

DNA Electrophoresis Lesson Plan

Primary Learning Outcomes:

Students will learn how to properly load a well in an agarose gel. Students will learn how to analyze the results of DNA electrophoresis. Students will learn how to use electrophoresis equipment and understand how electrophoresis works.

High School Georgia Performance Standards

SCSh2. Students will use standard safety practices for all classroom laboratory and field investigations.

SCSh3. Students will identify and investigate problems scientifically.

SCSh4. Students use tools and instruments for observing, measuring, and manipulating scientific equipment and materials.

SCSh6. Students will communicate scientific investigations and information clearly.

SCSh8. Students will understand important features of the process of scientific inquiry.

SB1. Students will analyze the nature of the relationships between structures and functions in living cells.

SB2. Students will analyze how biological traits are passed on to successive generations.

Middle School Georgia Performance Standards

S7CS2 Students will use standard safety practices for all classroom laboratory and field investigations.

S7CS3 Students will have the computation and estimation skills necessary for analyzing data and following scientific explanations.

S7CS4 Students use tools and instruments for observing, measuring, and manipulating equipment and materials in scientific activities.

S7CS6 Students will communicate scientific ideas and activities clearly.

S7CS9 Students will investigate the features of the process of scientific inquiry.

S7L2 Students will describe the structure and function of cells, tissues, organs, and organ systems.

Materials:

<i>Kit provides:</i>	<i>Teacher provides:</i>
2.5 g Agarose powder	
100 ml 10X TBE buffer	Distilled water
Small-range micropipettors	Microwave oven
Small-range micropipette tips	Flasks, for preparing agarose
Oven mitts	Ziploc bags (sandwich size)
7 Microcentrifuge tubes of 1x loading dye	Beakers
7 Microcentrifuge tube racks	
4 Electrophoresis units (including casting tray, 2 combs, and 2 running trays)	
DNA samples (if running 14 or fewer gels)	DNA samples (if running >14 gels - see ordering information below)
FastBlast stain (1 ml concentrate)	

If ordering DNA ladders, we suggest the following from New England Biolab (<http://www.neb.com/nebecomm/default.asp?>)

Quick-Load™ 100 bp DNA Ladder (Cat.# N0467S) - enough for 125 gel lanes - \$79

Quick-Load™ 2-Log DNA Ladder (Cat.# N0469S) - enough for 125 gel lanes - \$79

Quick-Load™ 1 kb DNA Ladder (Cat.# N0468S) - enough for 125 gel lanes - \$58*

***remember that you will need 2X the amount of one DNA ladder to be the suspect and the crime scene DNA. Excess ladder can be frozen for later use.**

You can also find EZ load DNA ladders from BioRAD (<http://www.bio-rad.com/>)

Materials to be assembled for a class of 28 students (7 groups of 4 students each):

Agarose gels (recipe follows)
1000 ml 1X TBE buffer (recipe follows)
7 small-range micropipettors
Small-range micropipette tips
7 beakers, for waste
Loading dye, divided into 7 microcentrifuge tubes
7 microcentrifuge tube racks
4 electrophoresis units (each unit accommodates 2 groups), including trays and combs
4 DNA samples, each divided into 7 microcentrifuge tubes
Diluted FastBlast stain
Water bath, set to ~60° C

Duration of activity:

Prep time: approximately 30 - 45 minutes

Class time: Day 1, 1 full 55-minute period

Day 2, approx. 30 minutes for analysis and completion of lab

- If class time is limited, a teacher might want to have the students cast the gels the day before. While the gels cool the students could work on the micropipette lab. The gels can sit for a day in TBE buffer.

Advance preparation:

- The 10X TBE buffer must be diluted to 1X. 1000 ml of 1X buffer should be sufficient for all needs of a 7-group class. To dilute, add 100 ml of 10X buffer to 900 ml of distilled water. This will be used to dissolve the agarose and to fill the electrophoresis units.
- Agarose gel - make a 1% solution using 1X TBE running buffer (for example, 1 g / 100 ml 1X TBE running buffer) - *don't use water; the DNA will not separate properly!* 50 ml of solution will prepare 2 small gels, so 250 ml of solution (2.5 g agarose in 250 ml 1X TBE buffer) should be more than enough for 7 groups. To dissolve the agarose, heat in a microwave for a minute or two on medium power, removing

and swirling the solution after each minute of heating. The container in which you heat the agarose mixture should be twice as large as you need it to be. In other words if you are heating 250 ml, then it should be in a 500 ml container. When the agarose is fully melted into solution you will not see any floaties in the buffer. The agarose must stay warm before pouring to prevent solidifying; a water bath set to 60° C will accomplish this.

Additional tips:

- Use the link on the student intro page to demonstrate DNA fragments migrating through a gel, with the resulting fingerprint.
- Students will need much practice pipetting. You should perform the Micropipette Lab prior to performing this lab. The students will still need to practice loading the wells with loading dye prior to loading any samples on the gel.
- 45 minutes is the minimum run time allowable for good separation (30 minutes is adequate), though an hour or more would be ideal. In a typical 55-minute class, students will probably have just enough time to practice and begin the electrophoresis separation. The teacher will have to time the gels and remove them for staining.

Staining: Have each group of students label a Ziploc bag. Dilute the stain provided to a 1X concentration by taking 1 ml of stain and diluting with 99 ml water. Place the gel in a Ziploc bag with enough stain to cover the gel and leave it until the following day. On day 2, dump out the stain and analyze the gel without removing it from the bag.

- The TBE buffer in each electrophoresis unit may be reused for additional classes.
- Teachers might prefer to pour gels for the students to save time.

- Middle School Teachers should consider the abilities of their students when determining teacher preparation versus student preparation.
- After all electrophoresis is complete for the day, the electrophoresis units, gel trays, and combs need to be washed off with tap water and then rinsed with distilled water.

DNA Electrophoresis Lab

Origin of the word: Electrophoresis

Electro refers to the energy of electricity. *Phoresis*, from the Greek verb *phoros*, means "to carry across." Thus, gel electrophoresis refers to the technique in which molecules are forced across a span of gel using an electrical current.

What is electrophoresis?

Electrophoresis is a method that separates macromolecules--either nucleic acids (DNA) or proteins--on the basis of size, electrical charge, and other physical properties. So, then, how is electrophoresis used?

(<http://www.tvdsb.on.ca/westmin/science/sbioac/genetics/Electro.htm>)

When DNA is treated with special enzymes, it is cut into fragments, or pieces of varying lengths. These fragments are loaded into a depression, or well, in a gel bed and are separated using an electric current. Pieces of varying lengths will travel different distances in the gel. The result will be a pattern of bands, or DNA "fingerprint," that is unique to each individual.

We will be using DNA electrophoresis on the DNA samples collected at the crime scene. Each sample has already been treated with restriction enzymes. If the DNA fingerprint of one of our suspects matches that of the unknown DNA left at the crime scene, we have found our criminal! So, get ready, let's use science to catch the bad guy!

Lab Equipment Needed:

DNA samples

Gel tray

Micropipettors

Test tube rack

Gel comb

1X TBE buffer

Pipette tips

Electrophoresis unit

Procedure

1. Insert a plastic gel comb into the notches over the black band in the casting tray.
2. Pour the warm agarose into a small casting tray until it comes halfway up the teeth of the comb. Be CAREFUL NOT TO MOVE CASTING TRAY once the agarose has been poured!! The gel will become cloudy as it solidifies.
3. Once the gel is solid, gently remove the comb by pulling straight up, taking care not to rip the wells. *(It may be helpful to have a partner hold the tray down as you pull up on the comb.)*
4. Remove the tray from the casting bed. *(If any agarose has solidified on the bottom of the tray, wipe it off with a paper towel.)*
5. Place the tray between the pegs in the bottom of the gel box. *(The notched end where the comb had been should lie at the negative end.)*
6. Fill the gel box with 1X TBE buffer until it just covers the entire surface of the gel. (Try to pour at either end of the gel box, not directly over the gel.)

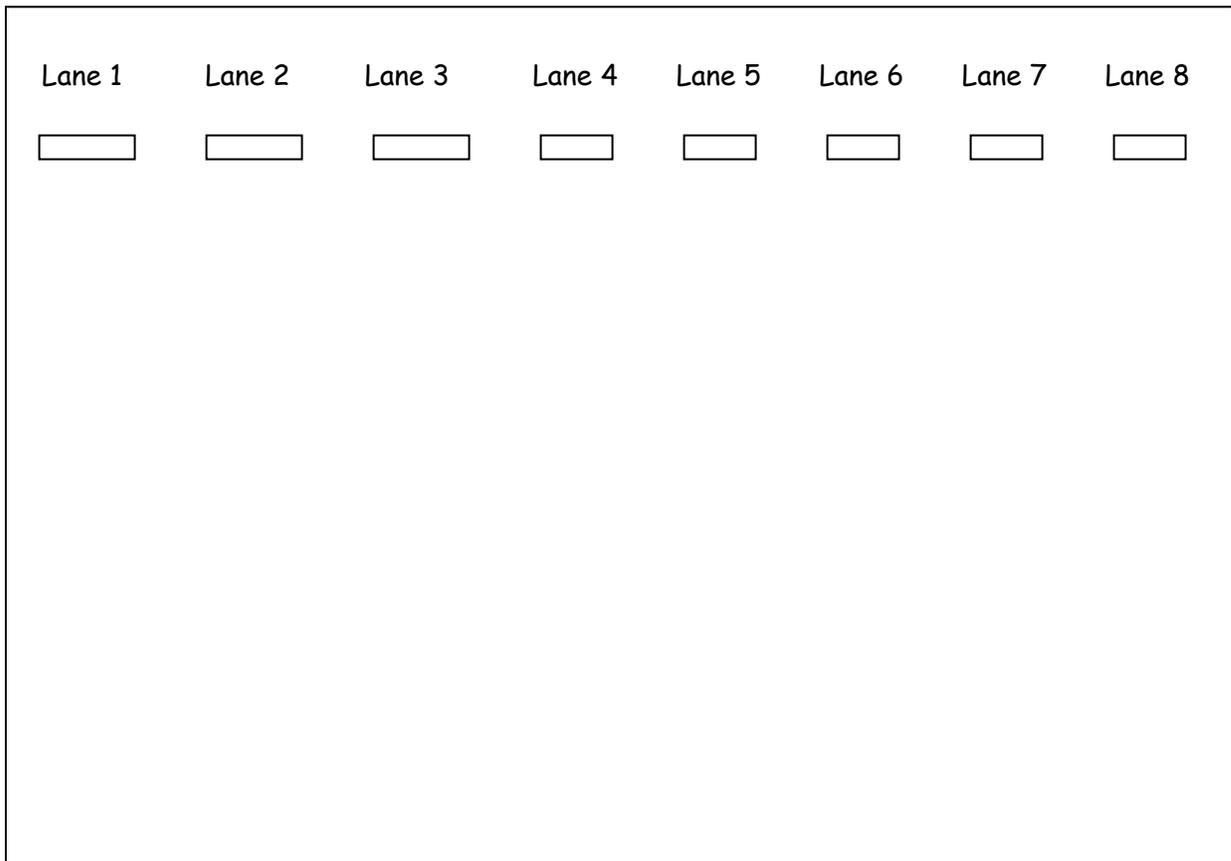
Loading Practice

- Set a small micropipettor to 10 μ l.
- Draw 10 μ l (microliters) of 1X loading dye into your pipettor. *(Make sure there are no air bubbles in the tip!)*
- Center the pipette over an outside well using two hands. *(Use only the outside wells for practice; the inside wells will be used for running your DNA samples!)*

- On a slight angle, dip the tip through the surface of the buffer and gently release dye into the well. (*The tip must be below the surface of the buffer but be CAREFUL not to push the tip through the bottom of the well.*)

7. You can use lanes 1, 2, 7, and 8 for loading practice. Load 10 μ l of the DNA/loading dye mixture into the assigned well. Label the gel diagram below as follows:

- a. In lane 3 load the crime scene DNA
- b. In lane 4 load suspect A's DNA
- c. In lane 5 load suspect B's DNA
- d. In lane 6 load suspect C's DNA



8. Once the samples are loaded, place the cover on the electrophoresis unit and connect the electrodes. Be careful not to move the electrophoresis unit once you load the samples.

9. Turn the voltage selection knob on the power supply to 100 volts and start the gel. You should allow the gel to run for 45 minutes. (If short on time the gel can be run for 30 minutes and you will get acceptable results.)
10. Watch the dye as it moves down the gel. This indicates that the unit is operating properly and that the DNA is moving through the gel. As a general rule you don't want the dye to run off the end of the gel.
11. Once the gel has run, unplug the power supply and remove the electrodes from the electrophoresis unit. Remove the top and inform the teacher that your gel is ready to stain.

Analysis and Conclusion

Now that the gel has been stained it is time to determine who is guilty of this crime! Look at your gel very carefully and match the DNA banding pattern from the evidence found at the crime scene to one of your suspects. When you find the matching pattern, that is the guilty person!

Optional analysis for high school students

Gel electrophoresis is a tool with many uses. Establishing DNA fingerprints is a forensics application. A research application would be to establish the size in base pairs (bp) of a particular DNA fragment. This is accomplished by adding a DNA size standard (not included with the kit) to one of the empty lanes of your gel. The DNA size standard contains several different DNA fragments of known size. The smaller fragments will travel a greater distance from the well than the larger fragments. This allows you to establish a "standard curve" for distance traveled vs. fragment size in bp. You can then use this graph to determine the size of unknown fragments based on the distance they traveled through the gel. The only special tool this requires is a piece of semi-log graph paper (shown below).

Size in bp's would be plotted on the y axis and distance traveled in mm on the x axis. To measure the distance a fragment traveled, start from the center of the well and measure to the center of the band. The y axis of the semi-log graph increases by an order of magnitude each time you transition from the lines that are close together to the ones that are farther apart. In other words if you start the y axis at 100 the next line would be 200, then 300 and as the lines got closer together you would reach 1000. The next line after 1000 would be 2000 and then 3000 and when you got to 10000 the line after that would be 20000 and so on. This allows you to get a linear graph from data that would not be otherwise. The x axis is just as any other x axis would be on any other graph because it increases by increments of one.

Once you have the standard curve established, you would make a best fit line to the data points by drawing a straight line that would come as close as possible to all the points. The distance traveled by a fragment of unknown size would then be determined in mm. Locate this distance on the x axis and make a line parallel to the y axis up until your line touches the standard curve. At that point make a line to the left that is parallel to the x axis until it touches the y axis and this will give you the size of the fragment.

