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Innocent or Guilty: A Lab on DNA Gel Electrophoresis

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INTRODUCTION

Description

This lesson, based on EDVOTEK Kit #109, "DNA Fingerprinting I: Identification of DNA by Restriction Fragmentation Patterns," presents a simulation of a DNA fingerprint (RFLP—Restriction Fragment Length Polymorphism).

The prelab section introduces the importance of DNA fingerprinting—a form of identification that is being accepted by both scientific and legal experts. The procedure is used in forensic work, paternity suits, missing-person cases, archeology, and animal breeding. The protocol for the lab is introduced.

The lab involves students preparing a gel for electrophoresis. DNA fragments, which have been predigested using two different restriction enzymes, will be run on a gel electrophoresis apparatus, and the results will be analyzed to determine which suspect committed the crime.

The post-lab section concentrates on the ethical implications of DNA fingerprinting.

Student Audience

This lesson is appropriate for average to gifted students, grades 8–12.

Goals for the Activity

The goals of this activity are to

- introduce students to agarose gel electrophoresis as a tool for separating DNA fragments,
- introduce the concept of DNA fingerprinting,
- give students practice in interpreting results,
- enable students to discuss the ethics of the procedure through reading and class discussion,
- allow students to identify personal values and understand the relationship between these values and ethical choices,
- help students understand the role of philosophical and ethical concerns and their relationship to biotechnology, and
- help students recognize and respect opposing viewpoints.

Recommended Placement in the Curriculum

This lesson would be appropriate as a culminating activity for a unit on human genetics, molecular biology, or microbial genetics.

STUDENT HANDOUT
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Scenario/Industrial Application

A newspaper account of a crime scene may be used, or a scenario such as the following may be used:

> Sixteen-year-old Michele Michael, a popular honor student and cheerleader, has been found murdered. She was reported missing by her family almost a year ago, and her decomposed body has been discovered in the desert by a hiker.
>
> The police, after an extensive investigation, have narrowed the list of possible suspects to two people. Bonita Ballantine, an older half-sister of Michele's, has a history of rivalry with her. Bonita was seen in public arguing with Michele about one week before her disappearance. Although her jealousy of Michele was well known, Bonita denies that she had anything to do with Michele's disappearance and murder.
>
> Brad Chadwick, Michele's ex-boyfriend, also denies having anything to do with Michele's disappearance and murder. However, Michele complained to a friend about two months after her breakup with Brad that she had seen him following her. She was growing increasingly uncomfortable with Brad's obsessive attention and planned to get a restraining order against him. Brad's friend has given him an alibi for the night Michele disappeared, but police feel he is still a prime suspect.
>
> Blood not belonging to the victim was found at the crime scene. Blood samples have been taken from each of the suspects. You are going analyze these samples (using a procedure similar to those used at actual forensic labs) and decide whether either of the suspects is the murderer.

Materials

Materials included in the EDVOTEK DNA Fingerprinting I Kit #109

- DNA sample from crime scene cut with Enzyme 1
- DNA sample from crime scene cut with Enzyme 2
- DNA sample from Suspect 1 cut with Enzyme 1
- DNA sample from Suspect 1 cut with Enzyme 2
- DNA sample from Suspect 2 cut with Enzyme 1
- DNA sample from Suspect 2 cut with Enzyme 2
- tube of practice gel loading solution
- bottle of UltraSpec-Agarose™ powder
- bottle of 50X concentrated electrophoresis buffer
- bottle of concentrated Methylene Blue Plus™ stain
- 10-mL pipet
- 100-mL graduated cylinder
- microtipped transfer pipets

Other materials

- horizontal gel electrophoresis apparatus
- DC power supply
- automatic micropipets and tips
- water bath (65°C)
- staining tray and net

- fluorescent light box or overhead projector
- microwave, hot plate, or burner
- pipet pumps or bulbs
- 250-mL flasks or beakers
- vinyl gloves and safety goggles
- aprons
- distilled or deionized water
- small plastic containers with covers or zipper-type plastic bags
- permanent marking pens
- Styrofoam® containers and ice
- plastic wrap
- (optional) microcentrifuge
- (optional) camera

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

When dealing with biological materials, take particular precautions as called for by the kit manufacturer or supplier.

While methylene blue is relatively harmless, it will stain clothes, skin, and other surfaces. Aprons, gloves, and goggles should be worn.

Dispose of used reagents according to local ordinances.

Procedure

DNA analysis in a forensic lab proceeds as follows: The first step is to extract high-molecular-weight, duplex DNA from blood, semen, skin, or hair roots. This step is followed by purification.

Restriction enzymes are then used to digest the DNA. Agarose gel electrophoresis is performed to separate the DNA fragments according to size. This is the procedure you will be performing during this lab, which uses predigested samples of nonhuman DNA.

Denaturation of the DNA separates the strands of the double helix. A Southern Blot transfers the results to a nylon or nitrocellulose filter. A radioactively labeled probe is added. It will hybridize to the complementary DNA so that the results can be exposed to X-ray film and be detected by autoradiography.

Procedure for performing the agarose gel electrophoresis

1. Pour agarose gel into the gel bed (if this step has not been performed by instructor).

2. After it sets, place agarose gel in the gel bed into an electrophoresis gel apparatus.

3. Add electrophoresis buffer to the gel apparatus until the gel is just covered.

4. With a clean pipet, place 5 μL of #1 DNA cut with enzyme #1 into well #1 of agarose gel.

5. With a clean pipet, place 5 μL of #1 DNA cut with enzyme #2 into well #2.

6. With a clean pipet, place 5 μL of #2 DNA cut with enzyme #1 into well #3.

7. With a clean pipet, place 5 μL of #2 DNA cut with enzyme #2 into well #4.

8. With a clean pipet, place 5 μL of crime scene DNA cut with enzyme #1 into well #5.

9. With a clean pipet, place 5 μL of crime scene DNA cut with enzyme #2 into well #6.

10. Place the top on the gel container and connect to a DC power source. Run the gel at 100–150 volts for 30 minutes or until the sample has almost completely crossed the gel.

11. Remove the gel from the chamber and carefully slide the gel into a container (with a cover).

12. Cover the gel with methylene blue stain for 10 minutes or until blue bands of DNA appear on the gel.

13. Pour the methylene blue stain into a stock bottle and rinse gel using distilled or deionized water. Do not use tap water for this step! Tap water washes the stain out completely and causes the DNA bands to fade. Rinse several times. Be careful not to drop or break the gel.

14. Place the gel on a light box and compare samples. Draw a picture of the gel in your lab notebook.

Questions

1. Whose blood was found at the crime scene (i.e., which suspect is guilty of the murder)? How do you know?

2. Why were two different restriction enzymes used in this lab?

3. Why was the methylene blue stain used?

4. Why is the gel box filled with TBE buffer instead of water?

5. What happens if the electrodes are reversed?

6. Who are the only individuals possessing the same DNA fingerprints?

7. What types of human cells can be used for this technique?

Suggested Reading

Cook, R. *Chromosome 6.* Berkley Publishing Group: New York, 1997.

Genetic Engineering: Opposing Viewpoints. Wekesser, C., Ed.; Greenhaven: San Diego, CA, 1996; Chapter 3, pp 120–147.

Ostrow, R.; Jackson, R. "DNA Database to Be Used to Fight Crime." *Los Angeles Times,* Oct 18, 1998.

Wambaugh, J. *The Blooding.* Bantam Books: New York, 1989. (This book describes how a British scientist develops DNA fingerprinting for forensic work and uses the technique to convict a suspect guilty of several rape/murder crimes in an English village.)

INSTRUCTOR NOTES
Innocent or Guilty: A Lab on DNA Gel Electrophoresis

Time Required
1 hour to discuss DNA fingerprinting and examine gel photos
2–3 hours for lab (students)
3–4 hours (instructor preparation)
1 hour for follow-up ethical discussion

Group Size
Plan on 1–6 students per kit; number of kits required depends on class size.

Materials
- EDVOTEK Kit #109, DNA Fingerprinting I, is available from EDVOTEK (800/338-6835, Fax 301/340-0582, *www.edvotek.com).* It is also available from Frey Scientific (catalog # G18886, 100 Paragon Parkway, Mansfield, OH 44903; 800/225-FREY).
  The kit contains a complete list of other materials needed to complete this lab (see the materials list in the Student Handout). Where the author's instructions call for use of a commercial kit and specific materials provided in that kit, we strongly recommend that you use the recommended materials to attain the desired results. When using a commercial kit, read and follow the instructions provided by the kit manufacturer. The author's procedure provided in this activity is not necessarily intended to duplicate or reproduce the manufacturer's instructions. Rather, the procedure has been provided by the author as a summary of the general steps to follow.

Scientific equipment listed under "Other materials" in the Student Handout is available from EDVOTEK (800/338-6835), Stratagene (800/424-5444), or a similar company.

Students will also need a lab notebook similar to the Cold Spring Harbor Lab Research Notebook (800/832-0034).

Safety, Handling, and Disposal
As the instructor, you are expected to provide students with access to SOPs, MSDSs, and other resources they need to safely work in the laboratory while meeting all regulatory requirements. Before doing this activity or activities from other sources, you should regularly review special handling issues with students, allow time for questions, and then assess student understanding of these issues.

When dealing with biological materials, take particular precautions as called for by the kit manufacturer or supplier. The EDVOTEK kit contains complete safety, handling, and disposal instructions. Material Safety Data Sheets are included for components used in the lab: 50X Electrophoresis Buffer, agarose, Methylene Blue Plus, and practice gel loading solution. The kit contains no human DNA and may be stored in a refrigerator. Dispose of used reagents according to local ordinances.

Points to Cover in the Pre-Lab Discussion
Components may be measured by students at lab stations, individual premeasured aliquots may be given to students, or materials may be dispensed from a central station.

If reagent aliquots become spread in a film around the sides or caps of the 1.5-mL tubes, you may either spin the tube in a balanced configuration in a microcentrifuge (if available) or tap the tubes with your fingers.

A constant-temperature water bath for incubating reactions can be made by maintaining a trickle flow of tap water into a Styrofoam box. Temperature may be monitored with a thermometer or an aquarium heater.

Very subtle damage to sample wells during casting and loading can cause gel defects. Good electrophoretic separation and straight, well-defined banding requires that DNA fragments of the same size enter the gel at essentially the same time against the vertical front face of the sample well. To prevent defects, take the following measures:

1. Gels should always be submerged in electrophoresis buffer prior to loading. The pipet tip is centered over the well and lowered only until it dips through the buffer surface. Then the DNA/loading dye solution is carefully expelled.

2. The comb should be pulled straight up from the gel. Wiggling or rocking the comb while removing it can deform the well. The casting tray should be held steady while the comb is pulled straight up.

3. Give the gel enough time to completely set.

4. The comb should be cleaned of any hardened agarose gel since any left-over material on a casting comb can cause defects.

5. A bent comb or loose casting tray notches can be corrected with masking tape.

6. Air bubbles and/or debris in a poured gel can cause defects. These should be removed before the gel sets.

Agarose gel can be cast a day or two before use. To prevent drying, keep it covered with TBE electrophoresis buffer in the covered electrophoresis chamber, a zipper-type bag, or a plastic container.

A fluorescent light box for viewing slides and negatives provides ideal illumination for the stained gels. An overhead projector may also be used. Cover the surface of the light box or projector with plastic wrap to keep liquid off the apparatus.

Photographing the gels after electrophoresis is desirable if the equipment is available. Photographs of DNA gels provide a permanent record of the experiment. Photographing allows time to analyze results critically.

Background

1. Review the structure of DNA and the process of Southern blotting that is done in actual DNA fingerprinting. Also, explain polymerase chain reaction (PCR), which is used to amplify DNA before the electrophoresis process. The following brief description may be used as a starting point:

Kary Mullis was awarded the Nobel Prize in 1993 for his work with PCR, which he developed during the 1980s. Through this technique, a specific piece of DNA may be targeted and then used to produce billions of copies in a short period of time.

2. Electrophoresis means "to carry with an electrical current." The different-sized pieces of DNA are carried different distances by an electric current. The smaller pieces travel the greatest distance in the gel.

3. The DNA molecule has an overall negative charge (due to the phosphate groups that alternate with the sugar, deoxyribose, to form the ladder structure of the double helix). The negatively charged DNA is attracted to the positively charged electrode when a current is supplied.

4. The DNA is "digested" by bacterial enzymes called restriction enzymes. Since this lab uses predigested DNA samples, the emphasis of the lab does not call for a lengthy discussion of the different types of restriction enzymes. However, you might want to expand the students' knowledge of restriction enzymes with another lab. (See "Extensions and Variations.")

5. Each individual has a unique DNA pattern. Agarose gel electrophoresis combined with methylene blue staining allows for a rapid analysis of the DNA fragments obtained by the digestion of the DNA by restriction enzymes.

6. DNA gels are read from left to right with the sample wells at the top. The area extending from the well in a vertical column down the gel is a "lane." Reading down the lane identifies fragments generated by a particular restriction reaction.

7. DNA fingerprinting has a 99% accuracy. The DNA in cells remains recognizable even after the death of the cell and over a long time period (e.g., dinosaur DNA).

8. If gel photos are available (Kit #109 contains diagrams), discuss their similarity to bar codes used to identify products in supermarkets.

Procedural Tips and Suggestions

Kit #109 contains diagrams and a step-by-step explanation of agarose gel electrophoresis that may be reproduced for student use. Following the procedure, conduct a class discussion of the ethics of DNA fingerprinting.

Sample Results

Kit #109 contains an idealized schematic of results, which may be reproduced for the students so that they may compare their gels to the schematic.

Plausible Answers to Questions

1. Whose blood was found at the crime scene (i.e., which suspect is guilty of the murder)? How do you know?
   The students should answer this based on their results.

2. Why were two different restriction enzymes used in this lab?
   There is no differentiation between the DNA from the two suspects cut by restriction enzyme #1. The differentiation occurs when the DNA is cut by restriction enzyme #2.

3. Why was the methylene blue stain used?
   Methylene blue stain was used to make the DNA fragments easier to see.

4. Why is the gel box filled with TBE buffer instead of water?
   If water were used, the electrophoresis would proceed very slowly. Water has a higher resistance than the TBE buffer when running at constant temperature. The TBE buffer is a better medium for the electrophoresis than water.

5. What happens if the electrodes are reversed?
   The fragments move in the opposite direction and thus off the near end of the gel very quickly.

6. Who are the only individuals possessing the same DNA fingerprints?
   Identical twins.

7. What types of human cells can be used for this technique?
   Blood, skin, hair, semen, etc.

Extensions and Variations

- Kit #225 ($95.00) "DNA Fingerprinting II: Usage of Restriction Enzymes in DNA Fingerprinting Analysis" (Intermediate). Kit #225 offers more student manipulation. It utilizes the crime scene format but includes undigested samples.

- Education Kit #14 is available from Stratagene (800/424-5444). This kit is similar to the EDVOTEK kit used in this lesson.

- The most hands-on lab is offered by California Lutheran University's Enriched Science Program (CLUES), Versions A, B, and C. More information may be obtained from California Lutheran University, Office of Science Outreach, 60 W. Olsen Road, Thousand Oaks, CA 91360-2787, or by calling 805/493-3385.

  Unit 3—Module 4
  Protocol A—Students perform restriction enzyme digests on DNA prior to electrophoresis steps and identify the unknown DNA.

  Protocol B—Students use DNA that has previously been digested with restriction enzymes. They compare their DNA's pattern with provided standard patterns. (This protocol is most like kit #109 used in this lesson.)

  Protocol C—Students use four dye mixtures to simulate DNA that has been digested with restriction enzymes. Agar is used to pour the gel instead of agarose.

References

"Background Information: DNA Print Identification Test." Lifecodes Corporation, 1990 [pamphlet].

Bloom, M.V.; Fryer, G.A.; Micklos, D.A. *Laboratory DNA Science: An Introduction to Recombinant DNA Techniques and Methods of Genome Analysis;* Benjamin/Cummings: Menlo Park, CA, 1996; pp 31–53.

Jacobs, P. "The Race to Crack the Gene Code." *Los Angeles Times,* Oct 29, 1998, 1, 22.

Micklos, D.A.; Freyer, G.A. *DNA Science: A First Course in Recombinant DNA Technology;* Cold Spring Harbor Laboratory: Plainview, NY, 1990; Chapters 4 and 7.

Thornton, J.I. “DNA Profiling: Forging a Stronger Link Between Evidence and Suspects.” *Chemical and Engineering News*, Nov 20, 1989, 18–30.

Walker, P.; Wood, E. *Crime Scene Investigations: Real-life Science Labs, Grades 6–12;* The Center for Applied Research in Education: New York, 1994.