

# Introduction to DNA Fingerprinting



## Introduction

Every organism on earth has its own unique set of DNA. DNA differences can easily be identified in a laboratory using a technique known as DNA Fingerprinting. Using restriction enzymes to cut the DNA at specific locations, different lengths of DNA are created based on the unique code of the organism. These different lengths of DNA can be observed using gel electrophoresis to separate the strands into observable bands. Every organism has a unique number and location of bands. The purpose of this lab is to introduce students to the technique of DNA fingerprinting by allowing them to make and load gels and see how the DNA will separate out into different bands.



**Grade Level:** 10 – 12

**Time Needed:** Three 40-minute class periods

## Learning Objectives

After completing this lesson, students will be able to:

1. Create agarose gels properly
2. Demonstrate how to load gels with DNA samples that have been cut with restriction enzymes
3. Demonstrate how to run a gel using an electrophoresis cell
4. Analyze the DNA bands created in the gel and interpret the results of the DNA fingerprinting

## Next Generation Science Standards (NGSS)

As a result of activities for grades 10-12, all students will learn content in these areas:

### Topics

- **LS1:** Structure & Function

### Performance Expectation

- **HS-LS3-1:** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring
- **HS-LS1-1:** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

### Dimension

#### Practices:

- Planning & carrying out investigations
- Analyzing & interpreting data
- Constructing explanations

#### Disciplinary Core Ideas:

- **LS1.A:** Structure & function
- **LS3.B:** Variation of traits

#### Cross-Cutting Concepts:

- Patterns
- Structure & function



## Materials

- (1) electrophoresis casting tray per group
- (1) six slot casting tray comb per group
- (1) 250ml beaker per group
- (3) hot plates
- (2) scales (accurate to 0.1 gram)
- (4-6) 5mL pipettes
- (4-6) electrophoresis chamber (depending on how many gels they hold)
- (4-6) 100mL graduated cylinder
- (3-4) pairs of heat-insulated gloves
- (1) piece of aluminum foil per group
- 1ml of 50X concentrated buffer
  - per group
- 0.4 grams of agarose powder per group
- 50ml of distilled water
- (4-6) electrophoresis power boxes
- (1) micropipette per group
- (5) micropipettes per group
- 400ml of buffer solution per group (15ml of 50x buffer w/ 385ml distilled water)
- (4-6) spatulas
- (1) Ziploc bag per group
- (1) spot plate per group
- (6) DNA samples per group

## Instructional Process



### Day 1 – Making the Agarose Gel

1. Set up stations around the room where students can go to mix the materials for the agarose gel, which include the following:
2. A weigh station with the agarose powder, scales, measuring scoop, and weigh boats
3. A station with the 50X Buffer solution, 5ml pipettes, and pipette pumps
4. A station with flasks of distilled water and graduated cylinders
5. A station with hot plates and insulated gloves to heat the mixtures
6. Each group will need a beaker and a piece of aluminum foil to cover the beaker.
7. Students will put 50mL of distilled water, 0.4g of agarose powder, and 1mL of 50X concentrated buffer into their 250ml beaker and cover with aluminum foil. Gently swirl the mixture to dissolve the powder completely.
8. Students will heat the mixture on the hot plates until the substance begins to boil. While the substance is heating, each group should assemble their casting trays. Make sure they place the comb in the end slot, not in the middle so the DNA has the most room to run. Once the agarose mixture begins to boil, remove it from the heat source and allow it to cool. When it has cooled to where the students can pick it up (approx 50<sup>o</sup> C) comfortably, have them pour the agarose into the casting trays.
9. When the agarose has solidified, have them remove the comb and carefully remove the gel from the tray. Have them place the gel into a Ziploc bag that is labeled with their names and hour.
10. Store the gels in the refrigerator overnight, until needed the next day.



## **Day 2 – Running the gels**

1. Have the electrophoresis chambers already set up with the buffer solution added and ready for the students to get started.
2. Be sure the students place the gel into the chamber wells positioned toward the positive end.
3. Be sure the gels are completely submersed in the buffer solution.
4. Load 15 $\mu$ l of each DNA sample into a separate well. Make sure the students get the sample into the well without pushing too far and damaging the gel.
5. The gels should be run on the highest possible voltage and for as long as the class period will allow.
6. At the end of the period, have the students take their gels out of the electrophoresis chamber using the spatula and place it back in the Ziploc bag to use the next day.
7. Store the gels overnight in a refrigerator.

## **Day 3**

1. Have each group get out their gels from the previous day.
2. Each student should draw a picture of the gel that accurately shows the band locations and lanes.
3. Students should complete the follow-up questions at the end of the lab.

## **Supplemental Content-Discussion Material while Gels are Running**

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1. Review the basic structure of a DNA molecule and the base pairing rules.
2. Discuss how restriction enzymes work and how they play a vital role in DNA Fingerprinting.
3. Explain the process of PCR and how it is used amplify small samples of DNA into larger useable samples.
4. Lead a class discussion on how DNA Fingerprinting is used currently in fields ranging from forensic science to agricultural science.

## **Follow Up Questions**

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1. What results from adding the restriction enzymes to the DNA? What would happen if you didn't add it to the samples before running the gels?
2. What is the charge of a DNA molecule? How do you know this after running the gels?
3. What does the distance a band moves through the agarose gel tell you about its size?
4. Why do you think DNA Fingerprinting is a powerful tool utilized by law enforcement to capture and convict criminals?
5. How do you think DNA fingerprinting is used in the field of agriculture?



# Introduction to DNA Fingerprinting

## Student Sheet

### Introduction

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### Materials

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- (1) electrophoresis casting tray per group
- (1) six slot casting tray comb per group
- (1) 250ml beaker per group
- (3) hot plates
- (2) scales (accurate to 0.1 gram)
- (4-6) 5mL pipettes
- (4-6) electrophoresis chamber (depending on how many gels they hold)
- (4-6) 100mL graduated cylinder
- (3-4) pairs of heat-insulated gloves
- (1) piece of aluminum foil per group
- 1ml of 50X concentrated buffer per group
- 0.4 grams of agarose powder per group
- 50ml of distilled water
- (4-6) electrophoresis power boxes
- (1) micropipette per group
- (5) micropipettes per group
- 400ml of buffer solution per group (15ml of 50x buffer w/ 385ml distilled water)
- (4-6) spatulas
- (1) Ziploc bag per group
- (1) spot plate per group
- (6) DNA samples per group



## Procedure

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### **Day 1 – Making the Gel**

1. Prepare the casting tray by closing off both ends with the rubber stoppers. Be sure to place the comb at the far end of the tray and not in the middle location.
2. Each group will need a 250ml beaker, a 100ml graduated cylinder, and a small square of aluminum foil (big enough to cover the mouth of the beaker).
3. First, add 50 mL of distilled water to the flask. You cannot use regular tap water for this lab; please use only the water located in the flasks labeled “distilled water” located around the room.
4. Next, add 1mL of 50X concentrated buffer to the same beaker. There is a station set up with pipettes, pumps, and the 50X buffer at a lab station.
5. Add 0.4 grams of agarose powder to the beaker and swirl gently to dissolve all of the powder completely.
6. Cover the beaker with the aluminum foil and place it on one of the hot plates located around the room. Heat the mixture until it just begins to boil. Using insulated gloves, remove the agarose mixture from the hot plate and allow it to cool.
7. When the beaker is cool enough to safely pick up, slowly pour the mixture into the casting tray. Be sure the casting tray is completely full to the top of the stoppers. Any extra agarose mixture should be poured in the trash, not down the drain.
8. Allow the agarose to cool completely, until the gel has completely set up and has become firm.
9. Remove the comb and one side of the rubber stoppers and slide the gel into your group’s Ziploc bag. Be sure to label the bag with your group members’ names and class hour.
10. Clean-up all glassware and materials, and return them to the proper locations.



### ***Day 2 – Running the Gel***

1. Quickly get out your gels from the day before and proceed to one of the electrophoresis chambers located around the room. **DO NOT** move the chambers; they are situated by power sources that are needed for today's activity.
2. The chambers should already be filled with diluted buffer. Carefully slip the gels into the chamber so that the wells are located toward the negative end and the majority of the gel is located toward the positive end. The gel should be completely submerged in the buffer. If not, add a little of the buffer solution to ensure the gel is completely covered.
3. Using the micropipettes, load 10  $\mu$ l of the tracking dye in lane 1 (furthest left). Continue loading each of the five DNA samples in the remaining wells using a different pipette tip each time. **DO NOT** cross-contaminate the samples.
4. When all the samples have been loaded, carefully place the lid onto the chamber and wait for the teacher turn the power supply on.
5. When the gels have finished running and the teacher has turned off the power, carefully remove the gel from the chamber using the spatula and place it back into your group's Ziploc bag.

### ***Day 3 – Analyze the Gel***

1. Carefully remove your gel from the Ziploc bag and place it on the table.
2. Accurately draw and label a diagram of your gel, noting the location and distance of each band in the lanes.
3. Answer the follow-up questions below.



## Follow Up Questions

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2. What is the charge of a DNA molecule? How do you know this after running the gels?
3. What does the distance a band moves through the agarose gel tell you about its size?
4. Why do you think DNA Fingerprinting is a powerful tool utilized by law enforcement to capture and convict criminals?
5. How do you think DNA fingerprinting is used in the field of agriculture?