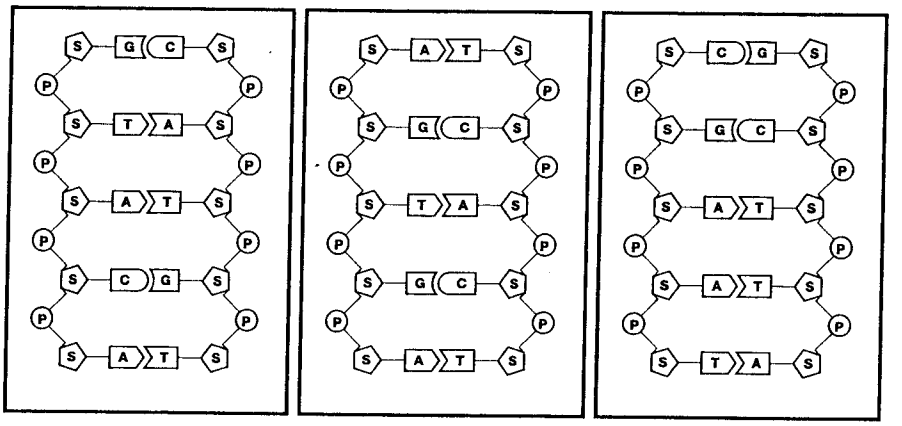
Lesson 1 Introduction to DNA Fingerprinting

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

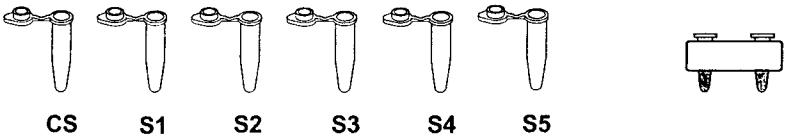
Digest the DNA Samples

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.

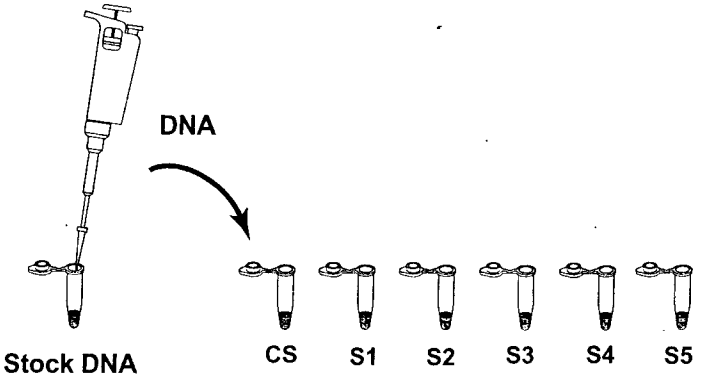


2. Locate the clear microtube that contains the restriction enzyme mix, labeled "ENZ".

   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).

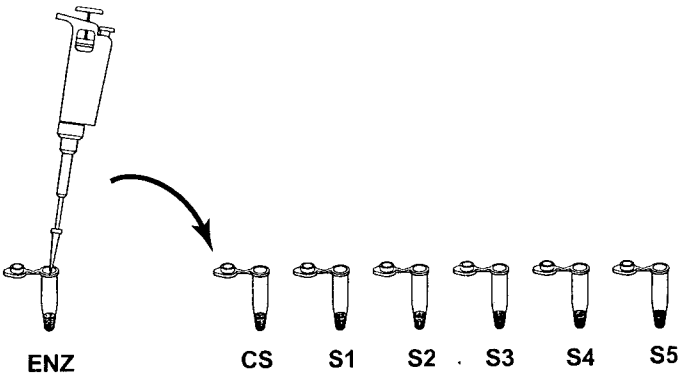
2) Is there any observable difference between the samples of DNA?

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.

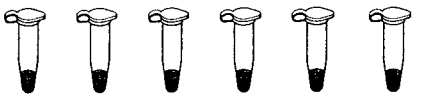


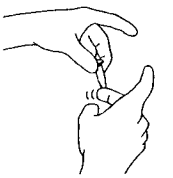
Now your DNA samples should contain:

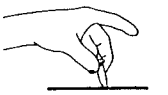
DNA Samples (10 µl each)	*Eco*RI/*Pst*I Enzyme Mix	Total Reaction Volume
Crime Scene [CS]	10 µl	20 µl
Suspect 1 [S1]	10 µl	20 µl
Suspect 2 [S2]	10 µl	20 µl
Suspect 3 [S3]	10 µl	20 µl
Suspect 4 [S4]	10 µl	20 µl
Suspect 5 [S5]	10 µl	20 µl

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.

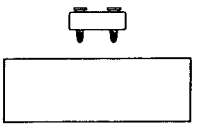



Flick


Tap

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.


Water bath

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	❑

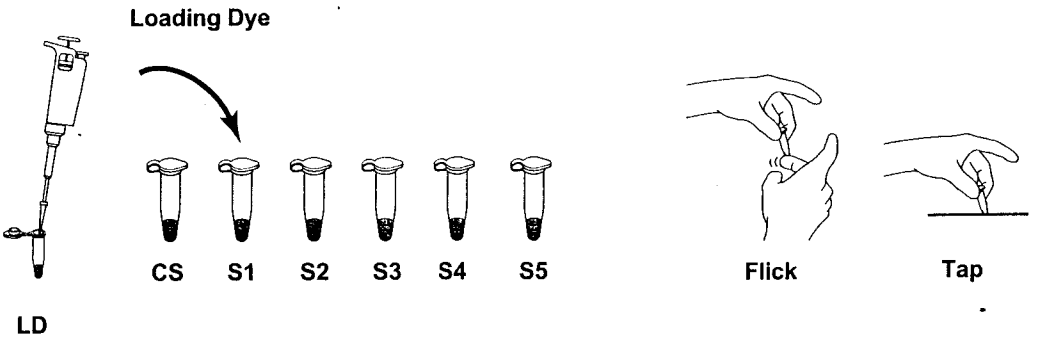
Lesson 3 Laboratory

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.

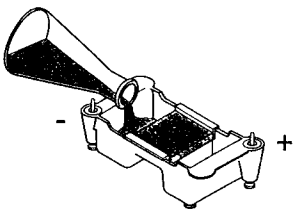
   Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube:

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

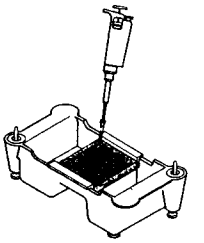
   Lane 3: S1, blue, 20 µl

   Lane 4: S2, orange, 20 µl

   Lane 5: S3, violet, 20 µl

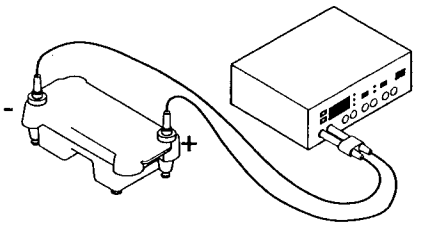
   Lane 6: S4, red, 20 µl

   Lane 7: S5, yellow, 20 µl



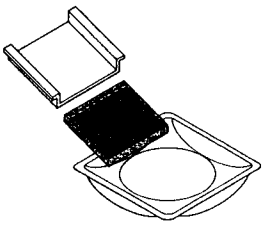
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect electrical leads to the power supply.

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

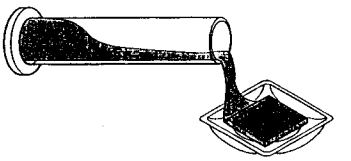


While you are waiting for the gel to run, you may begin the review questions on the following page.

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

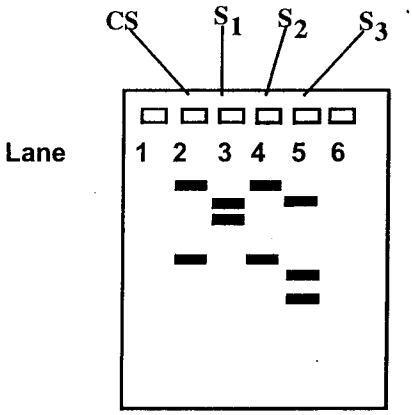
Unaided visual examination of gels indicates only the positions of the loading dyes and not the positions of the DNA fragments. DNA fragments are visualized by staining the gel with a blue dye. The blue dye molecules have a high affinity for the DNA and strongly bind to the DNA fragments, which makes 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

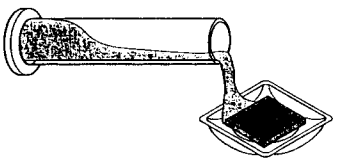
Student Workstations	Number	(✓)
Water for destaining gels	60 ml	❑
Millimeter ruler	1	❑
Linear graph paper	1	❑
Semi-log graph paper	1	❑

Instructor's Workstation

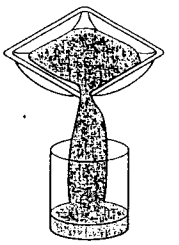
None required

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.

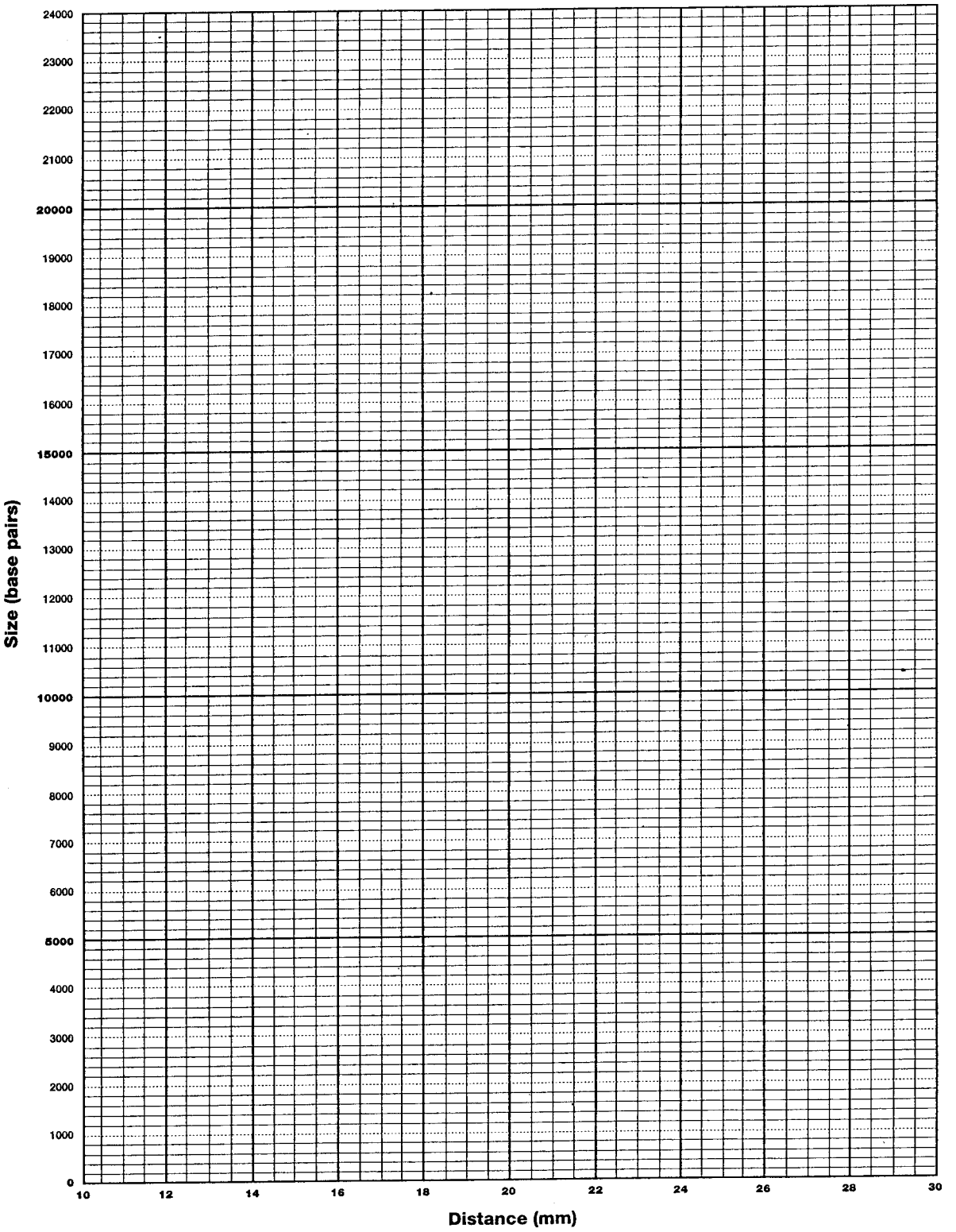
5. Compare the fragment sizes of the suspects and the crime scene.

   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												

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.