Case of the Crown Jewels

A DNA Restriction Analysis Laboratory Activity

Maryland Loaner Lab Teacher Packet

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www.towson.edu/cse

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Loaner Lab Overview

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 using the Maryland Loaner Lab must first complete the pre-laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

Supplied by Maryland Loaner Lab Program:

Description	Quantity	Comments	Return Directions
Teacher Binder	1	Background info, instructions, and student worksheet templates	Return
Gel Electrophoresis box	1	With lid, contains (6) combs, (6) trays, (12) stoppers and (2) slats.	Rinse, dry and return. **Refer to box for packing directions.**
Blue Light Transilluminator	1	For visualizing DNA bands	Wipe clean, dry and return
Power supply	1	With black cord	Return
Pyrex bottle with orange cap	1	To melt agarose for gels	Clean and return
Agarose Powder (0.84g)	1 tube	Shipped in bag labeled "Agarose supplies"	Discard tube, return labeled bag
SYBR Safe DNA Stain	1 tube	12 ul	Discard empty tube
2L Container	1	To dilute 10X TAE with dH_2O	Rinse and return
10X TAE Buffer	1 (150 ml) bottle	Follow directions in binder for dilution. If you requested multiple class sets: • Running buffer should be re-used for each class • An extra 150 ml 10X TAE buffer is supplied in case of spills & for making more gels	Rinse and return
20 µl Micropipettes	10	1 per group	Return
Micropipette tips (yellow box)	5 boxes	1 box per two groups (extra box sent if more than one class set requested)	Return boxes with unused tips
Foam microtube racks	10	1 per group	Return
DuraGel practice gels	11	1 per group (+ one for teacher)	Rinse, dry and return
Microcentrifuge	1	Used to spin samples down in tubes prior to setting out at student stations	Return
Graduated Cylinder (100 ml)	1	Used to measure and pour gels	Clean, dry and return
Description	**Quantity**	**Comments**	**Return Directions**

Loaner Lab Overview

Class set of "Crown Jewels Reagents"	1/class	• (10) RE (restriction enzyme) Student Tubes • (10) Prac Dye (Practice Dye) Student Tubes	Return
Class set of DNA "Crown Jewels DNA and LD Samples"	1/class	• (10) Crime Scene 'CS' Student DNA samples • (10) Suspect 1 'S1' Student DNA samples • (10) Suspect 2 'S2' Student DNA samples • (10) Loading Dye 'LD' Student Tubes • (1) Extra Loading Dye for Teacher Use **Refrigerate until use.**	Discard tubes, return labeled bag
Spatula	1	For moving gels to and from transilluminator boxes	Clean, dry and return
Insulated bag & freezer packs	varies	Holds DNA and all reagents & dyes	Return
Disinfectant Wipes	1 container	Used to cleanse equipment for return	Return
Laminated Micropipette Use & Practice Gel Exercise sheets (copies of S-5 & S-6)	10	Used to guide students through pre-laboratory skills activity	Return

Supplied by the Teacher:

Description	Quantity	Comments
Student worksheets	1/student	Copy from laminated template in binder (or can be found online)
Safety goggles	Enough for class	Each student working with the kit's contents should wear safety goggles
Gloves	≥1 pair/student	For student use with main laboratory activity
Scissors	5 pairs	1 per group
Rolls of Tape	5	1 per group
Poster Board	5 medium sheets	Use with pre-lab activity (can also use large sheets of paper)
Distilled Water (dH_20)	2000 ml	Used to dilute 10X TAE buffer. Will need an additional 1,350 ml if requesting more than one class set.
Lab Microwave or Hot Plate	1	To melt agarose gel
Waste Container	1/group	Use for discarded pipette tips, tubes, etc.

SAFETY: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

Next Generation Science Standards

Performance Expectations: Students' ability to complete the following performance expectation(s) will be supported by participation in this activity.

HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

Dimension	NGSS Code or citation	Corresponding student task in activity
Disciplinary Core Idea	LS3.B • The information passed from parents to offspring is coded in the DNA molecules that form the chromosomes. In sexual reproduction, chromosomes can sometimes swap sections during the process of meiosis (cell division), thereby creating new genetic combinations and thus more genetic variation. Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation.	Students explore how DNA sequences are unique to each individual person.
Practice	Modeling • Use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system.	Students will use a paper model to illustrate how restriction enzymes cut DNA.
	Analyzing and Interpreting Data • Analyze data using tools, technologies, and/or models (e.g., computational, mathematical) in order to make valid and reliable scientific claims.	Students will analyze the DNA banding pattern resulting from gel electrophoresis to determine if the DNA from a crime scene matches suspect DNA.
	Construct an explanation • Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects	After completing the paper DNA restriction enzyme activity, students construct an explanation about which suspect's DNA was found at the crime scene.
Crosscutting Concept	Patterns • Empirical evidence is needed to identify patterns.	Students will look for patterns in DNA fragments and use them as evidence when making a claim about whether the DNA left at a crime scene matches the suspect DNA.

Next Generation Science Standards

	Cause and Effect • Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects.	Students will use evidence from DNA patterns to suggest a cause and effect relationship between the DNA sample found at a crime scene and the DNA of suspects.

Nature of Science

Scientific Knowledge is Based on Empirical Evidence

- Science knowledge is based upon logical and conceptual connections between evidence and explanations.
- Science arguments are strengthened by multiple lines of evidence supporting a single explanation.

Connections to Common Core State Standards

English Language Arts/Literacy	RST.11-12.1
RST.9-10.1	RST.11-12.3
RST.9-10.3	RST.11-12.4
RST.9-10.4	RST11-12.7
RST9-10.7	

Case of the Crown Jewels

Introduction

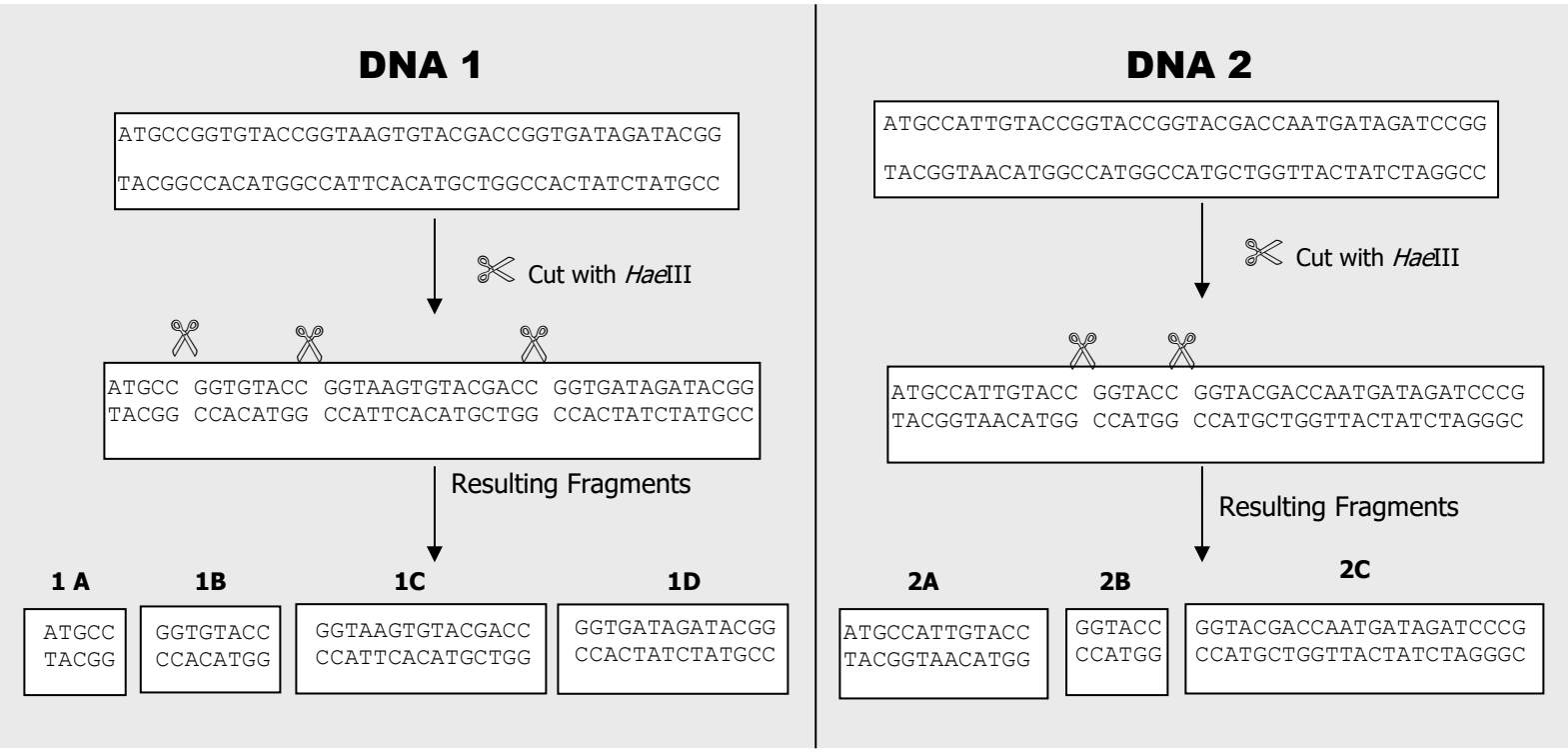
DNA restriction analysis is a technique with wide-ranging applications in medicine, research, and forensics. The Case of the Crown Jewels is an activity that simulates the basics of DNA fingerprinting, a technique used by forensic scientists, which relies on restriction analysis to analyze DNA evidence 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.
- Restriction enzymes, which are produced naturally by bacteria, cut DNA molecules at specific sites denoted by specific base sequences.
- Approximately 3000 different restriction enzymes have been identified and each cuts at different base sequences.

A **restriction digestion** occurs when a restriction enzyme cuts DNA molecules. The size of the fragments generated through restriction digestion will be unique to each molecule because of its difference in sequence. As shown in Figure 1, both DNA 1 and DNA 2 are cut with *Hae*III, an enzyme that cuts between the base pairs CC|GG on one strand and GG|CC on the opposite strand.

FIGURE 1: Restriction Digest of Two DNA Fragments



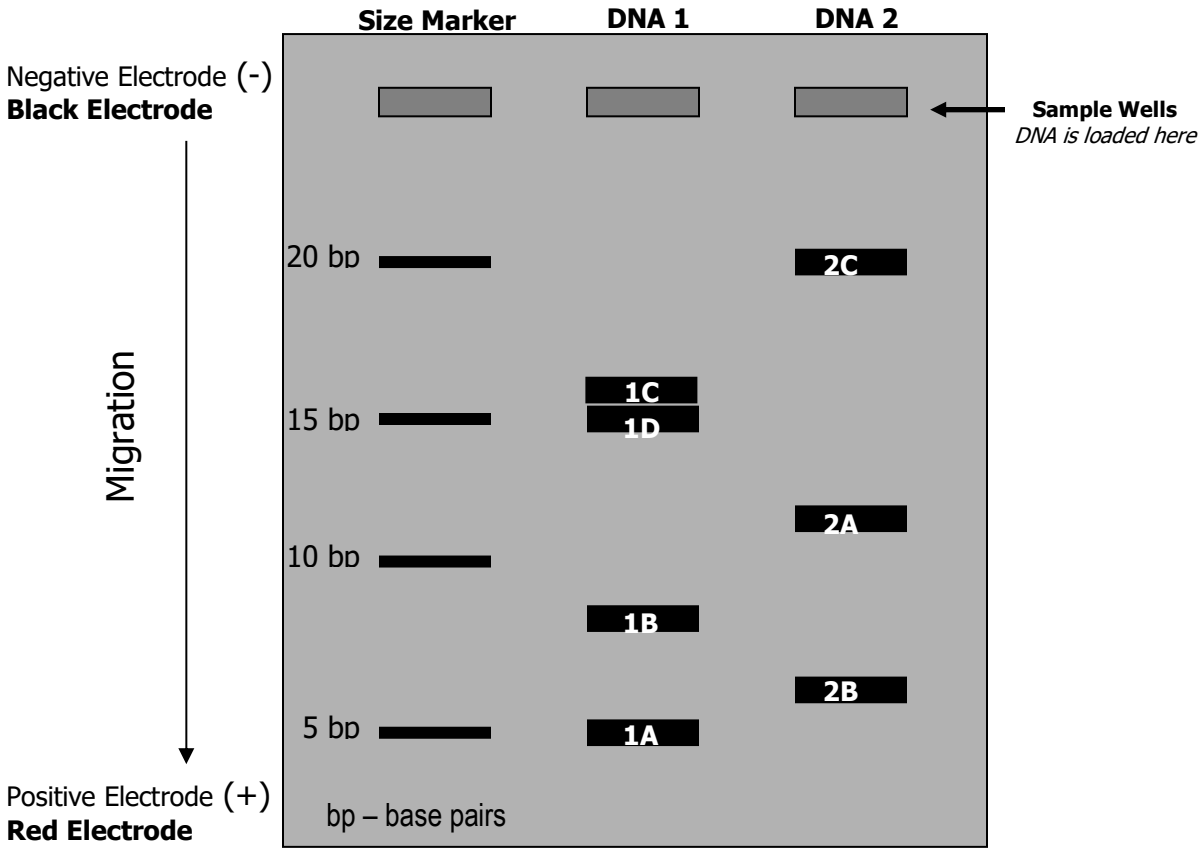
After being cut by restriction enzymes, DNA fragments remain mixed in solution and indistinguishable from one another. One way to distinguish between the different fragments created is to compare them by size. Different size fragments of DNA can be separated using gel electrophoresis.

Case of the Crown Jewels

Introduction

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 basic terms, negatively charged DNA will travel towards the positive electrode when exposed to an electric current (see Fig 2). This is because of the negative charge in the DNA's sugar-phosphate backbone that is attracted to the positive electrode. The positive electrode is colored red, and electrophoresis of DNA is always "running towards red". Smaller strands of DNA move more quickly through the gel matrix than longer strands, therefore, gel electrophoresis separates DNA according to size. The sizes of the DNA fragments can be determined by comparing them to a size marker (often called a "DNA ladder"), which has DNA fragments of known size which appear as a ladder with many rungs when run through a gel. By comparing the resulting pattern of the DNA fragments on the gel (looking at both the number of bands and the corresponding sizes), the different DNA molecules or samples can be differentiated. When using restriction analysis to analyze different DNA samples in forensic cases, many sets of tests are done to build what is called a "DNA Fingerprint" for individual samples. It is the matching of this "DNA Fingerprint" from a suspect to the crime scene DNA, which can either link or not link a suspect to a crime scene.

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



Case of the Crown Jewels

Introduction

Activity Overview

This lesson is organized into two parts – several pre-laboratory activities and a laboratory investigation.

Pre-laboratory Activities

1. **Concepts:** Students work in groups to simulate a DNA restriction analysis using paper DNA sequences and scissors to solve a fictional crime.

2. **Lab Skills and Techniques:** This investigation requires the precise use of the micropipette to accurately transfer small volumes. This lab protocol also requires students to load gels, which can be a challenge for students who have never loaded gels before. The ***Micropipette Use*** and ***Practice Gel Exercise*** allow the students to become familiar with micropipettes and to practice loading agarose gels before working with their DNA samples. After instruction on micropipette use and proper technique for loading gels, students practice loading gels with the micropipettes using practice gels. Once they are comfortable with both skills (pipetting and loading gels), students can begin the laboratory protocol.

Laboratory Activity

Students assume the role of a DNA technician to build evidence to solve a fictional crime. The students will run restriction digestion and DNA gel electrophoresis to compare the DNA fingerprints of two suspects with the sample collected from the crime scene. Restriction digestion is an important concept in this activity, but working with restriction enzymes presents many challenges in a classroom setting. Therefore, we send pre-digested DNA samples for students to use, but include the steps of adding "restriction enzyme" and allowing time for digestion in the student protocol. The "RE" tubes contain distilled water.

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Pre-laboratory Activity

NOTE: We strongly recommend that groups using the Maryland Loaner Lab first complete the pre-laboratory classroom activities. The concepts and technical skills introduced in the pre-laboratory activities are reinforced with the laboratory activity.

The purpose of the paper-based 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 different 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

Teacher Preparation (for 5 student groups, 5 students in each)

Photocopy the following documents:

Police Report
DNA Evidence Evaluation
DNA sequences
Final Report

Prepare 5 **poster-size** charts as shown at the bottom of the *DNA Evidence Evaluation* sheet.

Label 5 envelopes "Confidential Forensic Evidence".

Cut out the Crime Scene and four Suspect DNA sequences from the five photocopied *DNA Sequences* sheets. Place one set of five strips into each of the five appropriately labeled envelopes. Also place a copy of the *DNA Evidence Evaluation* sheet in each envelope.

Materials for each student group

One pair of scissors
One "Confidential Forensic Evidence" envelope which includes five strips of DNA sequence, for *Suspect #1, Suspect #2, Suspect #3, Suspect #4* and the *Crime Scene DNA,* and also includes a copy of the *DNA Evidence Evaluation* sheet.
One roll of tape
One copy of the *Police Report*
One copy of the *Final Report*
One poster-size chart as shown at the bottom of the *DNA Evidence Evaluation* sheet.

Pre-laboratory Engagement (10 minutes)

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

Pre-laboratory Activity

Pre-laboratory Exploration (30 minutes)

Tell the students that each group has an envelope which includes the evidence they'll need for their investigation of the crime. Each student will take one DNA sequence strip out of the envelope and be responsible for that one piece of evidence for the case. Ask the students to follow the instructions (the *DNA Evidence Evaluation* sheet) to try to solve the crime. The instructions will guide them through the process of a DNA restriction analysis. As students complete the activity as instructed, they will tape the resulting DNA fragments from each piece of evidence onto the poster-size chart to analyze the results. As facilitator, be prepared to assist the students and address any misconceptions. Encourage students to help each other but to work only on their DNA sequence evidence from their envelope.

Pre-laboratory Explanation (20 minutes)

Each group should have a copy of the *Final Report*. After each group has finished putting the DNA fragments on their chart, have each team fill out their *Final Report*. Lead a class discussion regarding their conclusions and the technique they employed to process the DNA evidence from the case. 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 does 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 used, DNA restriction analysis, provides indirect evidence regarding the similarity between particular sequences of DNA. 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

Have students write an entry in their laboratory notebooks, describing in their own words what they learned from the activity. Discuss briefly other applications for which DNA restriction analysis could be used, such as forensic identity/remains testing, paternity testing, and also the beginning steps of genetic engineering (such as modifying plant or bacteria cells to include new genes of interest).

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Laboratory Explanation

The purpose of the laboratory activity is to apply the concepts developed in the pre-laboratory activity to solve a fictitious crime. In this laboratory exercise, students will use DNA restriction analysis to determine if either of two suspects were at a fictitious crime scene.

The objectives of the laboratory component are as follows:

- Model the process of a DNA restriction analysis
- Perform a restriction digest and electrophoresis
- Analyze the results from completed 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 activity becomes a tool in the continuum of an ongoing problem rather than an isolated end in itself.

Developing the Concept: Pre-Laboratory Activity 1

Describe a crime scenario to the class from which one crime scene DNA sample and two suspects' DNA samples were obtained. Present three microcentrifuge tubes with DNA, one from the crime scene and one from each of the two suspects. Ask the class how DNA samples can be used as evidence in a case involving such a crime scenario. 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 activity. Ask if students can visually differentiate and compare the DNA in the microcentrifuge tubes. Guide the students to recognize that because the samples look exactly alike, a technique is needed to determine if either of the samples could match that found at the crime scene.

Developing Lab Skills: Pre-laboratory Activity

Before students perform the laboratory activity, discuss with students how to use micropipettes properly (see Micropipette Usage Instructions for Teachers section and the Micropipette Skills subsection). Students will practice using the micropipette by using practice dye in their micropipette tips. Because loading the wells of agarose gels can be difficult at first, allow students time to practice loading dye samples using the agarose practice gels. ***Micropipette Use*** and ***Practice Gel Exercise*** provide details for this activity.

The Laboratory Investigation

The students will perform a mock restriction digestion on real (pre-digested) DNA samples. Even though the restriction digest is a mock simulation, it will reinforce for students the actual steps necessary to digest, or cut, DNA using restriction enzymes. The protocol involves adding "Restriction Enzyme" to each of their three DNA sample tubes ("CS"= Crime Scene, "S1"= Suspect 1, and "S2"= Suspect 2). Restriction enzyme digests usually require a minimum of one hour to occur and take place in a 37°C water bath. This is to ensure complete digestion of the DNA. Our mock simulation only "requires" incubating at 37°C for 5 minutes for the "reaction" to occur. This reinforces for students that reactions take time, but is short enough to also fit within the time limitations of most classes.

In the laboratory protocol, students add loading dye ("LD") to each DNA sample before transferring the samples to the wells in the gel. Loading dye serves several purposes. First, it colors the DNA to help visualize the samples while loading into the wells. Second, loading dye increases the sample density to keep the samples in the wells while adding the running buffer. The DNA requires a very small amount (4 µl) of

Laboratory Explanation

loading dye, and students may run out if they do not micropipette correctly. A small amount of extra loading dye is included in your kit, but please watch students carefully to ensure they transfer the correct amount of loading dye to each sample.

Groups will use three wells in a gel to load 15 µl of their DNA samples. Once all gels have been loaded with the student samples the gels will be run for approximately 30 minutes at 100 volts in 1X TAE running buffer. The agarose gels act as a sieve to separate the different sized DNA fragments. A band of color will migrate down the gel, showing that the gel is running. This band is not the DNA. The DNA samples in the gels are invisible until placed on the Blue Light transilluminator.

Micropipette Usage Instructions for Teachers

NOTE: The Micropipette Use and Practice Gel Exercise may be performed the day of the laboratory activity, or any time in advance.

Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes. They are expensive and must be used with care. Their accuracy is dependent upon their proper use. Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume. Be sure that all students understand how to operate the micropipettes.

Basic Directions for Micropipette Use

Golden Rules for Pipetting

1. *Don't rotate the volume adjuster beyond the upper or lower range of the pipette as this can damage or break it.*
2. *Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette, which contaminates it, and can damage the mechanism inside.*
3. *Don't lay down a pipette that has a tip filled with liquid. If this happens, liquid can get inside the pipette, which contaminates the pipette, and can damage it.*
4. *Use new pipette tips between different samples to prevent contamination.*

Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1µl) or whole microliters in the smallest place so that you can read the scale correctly (it varies with different brands of micropipettes).

Drawing Up and Expelling Liquid

Micropipettes have two stops as you depress the plunger. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid.

To draw liquid into the pipette tip, depress the plunger control only to the first stop before dipping the tip into the liquid. Slowly release your thumb so the liquid can draw into the tip. NOTE: If you go to the second stop you will draw too much liquid into the micropipette tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume).

To expel liquid out of the micropipette tip, place the filled tip in the new tube or well then slowly compress your thumb to the second stop. Remove the tip while still compressing the plunger, then release your thumb. NOTE: Sometimes when filling the wells in gels, it is not necessary to go to the second stop as this pushes air after the liquid.

It is worthwhile to check each student for correct technique before beginning laboratory procedures requiring use of the pipettes.

Teacher Instructions for Micropipette Use and Practice Gel Exercise

Preparation of Student Stations for Micropipette Use and Practice Gel Exercise:

1 tube of "Prac Dye" (practice loading dye)
1 20-µl micropipette
micropipette tips (1 box for every 2 student stations)
1 practice loading gel (round gel)
Student sheets, S-5 and S-6 (laminated set included)

Practice Gel Exercise:

The purpose of this activity is to allow students to practice accuracy with the micropipettes and loading samples into the tiny wells of an agarose gel. We have supplied 10 practice gels (agarose gels cast into small, round petri dishes) so that each group of students can practice pipetting before handling their DNA samples. Students will use the tube of dye labeled "Prac Dye" (which stands for practice dye) for this activity. S-5 and S-6 (provided as a laminated class set and available in the student section) provides detailed instructions.

Micropipette Skills:

To teach micropipette skills, we recommend introducing students to the parts of the micropipette (plunger, volume control dial, volume window, tip release button, tip) and demonstrating how to hold the micropipette. Discuss the volume limits and relative amount with the students (i.e., a p20 micropipette only holds a maximum of 20 µl; 1000 µl is equivalent to 1 ml) Show students how to set the volume to the maximum and have them set their instruments to the maximum volume but caution to not exceed the maximum for fear of breaking the micropipette. Ask students to hold the micropipette and push down on the plunger with their thumb, feeling for two separate stops. After verifying that all students felt the first and second stops, ask students to change the volume to an amount near the minimum of the instrument, again reminding them to not go below the instrument's calibrated minimum volume. Have students again feel for the first and second stops, and ask them what changed. Students will notice there was less space between the open plunger and the first stop when the volume was reduced. Ask students to identify the measuring stop, the first stop or the second stop. At this point, students can move their volume to 4 µl and acquire a clean tip. Ask students to extract 4 µl of the practice dye then compare the volume in their micropipette to the group next to theirs. After student expel the 4 µl back into the practice dye tube, direct students to push to the second stop and extract the volume. Point out the difference in volume in the tip compared to the first extraction, and emphasize the importance of using the first stop to extract liquid for accurate measuring. Then have students change the volume setting to 15 µl for loading the practice gels.

Loading Gels Skills:

"Loading gels" (filling the wells of a gel) can be a challenging task, especially if one has never done it before. This is an opportunity for students to practice before they are asked to load the actual samples involved in the laboratory activity. Many people's hands shake a bit, so many people prefer to stabilize the micropipette while loading the gel. For example, some people like to rest their elbow on the counter while loading and steady the micropipette by bracing the pipette body against a finger of the other hand, while others like to place both elbows on the workbench and work with both hands to raise and lower the micropipette like a drill. Tell students to take their time, try different stabilizing positions, and practice filling a few different wells of the practice gels. Remind the students that this is for practice and not to get frustrated if they spill out of a well or accidentally tear the edge of the agarose gel. They can just try again.

An important thing to note about gels: the wells appear as holes, but they do not go all the way through the gel. They are more like indentations that do not go through completely to the bottom of the Petri dish.

This is why it is so important not to poke the micropipette tip through the bottom of the well, or else the liquid will seep into the bottom of the dish and not stay in the well.

Teacher Laboratory Preparation

Maryland Loaner Lab will supply reagents, equipment, and instruction for the laboratory activity for 10 groups. **Teachers must supply distilled water used for dilutions.**

Teachers must supply the students with the student handouts using the laminated master sheets in the back of this manual.

Prepare Student Stations (for 10 groups):

One foam microtube rack
One tube* each: "CS", "S1", "S2", "RE", and "LD"
One 20 µl micropipette
One box of micropipette tips (1 box/2 student groups)
One practice gel
One practice loading dye tube

*Use the microcentrifuge to spin down all DNA and dye samples; centrifuge for only a few seconds

Activity:	Time:
Preparing gels & student stations	60 min
Pre-lab paper activity	60 min
Pre-lab Skills (*Micropipette Use & Practice Gel Exercise*)	30 min
Laboratory Activity	90 min
Post-Lab Activity	10 min

Shared equipment for multiple groups:

One agarose gel for every two groups (each group has 3 wells, and each gel has 8 wells)
One gel electrophoresis chamber (gel box) for all 10 groups
One microcentrifuge for all groups (used to spin down DNA and dye samples for 2-4 seconds)

Electrophoresis: Agarose Gel Preparation and Directions for Running Gels

Step 1 – Prepare 1X TAE Buffer (for making agarose gels and for use as a running buffer)

Buffer, and not water, must be used to make and run the gels. The buffer supplies the necessary ions to conduct electricity. The buffer received in the kit is 150 ml of 10X Tris-Acetate-EDTA (TAE) packaged in a 150 ml bottle, and needs to be diluted with distilled water (dH_2O) to make a 1X concentrated solution.

Add the entire 150 ml of 10X TAE buffer to 1350 ml of distilled water in the 2-liter container provided and mix well. This dilutes the 10X TAE buffer to 1X TAE buffer of which 120 ml will be used to make the agarose gels for each class, up to three class sets. Use the remainder of the 1X TAE buffer in the gel box to run the gels. The gel box buffer will be reused between classes, but extra 10X TAE is provided to dilute to make additional class sets, if 4-5 class sets were requested.

Step 2 - Prepare a set of six 0.7% agarose gels (5 gels for the activity with 1 gel extra.)

Agarose gels and running buffer can be made several days in advance (store gels in refrigerator if available, but room temperature is acceptable if no refrigeration is not an option). This prep will make 5 small gels (with one extra in case of breakage). Each gel will have 8 wells and will accommodate two groups of students with 3 samples each. Before making the agarose solution, have casting trays prepared and ready to be used (see Step 3). The kit includes a sixth casting tray, for the extra gel.

a) Add the tube of 0.84 g agarose to the orange-capped glass bottle.

b) Add 120 ml of the diluted 1X TAE buffer from Step 1 to the bottle (do NOT use the graduated marks on the bottle to fill to 120 ml) and mix well with the agarose.

c) Dissolve the agarose in a microwave or on a hot plate. **The orange bottle cap must be removed before heating.** The power of the microwave may vary, but to prepare 120 ml of agarose it generally takes 1.5 - 2 minutes on high power. For best results, place the bottle in the microwave for one minute, stir and heat for 30 more seconds, and stir and heat another 30 seconds, if needed. **Do not overheat,** as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and swirl while checking, then continue heating as needed. Heating is done when the agarose in the bottom of the bottle is completely in solution and well mixed with no particulate matter visible.

d) Cool the agarose solution to about 60°C by placing the melted agarose in a 60°C water bath or by allowing it to stand at room temperature for several minutes. **Swirl occasionally** while it is cooling to avoid rapid cooling of the agarose in the bottom of the bottle, which will cause it to start to solidify. If the agarose cools and solidifies in the bottle, reheat in the microwave in 30 second intervals. The bottle of melted agarose solution is ready to be used when it is warm to the hand, but not too hot to handle (if it's too hot, it can warp the comb and gel tray).

e) Once the gel has cooled to ~60°C (still liquid, but cool enough to pour) add 12 ul of SYBR Safe DNA stain to melted agarose. Swirl to mix.

f) Once cooled, pour into gel trays with rubber dams and combs (see next step).

Step 3- Casting Agarose Gels

a) Place the rubber dams onto the ends of each gel tray (it is easiest to lay the rubber dam on a table and, holding the gel tray, carefully press it into one corner, then use your weight to "roll" the gel tray into the second corner and repeat with the other rubber dam; see Figure 3). **Use caution to prevent breaking the gel tray.**

Figure 3. Gel tray with dams and comb

b) Place the gel trays with rubber dams onto a flat surface.

c) Position the comb teeth down over the black mark. (**Use the large teeth only, as it will create 8 wells of the needed size**).

d) Swirl the mixture and slowly (to avoid air bubbles) pour 20 ml cooled agarose solution into the 5 casting trays using a graduated cylinder. Use a micropipette tip to pop any air bubbles.

e) After the gel has hardened (about 30 minutes), gently remove the comb. It is important that the gels have completely solidified before the comb is removed.

f) Being very careful so that the gel does not slide off the gel tray, remove the two rubber dams from each end of the gel tray.

g) The gels may be stored by placing them in a zip-lock bag or other plastic container with a little bit (~5 ml) of 1XTAE buffer for moisture. If storing for more than 1-2 hours, keep the gels out of the light, as the DNA stain is light sensitive. Refrigeration is best but not required. Be careful that the gels do not freeze as once frozen, they will need to be melted and poured again before use.

Step 4 –Prepare Electrophoresis Running Buffer

The gel boxes require approximately 1000 ml of running buffer. The buffer may be stored at room temperature or in a refrigerator.

Step 5 – Electrophoresis of the Samples (following student Laboratory Protocol)

a) The electrophoresis gel box holds all six gel trays (see Figure 4). The gel trays are labeled "1-6". Assign up to two student groups on one gel tray and assign three wells per group. Each gel has 8 wells, so assign wells #2-4 and wells #6-8 to the two student groups that are using each gel.

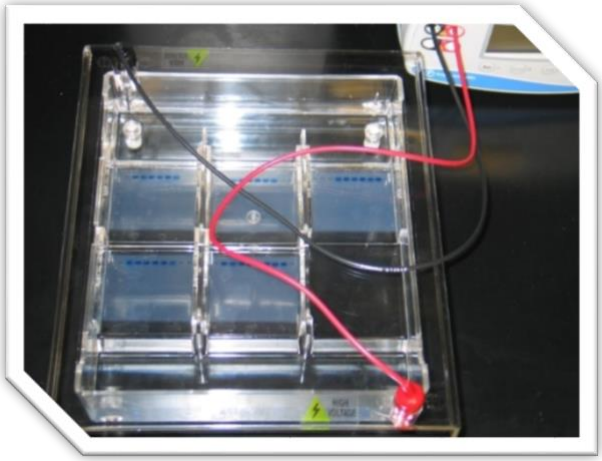


Figure 4. Gel box connected to power supply

b) After the students have prepared their samples by adding restriction enzyme, they will need to add 4 µl of loading dye (step 4 of student protocol). **Accurate pipetting is critical at this step**. If students are not pipetting correctly, they will not have enough loading dye for all of their samples. One of the most common mistakes is students will push the pipette plunger to the second stop when drawing up liquid. This results in them drawing up more than they intended, which will leave them short of loading dye for the rest of their samples. Note that we have included a limited amount of extra loading dye ("Teacher Stock LD" tube) that the teacher can use if students pipette incorrectly and run out of loading dye at their individual stations. If the samples get stuck on the sides of the tubes, you may tap the tubes on the workbench or centrifuge the samples to being all the liquid to the bottom of the tubes.

c) Next, the gels will be loaded dry at the students' tables. Students will load 20 µl of the DNA samples into their assigned wells. Finally, place the gel trays to the gel box (notice there is a notch at the top of the gel tray that fits or "locks" into place in the gel box). Be sure to **place the gel trays in the gel box so the ends containing the wells are closest to the black electrode**, or the samples will run backwards. This gel box holds two rows of gel trays so both rows must be oriented the same way in the gel box (see Figure 4).

d) Next, for the first class, slowly pour 1000 ml of the **1X TAE running buffer** into the bottom chamber of the gel box (nearest the red electrode). **Do not pour the buffer directly onto the gel** or the samples may come out of the wells. The gels in the trays need to be completely

submerged to run, but the top of the trays (sides) will be exposed out of the buffer while running. For subsequent classes, lower the loaded gels and trays directly into the running buffer.

e) Place the cover on the gel box, matching the black and red electrodes. Connect the gel box with lid to the power supply, again matching black and red electrodes to the colors marked on the ports of the power supply.

f) Follow the printed directions found on the top of the power supply to start the run. The voltage selector on the power supply should be set to 100V and the timer should be set for 30 minutes.

g) To confirm proper operation of the power supply, look for bubbles rising from the electrodes and verify that the samples are moving towards the red electrode ("running towards red").

h) When the gels are done, turn off the power supply and disconnect the lid of the gel box from the power supply.

i) Remove the gel trays from the box. Have each group take turns using the Blue Light transilluminator to visualize the gels.

j) Re-use the running buffer if you are performing this lab with multiple classes. When completely done with the running buffer, it may be poured down a sink drain. Used gels can be disposed of in the trash.

k) After use, the gel box and trays should be rinsed with tap water and allowed to air dry.

Step 6 – Interpretation of gels

After running, the pattern of DNA bands resulting from restriction analysis should be reviewed for a "match". Remind the students that a "match" consists of both the number of bands and their relative sizes being the same. A DNA sizing marker isn't run with this gel, so the size of the DNA fragments are based on their relative positions within the gel (larger DNA fragments are closest to the wells and the smallest are closest to the bottom of the gel, as they run faster). Results should show the DNA from Suspect 1 matching the Crime Scene DNA, as shown in **Figure 5**. If the student samples were not loaded in their entirety, it may be difficult to see all the individual bands of Suspect 2 (but it should be obvious that Suspect 2 does not match the Crime Scene DNA sample). A reminder to the students: if only one band of DNA is seen in a sample, then the restriction enzyme never saw the DNA sequence it recognizes and the DNA was never cut (hence, one piece of DNA).

The students should write their analysis in a lab notebook with evidence to support their results. Students should also answer the questions on the *Data/Observation Sheet*, including the gel diagram, which should contain their results. On the board, draw a blank sample gel similar to the one on page S-9 in the student sheets. Record the banding patterns from one of the class's gels. Use this to facilitate a discussion about how to interpret the results using questions such as:

- What can be inferred from the results of this test?
- What does a "match" consist of?
- Can you presume guilt of a crime by a match of a suspect and crime scene sample after DNA

restriction analysis?

- What could it mean if Suspect 1 and Suspect 2 have the same DNA pattern? (They could be identical twins, there could have been contamination of the samples, etc.)
- What would it mean if neither Suspect 1 nor Suspect 2 matches the Crime Scene DNA sample? (They could be "ruled out" as matches with the Crime Scene DNA sample.)

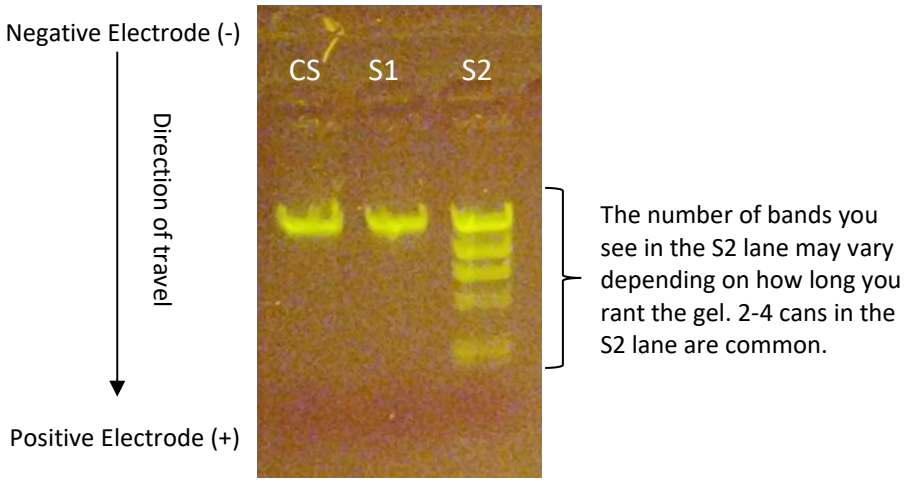


Figure 5. In this example, the sample from Suspect 1 (S1) matches the DNA found at the Crime Scene (CS). The DNA from Suspect 2 (S2) does not match the DNA found at the Crime Scene.

Student Worksheet Answer Key

I. Restriction Digest

10. What does the restriction enzyme do to the DNA?
The restriction enzyme cuts the DNA at specific locations, called restriction sites.

11. Why are the DNA samples and restriction enzyme incubated for 5 minutes?
Once the restriction enzyme is added to the DNA sample, it needs to incubate for 5 minutes to allow the restriction enzymes time to find the restriction sites and cut the DNA.

12. What will happen to the DNA if the enzyme did not find a restriction site? How many fragments will you have if the enzyme cuts the DNA two times?
If the restriction enzyme fails to find a restriction site, it will not cut the DNA, resulting in a single, uncut piece of DNA. If the enzyme cuts the DNA two times, it will produce three fragments of DNA.

II. Preparation of the Agarose Gel

13. What is the function of the agarose gel?
The agarose gel holds the DNA samples and serves as a sieve by which different-sized DNA fragments can be separated.

14. What is the function of the comb?
The comb forms the wells in the gel as the gel hardens.

15. Predict what would happen if you used 0.02 g of agarose instead of 0.2 g to make a gel. What effect would that have on the experiment? Would there be more or less separation of DNA fragments?
A gel made with 0.02 g of agarose would have a lower density than a gel made with 0.2 g. This would result in less separation amongst the DNA fragments, possibly resulting in an inability to distinguish the various DNA samples, CS, S1, and S2.

III. Preparation of the Gel Electrophoresis Box

16. Why is the gel in electrophoresis buffer?
The electrophoresis buffer conducts the electricity through the gel by providing ions by which the electricity can flow.

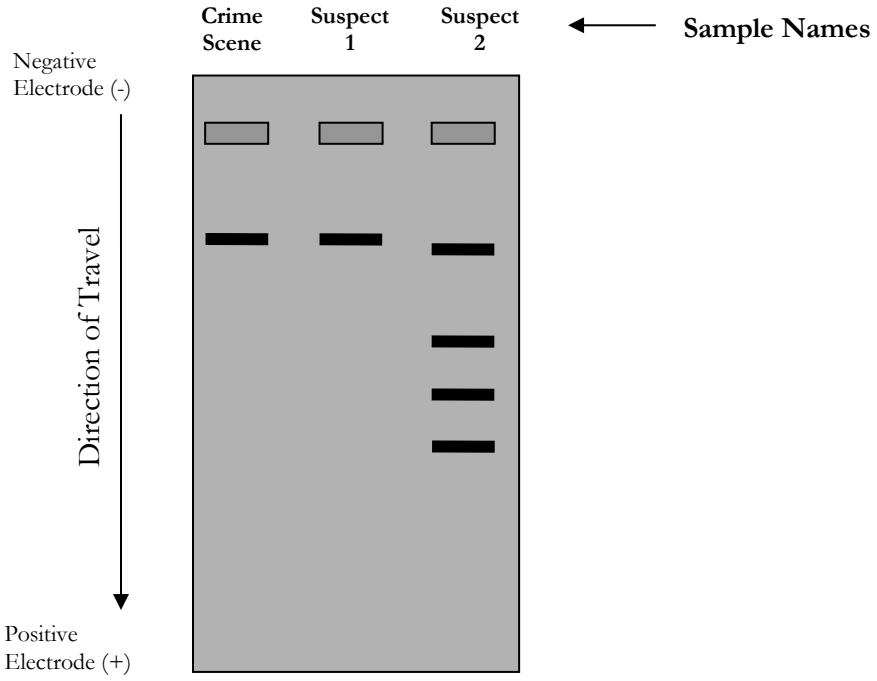
17. Describe what is occurring in the gel when the electric current is applied.
When current is applied to the gel, the negative charge of the DNA fragments causes them to migrate toward the positive electrode. The DNA fragments sort themselves according to length because the smaller DNA fragments are able to move more quickly, and thus further, in the gel than the larger DNA fragments due to the smaller fragments' ability to move around the holes in the gel more easily.

18. Predict what would happen if you put the wells of the agarose gel at the positive electrode.
If the wells were put at the positive electrode, the samples would not have much gel to move through and would migrate off of the gel when the current was applied.

Student Worksheet Answer Key

VI. RESULTS

19. Use this diagram to record the sample names and where you loaded each sample. Also record the results you observe after the gel finished running. Label the positive and negative electrodes and the direction the DNA traveled.



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 complete "Restriction Enzyme Worksheets 1 and 2 as reinforcement and review.

II 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, such as the school newspaper and photography club. Assign students to roles such as a jury member, prosecutor, defense, scientific expert and media. After all the evidence is collected, hold court in which each department presents their analysis of the evidence. Have the attorneys write a brief for the court and prepare testimony debating the strengths and weaknesses of the DNA evidence.

III Ask the students to write a letter to a friend who knows nothing about DNA restriction analysis describing their results.

IV 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, a second group as Suspect One, and the last group as 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:

Crime Scene	ACCGGTAT
Suspect One	CCGGATCA
Suspect Two	ACCGGTAT

Ask each group to form the DNA fragments that would be created if *Hae*III, 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.

V Engage the students in a discussion about genetic similarities between individual people, and between humans and other species. All humans are 99.9% similar in their DNA sequences, but we have over 3 billion bases in our genome, so <0.1% difference is still a sizable quantity of DNA that is different. We can use that difference to identify people in the case of a crime scene, bodily remains, paternity testing, etc. Student can compare genome sizes and chromosome numbers to compare humans and other species.

CITY POLICE DEPARTMENT
POLICE REPORT

INCIDENT DATA

Incident Type: Museum Theft
Complaint Status: Pending DNA Results

Processed by: Officer Joe Friday
Other Officers: Off. D. 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 window sill. We concluded that 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 the missing jewels. Having been a member of the prestigious ninja S.W.A.T. 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 from crime scene
DNA from four suspects

List of Procedures Used:
DNA extraction
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. Forensic scientists used DNA isolated from four suspects and compared them to the crime scene DNA using DNA restriction analysis.

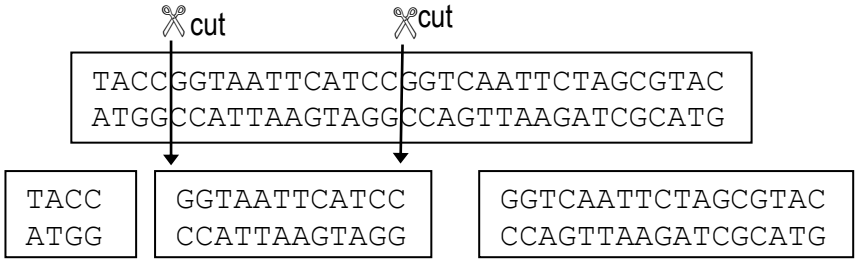
Results: See the *Final Report*.

DNA EVIDENCE EVALUATION

WORK ONLY ON YOUR DNA SAMPLE

1. Each team member should have one DNA sequence strip. Use your scissors (restriction enzymes) to cut your DNA sample only where you see this base pattern: Cut between the C and G as shown in this example:

CCGG
GGCC



Be sure to keep all the cut DNA fragments from one sample together; do not mix them up with other sample fragments.

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

GCTAATTCATCC CGATTAAGTAGG

Front side

12 base pairs

Back side

3. Following the example below, place your DNA sequences on the chart according to the number of base pairs. Be sure to put all the fragments from your sample in the proper column.

Crime DNA	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Number of Base Pairs (bp)
					32
					31
					30
					29
					28
					27
					26
					25
					24
					23
					22
					21
					20
					19
					18
					17
					16
					15
					14
					13
				12 bp	12
					11
					10
					9
					8
					7
					6
					5
					4
					3
					2
					1

4. Analyze the DNA fragments, by looking at both the number of fragments and their sizes, for all suspect and crime scene samples. Are there any suspects that match the crime scene DNA? Fill out the *Final Report* sheet with your conclusions.

Crime Scene DNA Crime Scene DNA Crime Scene DNA Crime Scene DNA Crime Scene DNA Crime Scene DNA Crime Scene DNA

GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG
CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

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

GTCTACGTAATCGTAGCCATCCGGACAGTGTGCACGATCGTACATGCTACGTG
CAGATGCATTAGCATCGGTAGGCCTGTCACACGTGCTAGCATGTACGATGCAC

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
CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA

GTCTCCATCCGGACTACCATACATCTGGTGTACCCGGTGATATCGTCCGGGTG
CAGAGGTAGGCCTGATGGTATGTAGACCACATGGGCCACTATAGCAGGCCCAC

CITY POLICE DEPARTMENT
FINAL REPORT

Names of Forensic Team Members:

_____

_____

_____

_____

_____

Was any suspect's DNA found at the crime scene? If so, whose?

_____

_____

Processing the evidence: Explain how your group came to its conclusion.

MICROPIPETTE USE

This pre-laboratory activity allows you to practice two lab skills necessary for this lab.

Micropipette Skills:

Laboratory science often involves working with very small volumes, often requiring careful measurement of less than one milliliter. The micropipette is a precision instrument used to accurately measure and transfer volumes up to 1 ml.

1 liter = 1,000 ml = 1,000,000 µl

How to use a Micropipette:

1. Adjust the pipette to 20 µl by turning the dial. Do not turn beyond the volume range for the pipette. Push down on the plunger, locating the first and second stops.
2. Adjust the pipette volume to 4 µl, and again find the first and second stops. What is different this time compare to the first time?
3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To practice micropipetting, keep the volume set to 4 µl. To draw liquid into the micropipette tip:
    a. Depress the plunger to the **first stop** to measure the desired volume and hold in that position.
    b. Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
    c. Draw the fluid into the tip by <u>slowly</u> releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
    d. To verify the correct volume was extracted, compare the volume in your micropipette tip with the group next to you.
5. To dispense the liquid:
    a. Place the tip into the container where the liquid is to be released, near the bottom.
    b. Slowly depress the plunger to the **second stop** to blow out all of the liquid in the tip. Keep the plunger depressed after dispensing the liquid until the micropipette tip is out of the container. This ensures that you do not suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just expelled.
    c. Eject the tip when done into a waste container by pressing the separate ejector plunger found on the side of the micropipette.
6. Repeat steps 4 and 5, but pipette incorrectly by going to the second stop in order to extract the liquid. Was a different volume drawn up into the micropipette tip, when compared with the amount drawn initially?
7. Change tips between liquids in order to prevent contamination.

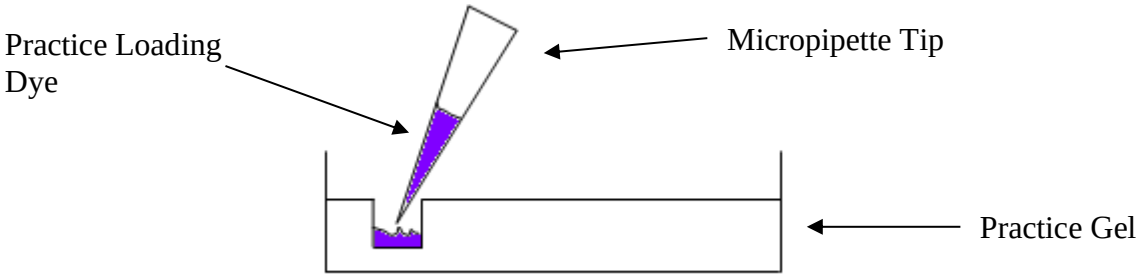
PRACTICE GELS EXERCISE

Loading Gels Skills:

"Loading gels" (filling the wells of a gel) can be a challenging task, especially if one has never done it before. This is an opportunity to practice before you load the actual samples involved in the laboratory activity. Many people's hands shake a bit, so people often prefer to stabilize the micropipette while loading the gel. For example, try resting your elbow on the counter while loading and steady the micropipette by bracing the pipette body against a finger of the other hand, or try placing both elbows on the workbench and work with both hands to raise and lower the micropipette like a drill. Take your time, try different stabilizing positions, and practice filling a few different wells of the practice gels. This is for practice so don't get frustrated if you spill out of a well or accidentally tear the edge of the agarose gel. Just try again!

Loading the practice gels

1. Adjust micropipette to 15 µl and gently tap a tip onto the micropipette.
2. Remove the lid of the practice agarose gel and make sure you can clearly see the wells. These well may appear as holes, but they are actually indentations that do not go completely through the gel.
3. Draw up 15 µl into of practice dye ("Prac Dye") into the micropipette tip.
4. Select a well to pipette the dye into.
5. Lower the tip filled with the dye into a well to be filled and gently expel the liquid into the well. Be careful not to poke through the bottom of the well or rip between the wells, or the liquid will not stay in the individual well you chose.



6. Look to see if all of the dye went into the well.
7. Repeat Steps 5-9 at least two or three times until you feel comfortable loading samples into a well. Each person in the group needs to practice loading wells in the practice gel. You do not need to change tips since you will be using the same liquid between group members.

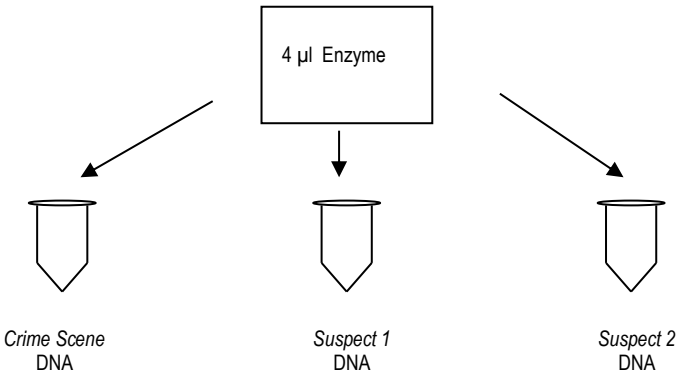
There are several tubes in your Crime Scene Kit:

Crime Scene DNA,**"CS"** Suspect 1 DNA, **"S1"** Suspect 2 DNA, **"S2**"
Restriction Enzyme, **"RE"** Loading Dye, **"LD"**

When working with micropipettes, be sure to change your pipette tip between samples to prevent contamination of your samples and your reagents. When you add a small amount of a reagent to your sample, be sure to add it to the sample at the bottom of your tube and pipette up and down once or twice to gently mix it.

2. **To set up a Restriction Enzyme Digest:** Add 4 µl of Restriction Enzyme **"RE"** to each of the three samples "CS", "S1", and "S2", by adding it to the DNA sample at the bottom of the tube. Remember to change your pipette tip for each sample.

3. Incubate the three samples at room temperature for **5** minutes for the enzymatic reaction to occur.



Assign Gel Trays and Sample Wells:

Gel Tray # _____

"CS" well #_____

"S1" well #_____

"S2" well # _____

4. Add 4 µl of loading dye **"LD"** to each of the three samples, "CS", "S1", and "S2", by adding it to the DNA sample at the bottom of the tube. Remember to change your pipette tip for each sample.

5. Centrifuge samples for 1 second. Note: when loading samples into the microcentrifuge, make sure to arrange them so the microcentrifuge is balanced.

6. Load 20 µl of each of the three samples, "CS", "S1", and "S2", into the wells in the gel you were assigned by your teacher. Remember to change your pipette tip for each sample.

7. After all samples have been loaded, your teacher will add electrophoresis buffer to the gel box and cover with the lid, connect the power supply to the gel box, plug it in, turn it on, and check it for the correct settings to be sure the gel runs properly. The gel will run for about 30 minutes at 100 volts.

8. After the gel is done running, your teacher will turn off the power supply and remove the cover to the gel box. Slide the gel off of the tray onto the Blue Light transilluminator and turn on.

9. Analyze the DNA bands for each sample. Remember to inspect both the number of bands and the different sizes. Complete the questions on the Data/Observation Sheets and record your results on the diagram.

CITY POLICE DEPARTMENT:
DATA/OBSERVATION SHEETS

I. RESTRICTION DIGEST

10. What does the restriction enzyme do to the DNA?

11. Why are the DNA samples and restriction enzyme incubated for 5 minutes?

12. What will happen to the DNA if the enzyme did not find a restriction site? How many fragments will you have if the enzyme cuts the DNA two times?

II. PREPARATION OF THE AGAROSE GEL

13. What is the function of the agarose gel?

14. What is the function of the comb?

15. Predict what would happen if you used 0.02 g of agarose instead of 0.2 g to make a gel. What effect would that have on the experiment? Would there be more or less separation of DNA fragments?

CITY POLICE DEPARTMENT:
DATA/OBSERVATION SHEETS

III. PREPARATION OF THE GEL ELECTROPHORESIS BOX

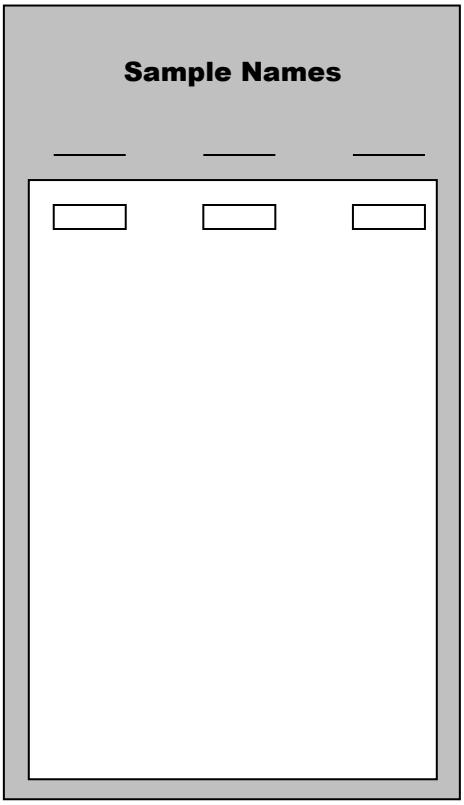
16. Why is the gel in electrophoresis buffer?

17. Describe what is occurring in the gel when the electric current is applied.

18. Predict what would happen if you put the wells of the agarose gel at the positive electrode.

VI. RESULTS

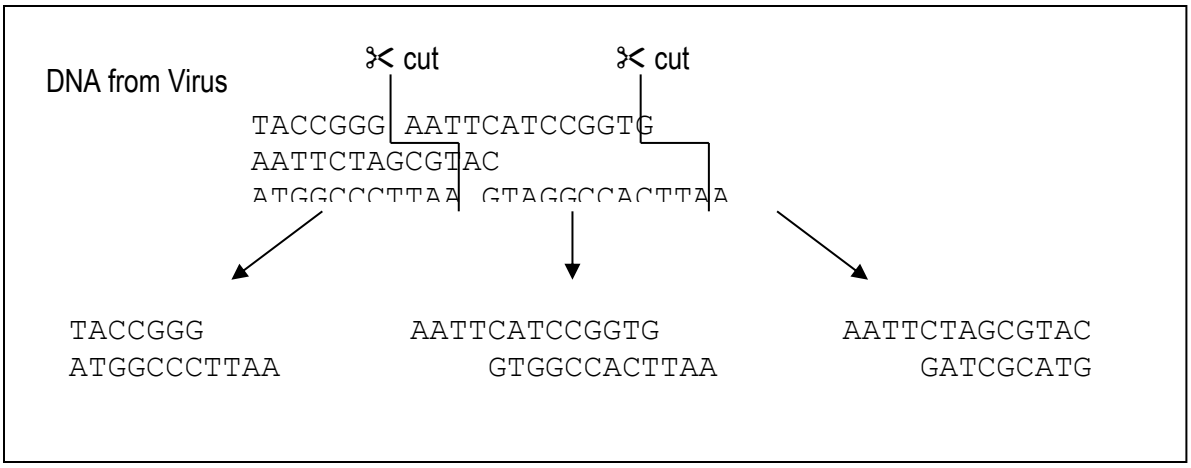
19. Use this diagram to record the sample names and where you loaded each sample. Also record the results you observed after the gel finished running. Label the positive and negative electrodes and the direction the DNA traveled.



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:

1. The chemical that cuts the DNA is called a restriction enzyme. Restriction enzymes cut the DNA into _____ .

2. The restriction enzyme used above is called *Eco*RI. *Eco*RI cuts DNA everywhere the base pattern_____ is found.

3. Another restriction enzyme is *Hae*III. It cuts DNA at the base sequence CCGG. It cuts between the C and G. Show the DNA fragments that would result if *Hae*III was used to cut the DNA fragment shown in the diagram above.

4. Do you think restriction enzymes could be used to cut DNA from other organisms?

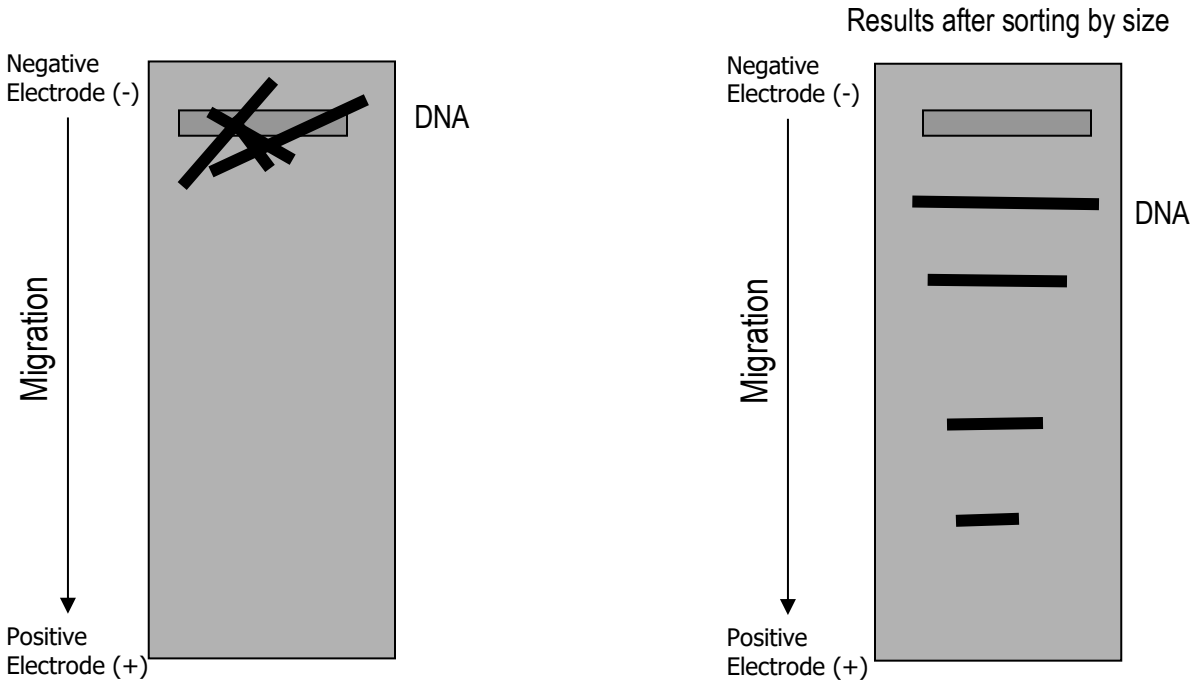
RESTRICTION ENZYME WORKSHEET 2

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.

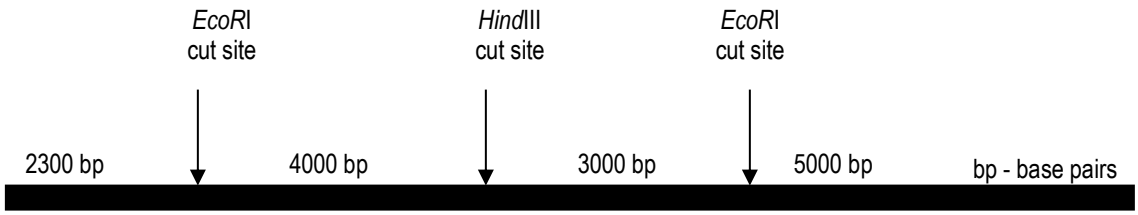
The DNA fragments need to be separated in order to be compared. The fragments are sorted by passing them through a gel. The gel acts like a screen, allowing small pieces of DNA to pass through more easily than large pieces, much like sifting rocks out of dirt. Electricity is used to move the DNA through 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



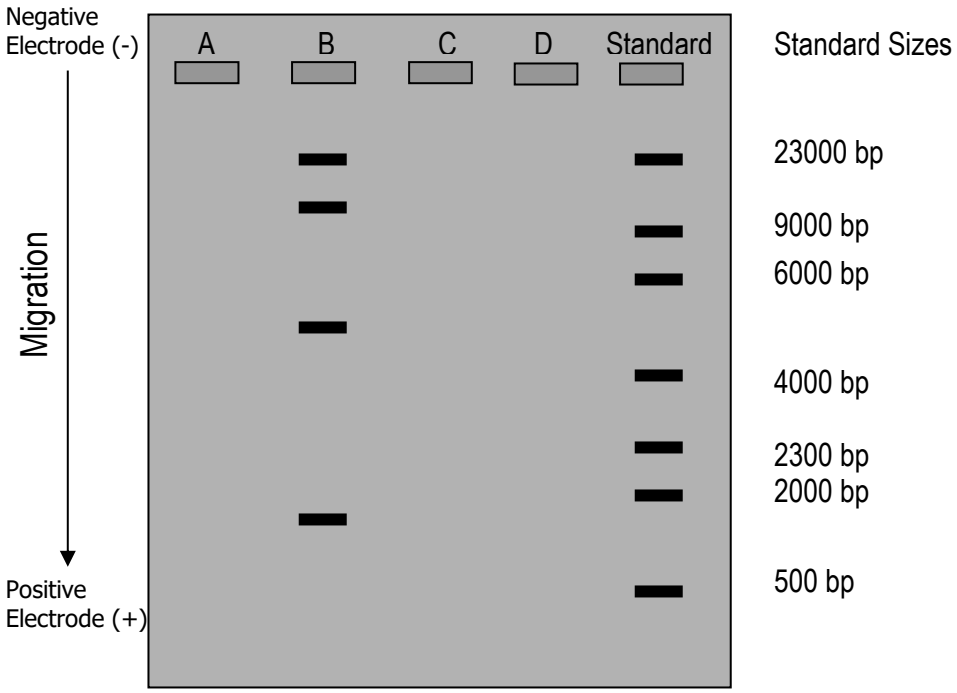
The process of sorting DNA fragments by size using a gel and electricity is called gel electrophoresis.

FIGURE 2: DNA Strand with specific *EcoR*I and *Hind*III sites



RESTRICTION ENZYME WORKSHEET 2

Use the gel box below to answer the following questions.



1. Next to each band in lane B, write the size of the DNA fragment that would be found in that lane.

2. Imagine the DNA strand shown in Figure 2 was cut with the restriction enzyme *Eco*RI 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.

3. Now assume the DNA was cut with both *Eco*RI and *Hind*III 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.