Case of the Crown Jewels

A DNA Restriction Analysis Laboratory Activity

Maryland Loaner Lab Teacher Packet

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Version 08.16.2011_nd

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STUDENT ACTIVITY HANDOUTS AND LABORATORY PROTOCOL

Police Report	S-1	*Used in the Pre-Laboratory Activity*
DNA Evidence Evaluation	S-2	*Used in the Pre-Laboratory Activity*
DNA Sequence	S-3	*Used in the Pre-Laboratory Activity*
Final Report	S-4	*Used in the Pre-Laboratory Activity*
Micropipette Challenge	S-5	*Used in the Pre-Laboratory Activity*
Crime Lab Laboratory Protocol	S-6	*Used in the Laboratory Activity*
Data/Observation Sheets	S-7	*Used in the Laboratory Activity*
Restriction Enzyme Worksheet #1	S-9	*Extension Activity*
Restriction Enzyme Worksheet #2	S-10	*Extension Activity*

Case of the Crown Jewels

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	Must Be Returned
Gel Electrophoresis Box	1	With lid	Yes
Power Supply	1	For use with gel electrophoresis box	Yes
Gel Trays	6	Use with gel trays	Yes
Gel Combs	6	To make 5 gels (+ one extra)	Yes
Gel Tray Ends	12	Used during gel prep.	Yes
Orange-capped Bottle	1	Used to make 5 gels (+ one extra)	Rinse and Return
Agarose Powder	1 baggie (0.84g)	Used to make 5 gels (+ one extra)	Return empty baggie
10X TAE Buffer	3 tubes (150 ml)	Follow Teacher Prep for dilution	Rinse and Return
2-liter Container	1	Follow Teacher Prep for dilution	Rinse and Return
20-ul Micropipettes	10	1 per group	Yes
Micropipette Tips	5 boxes	1 box per two groups	Yes
Staining Trays	2	Fits five gels	Rinse and Return
Carolina Blu™ Stain Kit	1	Dropper bottle and Stain bottle	Yes
Non-latex Gloves	1 pair	Use with gel stain	No
Restriction Enzyme	10 tubes ("RE")	1 per group **Refrigerate until use**	Yes
Crime Scene DNA	10 tubes ("CS")	1 per group **Refrigerate until use**	No
Suspect 1 DNA	10 tubes ("S1")	1 per group **Refrigerate until use**	No
Suspect 2 DNA	10 tubes ("S2")	1 per group **Refrigerate until use**	No
Sample Loading Dye	10 tubes ("LD")	1 per group **Refrigerate until use**	Yes, **Keep cold until returned**
Foam Microtube Racks	10	1 per group	Yes
Practice Gels	10 petri dishes	1 per group **Refrigerate until use**	Empty and Return
Practice Loading Dye	10 tubes	1 per group **Refrigerate until use**	Yes, **Keep cold until returned**
Microcentrifuge	1	Used to spin samples down in tubes	Yes
100-ml Graduated Cylinder	1	Used to pour gels	Yes
Disinfectant Wipes	1 container	Used to cleanse equipment for return	Yes

Supplied by the Teacher:

Description	Quantity	Comments
Test Tubes	30	Each group needs 1 empty and 2 with food colored water
Test Tube Rack	10	1 per group
Food Coloring	1 each color	*Micropipette Challenge*
Scissors	5 pairs	1 per group
Rolls of Tape	5	1 per group
Poster Board	5 medium sheets	Use with Pre-Laboratory activity (can also use large sheets of paper)
Distilled Water (dH_20)	2000 ml	Used to dilute 10X TAE buffer and destain gels
Lab Microwave or Hot Plate	1	To melt agarose gel

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

Case of the Crown Jewels

Maryland Science Core Learning Goals

These classroom and laboratory activities meet several of the Maryland Science Content Standards:

Goal 1.0 Skills and Processes

1.3.1	*The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques.* *

1.2.6	The student will identify appropriate methods for conducting and investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size, etc.).
1.2.7	The student will use relationships discovered in the lab to explain phenomena observed outside the laboratory.
1.3.1[NTB]	The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques. **
1.3.2	The student will recognize safe laboratory procedures. **
1.3.3[NTB]	The student will demonstrate safe handling of the chemicals and materials of science. **
1.3.4[NTB]	The student will learn the use of new instruments and equipment by flowing instructions in a manual or from oral direction. **
1.4.2	The student will analyze data to make predictions, decisions, or draw conclusions.
1.5.1	The student will demonstrate the ability to summarize data (measurements/observations).
1.5.4	The student will use tables, graphs, and displays to support arguments and claims in both written and oral communication.
1.5.5	The student will create and/or interpret graphics. (scale drawings, photographs, digital images, field of view, etc.)
1.7.1	The student will apply the skills, processes and concepts of biology, chemistry, physics, or earth science to societal issues.
1.7.5	Students will investigate career possibilities in the various areas of science. ***

Goal 3.0 Concepts of Biology

3.3.3	*The student will explain how a genetic trait is determined by the code in a DNA molecule.**

3.1.1	The student will be able to describe the unique characteristics of chemical substances and macromolecules utilized by living systems. (specifically, nucleic acids)
3.3.2	The student will illustrate and explain how expressed traits are passed from parent to offspring.

* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.
** All labs include **all** assessment limits/indicators for Goal 1.Expecation 3: The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.
*** The provided DVD, MdBio Tech Council of Maryland, *Careers in Biotechnology* addresses 1.7.5.

Case of the Crown Jewels

Introduction

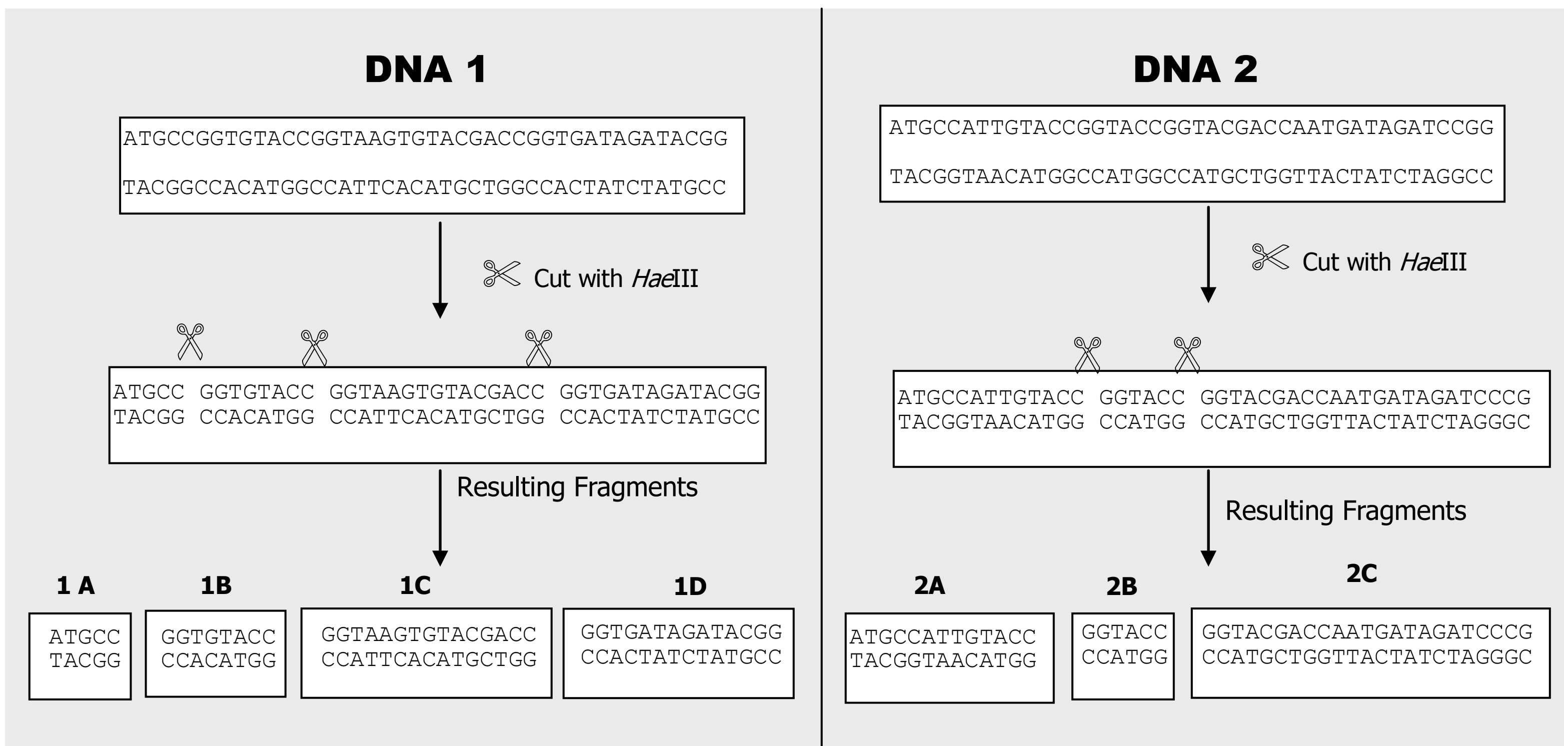
DNA restriction analysis is a technique with wide ranging applications in medicine, research, and forensics. The Case of the Crown Jewels is an activity that simulates the basics of DNA fingerprinting, a technique used by forensic scientists, which relies on restriction analysis to analyze DNA evidence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

When a restriction enzyme is used to cut different DNA molecules (in a restriction digestion) the size of the fragments generated 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

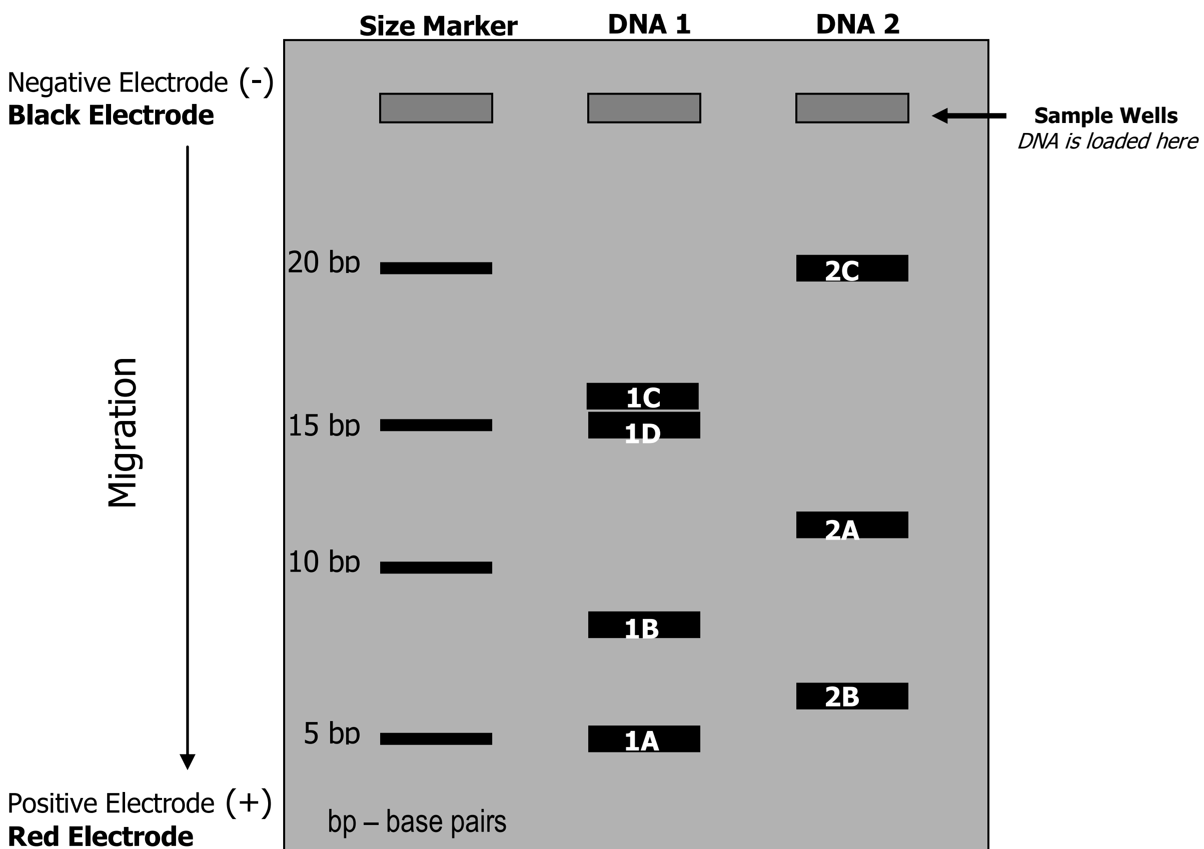


After being cut by restriction enzymes, DNA fragments remain mixed in solution and indistinguishable from one another. One way to distinguish between the different fragments created is to compare them by size. Different size fragments of DNA can be separated using gel electrophoresis.

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, DNA is negatively charged and in the presence of an electric current, DNA will travel according to size (smallest pieces first) towards the positive electrode as shown in Figure 2. The positive electrode is colored red, and electrophoresis of DNA is always "Running towards Red". 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 and when run on a gel appear as a ladder with many rungs. 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 or not. 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



This lesson is organized into two parts - a Pre-Laboratory activity and a laboratory investigation. During the Pre-Laboratory activity, students work in groups to simulate a DNA restriction analysis using paper DNA sequences and scissors to solve a fictional crime. Following the Pre-Laboratory activity, students work in the laboratory where they apply the concepts acquired in the Pre-Lab to compare two suspects' DNA found at a fictional crime scene. The restriction digestion in the laboratory investigation is a mock exercise due to the limitations of a loaner lab program (issues of keeping restriction enzymes frozen as needed, classrooms not being equipped with the necessary water baths, etc.). The actual DNA samples used in the lab are pre-digested with restriction enzymes.

Introduction

The laboratory investigation requires the use of micropipettes for accurately measuring small volumes. ***The Micropipette Challenge*** is an additional pre-laboratory activity included with this packet that will allow students practice in using this piece of equipment. If your students are unfamiliar with the use of micropipettes, provide additional time to do this activity before students perform the laboratory investigation. All students must be reminded about the proper usage of micropipettes, to prevent damage to the equipment and also to provide students with accurate results during the lab.

Another pre-laboratory activity allows the students to practice loading agarose gels, as it can be a difficult skill to initially acquire. The ***Practice Gel Loading Exercise*** instructs students with the proper technique used to load gels and gives them the opportunity to practice, before loading the samples involved in the laboratory investigation.

Case of the Crown Jewels

Pre-Laboratory Activity

NOTE: Groups 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.

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 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 that will be given to them.

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 the 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, DNA restriction analysis, provides indirect evidence that particular 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

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 to use DNA restriction analysis to determine if any 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 for the Laboratory Activity

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 whether 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 technique is needed to determine if either of the samples could match that found at the crime scene.

Additional pre-laboratory activities (to be performed in advance to the Laboratory Investigation)

Before students perform the laboratory activity, discuss with students how to use micropipettes properly (see Micropipette Challenge Explanation). Students will practice using the micropipette by performing ***The Micropipette Challenge*** prior to the lab. Because loading the wells of agarose gels can be difficult at first, students will be given an opportunity to practice loading dye samples into a practice agarose gel by performing the ***Practice Gel Loading Exercise.***

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). The "reaction" in the protocol will take place during the 5-minute incubation time. Restriction enzyme digests generally take place in a 37°C water bath for a minimum of an hour for the reaction to occur. This is to ensure complete digestion of the DNA.

Loading dye is added to the samples to help visualize the samples while loading into the gels, and the loading dye increases the sample density and keeps the samples in the wells while adding the running buffer. When the gels start to run, the loading dye also acts as a tracking dye and will start to separate into three dyes that each have their own sizes that correspond to the number of base pairs in a piece of DNA (blue corresponds to the largest number of base pairs, purple to an intermediate number, and yellow to the smallest number). This color separation allows researchers who are familiar with the actual sizes of the DNA fragments they are working with to know approximately where the DNA fragments are in the gel, so that they do not allow the fragments to run

off the gel. The yellow dye will come very close to the end of the gel (or will run off the end) after running 30 minutes. It is important for the students to understand that this is not stained DNA, it is the loading dye.

Groups will be assigned three wells in a gel to load their three DNA samples, all in the same order "CS", "S1", then "S2. Once all gels have been loaded with the student samples, they will be run for approximately 30 minutes at 100 volts. The agarose gels act as a sieve to separate the different sized DNA fragments. The DNA samples in the gels are invisible, but they will become stained with the Carolina Blu™, which is mixed into both the agarose gel and the electrophoresis running buffer. If the DNA bands are not dark enough after the run time is completed, an optional final Carolina Blu™ staining procedure can be performed to darken them. (see Teacher Preparation section)

Micropipette Challenge Explanation

NOTE: This activity 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 everyone understands how to operate the micropipettes.

Solution Preparation for Challenge

Make up the colored water solutions (yellow and blue) by adding food coloring to water (for 10 ml of water, add 1 drops of food coloring). Allow 1 ml of each colored solution for each student group.

Student Stations for Challenge

1 empty test tube test tube rack 20-ul micropipette micropipette tips
2 tubes of 1-ml each (pre-made yellow and blue solutions)

Basic Directions for Micropipette Use

Golden Rules of Pipetting

1. Don't rotate the volume adjuster beyond the upper or lower range of the pipette, this can damage it.

2. Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette 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 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 (0.1 µl) or whole microliters in the smallest place so that you can read the scale correctly (it varies with different brand micropipettes).

Drawing Up and Expelling Liquid

Micropipettes have 2 stops as you depress the plunger to expel liquid. 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 upon delivery. To draw liquid into the pipette tip depress the plunger control only to the first stop. If you go to the second stop you will draw too much liquid into the 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). When you are letting the liquid out of the tip then you go to the second stop. It is worthwhile to check each student for correct technique before beginning laboratory procedures requiring use of the pipettes.

Micropipette Challenge Explanation

Using the Micropipette:

1. Select the pipette that includes the volume range you will need.

2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.

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 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 slowly 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).

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. Be careful to not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
   c) Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depends on the brand of micropipette).

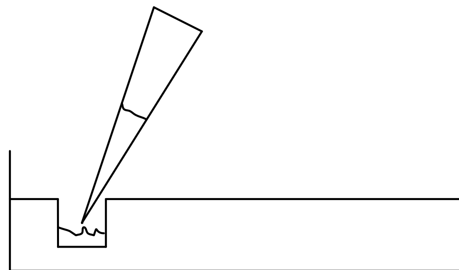
Practice Gel Loading Exercise

"Loading gels", or 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 are asked to load the actual samples involved in the laboratory activity. Take your time, figure out how you feel most comfortable doing this (example: some people like the rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remember, this is for practice so don't get frustrated if you spill out of a well or if you accidentally tear the edge of the agarose gel - try it again.

An important thing to note about gels: the wells appear as holes, but they really aren't. 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.

Loading the practice gels

1. Become familiar with the feel of the two stops on the micropipette by pressing on the plunger a few times. You should notice a difference in resistance and the amount of pressure required between the two stops. The first stop is used to suck the liquid into the tip and the second stop is to let the liquid completely out of the tip.
2. Adjust micropipette to 15 ul.
3. Make sure you gently tap a tip onto the end of the micropipette.
4. Remove the lid of the practice agarose gel and make sure you can clearly see the wells.
5. To suck up the practice loading dye into the tip, press down to the first stop outside of the tube then place your tip into the liquid dye and slowly lift up your thumb. (Be careful not to raise your hand while lifting your thumb or you'll get air bubbles and the volume will be incorrect)
6. Select a well to pipette the dye into.
7. Lower the tip filled with the dye into a well to be filled. 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.



8. To release the dye from the tip, press down to first stop and then keep pressing down until you reach the bottom of the second stop. While the second stop is still pressed in, lift up the micropipette so the tip is no longer in the well (or you may accidentally suck the liquid back into the tip).

9. Look to see if all of the dye went into the well.
   Repeat this 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.

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 following handouts:
Micropipette Challenge, Laboratory Protocol, and Data/Observation Sheets

Prepare Student Stations (for 10 groups):

One foam microtube rack
One tube each: "CS", "S1", "S2", "RE", and "LD"
One 20-ul 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 needed:
Preparing gels & student stations	30 minutes
Pre-Lab activity	60 minutes
Micropipette Challenge and Practice Gel Loading Exercise	30 minutes
Laboratory activity	90 minutes
Post-Lab activity	10 minutes

Shared equipment for multiple groups:

One agarose gel for every two groups (each group has 3 wells)
One gel electrophoresis chamber (gel box) for all 10 groups.

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 10X Tris-Acetate-EDTA (TAE) with 150 ml in three conical tubes, and needs to be diluted with distilled water (dH_2O) to make a 1X concentrated solution.

1. Add the entire 150 ml of 10X TAE buffer to 1350 ml of distilled water in the 2-liter container provided and mix well. From this now diluted 1X TAE buffer, 120 ml will be used to make the agarose gels and 1000 ml will be used as the electrophoresis running buffer.

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

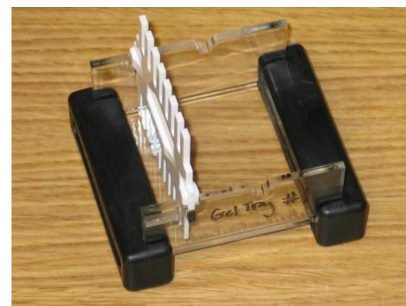
Agarose gels and running buffer should be made the night before use or the day of use, to prevent fading of the Carolina Blu™ stain. You can perform this step earlier, but a longer staining period may be required to ensure the experimental results are visible. This prep will make 6 small gels, one of which is extra. 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 one extra casting tray, which you will not need for this activity.

1. The glass bottle with 0.84 g agarose needs 120 ml of the diluted 1X TAE buffer from Step 1 (use the graduated marks on the bottle to fill to 120 ml). Add the buffer to the bottle and mix well with the agarose.
2. 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 to mix, then continue heating. The agarose must be completely in solution and well mixed. No particulate matter should be visible.

3. 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 (or reheating will be necessary). 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).
4. Add 5 drops of Carolina Blu™ stain (from the dropper bottle) to the bottle of 120 ml of the cooled agarose and swirl to mix. This stain is not toxic; however, it will stain your hands, so wear gloves. Immediately pour into gel trays with rubber dams (see next step).

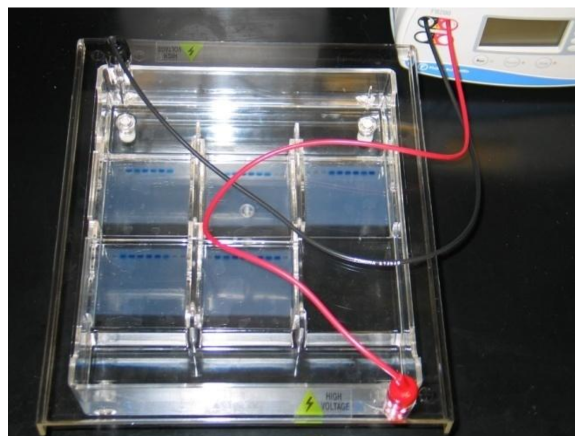
Step 3- Casting Agarose Gels



1. 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). **Use caution to prevent breaking the gel tray.**
2. Place the gel trays with rubber dams onto a flat surface.
3. Position the comb teeth down over the black mark (**Use the large teeth only, as it will create 8 wells of the needed size**).
4. 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 pipette tip to pop any air bubbles).
5. 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.
6. 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.
7. The gels may be stored by placing them in a zip-lock bag or other plastic container. Refrigeration is best, but not required.
8. Gels stored longer than two to three days tend to fade and the DNA may not be visible during electrophoresis. These gels are still usable, but will require longer staining after electrophoresis.

Step 4 –Prepare Electrophoresis Running Buffer

1. Measure out 1000 ml of 1X TAE buffer from Step 1, and add 49 drops of Carolina Blue and mix well. This now is the electrophoresis running buffer that will be used to run the gels. 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)

1. The electrophoresis gel box holds all six gel trays. The gel trays are labeled "1-6", with one being extra. 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.
2. 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, the teacher will very carefully pick up the gel trays and add them 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 picture).

3. Next, 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.
4. Once gels have been placed in the gel box and the running buffer added, be careful not to disturb the electrophoresis apparatus.
5. Place the cover on the gel box, matching the black and red electrodes.
6. 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.
7. 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.
8. To confirm proper operation of the power supply, look for bubbles rising from the electrodes and that the samples are moving in the proper direction ("running towards red")
9. When the gels are done, turn off the power supply and disconnect the lid of the gel box from the power supply.
10. Remove the gel trays from the box. Final DNA staining of the gels will be necessary for the bands to be dark enough to visualize.
11. Gently remove the gels from the trays and place them into the staining tray and cover them with the Carolina Blu™ Final Stain for 15 minutes. After staining and with gloves on, remove the gels and place them on a plastic surface or plastic wrap. **The used final stain should be poured back into its bottle and returned with the equipment (please mark on the bottle if you used it).** Place the gels back into the staining tray and rinse them off with **distilled water** and pour it out. Destain them by adding **distilled water** to the staining tray and allow them to sit for 15 minutes. Change the distilled water and repeat for a minimum of 15 minutes. It is best to destain the second time for longer than 15 minutes or the DNA bands may be difficult to see with the loading dye still present in the gels. The best way to destain is to leave the gels overnight in distilled water and to view the next day; the loading dye will be gone and the DNA bands will be easier to see. Destain can be poured down a sink drain. The DNA bands are best visualized when viewed against a white background or, even better, on a light box.
12. When done with the running buffer, it may be poured down a sink drain. Used gels can be disposed of in the trash.
13. 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 and staining, the pattern of DNA bands resulting from restriction analysis should be analyzed. The loading dye that separated into three colors while the gels ran should have faded during the final stain procedure. 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 3**. If the student samples were not loaded in their entirety and staining was incomplete, 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. To facilitate a discussion, choose a representative gel and put it on an overhead projector (don't leave for too long or DNA bands will fade). Highlight the bands projected on the board with a marker. Some sample questions could be:

- 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)

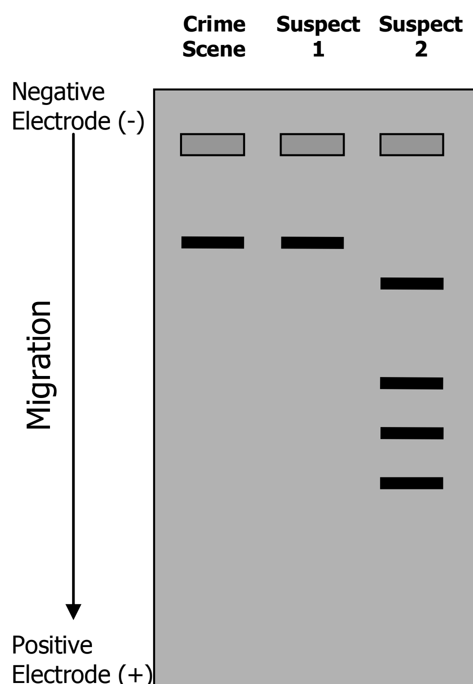


FIGURE 3: Restriction Analysis of DNA Evidence

Case of the Crown Jewels

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

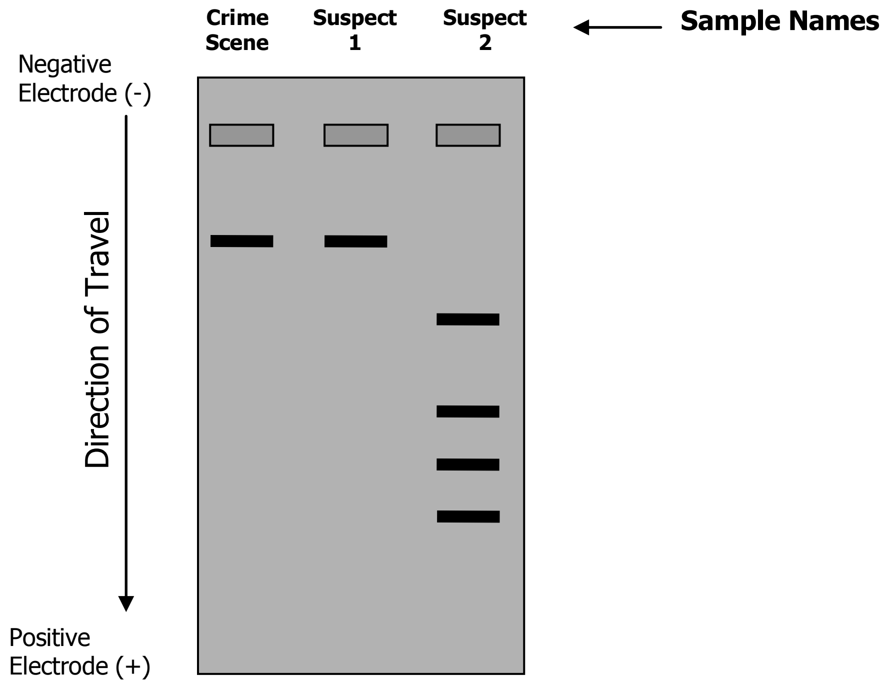
16. Why is the gel in electrophoresis buffer?
    The electrophoresis buffer conducts the electricity through the gel by providing ions by which the electricity can flow.

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.

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, 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.

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, 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 *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 how similar we are to one another, genetically speaking: that all humans are 99.9% similar in their DNA sequence. But share the fact that we have over 3 billion bases in our genome, so that <0.1% difference is still a "chunk" 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. Encourage students to look up information about how different humans are from different species such as monkeys, mice, fish, etc. (have them compare genome sizes and chromosome numbers).

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CITY POLICE DEPARTMENT
POLICE REPORT

INCIDENT DATA

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

PROPERTY

Property Code: Jewelry/Precious Metal
Name: Crown Jewels
Owner's Name: City Museum
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. 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 swats 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
Polymerase Chain Reaction

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*.

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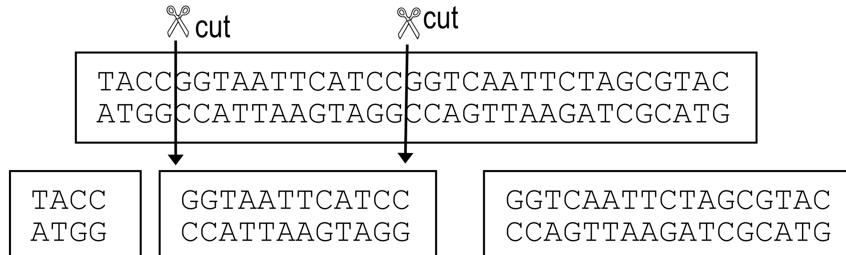
DNA EVIDENCE EVALUATION

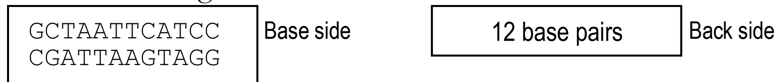
WORK ONLY ON YOUR DNA SAMPLE

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: CCGG GGCC
   Cut between the C and G as shown in this example:



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



3. An enlarged chart, like the one below, is provided for your group. Tape 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. Follow the example below:

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? Begin to 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

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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 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. **(1 liter = 1,000 ml = 1,000,000 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 tubes. One has blue food coloring and the other has yellow food coloring. Practice using the micropipettes by adding the amounts listed to an empty tube. Notice how very small the volumes really are. Discuss with your class what is considered good micropipette technique and how to use micropipettes properly.

Remember to change pipette tips between different color solutions, so not to contaminate them. (option: you could pipette all the yellow first, and then change the tip and pipette the blue last) Watch each other pipette and check each other's technique. Ask your teacher for help if you have questions about using the micropipettes.

Example:

Amount to add to tube	Color	Record the setting as it appears in the window
15 µl	blue	15.0

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	

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? _________________

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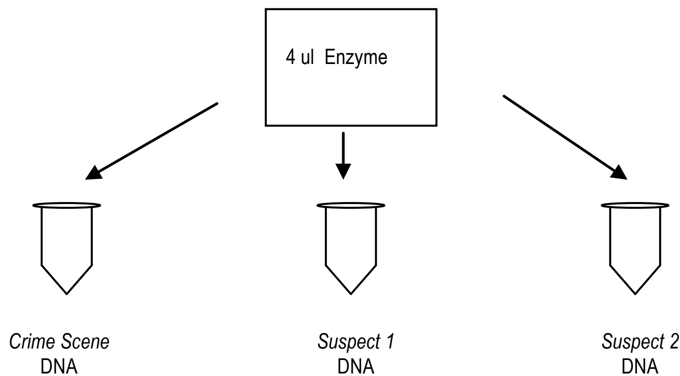
CITY POLICE DEPARTMENT
CRIME LAB LABORATORY PROTOCOL

1. **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 ul 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 3 ul 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 of gels you have been 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. If the gels need to be stained to make the DNA bands darker, your teacher will do this for your group.

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.

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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?

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.

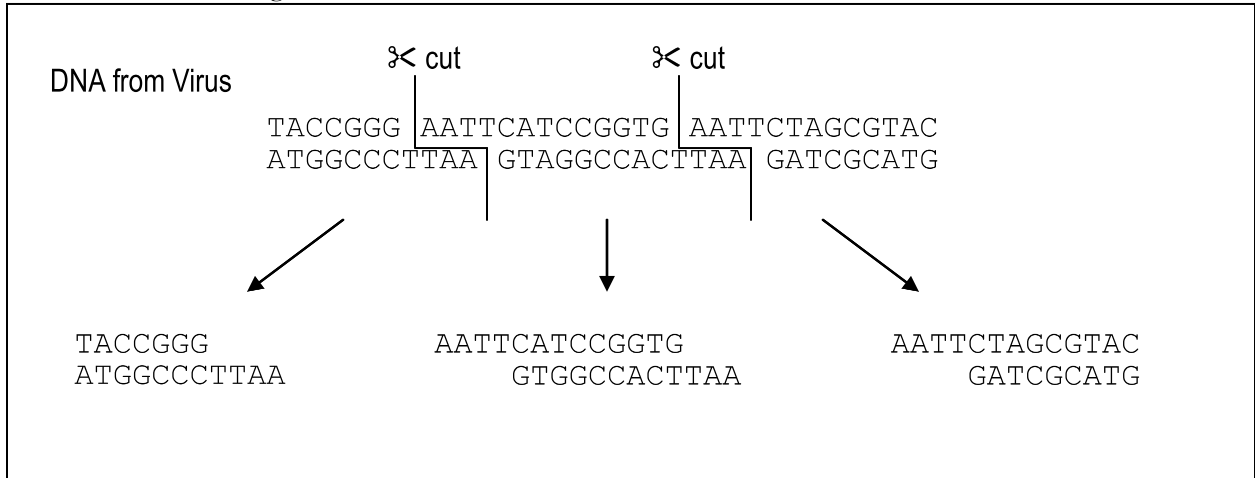
Sample Names

_____ _____ _____

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?

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?

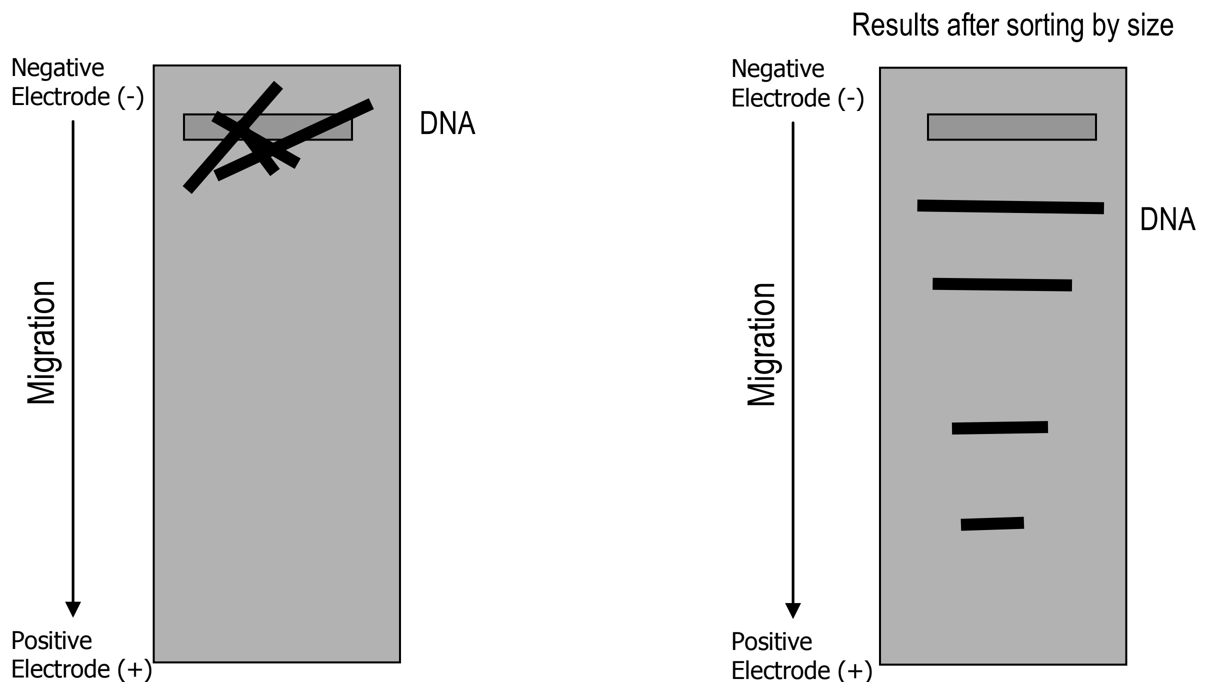
RESTRICTION ENZYME WORKSHEET 2

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.

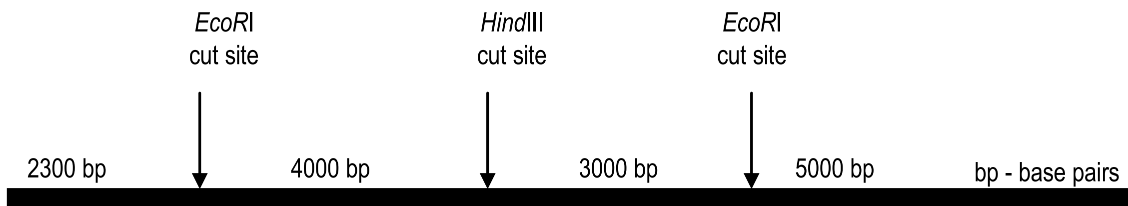
The DNA fragments need to be separated in order to be compared. The fragments are sorted by passing them through a gel. The gel acts like a screen, allowing small pieces of DNA to pass through more easily than large pieces, much like sifting rocks out of dirt. Electricity is used to move the DNA through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



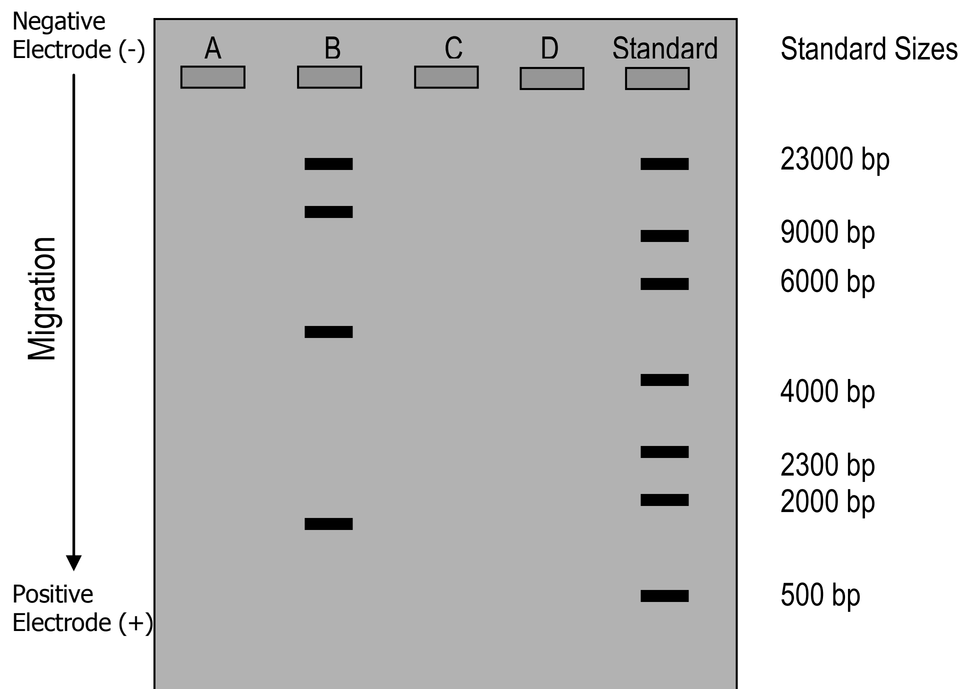
The process of sorting DNA fragments by size using a gel and electricity is called gel electrophoresis.

FIGURE 2: DNA Strand with specific *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.