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DNA Fingerprinting: The Great Cafeteria Caper

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INTRODUCTION

Description

Students will extract DNA from their own hair roots. A DNA fingerprinting simulation kit with standard DNA samples will also be used in this experiment. The DNA will be digested with a variety of restriction enzymes (e.g., B*am*H I and *Hin*d III). Students will run an electrophoresis gel to examine patterns of their DNA along with standard DNA. The experiment will be based on a crime scene scenario.

Student Audience

This experiment would be appropriate for advanced biology and chemistry labs in high school and college biology, microbiology, or chemistry classes.

Goals for the Activity

Students will

- explain the structure of DNA,
- explain how gel electrophoresis can be used to separate and visualize DNA,
- interpret sample results from gel electrophoresis,
- list applications of DNA science in our society, and
- explain the role of restriction enzymes in molecular biology.

Recommended Placement in Curriculum

This activity must follow a thorough theoretical explanation of the structure and functional roles of DNA and RNA. If time allows, plan to include a brief discussion of the ethical implications of DNA science.

STUDENT HANDOUT
DNA Fingerprinting: The Great Cafeteria Caper

Scenario

As you may know, the cafeteria is centrally located on our campus. It is a constant source of pride and joy for staff and students alike. You can imagine the surprise when a hypothetical cheeseburger was found with a hypothetical hair located between the burger and bun. The extremely hypothetical burger was served to an imaginary visiting dignitary. There are several suspects in this dastardly deed. The infamous hair was held as evidence, and DNA samples were obtained from various suspects. Your mission is to supply a hair sample, process all DNA samples, and interpret an electrophoresis gel.

You play the sleuth…and the suspect!

Purpose

Any cells (skin, blood, hair, semen, etc.) can supply DNA for analysis. The analysis that we will do is called "DNA fingerprinting." This technique allows scientists to compare DNA from various organisms (people, horses, bacteria) and identify a particular individual. You can extract the DNA and cut it with special enzymes called restriction enzymes that recognize specific base sequences on the DNA molecule. The pieces produced will be unique to each individual (except identical twins). The scientist (that's you) can then employ a tool called electrophoresis. Electrophoresis means "to carry with an electrical current." DNA pieces move through a gelatinous substance (agarose gel) carried by an electrical current. The DNA molecule has an overall negative charge due to the phosphate groups. Samples of DNA are loaded in small wells cast at one end of the gel and move with other anions away from the negative electrode toward the positive electrode. DNA fragments separate by size because the smaller pieces move farther through the agarose gel toward the positive electrode. (See Figure 1.) After separating the fragments, you turn off the current, remove the gel from the electrophoresis chamber, and stain the gel with a visible chemical that binds to DNA. The end product looks something like the bar code on a supermarket package. Each person should produce a unique pattern on the gel.

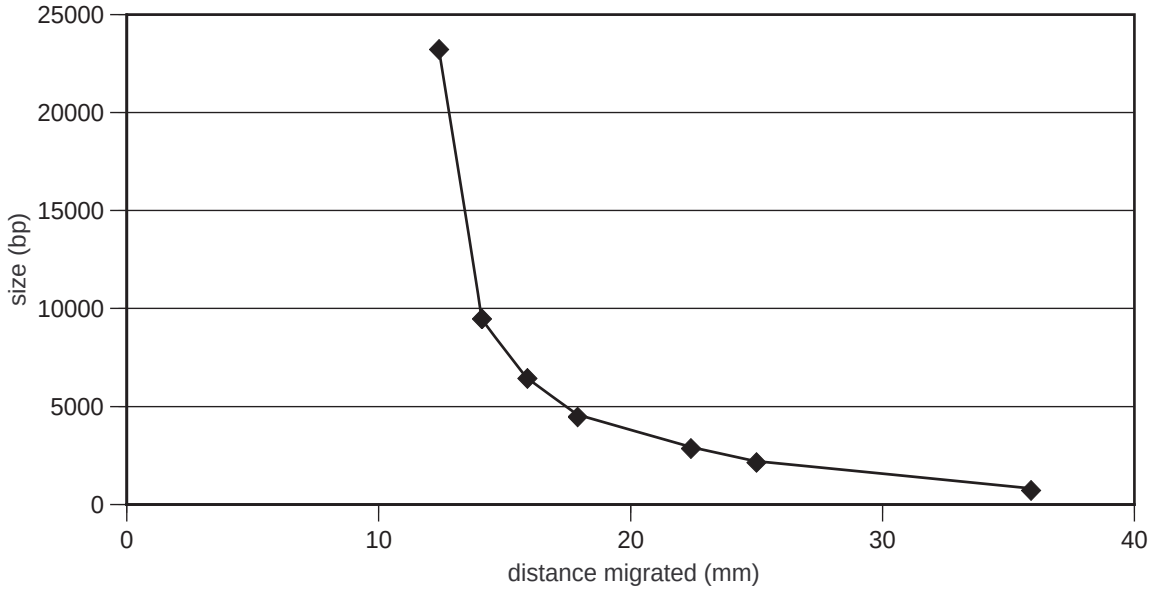

Figure 1: Distance of migration vs. DNA fragment size

Materials

- hair supplied by suspects
- dissecting microscope or hand lens
- razor blades
- forceps
- 1.5-mL locking tube
- micropipets (150 µL)
- proteinase K (100 µg/mL) in PBS buffer
- 37°C water bath
- 10% Chelex solution
- microcentrifuge
- DNA Fingerprinting Simulation Kit
- 6 precut plasmid DNA samples
- microcentrifuge tubes
- buffer solution
- enzyme solution (*Hin*d III)
- ice buckets
- agarose powder
- tris-borate-EDTA (TBE) buffer concentrate
- loading dye/stop solution
- methylene blue stain (0.025% suggested)
- capillary tubes with plungers
- storage bags for gels
- safety goggles
- lab coat or apron
- incubator or water bath
- markers
- beaker for used tips
- disposable gloves
- microcentrifuge
- staining tray
- distilled water
- electrophoresis equipment
- micropipetters and tips
- beaker for agarose
- camera and film
- masking tape
- test tube rack
- ethidium bromide (optional)

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.

Store restriction enzymes in a non-frost-free freezer at a constant temperature of -10 to -20°C. If a frost-free freezer must be used, store the enzymes in their Styrofoam® shipping container. Purified DNA can be stored in the refrigerator.

TBE buffer and methylene blue staining solutions can be reused for several labs. TBE buffer can be left in the electrophoresis chambers for a week.

If a UV light source is used, eye protection is necessary.

Ethidium bromide is a mutagen by the Ames assay. If ethidium bromide is used as the staining reagent, educators must research and adhere to proper handling and decontamination procedures.

Dispose of all used reagents according to local ordinances.

Procedure

I. Isolation of DNA from hair root for possible polymerase chain reactions (PCR)

1. Pull out four hairs from your head and observe them under a dissecting microscope or hand lens to confirm the presence of the hair sheath.

2. Use a razor blade to cut off each hair shaft just above the sheath material.

3. Use your forceps to transfer hair roots to a 1.5-mL locking tube labeled with your name.

4. Using a clean tip, add 150 µL proteinase K (100 µL/mL) in PBS buffer to the tube containing your hair roots. You may need to use the pipet tip to push or “wash” the hair roots to the bottom of the tube.

5. Place the sample tube into the 37°C water bath and incubate for 30 minutes. It is not necessary to lock the tube at this time.

6. Remove the sample tube to room temperature and use a clean pipet tip to add 150 µL 10% Chelex solution to the sample tube.

7. Lock your sample tube and place it in boiling water for 8 minutes.

8. Remove your sample tube and mix the contents by vortexing briefly.

9. Microcentrifuge the sample tube for 2 minutes. Be sure to balance the tubes.

10. Without disturbing the cell debris, transfer as much of the supernatant as possible to a fresh 1.5-mL tube labeled with your name.

11. The sample may be stored at -20°C until you are ready for polymerase chain reaction (PCR). PCR amplifies the amount of DNA found in a small sample. We will not be doing PCR at this time.

II. DNA restriction and electrophoresis

This section of the procedure is kit-dependent. This procedure is a suggested protocol with some details omitted. A reference for the outline was *Laboratory DNA Science* by Mark V. Bloom, Greg A. Freyer, and David A. Micklos.

Enzyme Restriction Digest

1. Use a permanent marker to label test tubes for DNA samples as indicated by the instructor.

2. Add the specified amount of DNA to each reaction tube. Touch the tip of the pipet to the side of the reaction tube near the bottom.

3. Use a fresh pipet tip to add a specified amount of buffer. Touch the tip of the pipet to a clean spot on the tube.

4. Use a fresh pipet tip to add a specified amount of restriction enzyme to the appropriate tubes.

5. You may need to add distilled water.

6. Close the top of the tube and pool the reagents. You may sharply tap the bottom of the tube on the lab bench or use the microcentrifuge.

7. Place the tubes in a 37°C water bath for approximately 20 minutes.

Cast Agarose Gel (suggested 0.8% agarose solution)

8. Close the ends of the gel casting tray and insert a clean well-forming comb. Place the tray so that the gel can set undisturbed.

9. Retrieve the agarose gel from the water bath and carefully pour the solution into the casting tray to a depth of about 5 mm (30-mL aliquot for some trays). Pour steadily in one corner to obtain a smooth gel.

10. When the gel has set (about 10 minutes), open the ends of the casting tray. Set the tray on the platform of the gel box so that the comb is at the negative (black) electrode.

11. Fill the electrophoresis box with TBE buffer (0.5X–1X). Pour buffer so that the liquid just covers the gel. Dimples indicate that more buffer should be added.

12. Carefully remove the comb by pulling it straight up and out of the gel. Readjust the buffer level if necessary.

Loading Gel and Electrophoresis

13. Remove restriction digests from the water bath.

14. Add 1 µL loading dye to each reaction tube. Sharply tap the tubes against the bench top to mix the solutions. If a microcentrifuge is available, spin the solutions to mix the contents.

15. Load 10 μL from each tube into a separate well in the gel. Take care not to damage the well with the pipet tip. Slowly depress the pipet plunger and keep the plunger depressed until the tip is withdrawn from the well and the buffer solution.

16. Close the top of the electrophoresis box and connect the leads from the power supply anode to anode (red-red) and cathode to cathode (black-black).

17. Turn on the power and set the voltage between 95–150 volts (as indicated by the instructor). Current can be detected by observing gas bubbles released from electrode wires.

18. Electrophorese for 40–60 minutes. Stop the current before the loading dye bands run off the end of the gel.

19. Turn off the power supply.

III. Stain and photograph the gel...ID the perpetrator!

1. Put on a pair of disposable gloves.

2. Flood the gel with 0.025% methylene blue solution and stain for 20–30 minutes.

3. After staining, decant as much of the staining solution as you can into a storage container for reuse.

4. Rinse the gel in running tap water and let it soak for 5 minutes in a couple of changes of fresh water.

5. View and photograph the gel as dictated by specific equipment.

6. Scan across the lanes to identify DNA fragments that have migrated the same distance down the gel. These DNA fragments are of similar size. Identify the perpetrator of the cafeteria crime according to your gel's evidence.

Questions

Please ask for clarification on any questions.

Internet assignment

1. Find two websites that discuss the application of DNA science in our society (medical, agricultural, or otherwise). Record the website addresses and summarize these discussions in two paragraphs. Finally, what is your opinion of each of these discussions (potential implications, ethical considerations, benefits, etc.)? This total discussion will get you 15 points for three clear, concise paragraphs.

DNA-based questions

2. Diagram the structural components of deoxyribonucleic acid (DNA). (5 points)

3. Define "gene." (4 points)

4. What chemical components of DNA are unique to each individual? (2 points)

5. What are restriction enzymes? (2 points)

Laboratory-based questions

6. What does RFLP stand for, and how was this concept used in this activity? (2 points)

7. What was the purpose of using the restriction enzymes in this activity? (2 points)

8. How and why could the techniques used in this lab be applied to paternity suits? (4 points)

9. What would happen to your gel if you forgot to add restriction enzymes? (2 points)

10. Why are the DNA fragments separated on the gel? (2 points)

Total of 40 Points

Suggested Reading

Anderson, W.F. "Gene Therapy," *Scientific American*. 1995, *273*(3), 124–128.

Bloom, M.V.; Freyer, 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.

Kolata, G. *Clone: The Road to Dolly and the Path Ahead;* William Morrow: New York, 1998.

Neufeld, P.J.; Colman, N. "When Science Takes the Witness Stand," *Scientific American*. 1990, *262*(5), 46–53.

INSTRUCTOR NOTES
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Time Required
This activity takes about 5 hours (two 2-hour lab sessions and one 1-hour lecture).

Group Size
This activity works best with a maximum of 16 students operating from eight stations.

Materials
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.

- hair supplied by suspects
- dissecting microscope or hand lens
- razor blades
- forceps
- 1.5-mL locking tube
- micropipets (150 µL)
- proteinase K (100 µg/mL) in PBS buffer
- 37°C water bath
- 10% Chelex solution
- microcentrifuge
- DNA Fingerprinting Simulation Kit
  This kit can be obtained from Stratagene Educational materials (800/424-5444) or Carolina Biological Supply (800/334-5551).
- 6 precut plasmid DNA samples
- microcentrifuge tubes
- buffer solution
- enzyme solution (*Hin*d III)
- ice buckets
- agarose powder
- tris-borate-EDTA (TBE) buffer concentrate
- loading dye/stop solution
- methylene blue stain (0.025% suggested)
- capillary tubes with plungers
- storage bags for gel
- safety goggles
- lab coat or apron
- incubator or water bath
- markers
- beaker for used tips
- disposable gloves
- microcentrifuge
- staining tray
- distilled water

- micropipetters and tips
- beaker for agarose
- camera and film
- masking tape
- test tube rack
- electrophoresis equipment
- ethidium bromide (optional)

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.

Store restriction enzymes in a freezer at a constant temperature of -10 to -20°C. If a frost-free freezer is used, store the enzymes in their Styrofoam shipping container. Purified DNA can be stored in the refrigerator.

TBE buffer and methylene blue staining solutions can be reused for several labs. TBE buffer can be left in the electrophoresis chambers for a week.

If a UV light source is used, eye protection is necessary.

Ethidium bromide is a mutagen by the Ames assay. If ethidium bromide is used as the staining reagent, educators must research and adhere to proper handling and decontamination procedures.

Dispose of all used reagents according to local ordinances.

Plausible Answers to Questions

Internet assignment

1. Find two websites that discuss the application of DNA science in our society (medical, agricultural, or otherwise). Record the website addresses and summarize these discussions in two paragraphs. Finally, what is your opinion of each of these discussions (potential implications, ethical considerations, benefits, etc.)? This total discussion will get you 15 points for three clear, concise paragraphs. *Student results will vary*.

DNA-based questions

2. Diagram the structural components of deoxyribonucleic acid (DNA).
   Look for deoxyribose and phosphate backbones with correctly paired cytosine, guanine, adenine, and thymine bases.

3. Define “gene.”
   A section of DNA with a particular function. Functions: 1) instructions for protein synthesis, 2) instructions for RNA synthesis, and 3) regulatory function.

4. What chemical components of DNA are unique to each individual?
   The order of the bases (cytosine, guanine, adenine, thymine).

5. What are restriction enzymes?
   Enzymes that break the DNA into segments of genetic material called restriction fragment length polymorphisms (RFLPs). The enzymes recognize certain base sequences in the intact DNA.

Laboratory-based questions

6. What does RFLP stand for, and how was this concept used in this activity?
   Restriction fragment length polymorphism is based on whether or not a sequence of bases (restriction site) is present on the DNA to be recognized by the restriction enzymes. The RFLPs are the fragments of DNA that are unique to the suspects in this caper. These pieces of DNA are identified by their unique migration pattern on the electrophoresis gel.

7. What was the purpose of using the restriction enzymes in this activity?
   These enzymes produce the unique fragments that migrate through the gel.

8. How and why could the techniques used in this lab be applied to paternity suits?
   The child and possible father could provide a DNA sample that, when subjected to the action of restriction enzymes, might provide similar DNA fragments. Based on examination of a stained gel, statistics are used to show a reasonable likelihood of whether the man in question is the child's father.

9. What would happen to your gel if you forgot to add restriction enzymes?
   The genomic DNA might be too large to migrate out of the well. (This may happen with the DNA extracted from hair.) You may also see a smear of DNA along the lane of the gel.

10. Why are the DNA fragments separated on the gel?
    The electrophoresis chamber sets up a positive and negative pole. The DNA has a negative charge, so it migrates slowly through the pores in the gel toward the positive pole. Fragment size is a factor in how far and fast the DNA can migrate.

Extensions and Variations

1. It would be possible to amplify DNA extracted from hair using polymerase chain reactions (PCR) techniques. An advanced class could use Southern hybridization to detect a specific restriction fragment.

2. Have the students discuss one or more of the following questions.
   a. Why can blood be drawn for a DNA analysis when the sample at the crime scene was hair?
   b. DNA fingerprinting became famous during the O.J. Simpson trial. Why is the collection and handling of samples so important?
   c. Could you separate proteins using any of the techniques in this lab?
   d. Have students watch the movie *Gattaca*. What aspects of this movie seem possible in our "not-so-distant future"? List three social implications addressed in this movie. Do you agree?

Reference

Bloom, M.V.; Freyer, 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.