis.

2. To understand that different **restriction enzymes** cut DNA at different sites to form fragments of different sizes. These fragments form **unique patterns** when using gel electrophoresis.

3. To estimate **size of DNA fragments** by comparing to a known ladder.

4. To demonstrate **proper techniques** of micropipetting, sample preparation, and gel electrophoresis.

Lesson Outline

1. **Engage**: Set the context for using gel electrophoresis using PowerPoint slides found on SEP Guides OR choose a scenario to engage students.

2. **Explain**: Have students read the **Background** to learn about the properties of DNA, gel electrophoresis, reagents and restriction enzymes.

3. **Explain**: Prepare students to perform gel electrophoresis using the **Tech Guide**.
   a. Review Learning Targets and Big Idea
   b. Have students create a flow chart for the procedures
   c. Demonstrate procedures and/or watch SEP videos from SEP Guides

4. **Explore**: Students perform the lab using the accompanying **Data & Analysis** sheet for recording observations, predictions, and data. (print 1 per student)

5. **Evaluate**: Assess students using the **Data & Analysis Sheet** as homework.
Prep Procedures

• Aliquot the DNA samples into labeled tubes and store in freezer.
• Aliquot the DNA ladder into labeled tubes and store in freezer.
• Aliquot Sample Loading Buffer into labeled tubes and store at room temperature.
• Aliquot STE into labeled tubes and store at room temperature.
• Dilute 50X TAE to 1X TAE. (20 ml of 50X TAE + 980 ml of distilled water = 1 liter)
• Aliquot 1X TAE into flasks/bottles for each lab station.

Freezer Box Reagents

☐ Marker I
☐ Marker II
☐ Marker III
☐ 1 Kb Ladder

Lab Station inventory

☐ Gel box
☐ Set of micropipets (P10, P20)
☐ Box of microtubes
☐ Tube rack
☐ Waste container
☐ 50 ml graduated cylinder
☐ Beaker
☐ 3 Boxes of Tips (P10, P20/200)
☐ 1X TAE in flask/bottle
☐ Permanent marker
☐ Gel staining tray
☐ Ruler
☐ Acetate sheets
☐ How to Make a DNA Gel Cards
☐ DNA Staining Protocol Cards
☐ Ziplock bags for gel storage
☐ Power supply (1 per 2 lab stations)

Shared Material

☐ 2-4 Reagent stations (on ice):
  ☐ STE
  ☐ DNA sample
  ☐ Sample Loading Dye
  ☐ DNA Ladder
☐ Sample loading buffer
☐ Microwave/hot plate
☐ Electronic balance
☐ Weigh boats
☐ Agarose
☐ Ice
☐ 1X TAE
☐ Distilled water
☐ DNA Stain (FastBlast or Fluorescent Stain)

Recommended Student Pre-Knowledge & Skills

• Ability to accurately measure using various micropipets.
• Experience with loading reagents into gels.
• Basic understanding of DNA structure and properties.
• Basic understanding of the interaction between different restriction enzymes and DNA.
• Basic understanding of how molecules are separated by gel electrophoresis.
Connections to NGSS

**This lesson supports:**

**Science and Engineering Practices**
- Analyzing and interpreting data
- Using mathematics and computational thinking

**Crosscutting Concepts**
- **Structure and function.** The way in which an object or living thing is shaped and its substructure determines many of its properties and functions.
- **Patterns.** Observed patterns of forms and events guide organization and classification, and they prompt questions about relationships and the factors that influence them.

**Disciplinary Core Ideas**
- **LS3.A:** Each chromosome consists of a single very long DNA molecule
- **PS2.B:** Attraction and repulsion between electric charges at the atomic scale explain contact forces between material objects.

**Clean Up**

Used tips, microtubes, and gloves can be disposed of in the trash. 1X TAE can be poured down the drain. Keep waste containers, and protocol cards at your lab station. Used agarose gels can be disposed of in the trash or kept for practice gels.

Pack the crates according to the kit packing diagram and inventory list.

**Bottlenecks:**

May occur around electronic balances, microwaves, centrifuges, reagents, or light boxes

**Stopping Points:**

- **After pouring gels:** Prepare and pour gels. Store in a Ziploc® bag with some 1X TAE buffer. Keep refrigerated until ready to use.
- **After preparing pre-cut samples:** Prepare samples by adding STE to pre-cut DNA samples then freezing at -20°C. When ready, thaw samples, add loading buffer, mix, and load.
- **After running for 10 minutes:** Run the gel for ~10 min then stop. Store in a Ziploc® bag with some 1X TAE buffer. Keep refrigerated until ready to continue running gel. Check that the gel is replaced in the gel box in the correct orientation.
- **After staining gels:** Run gels. Stain in Fast Blast, de-stain in tap water, wrap in plastic wrap, and store in the refrigerator.
- **After tracing bands in gel:** Have students trace DNA bands and record data in a table. If doing the semi-log plot, complete it another day when all students have their data tables ready.