DNA Fingerprinting and Gel Electrophoresis

Part of: **Dartmouth Rural STEM Educator Partnership**

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Driving Question Single big question addressed by this lesson. **How do we analyze DNA fragments by size to identify an unknown person?**	**Overview** Brief (1-2 sentence) description of the lesson. In this lesson, students identify a suspect using DNA electrophoresis, which separates DNA fragments based on size.
NGSS Standards MS-LS1-1. Conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells. **CCC's:** MS-LS1-2. Complex and microscopic structures and systems can be visualized, modeled, and used to describe how their function depends on the relationships among its parts, therefore complex natural structures/systems can be analyzed to determine how they function. **SEP's:** MS-LS1-2. Modeling in 6–8 builds on K–5 experiences and progresses to developing, using, and revising models to describe, test, and predict more abstract phenomena and design systems.	**Objectives** Through this lesson, students will (2-3 measurable objectives): ● Students will understand that DNA fingerprinting examines only a fraction of the genome at sites that vary between people. ● Students will explain the connection between shapes/sizes of molecules and movement through a gel. ● Students will describe how the electric current interacts with the negatively charged DNA.

Materials

What materials are needed to run the lesson?

- BlueGel Electrophoresis Unit with Built in Bioilluminator
  - Power Supply
  - Anti-fog spray with microfiber cloth
  - Gel casting trays with combs
  - Storage bag for kit
  - https://www.minipcr.com/product/bluegel-bundle-8/
- SeeGreen All-in-One Agarose Tablets
  - https://www.minipcr.com/product/seegreen-agarose-tabs-20-tablets/

- Pipettes 10 uL fixed volume
- 2-200 uL pipette tips
- BioRad Lambda DNA Kit Refill
    - https://www.bio-rad.com/en-us/sku/1660011EDU-lambda-dna-kit-refill?ID=1660011EDU
    - Note: This will advise you to store in a refrigerator. You can do this to extend shelf life. DNA is also quite stable at room temperature for days to weeks.
- 50 mL beaker
- Pot holder
- 0.5 mL tubes
- Tube labels (stickers or tape and pen, sharpie)
- Tube rack
- 1 liter graduated cylinder
- 1 gallon jug (can be recycled/reused)
- Quart Size Ziploc bags and/or cling wrap
- Access to distilled or tap water, outlet, microwave
- Lab notebook -> for record keeping as desired by the teacher

Preparation

What needs to be prepared before the lesson?
Introduction to gel electrophoresis - Amoeba Sisters
Use in crime investigation

Prep for Day Two

The DNA samples from the BioRad Lambda DNA kit will need to be prepared as follows:
Use clean tips for all

1. To each of the 4 tubes containing DNA (labeled "DNA Size Standard," "PstI lambda DNA digest," "EcoRI lambda DNA digest," and "uncut lambda DNA"), add 20 uL of the "Sample Loading Buffer"
    a. You will have to use your fixed volume 10 uL pipette twice to add 20 uL.
    b. You will have an excess of the Sample Loading Buffer left over.
    c. Warning: The sample loading buffer will stain things blue.
    d. Invert tubes several times to mix to a uniform blue color.
2. RECOMMENDED: These 4 DNA samples can then be distributed to the 0.5 mL tubes for student use. Each group needs 10 uL of each sample to load. You can distribute with your 10 uL fixed volume pipette. Assign each of the four DNA samples a letter, A-D.
    a. Each student group should get 4 known samples labeled A, B, C, D
    b. Each student group will then get an unknown sample, which is a duplicate of one of the known.
        i. Any one of A, B, C, or D can be used as the unknown. In fact, it might be useful for different student groups to have different unknowns.

The TBE powder will need to be dissolved to produce running buffer ahead of time:

1. Measure 3 liters of distilled or tap water (using the 1 liter graduated cylinder) into a 1 gallon jug (can use recycled / rinsed out milk jug). Measurements can be approximate.
2. Add the entire container of TBE powder (25.5 g) to the 3 liters of water.
3. Mix by shaking. May require intermittent shaking over the course of about 15 minutes
    a. It is normal for some powder to remain undissolved. Small amounts of undissolved powder will not affect performance.

b. Can store at room temperature for months, once it becomes cloudy, discard. Safe to dispose down the drain.

Background information

Any background information that may be helpful to the teachers?

The objective of this activity is for students to match an unknown DNA sample to one of four reference DNA samples by the banding pattern/DNA fingerprint on gel electrophoresis.

DNA samples gathered at crime scenes are sent to labs for processing. At the lab, specific sections of DNA known to vary between people are chosen for analysis; we do not compare the entire sample (the genome is HUGE). Chosen sections of DNA are isolated from the sample and replicated/amplified to take a tiny sample and create enough to analyze.

To compare chosen sections of DNA from one person to another, we cut the DNA with restriction enzymes. Restriction enzymes cut at specific sequences (specific order of A/T/C/G bases). These cuts allow us to isolate the variable parts of the DNA. At these variable parts, people have different numbers of short tandem repeats (STR) which make their DNA fragments different sizes. Short tandem repeats are essentially a few nucleotides that repeat over and over. The number of repeats varies by person, so we can use a pattern of DNA fragment sizes to identify people.

We visualize this pattern of DNA fragment sizes using gel electrophoresis. Gel electrophoresis involves running an electrical current through a gel or matrix. The electric current "pulls" the negatively charged DNA with it through the matrix. Larger pieces of DNA travel smaller distances because they get caught up more by the matrix. Smaller pieces of DNA travel larger distances.

We can match the pattern of DNA fragments to a specific person.

All of this background is summarized in a powerpoint presentation with video linked to show to students to frame this lesson

Story line to frame the lesson

The local forensics lab is reaching out to your classroom for help with identifying a suspect in a recent robbery. The suspect stole packages from the local post office. In breaking the window, they must've cut themselves, as several drops of blood were recovered from the scene. Four suspects were identified from security cameras. Each suspect has given a blood sample after law enforcement secured warrants. Unfortunately, the blood types from all four suspects and the unknown sample from the crime scene are the same (type O+ is the most common in the United States, at about 37% of people).

To identify the suspect, we must analyze DNA from the white blood cells in the samples. The forensics lab was able to isolate the DNA from the white blood cells. To avoid the expense and time of sequencing the entire DNA samples (the human genome is about 3 billion base pairs long), they've decided to compare "DNA fingerprints." Restriction enzymes cut at specific sequences in the DNA to create fragments of unique sizes; the pattern of uniquely sized pieces can be considered DNA fingerprints.

The local forensics lab was able to analyze the blood samples and create the DNA fingerprints, but they weren't able to run the fingerprints on a gel to identify whose blood was left at the scene. They've sent you the four samples from the suspects, labeled A, B, C, and D as well as the sample from the scene

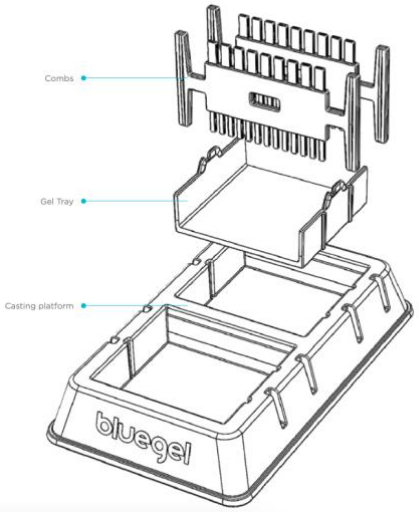
labeled unknown. Using your gel electrophoresis apparatus, can you match the DNA fingerprint from the scene to one of the suspects?

Lesson

Detailed description of the lesson with sub-headings as needed

Day One: Pouring gels

- Introduce the scenario and the technique of gel electrophoresis using the provided gel electrophoresis powerpoint
- Assign students to groups of 2-4 students per gel electrophoresis apparatus
- Each group will need a gel casting platform with a gel tray and comb, a SeeGreen All-in-One Agarose tablet, a 50 mL beaker, and access to a microwave and pot holders.
    - Note: the combs are stored clipped to the bottom of the casting apparatus.

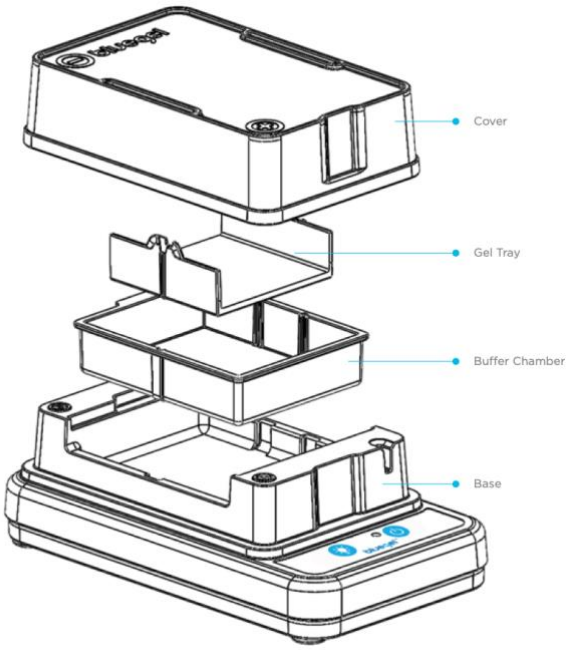


- Have the group assemble their casting platform with the 9-tooth combs inserted into the far end of both trays (combs can be inserted into the middle or far end, by inserting them into the far end, you give your samples more gel space to run across).
- Add about 40 mL of distilled or tap water to the beaker, using the lines to measure.
- Add your SeeGreen All-in-One Agarose tablet to the 40 mL of water in the beaker. Swirl around for a few minutes until the tab is dissolved.
- Microwave the beaker for about 30-40 seconds, until the powder is dissolved into a clear solution.
    - Be very careful and use the pot holder.
- Pour the heated solution into the casting trays. Should have enough to fill both gels from one beaker, although each group will only need one gel for day two. This should give a comfortable excess of gels in case of mishaps with one group or another.
- The gel will take about 30 minutes to fully solidify. Gels can be either covered in cling wrap or placed in ziplock baggies to prevent drying out. If the gels are not yet solid, they can be covered in cling wrap while still in the casting apparatus and left there overnight.
    - Try to minimize moving the gels while they are solidifying. Consider having students pour them where you want to leave them to set.

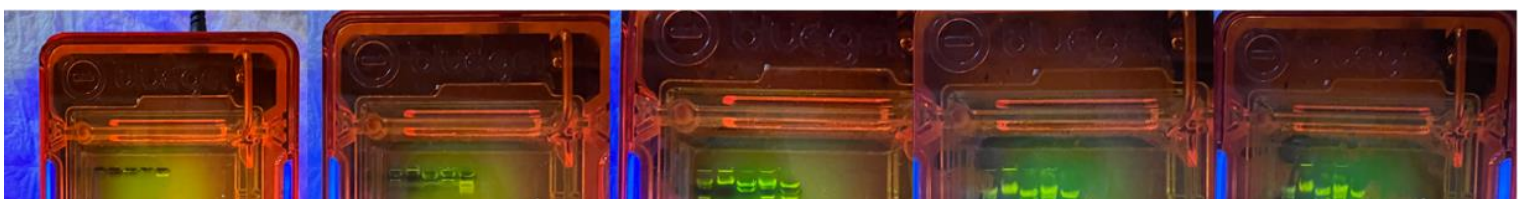
Day Two: Running gels

- Get started right away with loading and running the DNA gels. While they are running, you can review some of the key points from day one, perhaps using select slides from the powerpoint.
- Students will need: their gel casting apparatus from day one, their gel running apparatus, a fixed 10 uL pipette with tips, a rack for their 5 tubes, a 50 mL beaker
    - They should each be within reach of an outlet.

- Have students remove the combs from their gels. If they have two gels, have them select the most uniform gel to run today.
- Assemble the gel running apparatus: Base, Buffer chamber, gel tray with the cast gel in it. ***The wells should be closest to the (-) end of the running chamber***



- Using the 50 mL beakers, give each group about 40 mL of TBE buffer. Students should pour this buffer into the buffer chamber. The gel should be submerged; the wells in the gel should be filled with buffer. The 40 mL is approximate, add more as needed.
- Give each group of students the 4 vials of suspect DNA and 1 vial of DNA from the scene (should be premixed with the blue loading dye, warn the students that the dye can stain).
- Have the students load 10 uL of sample per well. They should have 5 wells filled with samples. Make sure they record which order they loaded the samples.
- Once samples are loaded, prepare the cover by spraying with the ClearView Spray on the inside of the cover. Spread the spray, but do not wipe it off, with the microfiber cloth. Place the cover on the unit.
- Plug the power cord into the gel running unit, then into an outlet. Push the power button (the green LED will light up to indicate it is running). Bubbles will form on the electrodes.
- Turn on the lightbulb button and consider dimming the lights or using the Fold-A-View to peer in.
    - The orange cover works to filter out excess blue light, the DNA interacts with the SeeGreen tablets to fluoresce.
- The gels can run up to 60 minutes, patterns can be seen clearly around 20 minutes.
    - Remember - the students should have one of these four in duplicate, so they can match the scene of the crime DNA to one of the suspect DNA samples. Ignore the labels on the tubes as they come.
    - If fogging becomes a problem, you can turn off the unit, unplug the unit, take off the cover, wipe the condensation, reapply the ClearView Spray, replace the cover, plug back in the unit, and resume running.
    - Actual photos of running gels (in this example, the unknown in lane 1 matches suspect 3 in lane 4).



- Students can document their findings either by taking photos or by sketching the gel results. Students are encouraged to watch the separation in real-time by leaving the light on the entire time.

Clean-up: all liquids are safe to go down the drain. The tubes, pipette tips, extra DNA samples, and the gels can all be thrown away in regular trash. Rinsing the casting trays and gel running apparatus with water (without submerging the electronics) is recommended for longevity of the units. Each BlueGel comes with a storage bag for the units.

Evidence of Learning

How will students demonstrate their learning? Exit questions?

Students should be able to answer the following questions on an exit ticket:

1. Draw your gel electrophoresis results, labeling the top of the gel, the bottom of the gel, and which sample is in each lane.
2. Which suspect matches the DNA from the crime scene?
3. Which would travel further on a DNA gel, a large piece of DNA or a small piece of DNA?
4. DNA travels towards the positively charged electrode in DNA gel electrophoresis because the DNA itself is (circle one): positively charged negatively charged

Extensions

How could this lesson be extended?

The gel electrophoresis units can be used to analyze any DNA samples.

A DNA ladder, which consists of DNA fragments of known size / molecular weight, could be included in one of the empty lanes. This would allow students to estimate the specific sizes of the DNA fragments in each sample.

Students are loading 10 uL of sample per well. What is a uL or microliter?

Glossary of terms

Include a glossary of terms if needed.

DNA fingerprint: an identifying pattern of DNA size fragments
Gel electrophoresis: a method to separate DNA fragments based on size
uL (microliter): one millionth of a liter, measure of volume
Restriction enzyme: a protein that cuts at specific sequences in DNA
Short tandem repeats (STR): DNA sequences of a variable number of repeats of a 1-6 base pair unit

Appendices

References or other materials as needed.

https://www.minipcr.com/wp-content/uploads/bluegel-Users-Manual-290321.pdf

https://www.youtube.com/watch?v=7onjVBsQwQ8
https://www.youtube.com/watch?v=eXE2bEd04RI