DNA Fingerprinting

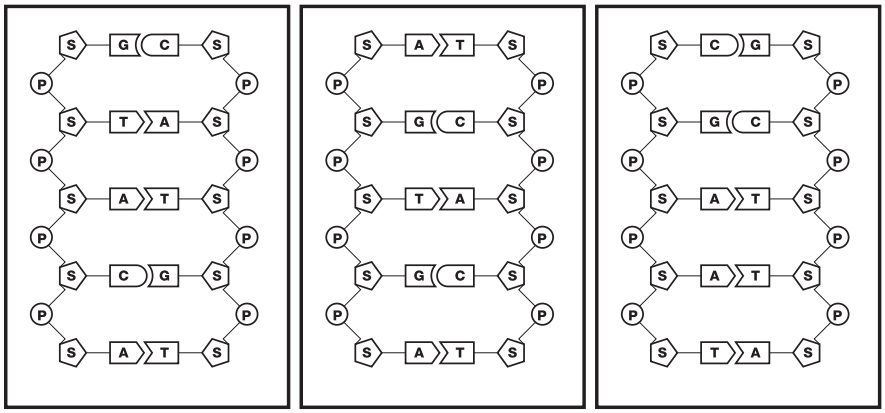
Student Manual

Contents

Lesson 1 Introduction to DNA Fingerprinting

You are about to perform a procedure known as DNA fingerprinting. The data obtained may allow you to determine if the samples of DNA that you will be provided with are from the same individual or from different individuals. For this experiment it is necessary to review the structure of DNA molecules.

The Structure of DNA



The schematics above represent a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows:

Side Chains

S = Five carbon **SUGAR** molecule known as deoxyribose

P = **PHOSPHATE** molecule composed of a phosphorous and oxygen atoms

DNA Nucleotide Bases:

A = adenine **C** = cytosine **G** = guanine **T** = thymine

Analysis of the three DNA samples above (see next page) might help us detect similarities and differences in samples of DNA from different people.

Lesson 1 Introduction to DNA Fingerprinting

Consideration 1 What is the structure of DNA?

1. Compare the "backbone" of the sugar-phosphate arrangement in the side chains of all three figures. Are there any differences?

2. In the above figure, do all three samples contain the same bases? Describe your observations.

3. Are the bases paired in an identical manner in all three samples? Describe the pattern of the base pair bonding.

4. In your attempt to analyze DNA samples from three different individuals, what conclusions can you make about the similarities and differences of the DNA samples?

5. What will you need to compare between these DNA samples to determine if they are identical or non-identical?

Lesson 2 Restriction Digests of DNA Samples

Consideration 2 How can we detect differences in base sequences?

At first sight, your task might seem rather difficult. You need to determine if the ***linear*** base pair ***sequence*** in the DNA samples is identical or not! An understanding of some relatively recent **developments in recombinant DNA technology** might help you to develop a plan.

In 1968, Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore, discovered a group of enzymes in bacteria, which when added to **any** DNA will result in the breakage [**hydrolysis**] of the sugar-phosphate bond between certain specific nucleotide bases [**recognition sites**]. This causes the double strand of DNA to break along the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are **restriction endonucleases**.

[Can you figure out why they are called restriction endonucleases?]

Two common restriction endonucleases are *Eco*RI and *Pst*I which will be provided to you in this lab procedure. To better understand how *Eco*RI and *Pst*I may help you in performing your DNA fingerprinting test, first you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA :

The line through the base pairs represents the sites where bonds will break if a restriction endonuclease recognizes the site **GAATTC**. The following analysis questions refer to how a piece of DNA would be affected if a restriction endonuclease were to "cut" the DNA molecule in the manner shown above.

1. How many **pieces** of DNA would result from this cut? _____

2. Write the **base sequence** of both the left and right side DNA fragments.

   Left: **Right:**

3. What differences are there in the two pieces?

4. DNA fragment size can be expressed as the number of base pairs in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].

   a) The smaller fragment is _____ base pairs (bp).

   b) What is the length of the longer fragment? _____

5. Consider the two samples of DNA shown below - single strands are shown for simplicity:

Sample #1

C A G T G A T C T C G A A T T C G C T A G T A A C G T T

Sample #2

T C A T G A A T T C C T G G A A T C A G C A A A T G C A

If both samples are treated with a restriction enzyme [recognition sequence **GAATTC**] then indicate the number of fragments and the size of each fragment from each sample of DNA.

Sample # 1 **Sample # 2**

# of fragments:_____ # of fragments:_____

List fragment size in order: largest ——> smallest

Sample # 1 **Sample # 2**

Lesson 2 Restriction Digestion of DNA Samples

Laboratory Procedure

Upon careful observation, it is apparent that the only difference between the DNA of different individuals is the linear sequence of their base pairs. In the lab, your team will be given 6 DNA samples. Recall that your task is to determine if any of them came from the same individual or if they came from different individuals.

Thus far your preliminary analysis has included the following:

- The similarities and differences between the DNA from different individuals.
- How restriction endonucleases cut [hydrolyze] DNA molecules.
- How adding the same restriction endonuclease to two samples of DNA might provide some clues about differences in their linear base pair sequence.

Now that you have a fairly clear understanding of these three items you are ready to proceed to the first phase of the DNA fingerprinting procedure—performing a restriction digest of your DNA samples.

Your Workstation Check (✔) List

Make sure the materials listed below are present at your lab station prior to beginning the Lab.

Student workstations (8)	**Number**	**(✔)**
Pipet tips	15	❑
*Eco*RI/*Pst*I enzyme mix (ENZ)	1 tube (80 µl)	❑
P-10 or P-20 micropipet	1	❑
Color coded microtubes:		
green, blue, orange, violet, red, yellow	1	❑
Lab marker	1	❑
Waste container	1	❑
Styrofoam microtube rack	1	❑
Ice bucket with ice	1	❑
Instructors workstation		
Crime Scene DNA	1 vial	❑
Suspect 1 DNA	1 vial	❑
Suspect 2 DNA	1 vial	❑
Suspect 3 DNA	1 vial	❑
Suspect 4 DNA	1 vial	❑
Suspect 5 DNA	1 vial	❑
Incubator or bath—(37 °C)	1/class	❑

Lesson 2 Laboratory

Digest the DNA Samples

1. Label reaction tubes.

   A. Obtain one each of the the following colored microtubes. Label the 5 colored microtubes as follows:

   Green CS (crime scene)

   Blue S1 (suspect 1)

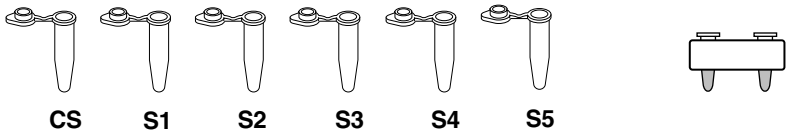
   Orange S2 (suspect 2)

   Violet S3 (suspect 3)

   Red S4 (suspect 4)

   Yellow S5 (suspect 5)

   Put your name and period number on the tubes! The restriction digests will take place in these tubes. These tubes may now be kept in your rack.

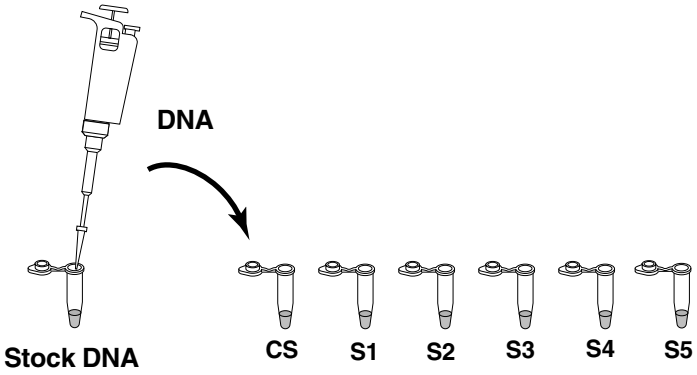


2. Locate the clear microtube that contains the restriction enzyme mix, labeled "ENZ".

   ENZ = Enzyme mix

3. Obtain your DNA samples.

   Using a fresh tip for each sample, transfer 10 µl of each DNA sample from the colored stock tubes into each of the corresponding labeled colored tubes.



Observations

1) Describe the samples of DNA (physical properties).

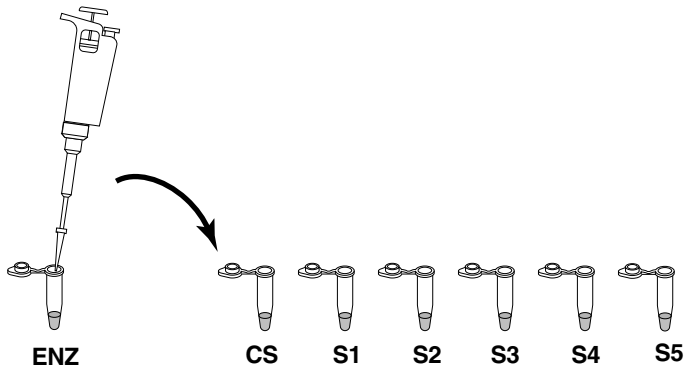
2) Is there any observable difference between the samples of DNA?

3) Describe the appearance of the restriction endonuclease mix.

4) Combine and react.

   Using the micropipet, and a new pipet tip for each sample, transfer 10 µl of the enzyme mix "ENZ" to each reaction tube as shown below.

   Note: Change tips whenever you switch reagents, or, if the tip touches any of the liquid in one of the tubes accidentally. When in doubt, change the tip! DNA goes in the tube before the enzyme. Always add the enzyme last.

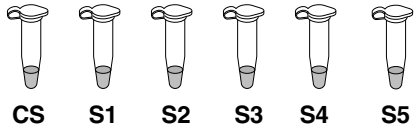


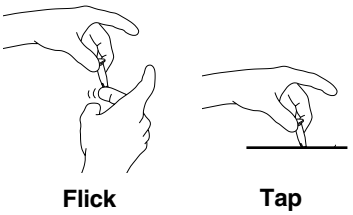
Now your DNA samples should contain:

DNA Samples (10 µl each)	*Eco*RI/*Pst*I Enzyme Mix	Total Reaction Volume
Crime Scene [CS]	10 µl	20 µl
Suspect 1 [S1]	10 µl	20 µl
Suspect 2 [S2]	10 µl	20 µl
Suspect 3 [S3]	10 µl	20 µl
Suspect 4 [S4]	10 µl	20 µl
Suspect 5 [S5]	10 µl	20 µl

5. Mix the contents.

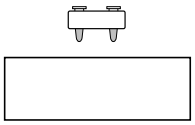
   Close the caps on all the tubes. Mix the components by gently flicking the tubes with your finger. If there is a centrifuge available, pulse the tubes for two seconds to force the liquid into the bottom of the tube to mix and combine reactants. (Be sure the tubes are in a **BALANCED** arrangement in the rotor). If your lab is not equipped with a centrifuge, briskly shake the tube (once is sufficient) like a thermometer. Tapping the tubes on the lab bench will also help to combine and mix the contents.





6. Incubate the samples.

   Place the tubes in the floating rack and incubate them at 37 °C for 45 minutes. Alternatively, the tubes can be incubated in a large volume of water heated to 37 °C and allowed to slowly reach room temperature overnight. After the incubation, store the DNA digests in the refrigerator until the next lab period.



Lesson 2 Restriction Digestion of DNA Samples

Review Questions

1. Before you incubated your samples, describe any visible signs of change in the contents of the tubes containing the DNA after it was combined with the restriction enzymes.

2. Can you see any evidence to indicate that your samples of DNA were fragmented or altered in any way by the addition of *Eco*RI/*Pst*I? Explain.

3. In the absence of any visible evidence of change, is it still possible that the DNA samples were fragmented? Explain your reasoning.

4. (Answer the next day)

   After a 24 hour incubation period, are there any visible clues that the restriction enzymes may have in some way changed the DNA in any of the tubes? Explain your reasoning.

Lesson 3 Electrophoresis and Staining of DNA Samples

Consideration 3 How can we detect the position of *Eco*RI and *Pst*I restriction sites on our DNA samples?

Since we are attempting to detect changes at the molecular level, and there are no visible clues for us to analyze, this task might seem beyond our capabilities and impossible to do. Let's see if we can figure this out. One way to determine the location of restriction sites might be to determine the following:

1) How many different sizes of DNA fragments are in each sample?

2) What are the relative sizes of each fragment?

Therefore, you must somehow get evidence to answer the following question: Do the *Eco*RI and *Pst*I restriction sites occur at the same locations in any of the DNA samples?

The following facts will be helpful to you in your attempt to determine the actual range of DNA fragment sizes in your samples.

Restriction Digestion Analysis

The 3-dimensional structure of restriction enzymes allows them to attach themselves to a double-stranded DNA molecule and slide along the helix until they recognize a specific sequence of base pairs which signals the enzyme to stop sliding. The enzymes then digest (chemically separate) the DNA molecule at that site—called a "restriction site"—acting like molecular scissors, they cut DNA at a specific sequence of base pairs.

If a specific restriction site occurs in more than one location on a DNA molecule, a restriction enzyme will make a cut at each of those sites resulting in multiple fragments. The length of each fragment will depend upon the location of restriction sites contained within the DNA molecule.

When restriction enzymes are used to cut a long strand of DNA, fragments of varying sizes may be produced. The fragments can be separated and visualized using a process known as **agarose gel electrophoresis.** The term electrophoresis means to *carry with electricity*.

Agarose Gel Electrophoresis

Electrophoresis separates DNA fragments according to their relative size. DNA fragments are loaded into an agarose gel slab, which is placed into a chamber filled with a conductive liquid buffer solution. A direct current is passed between wire electrodes at each end of the chamber. DNA fragments are negatively charged, and when placed in an electric field will be drawn toward the positive pole. The matrix of the agarose gel acts as a molecular sieve through which smaller DNA fragments can move more easily than larger ones. Over a period of time smaller fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in single "bands" of DNA.

An analogy: Equate this situation to your classroom in which all the desks and chairs have been randomly scattered around the room. An individual student can wind his/her way through the maze quickly and with little difficulty, whereas a string of four students holding hands would require more time and have difficulty working their way through the maze of chairs. Try it!

Lesson 3 Electrophoresis of DNA Samples

Laboratory Check (✔) List

Student workstations	Number/Station	(✔)
Agarose gel	1	❑
Digested DNA samples	5	❑
DNA sample loading dye "LD"	1	❑
Marking pen	1	❑
Pipet tips	1 box	❑
P-10 or P-20 micropipet	1	❑
Lab marker	1	❑
Waste container	1	❑
Styrofoam microtube rack	1	❑
Gel box and power supply	1	❑
Gel staining tray	1	❑
*Hin*dIII DNA size markers "M"	1	❑
Instructors workstation		
1x TAE electrophoresis buffer	275 ml gel/box	❑
Bio-Safe DNA stain—1x solution	500 ml	❑

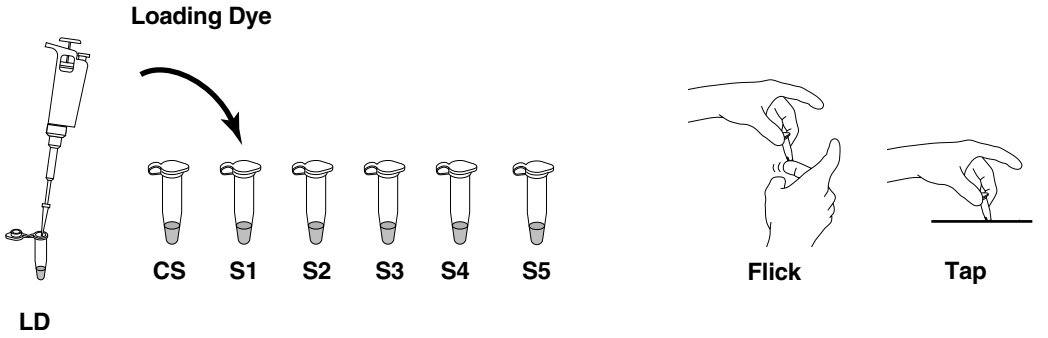
Lesson 3 Laboratory

Electrophoresis of DNA Samples

1. Obtain a prepoured agarose gel from your teacher, or if your teacher instructs you to do so, prepare your own gel.
2. After preparing the gel, remove your digested samples from the refrigerator.

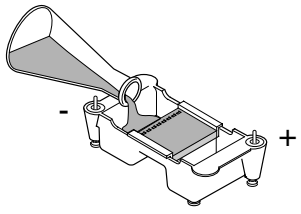
   Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube:

DNA Samples	Loading dye
Crime Scene [CS]	5 µl
Suspect 1 [S1]	5 µl
Suspect 2 [S2]	5 µl
Suspect 3 [S3]	5 µl
Suspect 4 [S4]	5 µl
Suspect 5 [S5]	5 µl



   Close the caps on all the tubes. Mix the components by gently flicking the tubes with your finger. If a centrifuge is available, pulse spin the tubes to bring the contents to the bottom of the tube. Otherwise, tap the tubes upon a table top.

3. Place the casting tray with the solidified gel in it, into the platform in the gel box. The wells should be at the (-) cathode end of the box, where the black lead is connected. Very carefully, remove the comb from the gel by pulling it straight up.
4. Pour ~ 275 ml of electrophoresis buffer into the electrophoresis chamber. Pour buffer in the gel box until it **just covers** the wells.



5. Locate your lambda *Hin*dIII DNA size marker in the tube labeled "M".

   Gels are read from left to right. The first sample is loaded in the well at the left hand corner of the gel.

6. Using a separate pipet tip for each sample, load your gel as follows:

   Lane 1: *Hin*dIII DNA size marker, clear, 10 µl

   Lane 2: CS, green, 20 µl

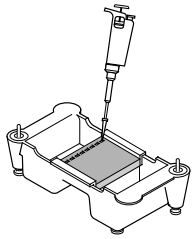
   Lane 3: S1, blue, 20 µl

   Lane 4: S2, orange, 20 µl

   Lane 5: S3, violet, 20 µl

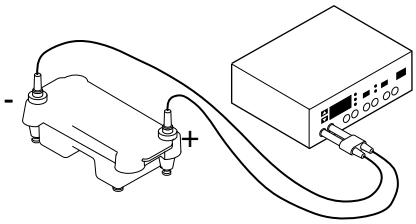
   Lane 6: S4, red, 20 µl

   Lane 7: S5, yellow, 20 µl



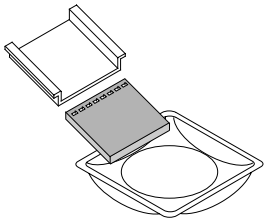
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect electrical leads to the power supply.

8. Turn on the power supply. Set it for 100 V and electrophorese the samples for 30–40 minutes.

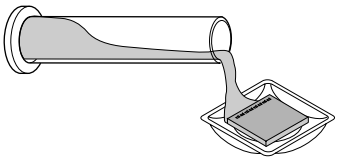


While you are waiting for the gel to run, you may begin the review questions on the following page.

9. When the electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery! Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.



10. Pour 60 ml of Bio-Safe DNA stain into your plastic staining tray, cover with plastic wrap, and let the gel stain overnight, shaking intermittently if no rocking platform is available.



Lesson 3 Electrophoresis of Your DNA Samples

Review Questions

1. The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. DNA molecules are negatively charged. To which electrode pole of the electrophoresis field would you expect DNA to migrate? (+ or -)? Explain.

2. What color represents the negative pole?

3. After DNA samples are loaded into the sample wells, they are "forced" to move through the gel matrix. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel most quickly? Explain.

4. Which fragments (large vs. small) are expected to travel the shortest distance from the well? Explain.

Lesson 4 Drying Gels and Analyzing the DNA Patterns

Consideration 5 Are any of the DNA samples from the suspects the same as an individual at the crime scene?

Take a moment to think about how you will perform the analysis of your gel. In the final two steps, you will:

A. Visualize DNA fragments in your gel.

B. Analyze the number and positions of visible DNA bands on your gel.

Making DNA Fragments Visible

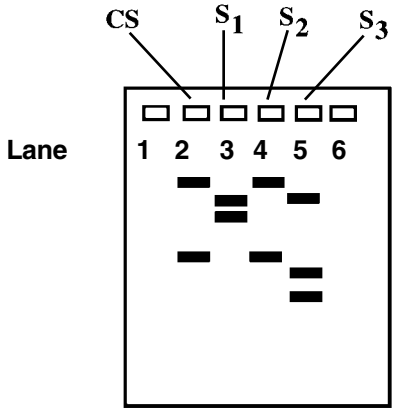
Unaided visual examination of gels indicates only the positions of the loading dyes and not the positions of the DNA fragments. DNA fragments are visualized by staining the gel with a blue dye. The blue dye molecules have a high affinity for the DNA and strongly bind to the DNA fragments, which makes them visible. These visible bands of DNA may then be traced, photographed, sketched, or retained as a permanently dried gel for analysis.

The drawing below represents an example of a stained DNA gel after electrophoresis. For fingerprinting analysis, the following information is important to remember:

- Each lane has a different sample of DNA

- Each DNA sample was treated with the same restriction endonucleases.

With reference to the numbered lanes, analyze the bands in the gel drawing below, then answer the questions on the following page.



Lesson 4 Questions

1. What can you assume is contained within each band?

2. If this were a fingerprinting gel, how many samples of DNA can you assume were placed in each separate well?

3. What would be a logical explanation as to why there is more than one band of DNA for each of the samples?

4. What caused the DNA to become fragmented?

5. Which of the DNA samples have the same number of restriction sites for the restriction endonucleases used? Write the lane numbers.

6. Which sample has the smallest DNA fragment?

7. Assuming a circular piece of DNA (plasmid) was used as starting material, how many restriction sites were there in lane three?

8. Which DNA samples appear to have been "cut" into the same number and size of fragments?

9. Based on your analysis of the gel, what is your conclusion about the DNA samples in the photograph? Do any of the samples seem to be from the same source? If so, which ones? Describe the evidence that supports your conclusion.

Lesson 4 Analyzing the DNA Patterns

Laboratory Procedure

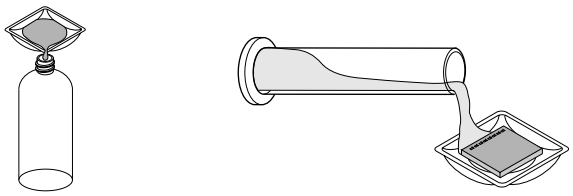
Student Workstations	Number	(✔)
Water for destaining gels	60 ml	❑
Millimeter ruler	1	❑
Linear graph paper	1	❑
Semi-log graph paper	1	❑

Instructor's Workstation

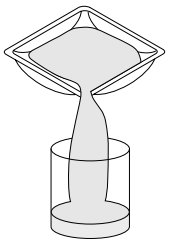
None required

Gel Staining and Destaining Steps

1. Pour off the Bio-Safe DNA stain into a bottle or another appropriate container and destain the gel with 60 ml of water for ~15 minutes.



2. Pour the water out of the staining tray. Ask the instructor how to properly dispose of the stain.



3. Trim away any empty lanes of the gel with a knife or razorblade. Let the gel dry on the hydrophilic side of a piece of gel support film or in your staining tray on your lab bench for 3–5 days. When the gel is dry, tape it into your lab notebook for a permanent record.

Quantitative Analysis of DNA Fragment Sizes

If you were on trial, would you want to rely on a technician's eyeball estimate of a match, or would you want some more accurate measurement?

In order to make the most accurate comparison between the crime scene DNA and the suspect DNA, other than just a visual match, a quantitative measurement of the fragment sizes needs to be created. This is done below:

1. Using the ruler, measure the migration distance of each band. Measure the distance in millimeters from the bottom of the loading well to each center of each DNA band and record your numbers in the table on the next page. The data in the table will be used to construct a standard curve and to estimate the sizes of the crime scene and suspect restriction fragments.

2. To make an accurate estimate of the fragment sizes for either the crime scene or the suspects, a standard curve is created using the distance (x-axis) and fragment size (y-axis) data from the Lambda/*Hin*dIII size marker. Using both linear and semi-log graph paper, plot distance versus size for bands 2–6. On each graph, use a ruler and draw a line joining the points. Extend the line all the way to the right hand edge of the graph.

   Which graph provides the straightest line that you could use to estimate the crime scene or the suspects' fragment sizes? Why do you think one graph is straighter than the other?

3. Decide which graph, linear or semi-log, should be used to estimate the DNA fragment sizes of the crime scene and suspects. Justify your selection.

4. To estimate the size of an unknown crime scene or suspect fragment, find the distance that fragment traveled. Locate that distance on the x-axis of your standard graph. From that position on the x-axis, read up to the standard line, and then follow the graph line to over to the y-axis. You might want to draw a light pencil mark from the x-axis up to the standard curve and over to the y-axis showing what you've done. Where the graph line meets the y-axis, this is the approximate size of your unknown DNA fragment. Do this for all crime scene and suspect fragments.

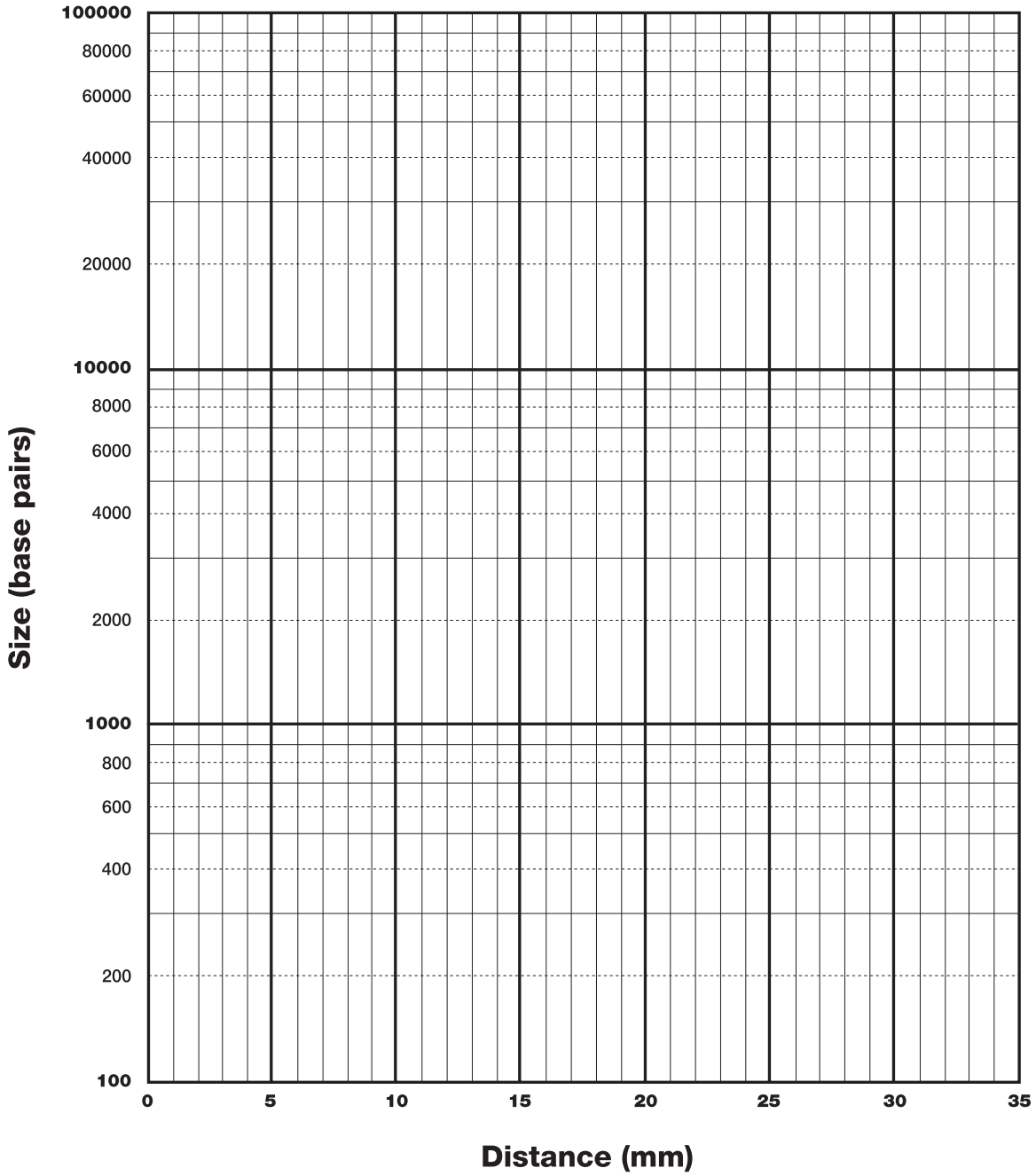
5. Compare the fragment sizes of the suspects and the crime scene.

   Is there a suspect that matches the crime scene?

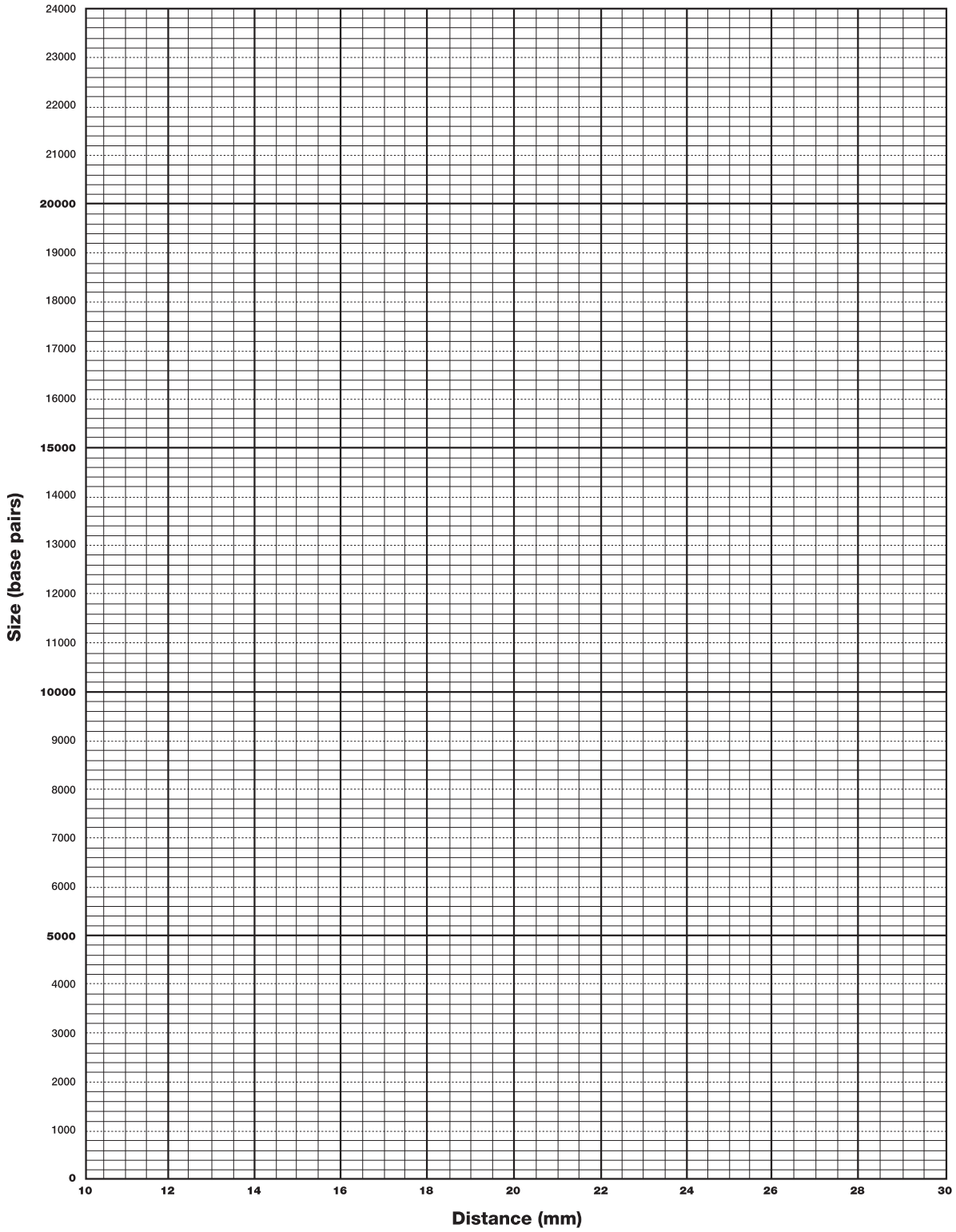
   How sure are you that this is a match?

	Lambda/*Hin*dIII size marker		**Crime Scene**		**Suspect 1**		**Suspect 2**		**Suspect 3**		**Suspect 4**		**Suspect 5**	
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)
1		23,130												
2		9,416												
3		6,557												
4		4,361												
5		2,322												
6		2,027												

Semi-Log Graph Paper



Graph Paper


Size (base pairs)
24000
23000
22000
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10
12
14
16
18
20
22
24
26
28
30
Distance (mm)

Lesson 4 Analyzing the DNA Patterns

Interpretation of Results

Attach a photo, photocopy, or your actual dried gel in this space. Indicate which sample is in each well.

1. What are we trying to determine? Restate the central question.

2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

3. What caused the DNA to become fragmented?

4. What determines where a restriction endonuclease will "cut" a DNA molecule?

5. A restriction endonuclease "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at **that location**?

6. Do any of your suspect samples appear to have *Eco*RI or *Pst*I recognition sites at the same location as the DNA from the crime scene?

7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

Appendix A

Alternative DNA Fingerprinting Scenarios!

DNA typing, DNA profiling, and DNA fingerprinting are all names for the same process, a process which uses DNA to show relatedness or identity of individual humans, plants, or animals. DNA typing has become the subject of much debate and interest because of its uses for forensics analysis in prominent criminal cases such as the O. J. Simpson case. The applications of DNA typing, however, are much broader than forensic science alone and are having a profound impact on our society.

DNA typing is used in forensics, anthropology, and conservation biology not only to determine the identity of individuals but also to determine relatedness. This process has been used to free innocent suspects, reunite children with their relatives, identify stolen animals, and prove that whale meat has been substituted for fish in sushi. It is used in times of war to help identify the remains of soldiers killed in combat. It is also being used to find genetic linkages to inherited diseases. In addition, scientists are learning a great deal about our evolutionary history from DNA analysis.

Each of the following paragraphs describes a scenario in which DNA has been used to show how individuals are related to each other, or to show that a person is (or is not) the perpetrator of a crime. These scenarios provide a context for using DNA typing for use in teaching molecular biology, conservation biology, and biotechnology. Have your students research a scenario that is interesting to them and present their findings to the class.

1. **Food identification (endangered species identification).**

   The purity of ground beef (or impurity) has been proven using DNA typing. Hamburger has been shown to often be a mixture of pork, and other non-beef meats. Using portable testing equipment, authorities have used DNA typing to determine that the fish served in sushi was really meat from whales and dolphins. These are, many times, endangered species that are protected by international law.

2. **Accused and convicted felons set free because of DNA typing.**

   A man imprisoned for 10 years was released when DNA testing, unavailable when he was convicted, was used to show that he could not have been the rapist. Statistics show that about one-third of all sexual assault suspects are freed as a result of DNA testing.

3. **Identifying of human remains.**

   Scientists have used DNA typing to confirm that the body in the grave was (or was not) the person that was supposed to be there. Bones found in Russia are believed to be those of the Romanovs, Russia's last imperial family. Czar Nicholas II and his family were executed by the Bolsheviks in 1918. Experts from around the world have been studying the bones to match skulls, teeth, and other features with photographs. DNA from the bones will be compared to that of known descendants to determine whether the bones do indeed belong to the Czar and his family.

4. **Determining relatedness of humans.**

   DNA typing has shown that the 5000 year old Ice Man found in a melting glacier is most closely related to modern Europeans. ("Iceman Gets Real." Science, Vol. 264:1669. June 17, 1994.) The DNA typing evidence also "removes all the suspicions that the body was a fraud—that it had been placed on the ice" says Svante Paabo of the University of Munich. (Science, Vol. 264:1775. June 17, 1994).

5. **Studying relatedness among ancient peoples.**

   DNA found at archeological sites in western Montana is being used to help determine how many related groups of people (families) lived at a particular site. (Morell, Virginia. "Pulling Hair from the Ground." Science, Vol. 265:741-745 August 1994.)

6. **DNA testing of families.**

   DNA testing of families has been used in Argentina and El Salvador to identify the children of at least 9,000 citizens of these countries who disappeared between 1975 and 1983, abducted by special units of the ruling military and police. Many of the children born to the disappeared adults were kidnapped and adopted by military "parents" who claimed to be their biological parents. After genetic testing of the extended family revealed the true identity of a child, the child was placed in the home of its biological relatives. It was feared that transferring a child from its military "parents" who were kidnappers, but who had reared the child for years, would be agonizing. In practice, the transferred children became integrated into their biological families with minimal trauma.

7. **Identifying organisms that cause disease.**

   Eva Harris, a UCSF scientist, is helping scientists in Nicaragua and Ecuador to learn to use DNA technology to detect tuberculosis, and identify the dengue virus and various strains of Leishmania. Other available tests cause waits of many weeks while disease organisms are cultured and sent to foreign labs to be identified. (Marcia Barinaga, "A Personal Technology Transfer Effort in DNA Diagnostics." Science, 266:1317-1318. Nov. 25, 1994.)

8. **Identifying birth parents (paternity testing).**

   Girls in Florida were discovered to have been switched at birth when one girl died of a hereditary disease. The disease was not in her family, but was known to be in the family of another girl, born in the same hospital and about the same time she was born.

9. **Proving paternity.**

   A woman, raped by her employer on Jan. 7, 1943, her 18th birthday, became pregnant. The child knew who her father was, but as long as he lived, he refused to admit being her father. After the man died, DNA testing proved that she was his daughter and she was granted a half of his estate. ("A Child of Rape Wins Award from Estate of Her Father." New York Times, July 10, 1994.)

10. Determining effectiveness of bone marrow transplants.

"DNA fingerprinting can help doctors to monitor bone marrow transplants. Leukemia is a cancer of the bone marrow and the diseased marrow must be removed. The bone marrow makes new blood cells, so the leukemia sufferer will die without a transplant of healthy marrow. Doctors can quickly tell whether the transplant has succeeded by DNA typing of the patient and the donor. If the transplant has worked, a fingerprint from the patient's blood shows the donor's bands. But if the cancerous bone marrow has not been properly destroyed, then the cancerous cells multiply rapidly and the patient's own bands predominate." ("Our Ultimate Identity Card in Sickness and in Health," in "Inside Science", New Scientist, Nov. 16, 1991.)

11. Proving relatedness of immigrants.

DNA fingerprinting has been used as proof of paternity for immigration purposes. In 1986, Britain's Home Office received 12,000 immigration applications from the wives and children of Bangladeshi and Pakistani men residing in the United Kingdom. The burden of proof is on the applicant, but establishing the family identity can be difficult because of sketchy documentary evidence. Blood tests can also be inconclusive, but DNA fingerprinting results are accepted as proof of paternity by the Home Office. (DNA fingerprints, source unknown: Based on A. J. Jeffreys, *et al.*, "Positive Identification of an Immigration Test-Case Using Human DNA Fingerprints." Nature, 317:818-819, 1985.)

12. Confirming relatedness among animals.

Scientists who extracted DNA from the hair of chimpanzees throughout Africa now have evidence that there might be a third species of chimpanzee. At the same time they have learned things about chimp behavior and kinship patterns that would have once taken years to theorize. They discovered a group of chimps living in western Africa to be genetically distinct from the chimps living in other parts of Africa, suggesting that the group may be an endangered species. The have discovered that male chimps living in a given area are often as closely related as half-brothers, and many so-called sub-species may all be part of a single species. The male chimps' relatedness may explain why, unlike other primates, the males are quite friendly to each other.

13. DNA testing of plant material puts murderer at the scene.

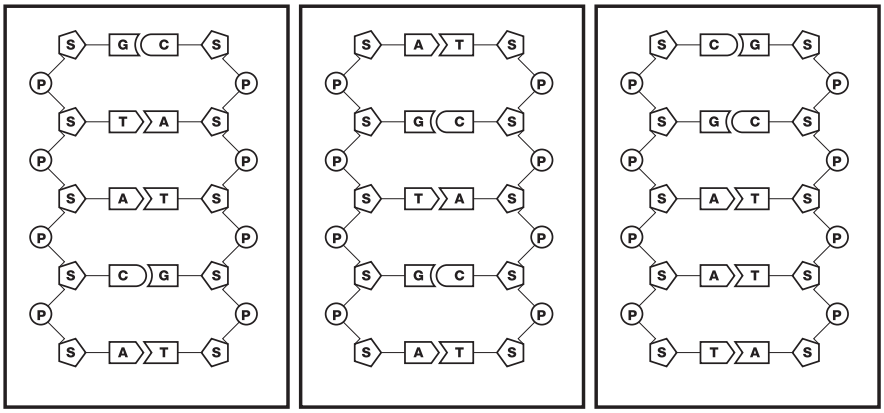
Two small seed pods caught in the bed of his pick-up truck put an accused murderer at the murder scene. Genetic testing showed that DNA in the seed pod exactly matched the DNA of a plant found at the scene of the murder. The accused had admitted he had given the victim a ride, but he denied ever having been near the crime scene.

Appendix B

Prelab Activity 1 A Review of Restriction Enzymes

DNA consists of a series of nitrogen base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar and phosphate backbone. The four different nitrogen bases are **adenine**, **thymine**, **guanine** and **cytosine**. (**A**, **T**, **G**, and **C**: Remember the base-paring rule is A-T and G-C). Refer to Figure 1 to review the structure of a DNA molecule.

Fig. 1. The Structure of DNA



If a **segment** of DNA is diagrammed without the sugars and phosphates, the base-pair sequence might appear as:

Read to the right----> A C T C C G T A G A A T T C....>

<....T G A G G C A T C T T A A G <----Read to the left

Look at the linear sequence of bases (As, Ts, etc.) on each of the strands:

- Describe any pattern you might see in the upper sequence of bases.

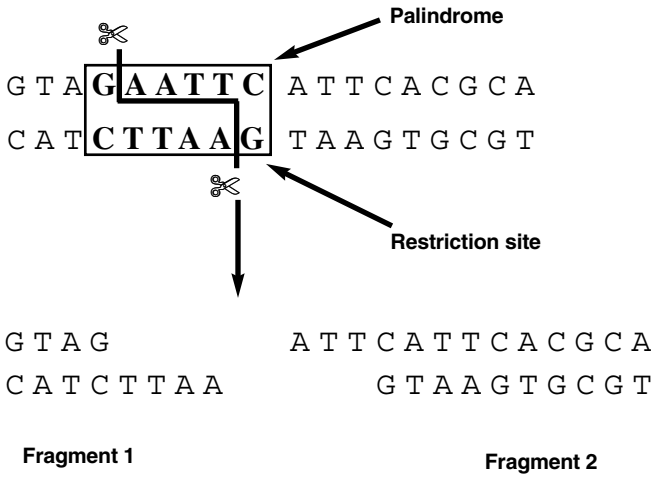
- Compare the bases in the upper portion of the molecule to those in the lower portion. Describe any relationship you can see.

- Now look at the upper sequence of bases and compare it to the lower. Do you notice any grouping of bases that when read to the right and read to the left are exactly the same order?

You may have discovered that the base sequence seems to be arranged randomly and that the two strands seem to complement each other; As are paired with Ts, etc. You may have also noticed that a portion of the top strand GAATTC (read to the right) has a counterpart in the lower strand CTTAAG (read to the left). Similar sequences are AAGCTT and TTCGAA; and CTGCAG and GACGTC. These sequences, called **palindromes**, are quite common along the DNA molecule.

A major "enemy" of bacteria are viruses called bacteriophages, such as lambda. These viruses infect bacteria by injecting their own DNA into bacteria in an attempt to take over the operations of the bacterial cell. Bacteria have responded by evolving a natural defense (called restriction enzymes) to cut up and destroy the invading DNA. These enzymes search the viral DNA looking for certain palindromes (GAATTCs, for example) and cut up the DNA into pieces at these sites. The actual place in the palindrome where the DNA is cut is called a **restriction site**.

Look at the DNA sequence below:



A restriction enzyme cut the DNA between the G and the A in a GAATTC palindrome.

- How many base pairs are there to the left of the "cut"?

- How many base pairs are there to the right of the "cut"?

- Counting the number of base pairs, is the right fragment the same size as the left fragment?

- How could you describe fragment size in reference to the number of base pairs in the fragment?

An important fact to learn about restriction enzymes is that each one only recognizes a specific palindrome and cuts the DNA only at that specific sequence of bases. A palindrome can be repeated a number of times on a strand of DNA, and the specific restriction enzymes **will cut all those palindromes** at their restriction sites.

The table below shows three kinds of palindromes that may be present in a strand of DNA along with the specific enzyme that recognizes the sequence.

Palindrome on the DNA molecule	**Name of enzyme that recognizes the palindrome**
G A A T T C	*Eco*RI
A A G C T T	*Hin*dIII

If the **GAATTC** palindrome is repeated four times on the same piece of DNA, and the restriction enzyme that recognizes that base sequence is present.

- How many DNA fragments will be produced?

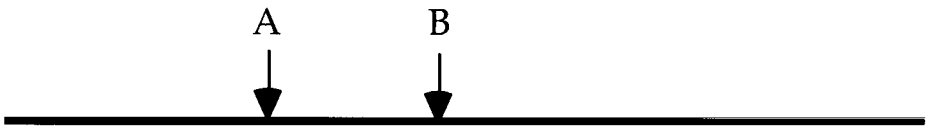
- If the **GAATTC** palindrome repeats are randomly spaced along the DNA strand, then what can you say about the size of the fragments that will be produced?

Let's summarize what we learned so far.

- **The base sequence in one strand of DNA can have a palindrome in the other strand. (GAATTC and CTTAAG).**
- **Palindromes can be detected by restriction enzymes.**
- **Restriction enzymes cut the palindromes at restriction sites.**
- **A restriction enzyme only recognizes one specific kind of palindrome.**
- **Cutting DNA at restriction sites will produce DNA fragments.**
- **Fragment sizes can be described by the number of base pairs they contain.**

Applying what you have learned.

- If a linear DNA molecule had the restriction sites A and B for a specific palindrome, how many fragments would be produced?



- Number each fragment.

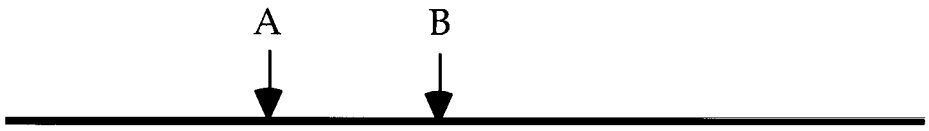
- Which fragment would be the largest?

- Which fragment would be the smallest?

- Draw a DNA molecule that has 5 randomly spaced restriction sites for a specific palindrome. How many fragments would be produced if they were each cut by a restriction enzyme?

- Label each fragment.

- Rank them in order of size from largest to smallest.



In this diagram, A and B are different palindrome sequences on a DNA strand. Only the restriction enzyme that recognizes site B is present.

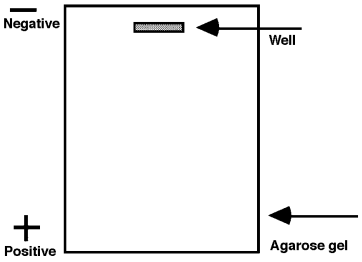
- Explain why only two fragments would be produced.

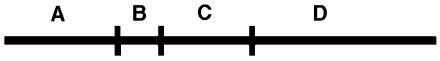
Prelab activity 2 Review of Electrophoresis

How can one see the DNA fragments?

Agarose gel electrophoresis is a procedure that can be used to separate DNA fragments. DNA is a molecule that contains **many negative electrical charges**. Scientists have used this fact to design a method that can be used to separate pieces of DNA. A liquid solution containing a mixture of DNA fragments is placed in a small well formed into the gel. (The gel looks like Jello™ dessert). Electricity causes the molecules to move. Opposite electrical charges attract each other; **negative** (-) **charges move towards the positive** (+) **charge.** Imagine the gel as a "strainer" with tiny pores that allow small particles to move through it very quickly. The larger the size of the particles, however, the slower they are "strained" through the gel. After a period of exposure to electricity, the fragments will sort themselves out by size. **Fragments that are the same size will tend to move together** through the gel. The group will tend to form concentrations, called **bands,** of pieces that are all the same size.

A linear piece of DNA is cut into 4 fragments as shown in the diagram. A solution of the 4 fragments is placed in a well in an agarose gel. Using the information given above, draw (to the right) how you think the fragments might separate themselves. Label each fragment with its corresponding letter.





- Have your teacher check your diagram before you proceed.

- Where would the larger fragments—those with the greater number of base pairs—be located; toward the top of the gel or the bottom? Why?

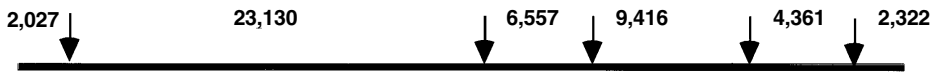
- Suppose you had 500 pieces of each of the four fragments, how would the gel appear?

- If it were possible to weigh each of the fragments, which one would be the heaviest? Why?

- Complete this rule for the movement of DNA fragments through an agarose gel:

  The larger the DNA fragment, the ...

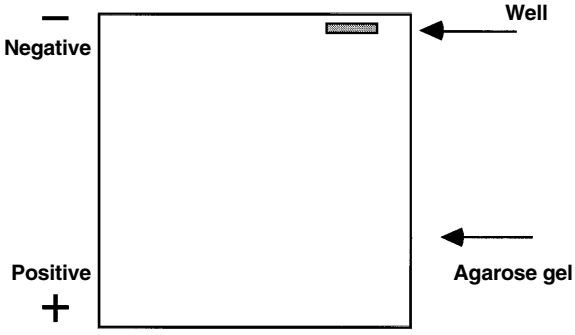
This diagram represents a piece of DNA cut with *Hin*dIII at each of the restriction sites pointed to by the arrows. The numbers represent the number of base pairs in each fragment.



- How many fragments were produced by the restriction enzyme *Hin*dIII?

On the gel diagram at the right, show how you believe these fragments will sort out during electrophoresis.

- Label each fragment with its correct number of base pairs.



Appendix D: Plasmid DNA and Restriction Enzymes

The Crime Scene and Suspect DNA samples in this kit do not contain human DNA but consist of plasmid DNA isolated from bacteria. Plasmids are small, circular pieces of DNA that can replicate inside bacterial cells. In nature, bacteria evolved plasmids containing genes that enabled them to survive antibiotics produced by other microorganisms in the environment. This antibiotic resistance gave the bacteria with plasmids a selective advantage over their competitors. Bacteria were able to pass the beneficial plasmid DNA to other bacteria via conjugation.

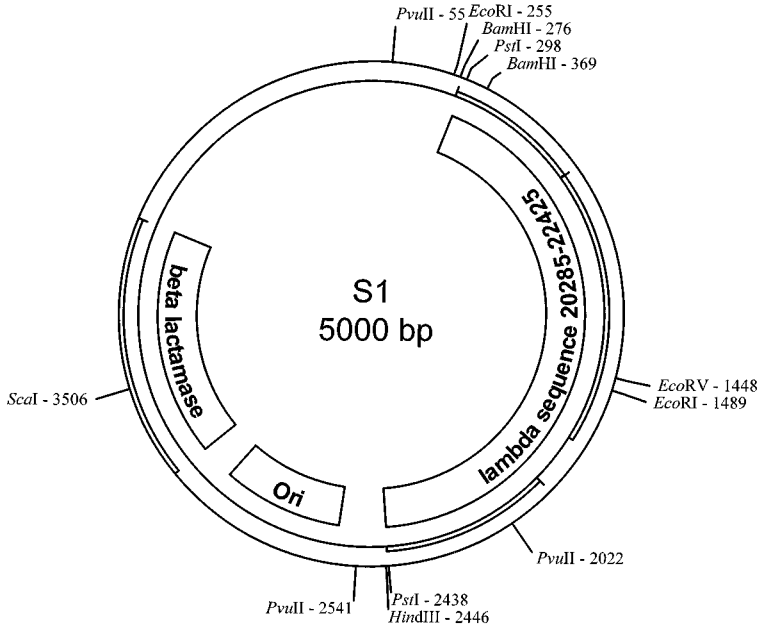
Scientists have taken advantage of plasmid DNA because its small size makes it easy to purify, and it can be reintroduced into bacterial cells using a procedure called transformation. Scientists have also benefited from another natural, bacterial defense mechanism: the restriction enzyme. Bacteria evolved enzymes to destroy DNA from invading viruses, or bacteriophages, when they inject their DNA. Restriction enzymes recognize specific DNA sequences within the phage DNA and then cut, or restrict, the DNA at that site. The fragmented phage DNA can no longer pose a threat to bacterial survival. Once purified in the laboratory, these restriction endonucleases (nuclease = enzyme that cuts, endo = within, nucleic acids) are named for the bacteria from which they were isolated. For example, *Eco*RI was isolated from *Escherichia coli.* Purified restriction enzymes can then be used in the laboratory to cut DNA isolated from any source at completely predictable sites.

After plasmids are cut with a restriction enzyme, they can be joined to foreign DNA, from any source, that has been cut with the same enzyme. The resulting hybrid DNA can then be transformed into bacterial cells. The hybrid plasmids can perpetuate themselves in bacteria just as before, except that the foreign DNA that was joined to them is also being perpetuated. Every hydrid plasmid now contains a perfect copy of the piece of foreign DNA joined to it. We say that the foreign piece of DNA has been cloned, and the plasmid DNA that carried it is called a vector.

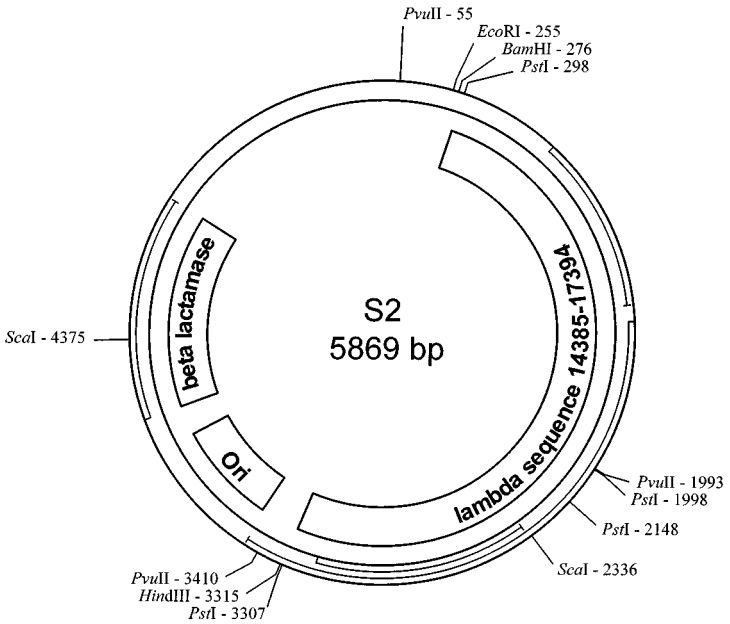
The Crime Scene and Suspect DNA samples in this kit were created by joining *Pst*I-digested bacteriophage lambda DNA with *Pst*I-digested plasmid vector pTZ18U. Recombinant plasmids were selected that gave distinct, striking banding patterns, or restriction fragment length polymorphisms (RFLPs again!), when digested with the restriction enzymes *Pst*I and *Eco*RI and analyzed on an agarose gel.

Complete restriction maps of each of the Crime Scene and Suspect plasmids, the parent vector pTZ18U, and the donor lambda phage are included for further classroom discussion and exploration. Try this: predict the number of base pairs in the S4 and S5 plasmids, based on your gel results. How do these sizes compare with the number of base pairs indicated on the S4 and S5 plasmid maps? How can you explain the discrepancy? How could you get a more accurate estimate of the plasmid sizes using restriction analysis and agarose electrophoresis? (Hint: perhaps other restriction enzymes would generate different banding patterns on the gel.) Which enzymes would you choose?

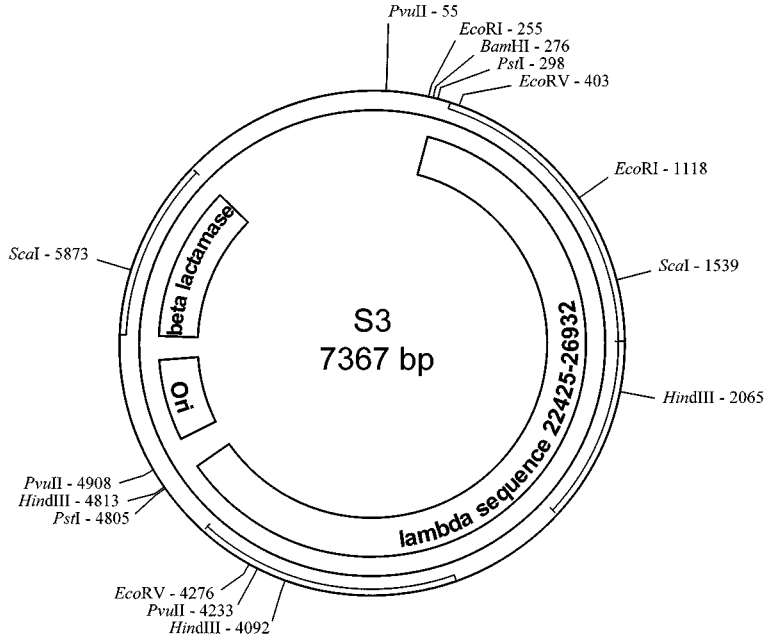
Plasmid Maps



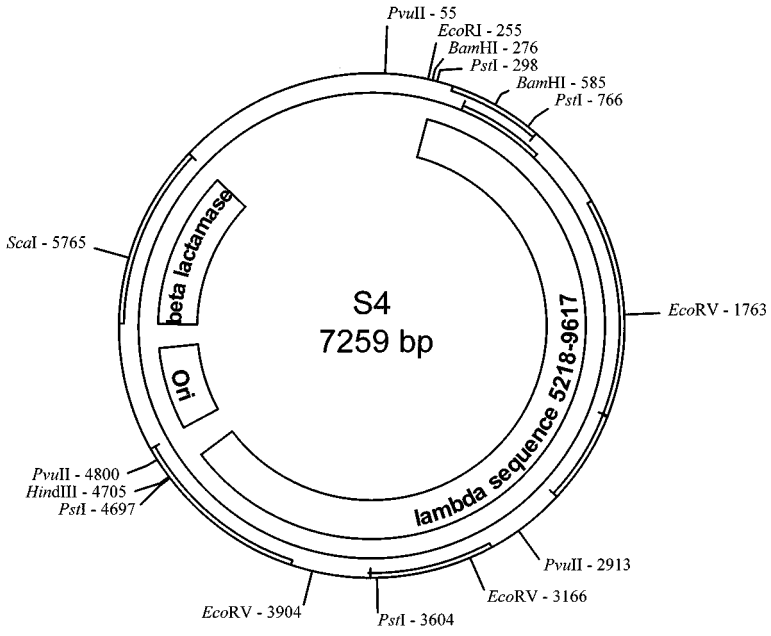
Suspect 1 DNA Sample



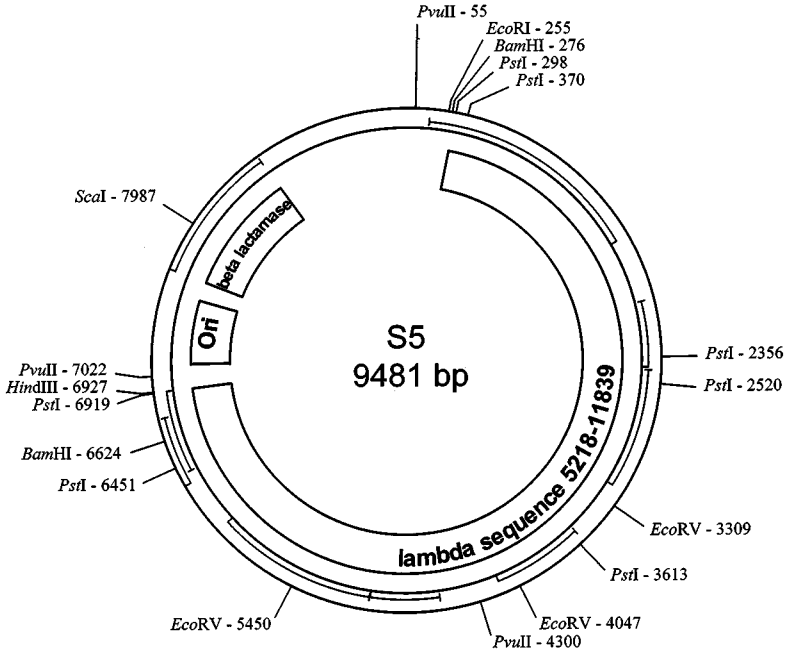
Suspect 2 DNA Sample



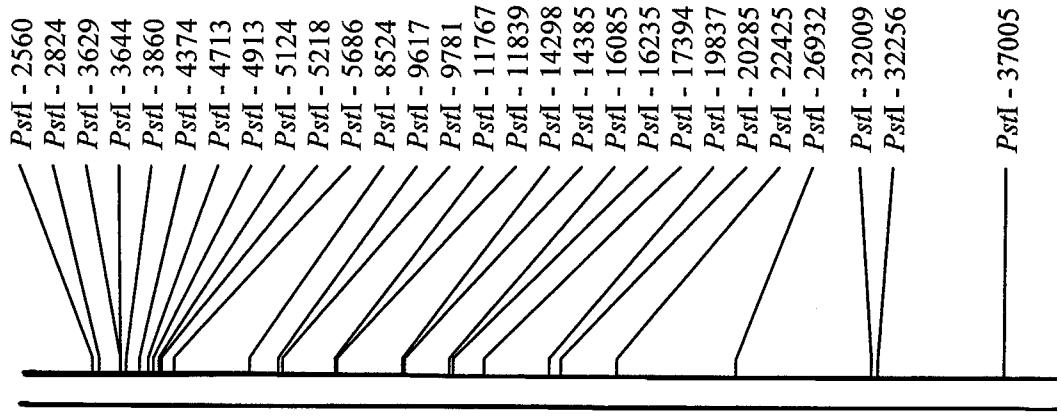
Crime Scene/Suspect 3 DNA Sample



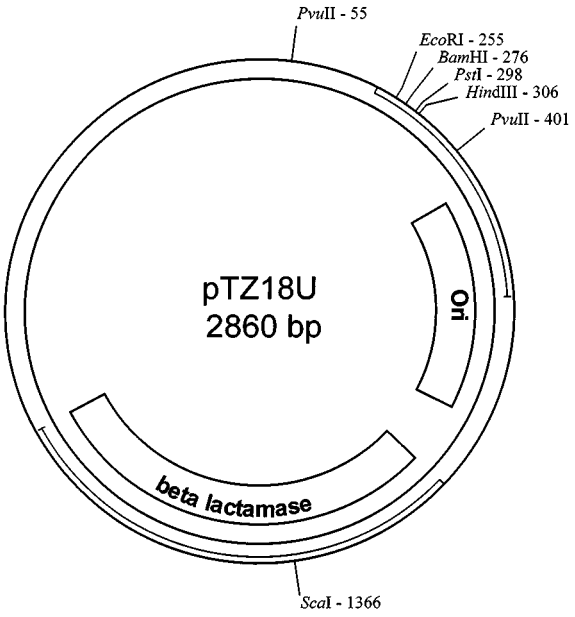
Suspect 4 DNA Sample



Suspect 5 DNA Sample



lambda bacteriophage genome
48502 bp



Plasmid Parent Vector

BIO-RAD

Bio-Rad Laboratories

Life Science Group

Website *www.bio-rad.com* ***Bio-Rad Laboratories Main Office*** *2000 Alfred Nobel Drive, Hercules, CA 94547, Ph. (510) 741-1000, Fx. (510)741-5800*
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Finland *Ph. 358 (0)9 804 2200, Fx. 358 (0)9 804 1100* ***France*** *Ph. 01 43 90 46 90, Fx. 01 46 71 24 67* ***Germany*** *Ph. 089 318 84-0, Fx. 089 318 84-100*
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