

the

Case of the Crown Jewels

A DNA Restriction Analysis Laboratory Activity

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The MdBioLab is sponsored by:

Fisher Scientific, Inc.
SAIC

NIH Foundation
Bio-Rad

Montgomery County, MD

Case of the Crown Jewels

For Groups Visiting the MdBioLab

The Case of the Crown Jewels has two parts:

- A classroom activity that allows students to explore how the unique sequence of bases in DNA can be used to identify individuals.
- A laboratory activity that allows students to use DNA restriction analysis to determine if one of the two suspects were at a fictitious crime scene.

Teachers and students who will be performing *The Case of the Crown Jewels* laboratory activity on the MdBioLab must first complete the pre-laboratory classroom activity before visiting the mobile lab. The conceptual aspects of the curriculum will be reinforced with the laboratory activity on the mobile lab.

MdBioLab will supply all reagents, equipment and instruction for the laboratory activity. Teachers and students will be required to supply their own goggles and copies of the following handouts:

Micropipette Challenge	Page 16
Data/Observation Sheet	Page 17

The MdBioLab is a state-of-the-art mobile laboratory for high school teachers and students designed to bring new opportunities in science education and training to schools interested in expanding their current science curricula. This educational tractor-trailer and instructors are capable of hosting 32 students in four different hands-on, cutting-edge laboratory investigations.

Case of the Crown Jewels

MSDE Science Core Learning Goals

These classroom and laboratory activities meet several of the MSDE Science Core Learning Goals:

Goal 1: Skills and Processes: The student will demonstrate ways of thinking and acting inherent in the practice of science. The student will use the language and instruments of science to collect, organize, interpret, calculate, and communicate information.

3. **Expectation:** The student will carry out scientific investigations effectively and employ the instruments, systems of measurement and material of science appropriately.

Indicators of Learning:

- 1) The students will develop skills in using lab and field equipment to perform investigative techniques
- 2) The student will demonstrate safe handling of the chemicals and materials of science appropriately
- 3) The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction

4. **Expectation:** The student will demonstrate that data analysis is a vital aspect of the processes of scientific inquiry and communication.

Indicators of Learning:

- 1) The student will use analyzed data to evaluate a hypothesis
- 8) The student will use models and computer simulations to represent systems

5. **Expectation:** The student will use appropriate methods for communicating in writing and orally the processes and results of scientific investigation.

Indicators of Learning:

- 1) The student will demonstrate the ability of summarize data, investigate results, scientific concepts and processes through drawing, written and/or oral communication
- 2) The students will use tables, graphs, and charts to display data in making arguments and claims in both written and oral communication
- 3) The student will create and interpret scale drawings
- 5) The student will read a technical report and interpret it appropriately

Goal 3: Concepts of Biology: The student will demonstrate the ability to use the scientific skills and processes and major biological concepts to explain the uniqueness and interdependence of living organisms, their interactions with the environment, and the continuation of life on earth.

5. **Expectation:** The student will explain the mechanism of evolution change.

Indicators of Learning:

The student will estimate degrees of kinship among organisms or species

Goal 4: Concepts of Chemistry: The student will demonstrate the ability to use scientific skills and processes to explain composition and interactions of matter in the world in which we live.

5. **Expectation:** The student will explain how the observation of the properties of matter forms the basis for understanding its structure and changes in its structure.

Indicators of Learning:

- 1) The student will select and use appropriate devices to measure directly or indirectly the length, mass, volume, or temperature of a substance.

Case of the Crown Jewels

Introduction

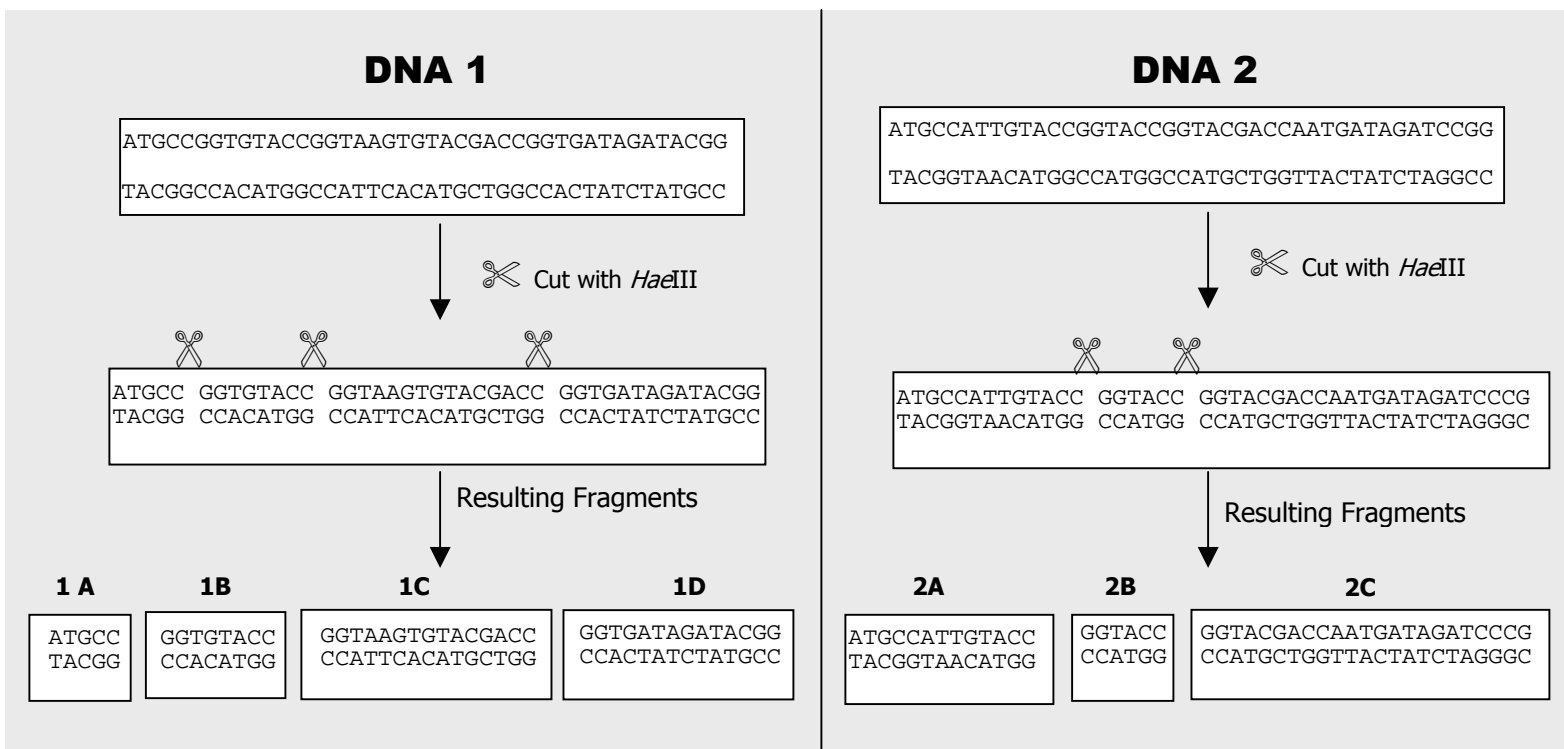
DNA restriction analysis is a technique with wide ranging applications in medicine, research, and forensics. The Case of the Crown Jewels is an activity that simulates the DNA fingerprinting process used by forensic scientists, which relies on restriction analysis to analyze DNA evidence from a fictional crime scene.

DNA restriction analysis is based on the following assumptions:

- DNA molecules can be identified by a difference in the sequence of bases
- Enzymes, which are produced naturally by bacteria, cut DNA molecules at specific sites denoted by base sequences

When a restriction enzyme is used to cut different DNA molecules, the size of the fragments generated will be unique to each molecule. As shown in Figure 1, both DNA 1 and DNA 2 are cut with *Hae*III, an enzyme that cuts between the base pairs GG | CC and CC | GG.

FIGURE 1: Restriction Digest of Two DNA Fragments

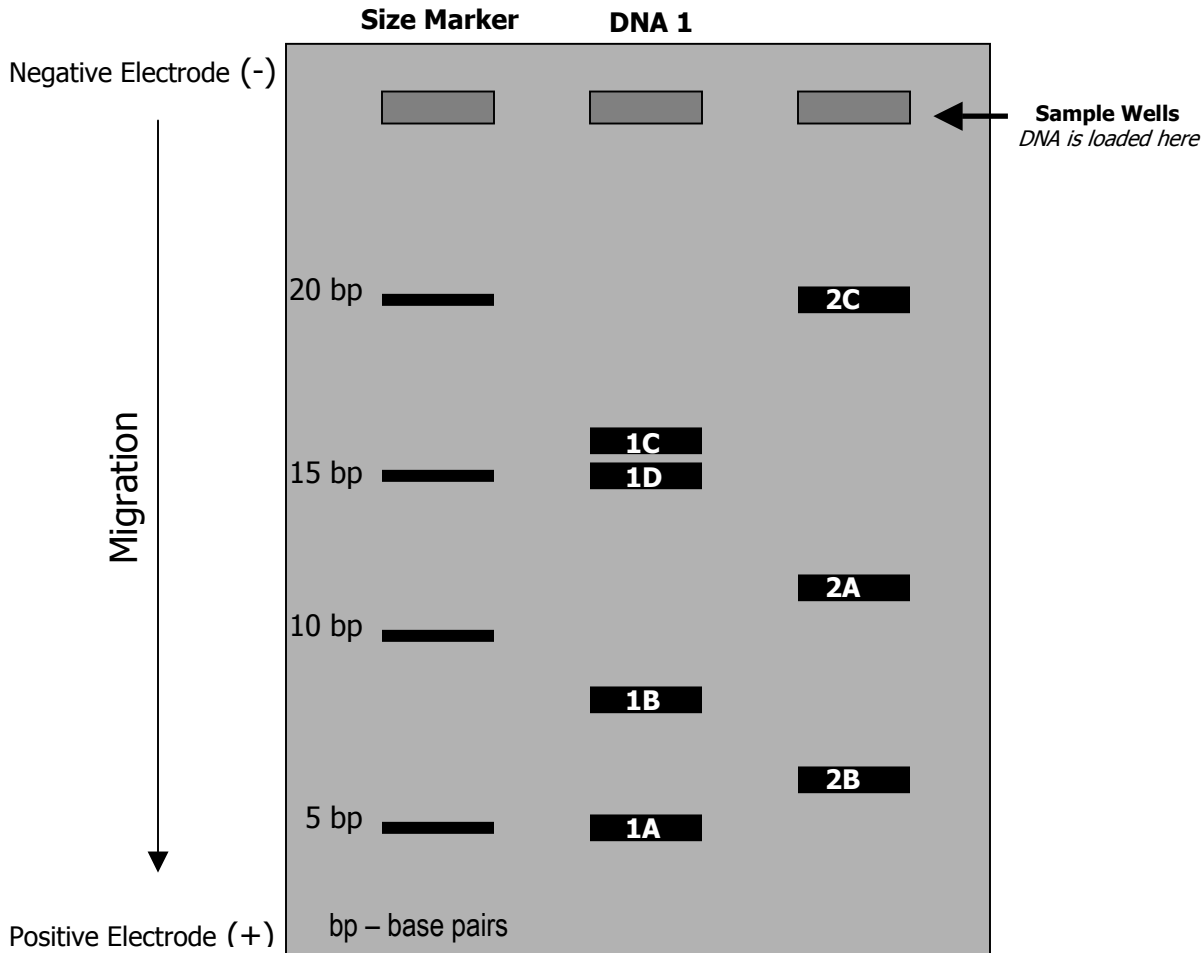


After being cut by restriction enzymes, DNA fragments remain mixed in solution and indistinguishable from one another. One way to distinguish between the different fragments created is to compare them by size. Different size fragments of DNA can be separated using gel electrophoresis.

Gel electrophoresis is a technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric field. In non-technical terms, DNA is negatively

charged - thus, when in the presence of an electric current, DNA will travel according to size (smallest pieces first) towards the positive electrode. By comparing the resulting pattern of DNA fragments on the gel, the DNA strands can be differentiated.

FIGURE 2: Gel Electrophoresis of Fragments from Restriction Digest with *Hae*III



This lesson is organized into two parts - a pre-laboratory and a laboratory investigation. During the pre-laboratory, students work in groups to simulate DNA restriction analysis using paper DNA strands and scissors to solve a fictional crime. Following the pre-laboratory, students work in the laboratory where they apply the concepts acquired in the pre-laboratory to compare two suspects' DNA found at a fictional crime scene.

Case of the Crown Jewels

Pre-Laboratory Activity

NOTE: Groups visiting the MdBioLab must first complete the pre-laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity on the mobile lab.

The purpose of the pre-laboratory activity is to explore how the unique sequence of bases in DNA can be used to identify individuals. It provides students the opportunity to investigate the application of restriction enzymes and gel electrophoresis to generate evidence of similarities or differences among DNA molecules. The objectives of the pre-laboratory are:

- Identify a need for DNA restriction analysis
- Model the concept of DNA restriction analysis
- Apply DNA restriction analysis to the identification of DNA fragments
- Work cooperatively to analyze the results of the DNA restriction analysis

Pre-laboratory Materials

Five scissors

Five sets of envelopes labeled *Suspect #1*, *Suspect #2*, *Suspect #3*, *Suspect #4* and *Crime Scene DNA*

Instructions for each envelope (labeled *DNA Instructions*)

Five sets of base sequences (labeled *DNA Strips*)

Five rolls of tape

Five poster-size charts as shown on *DNA Instructions* sheet

Pre-laboratory Engagement (10 – 15 minutes)

Organize students into five groups (no more than five students per group). Tell the students that they are now part of a forensics team. Have each team read one copy of *The Case of the Crown Jewels: Police Report* and tell them that their job is to solve the crime on the basis of the evidence that will be given to them.

Pre-laboratory Exploration (30 – 40 minutes)

Prepare one set of five evidence envelopes (see pre-laboratory materials) for each group by cutting out the DNA base fragments into *DNA Strips* and putting the cut outs of the DNA sequences in their respective envelopes with a *DNA Evidence Evaluation* sheet. Provide the students with the evidence needed for their investigation of the crime by providing a set of evidence envelopes for each group, one envelope per student. Tell the students to follow the instructions to try to solve the crime. The instructions will guide them through the process of DNA restriction analysis. As facilitator, be prepared to assist the students and address any misconceptions. Encourage students to help each other but to work only on the DNA fragment in their envelope.

Provide each group with a poster-size chart like that shown on page two of the *DNA Evidence Evaluation*. As students complete the activity as instructed, they will tape the resulting DNA fragments on the poster-size chart.

Pre-laboratory Explanation (20 – 30 minutes)

Provide a *Final Report* handout to each group. After each group has finished putting the DNA fragments on the chart, have each team fill out the *Final Report*. Lead a class discussion regarding their conclusions and the process they employed to obtain evidence based on DNA. Possible discussion questions could be:

- This process is often referred to as DNA fingerprinting. Why do you think this term is used?
- Why use DNA as evidence?
- What purpose do the restriction enzyme serve?
- Does a match of the suspect DNA fragments with the crime DNA fragments mean the suspect is guilty? Why or why not?

Emphasize that the distinguishing characteristic of DNA is the sequence of nucleotide bases. Note that the technique modeled does not sequence the DNA. The technique, DNA restriction analysis, provides indirect evidence that sequence of DNA samples are the same or different from one another. If the restriction enzymes cut the DNA sample into identical size fragments, the DNA samples are probably the same. If the restriction enzymes cut the DNA samples into different size fragments, the DNA samples are probably different.

Assessment

Give an index card to each student. Have them describe in their own words what they learned from the activity and discuss briefly other applications for which DNA restriction analysis could be used.

Case of the Crown Jewels

Laboratory Explanation

NOTE: MdBioLab will supply all reagents, equipment and instruction for the laboratory activity for groups visiting the mobile lab. Teachers and students will be required to supply their own goggles and copies of the following handouts:

Micropipette Challenge	Page 16
Data/Observation Sheet	Page 17

If a teacher would like to perform this laboratory activity in their classroom, information on preparing the solutions is included at the end of this document.

The purpose of the laboratory component of this unit is to apply the concepts developed in the pre-laboratory to use DNA restriction analysis to determine if one of the two suspects were at a fictitious crime scene. The objectives of the laboratory component are as follows:

- Model the process of DNA restriction analysis
- Perform a restriction digest and electrophoresis
- Analyze the results of the competed DNA gels

Before proceeding with the laboratory investigation, it is necessary to make a logical connection to the concepts developed in the pre-laboratory. In doing so, the laboratory component becomes a tool in the continuum of an ongoing problem rather than an isolated end in itself. The transitional activity that follows links the pre-laboratory concepts to the ensuing laboratory investigation.

Developing the concept for the technique

Describe a crime scenario to the class with one crime sample and two suspects. Present three microcentrifuge tubes with DNA from the crime and two suspects. Ask the class how DNA can be used to obtain evidence in the case. Pursue a line of questioning which facilitates a discussion of how the DNA samples can be differentiated, i.e. "How are we going to distinguish one DNA sample from another?" Students should be reminded to reflect on the pre-laboratory exercise. Ask whether students can tell if either suspect's DNA is the same as that found at the crime scene by visually inspecting it. Because the samples look exactly alike, a tool is needed to determine if either of the samples could match that found at the crime scene.

Overview of Laboratory Protocol

Each student receives three DNA samples, one from the crime scene and one from each of the two suspects. Tell students that they will now apply the concepts they have learned in the pre-laboratory activity to the real DNA in the laboratory. The crime samples need to be prepared by the instructor before beginning the laboratory activity; instructions for the crime samples follow the laboratory overview protocol.

Step 1 - Mixing the DNA with restriction enzyme

Add the DNA, water, buffer and enzyme together in a microcentrifuge tube and spin in a microcentrifuge for five seconds to collect the contents at the bottom of the tube. Incubate the samples at 37°C.

NOTE: This may be a stopping point. Samples may be removed from the incubator and stored at 2 to 8°C (refrigerated) for up to 24 hours. Samples may be stored at -15 to -25°C (frozen) for up to one week.

Step 2 - Prepare a 1 % agarose gel

If available, use EGels to run the samples. EGels are convenient pre-prepared agarose gels that come with blue stain or ethidium bromide. Follow the manufacturer's recommendations for loading and running the gels.

If EGels are not available, prepare a one percent agarose gel by combining 1 g of agarose for every 100 mL of 1X TAE. Heat in a microwave until the agarose has completely dissolved. Add ethidium bromide if that is the stain the class will use (10 mg/mL) to a final concentration of 0.2 µg/mL and mix. If methylene blue will be used to stain the gel (see step 5), do not add ethidium bromide to the agarose.

Allow the agarose to cool to approximately 55 °C. Prepare and seal the ends of the gel mold according to the manufacturer's instructions. Also position the desired comb to cast the wells. Pour the cooled, liquefied agarose into the gel mold and allow it to solidify. Gels should be 5 - 8 mm thick.

After the gel has solidified place it in the electrophoresis chamber and carefully remove the comb. Add a sufficient volume of the 1X TAE buffer used to make the gel to the electrophoresis chamber to cover the gel by 1 - 2 mm.

Step 3 - Prepare and load the DNA samples

Add loading dye to the DNA samples and place in a 65°C heating block for five minutes. Put 15 µL of the crime DNA and 15 µL of each suspect DNA into separate wells on the gel.

Loading dye is not necessary if the samples are run on the blue stain EGel.

Step 4 - Electrophorese the samples

Connect the cables and run the gels at 100 volts until the blue portion of the loading dye migrates about 30 mm from the wells.

Step 5 - View the results of gel electrophoresis

If the blue stain EGels are used, results should be visible without a light. If ethidium bromide is used, a UV light must be used to see the DNA bands on the gel.

Methylene blue may also be used to stain the gel. To stain manually, place the gel in a plastic container, which is a little larger than the gel. Add enough 0.025 percent methylene blue solution to cover the gel about one quarter of an inch. Stain the gel for 20 - 30 minutes. Carefully pour off as much of the methylene blue solution as possible; the entire gel will appear deep blue. Rinse the gel in running tap water. Let the gel soak covered with water for approximately 10 minutes. Rock the tray occasionally to help destain the gel. Repeat three to four times. The DNA bands will become more distinct as the gel destains.

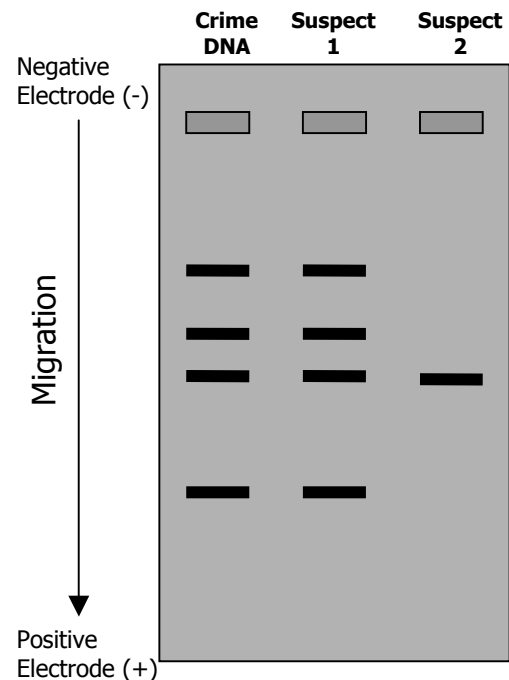
Photograph or sketch the gel (gels may also be viewed on an overhead projector). Gels can be stored in plastic zip lock bags in the refrigerator.

Step 6 - Interpretation

After staining, the pattern of DNA bands resulting from restriction analysis from one suspect will match the crime scene, as shown in Figure 3. Results may vary. Students write their analysis in a lab notebook with evidence to support their results. To facilitate discussion, choose a representative gel and put it on an overhead projector. Highlight the bands projected on the board with a marker. Some sample questions could be:

- What can be inferred from the results of the tests?
- Can you presume guilt by showing that the bands of DNA match after restriction analysis?

FIGURE 3: Restriction Analysis of DNA Evidence



CITY POLICE DEPARTMENT

POLICE REPORT

INCIDENT DATA

Incident Type: Museum Theft
Complaint Status: Pending DNA Results
Processed by: Officer Joe Friday
Other Officers: Officer Dee Enae
Officer Ligase

PROPERTY

Property Code: Jewelry/Precious Metal
Owner's Name: City Museum
Name: Crown Jewels
Value: \$1,000,000

BURGLARY DATA

Method of Entry: Unlawful Entry through broken window

Narrative: The crown jewels were allegedly stolen from the City Museum. Once on the scene I noted that the only window in the room was broken. Officer Ligase approached me and said that there were no prints or any apparent evidence left at the crime scene. However, upon further inspection of the window, my partner, Dee Enae, noticed that there was some blood on the sill. The thief had cut himself on the broken glass. The blood sample was collected and sent to the crime lab via the messenger, R. Renee, who gave the package to the technician Edna N. Zime.

SUSPECT DATA

Suspect Number: 1

Name: Pockets Peterson

Brief Description of Suspicion: A widely known and successful crime thief. Peterson has been known to brag that he could get by any security system. He said he would prove it by someday taking the crown jewels. No stone has been known to have higher security.

Suspect Number: 2

Name: Cruella "The Cat" Blanchard

Brief Description of Suspicion: Owns the largest private collection of precious stones in the world. She has offered millions of dollars for them. Having been a member of the prestigious ninja swat team, she has the talent and guts to pull off such a crime.

Suspect Number: 3

Name: Professor Angstrom

Brief Description of Suspicion: Past curator of the museum that housed the crown jewels. He was recently fired from his job and replaced by the boss's niece. His motive may be revenge.

Suspect Number: 4

Name: The Resident Scientist

Brief Description of Suspicion: Credited for discovery of the crown jewels. She claims they are rightfully hers.

CRIME LAB DATA

Crime Lab Investigator: Edna N. Zime

Evidence Messenger: R. Renee

List of Evidence Received: Plastic bag with Blood
DNA from four suspects

List of Procedures Used: DNA Extraction
Polymerase Chain Reaction
DNA restriction analysis

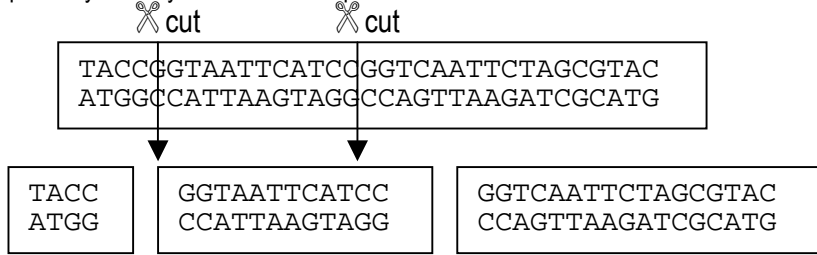
Narrative: After receiving the package with the plastic bag marked *Crime Scene*, the crime scene DNA was extracted from the blood sample in the bag. Because the sample was so small the DNA was amplified using the polymerase chain reaction. Lab assistants used DNA isolated from four suspects and compared them to the crime scene DNA using DNA restriction analysis.

Results: See attached *DNA Results Poster* from lab assistants

CONFIDENTIAL DNA EVIDENCE EVALUATION

WORK ONLY ON YOUR DNA SAMPLE

- Turn your paper strip with the DNA base sequences over so the side with the bases is facing you. Use your scissors (restriction enzymes) to cut your DNA samples only where you see this base pattern: CCGG. Cut between the C and G as shown in this example:



- Count the number of base pairs (bp) in each piece of DNA that you have created. A base pair consists of two complementary bases. Record the number of base pairs in each piece on the blank side of the DNA fragment.



- An enlarged chart like the one below is available for your group. Tape your DNA sequences on the chart according to the number of base pairs. Be sure to put your sample in the proper column. Follow the example below:

Crime DNA	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Number of Base Pairs (bp)
					30
					29
					28
					27
					26
					25
					24
					23
					22
					21
					20
					19
					18
					17
					16
					15
					14
					13
					12
					11
					10
					9
					8
					7
					6
					5
					4
					3
					2
					1

12 bp

Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA
GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG
CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCCTATCGACTATCGAGGCCAC

Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA
GTCCCAGCCGGACCGTACCGGTAGATCAGCCGGTAGATTGATAGCGTGATGTG
CAGGGTCGGCCTGGCATGGCCATCTAGTCGGCCATCTAACTATCGCACTACAC

Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA
GTCTACGTAATCGTAGCCATCCGGACAGTGTGCACGATCGTACATGCTACGTG
CAGATGCATTAGCATCGGTAGGCCCTGTCACACGTGCTAGCATGTACGATGCAC

Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA
GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG
CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCCTATCGACTATCGAGGCCAC

Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA
GTCTCCATCCGGACTACCATAACATCTGGTGTACCCGGTGATATCGTCCGGGTG
CAGAGGTAGGCCCTGATGGTATGTAGACCACATGGGCCACTATAGCAGGCCAC

CITY POLICE DEPARTMENT FINAL REPORT

Names of Team Members:

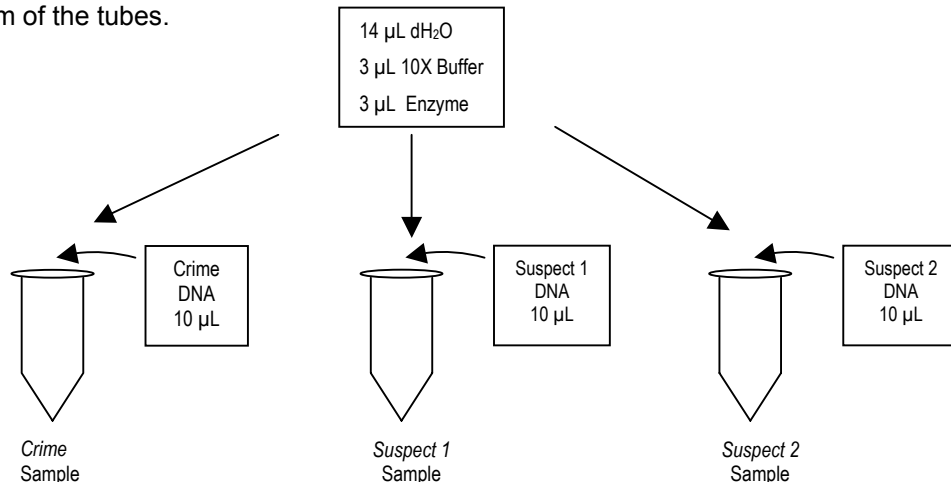
Name of the person who's DNA was found at the crime scene:

Evidence: Explain how you came to your conclusion (you may include diagrams and explanations):

CITY POLICE DEPARTMENT CRIME LAB LABORATORY PROTOCOL

PART ONE – MIXING THE DNA WITH RESTRICTION ENZYMES

- 1.1 Add 10 μL (microliters) of the DNA from the *Crime* sample to the empty tube labeled *crime*.
- 1.2 Add 10 μL of the DNA from the *Suspect 1* sample to the empty tube labeled *susp-1*.
- 1.3 Add 10 μL of the DNA from the *Suspect 2* sample to the empty tube labeled *susp-2*.
- 1.4 Add 14 μL of dH_2O from the tube labeled *dH₂O* to each of the three tubes.
- 1.5 Add 3 μL of 10X buffer from the tube labeled *10X* to each of the three tubes.
- 1.6 Add 3 μL of enzyme from the tube labeled *Enzyme* to each of the three tubes.
- 1.7 Centrifuge all of the tubes for five second in the centrifuge to collect the contents at the bottom of the tubes.



- 1.8 Write the letter of your tube rack on your data sheet. Put the rack containing your tubes into the incubator at 37°C for 30 minutes.

PART TWO – PREPARATION OF SAMPLES FOR ELECTROPHORESIS

- 2.1 Remove your DNA samples from the incubators
- 2.2 Add 5 μL of loading dye to each of the three tubes. Centrifuge tubes for five seconds to collect the contents at the bottom of the tube.

PART THREE – LOADING AND RUNNING THE AGAROSE GEL

- 3.1 Follow the guidelines from the instructor for setting up your agarose gel. Load 15 μL of the *Crime* sample into a well. Keep record of the well you loaded on your data sheet
- 3.2 Load 15 μL of the *Suspect 1* sample into a well. Keep a record of the well you loaded on your data sheet.

- 3.3 Load 15 μL of the *Suspect 2* sample into a well. Keep a record of the well you loaded on your data sheet.
- 3.4 After all samples have been loaded close the lid of the gel box.
- 3.5 The gel should be run at 100V for approximately 45 minutes. Remember samples should always “**Run to Red**” or towards the Red electrode.

PART FIVE – GEL STAINING AND ANALYSIS

- 4.1 Turn the power off and disconnect the cables from the electrophoresis box.
- 4.2 Open the lid of the electrophoresis box and gently lift the casting tray out of the gel box.
- 4.3 Follow your instructor’s guidelines for viewing the results of your gel electrophoresis. Observe the banding patterns on your gel and record the results on your data sheet.

MICROPIPETTE CHALLENGE

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1 μL . You can imagine that it would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Micropipettes differ in the volume of liquid they can accurately measure.

To help you become accustomed to using micropipettes you will be given two microcentrifuge tubes. One has blue food coloring; the other has yellow food coloring. Practice using the micropipettes by adding the amounts listed to an empty tube.

Example:

Amount to add to tube	Color	Record the setting as it appears in the window
15 μL	blue	150

Add the following amounts to an empty tube – all of the amounts will be added to the same tube:

Amount to add to tube	Color	Record the setting as it appears in the window
12 μL	yellow	
5 μL	blue	
8 μL	yellow	
3 μL	blue	
2 μL	yellow	
5 μL	blue	
15 μL	yellow	

How many microliters (μL) should you have in the test tube when you are done? _____

How many milliliters (mL) should you have in the test tube when you are done? _____

Compare the amount of the liquid in your tube with a classmate's tube. Is it the same or different? If the amounts are different, try to figure out why. Watch each other pipette and check each other's technique. Ask an instructor for help if you have questions about using the micropipette properly.

CITY POLICE DEPARTMENT: **DATA/OBSERVATION SHEET**

I. RESTRICTION DIGEST

1. What does the restriction enzyme do to the DNA?
2. Why are the DNA samples put into the incubator?
3. Predict what will happen to the DNA you prepared in part I while it is in the incubator.

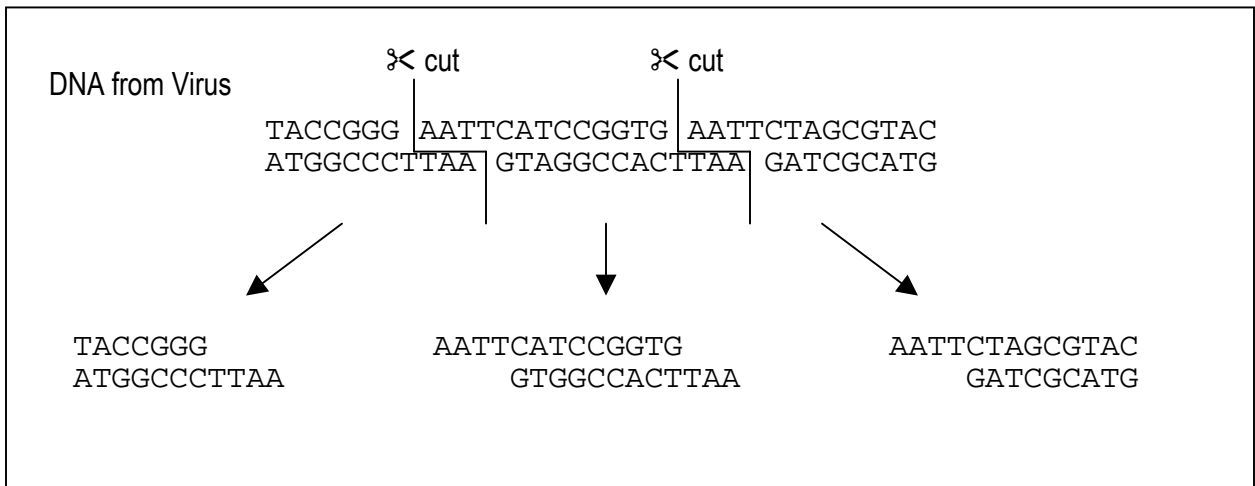
II. PREPARATION OF THE AGAROSE GEL

1. What is the function of the Agarose gel?
2. Predict what would happen if you used 0.02 g of Agarose instead of 0.2 g. What effect would that have on the experiment?
3. What is the function of the comb?

RESTRICTION ENZYME WORKSHEET 1

NAME: _____

A natural enemy of bacteria is a virus. To defend them when attacked by a virus, bacteria use chemical weapons that breakup the DNA of the virus. The action of these chemicals on the viral DNA is shown in the diagram below:



Use the diagram above to complete the sentences or answer the questions below:

- The chemical that cuts the DNA is called a restriction enzyme. Restriction enzymes cut the DNA into _____.
- The restriction enzyme used above is called *EcoRI*. *EcoRI* cuts DNA everywhere the base pattern _____ is found.
- Another restriction enzyme is *HaeIII*. It cuts DNA at the base sequence CCGG. It cuts between the C and G. Show the DNA fragments that would result if *HaeIII* was used to cut the DNA fragment shown in the diagram above.
- Do you think restriction enzymes could be used to cut DNA from other organisms?

5. The words BOB and MADAM are called palindromes. What are palindromes? (hint: spell the words backwards)

6. What do palindromes have to do with the way restriction enzymes cut DNA?

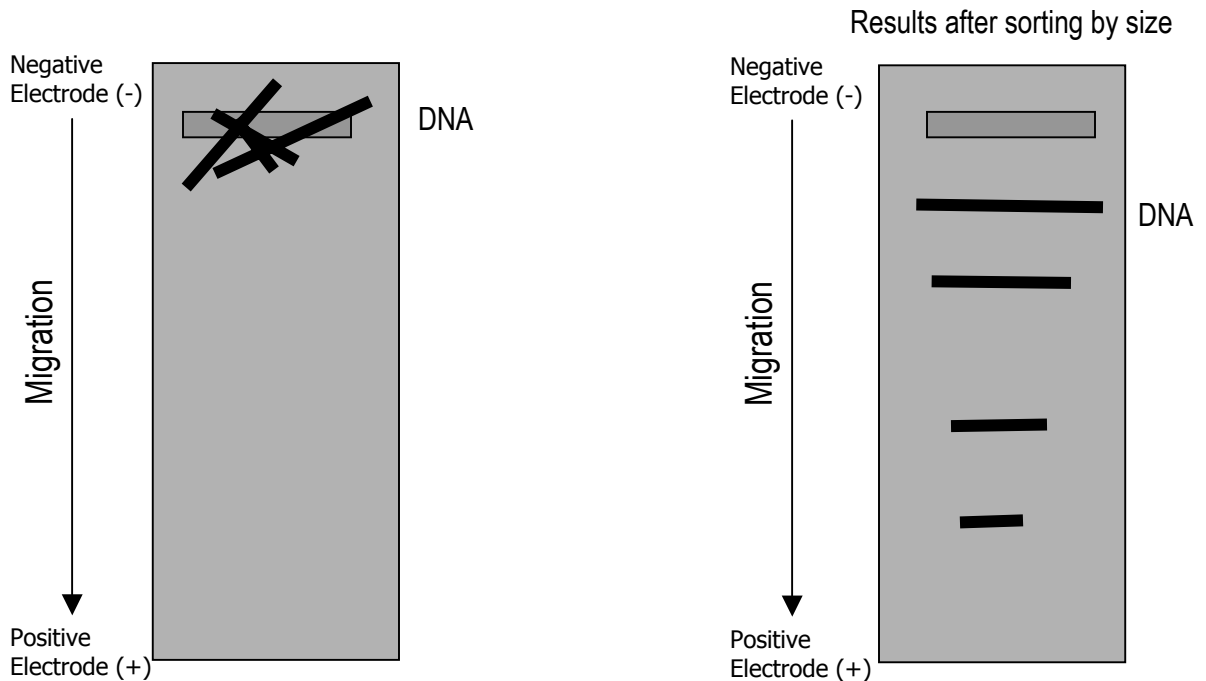
RESTRICTION ENZYME WORKSHEET 2

NAME: _____

Restriction enzymes are important tools for the researcher. Since each DNA molecule is unique, it will produce unique fragment sizes when cut by a restriction enzyme. These fragments can be used to identify DNA molecules.

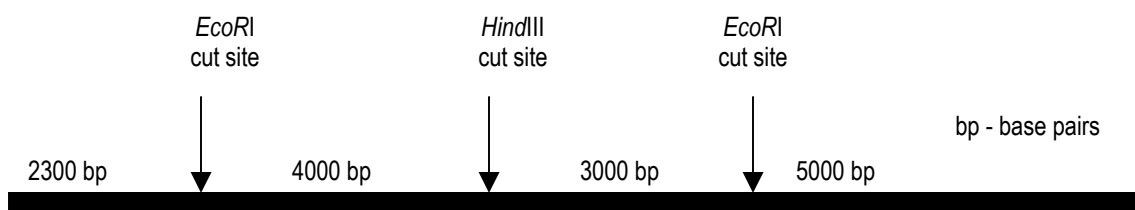
The DNA fragments need to be separated in order to be compared. The fragments are sorted by passing them through a gel. The gel acts like a screen, allowing small pieces of DNA to pass through more easily than large pieces, much like sifting rocks out of dirt. Electricity is used to move the DNA through the gel matrix. Since DNA has a negative charge when it is placed in an electric field, it migrates toward the positive pole.

FIGURE 1: Gel Electrophoresis sorting fragments of DNA by size

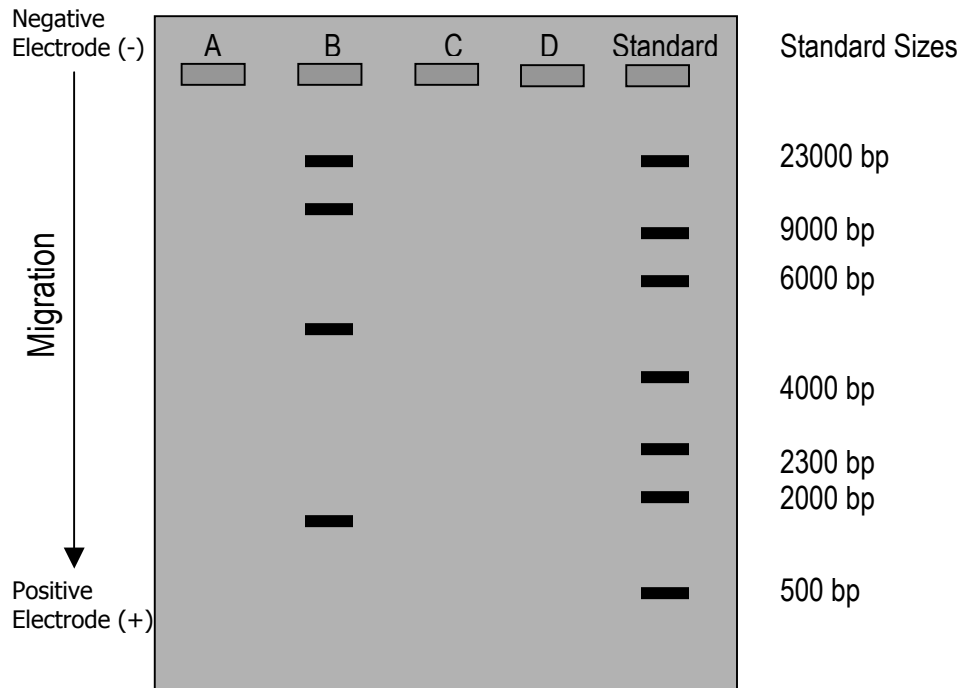


The process of sorting DNA fragments by size using a gel and electricity is called gel electrophoresis.

FIGURE 2: DNA Strand with specific *EcoRI* and *HindIII* sites



Use the gel box below to answer the following questions.



- Next to each band in lane B, write the size of the DNA fragment that would be found in that lane.
- Imagine the DNA strand shown in Figure 2 was cut with the restriction enzyme *EcoRI* and placed in well C. Draw the bands in lane C as they would appear after electrophoresis. Next to each band indicate the size of the DNA in base pairs.
- Now assume the DNA was cut with both *EcoRI* and *HindIII* and the DNA fragments were placed in well A. Draw the bands that would result after electrophoresis in lane A. Next to each band indicate the size in base pairs.

Case of the Crown Jewels

Laboratory Preparation

NOTE: The following information contains instructions to order the reagents and supplies needed to prepare the solutions and perform this laboratory activity.

Materials

Item	Company	Phone Number	Catalog Number	Quantity
Lambda DNA	Fisher Scientific	800-955-1177	CDS1120216	80 µg
Lambda DNA/cut with EcoRI	Fisher Scientific	800-955-1177	S1120220	25 µg
HindIII with 10X Buffer	Fisher Scientific	800-955-1177	BP3372-1	5000 units
Loading Dye	Fisher Scientific	800-955-1177	BP645-1	1 mL

Crime Sample Preparation

1 Lambda DNA

- 1.1 Place the tube of Lambda DNA purchased from the supplier in a 65°C heating block for five minutes. Remove and invert several times to mix. Allow the tube to cool to room temperature. Determine the concentration of the Lambda DNA (see the label on the tube). The final concentration of the lambda DNA should be 0.175 µg/µL. Calculate and perform a dilution using the following formula:

$$(\text{Final concentration}) \times (\text{Final volume}) = (\text{Initial concentration}) \times (\text{Initial volume})$$

Final concentration	0.175 µg/µL
Final volume	1000 µL
Initial concentration	Concentration of the lambda DNA from the supplier
Initial volume	Total volume of the lambda DNA from the supplier

For example:

$$X = \frac{(0.175 \mu\text{g}/\mu\text{L}) (1000 \mu\text{L})}{(\text{Concentration of lambda DNA from the supplier})}$$

Where X = volume of the lambda DNA that will be diluted to 0.175 µg/µL in a final volume of 1000 µL with distilled water (dH₂O).

- 1.2 After determining the amount of lambda DNA from the supplier to use, subtract that amount from 1000 to determine the total volume of water to add.

For example - If the tube of lambda DNA has a concentration of 0.533 µg/µL of DNA, then the calculation would be as follows:

$$(0.175 \mu\text{g}/\mu\text{L}) (1000 \mu\text{L}) = (0.533 \mu\text{g}/\mu\text{L}) X$$

$$X = \frac{(0.175 \mu\text{g}/\mu\text{L}) (1000 \mu\text{L})}{(0.533 \mu\text{g}/\mu\text{L})}$$

$$X = 328 \mu\text{L}$$

$$Y = 1000 \mu\text{L} - 328 \mu\text{L}$$

$$Y = 672 \mu\text{L dH}_2\text{O}$$

Therefore, 328 μL of the purchased lambda DNA would be mixed with 672 μL of dH_2O . This mixture is the stock lambda DNA to be used later in the experiment. Store the lambda DNA at -15 to -25°C until use.

2 Lambda DNA cut with EcoRI

- 2.1 Determine the concentration of the Lambda DNA/EcoRI (see the label from the supplier).
- 2.2 Using the formulas in section 1, dilute the Lambda DNA/EcoRI to a final concentration of $0.175 \mu\text{g}/\mu\text{L}$.
- 2.3 Store the diluted Lambda DNA/EcoRI at -15 to -25°C until ready to use.

3 Aliquoting student samples

- 3.1 Set-up seven microcentrifuge tubes for each pair of students doing the lab. Label and add the following to each set of tubes:

Crime Scene DNA	15 μL of lambda DNA
Suspect 1	15 μL of lambda DNA
Suspect 2	15 μL of lambda DNA/EcoRI
10X Restriction Buffer	15 μL of 10X Buffer (<i>supplied with the enzyme</i>)
Enzyme	12 μL of HindIII
Dye	20 μL of loading dye
dH_2O	1 mL of distilled water

- 3.2 These tubes are to be shared between two students. The tubes should be stored at -15 to -25°C until ready to use.

4 Laboratory station set-up

- 4.1 Prepare the laboratory stations; two students can work at each station. Place the following at each station:

Crime Scene Kit (with reagents)
Pipet tips for 2 - 20 μL pipet
Tube rack with empty tubes labeled *Crime*, *Susp-1*, and *Susp-2*
2 - 20 μL pipet

Case of the Crown Jewels

Extension Activities

The following extension activities may be used to reinforce the concepts introduced during the pre-laboratory activity and the laboratory activity.

- I Students may use the “Micropipette Challenge” to familiarize themselves with using a micropipette. It is recommended to include this activity with the laboratory activity.
- II Students may complete “Restriction Enzyme Worksheet 1 and 2” as reinforcement and review.
- III Stage a mystery in the school such as the theft of the school mascot. Include as part of the evidence DNA from the crime scene and suspects. Other clues may involve the chemistry, English and history departments. If possible, invite the participation of other school communities, i.e. the school newspaper and photography club. Assign students to role as a jury, prosecutor, defense, scientific expert and media. After all the evidence is collected, hold court in which each department presents the analysis of its evidence. Have the attorneys write a brief for the court and prepare testimony debating the strengths and weaknesses of the DNA evidence.
- IV Ask the students to write a letter to a friend who knows nothing about DNA restriction analysis describing their results.
- V Electrophoresis role-play: A role-play can be used to reinforce the concepts of restriction digestion and electrophoresis. Divide the class into three equal groups and have the students come to the front of the room, standing together as a group. Each group represents a single stranded DNA molecule and each person in a group represents a nucleotide. Model phosphate bonding by instructing the students to lock arms. Designate one group the crime sample, one group suspect one, and the other group suspect two. Hand each person a piece of paper with *A*, *C*, *T* or *G* written on it. Be sure to arrange the groups in the following order:

Group One	ACCGGTAT
Group Two	CCGGATCA
Group Three	ACCGGTAT

Ask each group to form the DNA fragments that would be created if *HaeIII*, the enzyme that cuts between the C and G in the pattern CCGG, cut them. Point out that the fragments are still mixed together after cutting and challenge the students to determine how to separate the pieces. Illustrate this concept by telling the class to imagine the classroom as an electrical field with the positive pole at the back of the room and the negative pole at the front of the room. Put the DNA groups at the negative end and ask the student to predict how the DNA would react in the electric field. Remind students that the DNA has a net negative charge and will, therefore, be attracted to the positive pole. The smaller resulting fragments should move more quickly to the positive pole than those DNA strands that are large. Pretend to turn on the electricity and have the students imitate the migration of the DNA fragments. Ask them to determine which suspect DNA is the same as the crime sample.

Bioscience... Real Jobs, Real People

Post-Laboratory Activity

Bioscience: Real Jobs, Real People follows four high school students to various Maryland bioscience companies on "Bioscience Career Day." During the nine-minute film students interview scientists who work in a broad range of careers such as research, development, management, and manufacturing. Not only are their day to day responsibilities explained but each scientist also discusses universal truths about pursuing a career in the industry.

Teachers received this nine-minute film and accompanying lesson plan in the fall of 2000. The lesson plan can also be downloaded from the MdBioLab web site (<http://www.mdbiolab.org>). If you are unable to locate the video *Bioscience... Real Jobs, Real People*, please contact MdBio at info@mdbio.org to find out who received the video at your school.