Biotechnology Explorer

DNA Fingerprinting Kit

Instruction Manual

Catalog Number
166-0007-EDU

www.explorer.bio-rad.com

Lyophilized reagents can be stored at room temperature. Store DNA markers at 4 ºC, or colder within 4 weeks of arrival.

BIO-RAD

Can DNA evidence solve human problems?

DNA fingerprinting is now used routinely to solve crimes. In recent years, news stories have reported how miniscule amounts of DNA have been used to identify individuals involved in incidents even many years in the past, as well as exonerate innocent people from incrimination.

The power of DNA as a tool for individual identification captures students' imaginations. This activity provides in-depth instruction about how restriction enzymes cleave DNA, how electrophoresis is used to separate and visualize DNA fragments, and how these techniques can be combined to obtain a DNA fingerprint. Principles of restriction analysis, plasmid mapping and DNA fragment size determination can also be documented with this kit.

Open the door to rich discussions about scientific, ethical, and legal implications of DNA profiling. DNA fingerprinting is used in medical and forensic procedures, as well as in paternity determinations to discern genetic relationships between individuals at the molecular level. This kit allows students to play the role of a forensic scientist and make a positive ID. That is, to simulate using real DNA as evidence and figure out for themselves: "Who done it?"

In this activity, students analyze six different samples of plasmid DNA. One sample collected from a hypothetical "crime scene" and five samples obtained from "suspects" are digested with two restriction enzymes. The resulting DNA fragments are separated and visualized in agarose gels using Bio-Rad's Bio-Safe DNA staining solution. Based on the restriction fragment patterns, students compare the evidence and match one of the suspects' DNA to the sample collected at the crime scene.

As an alternative to the classical human forensic applications for this kit, have your students imagine they are high tech pathologists investigating an outbreak of an aggressive infectious disease that has never been seen before. The Centers for Disease Control and Prevention suspects that a new strain of bacteria has arisen that not only is the cause of the new disease, but also has acquired multiple resistance plasmids from some other bacterial strains. Their job is to develop a DNA diagnostic tool for identifying the culprit plasmids. They decide to use restriction enzyme analysis and "DNA electrophoresis fingerprinting" to identify and distinguish different suspect plasmids and track their spread through the environment. DNA from the cultures of a number of stricken patients has been isolated. Have your students identify the new killer bug before the pathogen gets out into the general population and starts a true epidemic!

We strive to continually improve our Biotechnology Explorer kits and curricula. Please share your stories, comments and suggestions!

Ron Mardigian
Dr. Patti Taranto
Bio-Rad Laboratories
ron_mardigian@bio-rad.com

patti_taranto@bio-rad.com

1-800-424-6723

DNA Fingerprinting Curriculum

Intended Audience

This investigation is intended to be used by any high school or college student, independent of the degree of prior familiarity with the chemistry of nucleic acids.

Goals of the Curriculum

That all students who participate in this investigation:

1) Become challenged by the task and intrigued by the methodology of the investigation.
2) Develop an understanding of some of the basic scientific principles involved in DNA fingerprinting.
3) Weigh evidence and be able to analyze and interpret the data that is generated in this investigation with clarity and confidence.
4) Have a clear understanding of the thought processes involved in scientific work.
5) Develop the curiosity and confidence to further explore questions and issues involving scientific investigations.

Teaching Strategies

This curriculum is designed to simulate human forensic testing but can also be used to simulate a wide range of applications for genetic analysis. The actual scenario employed is up to the discretion of the instructor. (Refer to alternative scenarios in Appendix A).

The analysis sections of this investigation are intended to guide students through the process of discovering and understanding concepts that are of significance to the procedures and the analysis of the data at each step along the way. It is hoped that this approach (as compared to the teacher giving the students all of the background information) will make the entire investigation more comprehensible to a greater number of students. So long as the teacher has the opportunity to check on the progress and levels of understanding of each group, some degree of self pacing is possible, if so desired. We have found that this approach allows a larger number of the diverse population of students we work with to experience the goals that have been identified above.

The curriculum for this activity was developed in collaboration with:

Len Poli and Russ Janigian:
S.F. Base - Biotechnology Program
San Francisco

Table of Contents

Kit Inventory: Check (✔) List

Components Provided in this Kit	Class Kit	(✔)
1. Crime Scene (CS) DNA with buffer, lyophilized, 60 µg	1 vial	❑
2. Suspect 1 (S1) DNA with buffer, lyophilized, 60 µg,	1 vial	❑
3. Suspect 2 (S2) DNA with buffer, lyophilized, 60 µg	1 vial	❑
4. Suspect 3 (S3) DNA with buffer, lyophilized, 60 µg	1 vial	❑
5. Suspect 4 (S4) DNA with buffer, lyophilized, 60 µg	1 vial	❑
6. Suspect 5 (S5) DNA with buffer, lyophilized, 60 µg	1 vial	❑
7. *Eco*RI/*Pst*I, restriction enzyme mix, lyophilized, 1800 units	1 vial	❑
8. Sterile water, 2.5 ml	1 vial	❑
9. Lambda *Hin*dIII DNA markers (0.2 µg/µl), 100 µl	1 vial	❑
10. DNA sample loading dye	1 vial	❑
11. DNA staining solution (500x) 1 ml	1 vial	❑
12. Microtubes, 1.5 ml, assorted colors		
clear	30	❑
green	10	❑
blue	10	❑
orange	10	❑
violet	10	❑
red	10	❑
yellow	10	❑
13. Agarose, 5 g	1	❑
14. TAE buffer (50x) 100 ml	1	❑
15. Foam test tube racks	16	❑
16. Gel staining trays	10	❑

Accessories Not Included in this Kit	No.	(✔)
Micropipet, 2-20 µl (catalog number 166-0506-EDU)	1–8	❑
Pipet tips - 1 box, 5 racks of 200 (catalog number 223-9338-EDU)	1	❑
Electrophoresis chamber (catalog number 170-4406-EDU)	1–8	❑
Power supply (catalog number 170-5050-EDU)	1–2	❑
Permanent markers	1	❑
Microwave oven	1	❑
Distilled water	1	❑
250 ml Erlenmeyer flask for microwaving agarose	1	❑
500 ml flask or beaker for DNA stain	1	❑
Ice bucket with ice	1	❑
Optional Accessories		
Microcentrifuge (catalog number 166-0503-EDU)	1	❑
37 °C water bath (catalog number 166-0504-EDU)	1	❑
Gel Bond gel drying sheets (catalog number 170-2984-EDU)	1	❑

Background Information for the Instructor

Introduction

Technicians working in forensic labs are often asked to do DNA profiling or "fingerprinting" to analyze evidence in law enforcement cases and other applications.[1] DNA fingerprinting may involve polymerase chain reaction (PCR[2]) amplification to analyze minute quantities of DNA or restriction fragment length polymorphism (RFLP[3]) analysis, if large amounts of DNA are recovered. A step in human RFLP analysis requires the student to compare band patterns produced by cleavage of DNA samples when separated on an agarose gel. The patterns in this exercise are produced from one sample that represents DNA taken at the crime scene and five samples obtained from suspects in the case. It may be important for you to point out to your students that this laboratory exercise **models** the more elaborate technique that is performed on complex human DNA samples.

Restriction Enzymes

Restriction enzymes sit on a DNA molecule and slide along the helix until they recognize specific sequences of base pairs that 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, cutting 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. Therefore, if a given linear piece of DNA is cut with a restriction enzyme whose specific recognition code is found at two different locations on the DNA molecule, the result will be three fragments of different lengths. If the given piece of DNA is circular and is cut with a restriction enzyme whose specific recognition code is found at two different locations on the DNA molecule, the result will be two fragments of different lengths. The length of each fragment will depend upon the location of restriction sites on the DNA molecule.

When restriction enzymes are used to cut strands of circular plasmid DNA, such as the samples included in this kit, fragments of varying sizes are produced. DNA that has been cut with restriction enzymes can be separated and observed 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 would be to 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.

DNA Fingerprinting

Each person has similarities and differences in DNA sequences. To show that a piece of DNA contains a specific nucleotide sequence, a radioactive complementary DNA probe can be made that will recognize and bind that sequence. Radioactive probes allow molecular biologists to locate, identify, and compare the DNA of different individuals. This probe can be described as a "radioactive tag" that will bind to a single stranded DNA fragment and produce a band in a gel or a band on a piece of nylon blotting membrane that is a replica of the gel (also known as a Southern blot). Because of its specificity, the radioactive probe can be used to demonstrate genotypic similarities between individuals. In DNA fingerprinting, the relative positions of radiolabeled bands in a gel are determined by the size of the DNA fragments in each band. The size of the fragments reflect variations in individuals' DNA.

We are rapidly getting beyond the scope and intention of this manual. For more detailed information, we recommend a review of the references listed on page 7.

The evidence needed for DNA fingerprinting can be obtained from any biological material that contains DNA: body tissues, body fluids (blood and semen), hair follicles, etc. The DNA analysis can even be done from dried material, such as blood stains or mummified tissue. If a sample of DNA is too small it may be amplified using PCR techniques. The DNA is then treated with restriction enzymes that cut the DNA into fragments of various length.[4]

Restriction Digestion of DNA

Because they cut DNA, restriction enzymes are the "chemical scissors" of the molecular biologist. When a *particular* restriction enzyme "recognizes" a *particular* four - or six -base pair (bp) **recognition sequence** on a segment of DNA, it cuts the DNA molecule at that point. The recognition sequences for two commonly-used enzymes, ***Eco*RI** and ***Pst*I**, are shown below. The place on the DNA backbones where the DNA is actually cut is shown with a (✄) symbol:

For the enzyme: ***Eco*RI**

For the enzyme: ***Pst*I**

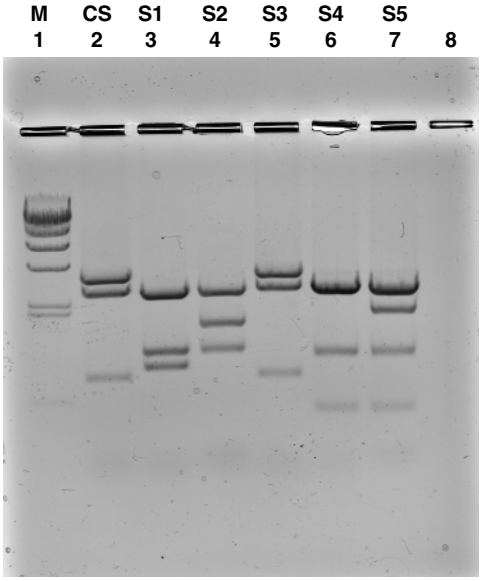
Like all enzymes, restriction enzymes function best under specific buffer and temperature conditions. The proper restriction enzyme buffer has been included with the DNA sample, so that when the rehydrated DNA and enzymes are mixed, the ideal conditions are created for the enzymes to function optimally. The final reaction buffer consists of 50 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DDT, pH 8.0, which is the ideal condition for *Eco*RI and *Pst*I enzymes to function.

Visualizing DNA Restriction Fragments

DNA is colorless so DNA fragments in the gel can't be seen during electrophoresis. A blue loading buffer, containing two blue loading dyes, is added to the DNA solution. The loading dyes do not stain the DNA but make it easier to load the gels and monitor the progress of the DNA electrophoresis. The dye fronts migrate toward the positive end of the gel, just like the DNA fragments. The "faster" dye co-migrates with DNA fragments of approximately 500 bp, while the "slower" dye co-migrates with DNA fragments approximately 5 kb in size.

Staining the DNA pinpoints its location on the gel. When the gel is immersed in a dilute solution of Bio-Safe DNA stain, the dye molecules attach to the DNA molecules trapped in the agarose gel. To enhance contrast and to easily visualize the DNA bands, excess background stain can be removed from the gel by destaining the gel with water. When the bands are visible, your students can compare the DNA restriction patterns of the different samples of DNA.

The gel below shows the DNA pattern that will be obtained by your students following electrophoresis. The DNA from the crime scene has been labeled CS, that from Suspect #1, S1 and so on. The DNA from the crime scene is placed in lane 2; one suspect's DNA is placed in each of lanes 3, 4, 5, 6 and 7. Lane 1 contains *Hin*dIII DNA size markers. By convention, the lanes are numbered from the top left. The students' task is to look at the DNA banding patterns and see if any of the suspects' bands match those of the DNA found at the crime scene.



It's easy to see that the DNA taken from the crime scene and the DNA from S3 is identical. You may want to point out how "strong or weak" this evidence is in convicting a suspect. The DNA evidence may place the suspect at the scene, but other evidence may be needed to prove him or her guilty![5,6]

You may point out to your students that this is a simulation. In actual DNA fingerprinting, technicians analyze much larger segments of DNA and many more bands and lanes are produced. These technicians are looking for a specific DNA segment, common to a given population, that will produce a unique banding pattern for each individual.

Reliability of DNA Evidence

Two major factors affecting the reliability of DNA fingerprinting technology in forensics are population genetics and genetic statistics. In humans there are thousands of RFLP loci or DNA segments that can be selected and used for fingerprinting analysis. Depending on demographic factors such as ethnicity or geographic isolation, some segments will show more variation than others.

Some populations show much less variation in particular DNA segments than others. The degree of variation will affect the statistical odds of more than one individual having the same sequence. If 90% of a given population has the same frequency in its DNA fingerprinting pattern for a certain DNA segment, then very little information will be attained. But if the frequency of a DNA pattern turning up in a population for a particular segment is extremely low, then this segment can serve as a powerful tool to discriminate between individuals in that population. Different populations show different patterns in their genotypes due to the contributions made to their individual gene pools over time.

Therefore, in analyzing how incriminating the DNA evidence is, one needs to ask the question:

"Statistically how many people in a population may have the same pattern as that taken from a crime scene: 1 in 1,000,000? 1 in 10,000? Or, 1 in 10?"

References

1. DNA Profiling Fast Becoming Accepted Tool For Identification, Pamela Zurer, *Chemical and Engineering News*, Oct. 10, 1994.
2. PCR means polymerase chain reaction; it is a technique used to amplify small amounts of DNA (in this case so that further analysis of the DNA can occur).
3. RFLP means restriction fragment length polymorphisms..."riff-lips" in biotech jargon...Pieces of DNA are cut with restriction enzymes into fragments of various lengths. Individuals possess variable restriction recognition sites so that two pieces of DNA from separate sources may have different fragment lengths when their DNA is cut by the same enzyme.
4. An excellent resource for the classroom teacher is Genetic Fingerprinting, Pauline Lowrie and Susan Wells, *New Scientist*, 16 November 1991.
5. Is DNA Fingerprinting ready for the courts?, William C. Thompson and Simon Ford, *New Scientist*, March 31, 1990.
6. When Science Takes the Witness Stand, Peter Neufeld and Nevelle Coleman, *Scientific American*, Vol. 262: 5, May 1990.

Implementation Timeline

There are five student lessons in this fingerprinting curriculum. All lessons are designed to be carried out in consecutive 50 minute periods. All lessons include:

- A series of prelab considerations for students
- An active student investigation
- Questions for analysis and interpretation of lab results

Student Schedule

Lesson 1:	**Introduction to DNA Fingerprinting**
Activity	Lecture and discussion Prelab Considerations 1 and 2
Lesson 2:	**Restriction Digest of DNA Samples**
Activity	Pour gels; perform the restriction digests Complete preliminary analysis and review questions
Lesson 3:	**Electrophoresis of DNA Samples**
Activity	Load and run gels; stain gels overnight Do analysis and review questions
Lesson 4:	**Analysis and Interpretation of Results**
Activity	Destain gels Do analysis questions Generate standard curve Discuss results and weigh evidence

Instructor's Advance Preparation Overview

This sections outlines the recommended schedule for advanced preparation on the part of the instructor. A detailed Advance Preparation Guide is provided on pages 11–15.

Activity	When	Time required
Read Fingerprinting manual	Immediately	1 hour
Prepare electrophoresis TAE buffer and pour agarose gels	Prior to or during Lesson 2	1 hour
Rehydrate lyophilized DNA/ buffer samples and enzyme mix and aliquot	Prior to Lesson 2	20 minutes
Prepare Bio-Safe DNA stain	Prior to Lesson 2	10 minutes
Set up workstations	The day of student labs	10 minutes/day

Workstation Check (✓) List

Student Workstations: Materials and supplies that should be present at each student workstation prior to beginning each lab experiment are listed below. The components provided in this kit are sufficient for 8 student workstations.

Instructor's (Common) Workstation: A list of materials, supplies, and equipment that should be present at a common location that can be accessed by all student groups is also listed below. It is up to the discretion of the teacher as to whether students should access common buffer solutions/equipment, or whether the teacher should aliquot solutions and operate equipment.

Lesson 2 Restriction Digests of DNA Samples

Student Workstations	**Number/Station**	**(✔)**
*Eco*RI/*Pst*I enzyme mix	1 tube (80 µl)	❑
Pipet tips	15 tips	❑
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	❑
Instructor's Workstation		
Crime Scene DNA with buffer, rehydrated	1 vial	❑
Suspect 1 DNA with buffer, rehydrated	1 vial	❑
Suspect 2 DNA with buffer, rehydrated	1 vial	❑
Suspect 3 DNA with buffer, rehydrated	1 vial	❑
Suspect 4 DNA with buffer, rehydrated	1 vial	❑
Suspect 5 DNA with buffer, rehydrated	1 vial	❑
Incubator or bath - (37 °C)	1/class	❑
Molten agarose (See Advance Prep)	35–40 ml/gel	❑
Gel trays	1/station	❑
Lab tape for gel trays	1/station	❑

Protective eye goggles should be worn in the laboratory at all times.

Proper safety precautions, such as no eating or drinking, should always be practiced.

Lesson 3 Electrophoresis of DNA Samples

Student Workstations	Number/Station	(✔)
Agarose gel	1	❑
Digested DNA samples	5	❑
DNA sample loading dye	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	❑

Instructor's Workstation

1x TAE Electrophoresis buffer	275 ml gel/box	❑
Bio-Safe DNA stain - 1x solution	500 ml	❑
*Hin*dIII DNA markers	1	❑

Lesson 4 Analysis of Results

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

Instructor's Workstation

None required

Instructor's Advanced Preparation for Labs

This section describes the preparation that needs to be performed by the instructor before each laboratory. An estimation of preparation time is included in each section.

Lesson 2 (Lab) Restriction Digests of DNA Samples

Advance Preparation

Objectives: Rehydrate DNA/buffer samples and restriction enzymes
Aliquot restriction enzymes
Set up student and instructor workstations
Pour agarose gels, or, **if you have your students pour their own gels during the lab, prepare the agarose ahead of time. Agarose, once prepared, may be kept in a water bath set at 50–55 °C until used by the students.**
Set temperature of 37 °C for water bath

Time required: Thirty minutes to 1 hour (will vary depending on how you choose to prepare agarose gels)

What's required: Electrophoresis gel boxes, casting trays and combs
Electrophoresis buffer (50x TAE)
Agarose powder
16 clear microtubes

Procedures

1. Rehydrate samples:

   Note: All of the DNA and enzyme vials should contain a white residue, which may appear as a loose powder in the DNA vials. The lyophilized DNA samples have color-coded labels on clear glass vials. The lyophilized *Eco*RI/*Pst*I enzyme mix is in an amber vial.

   A. To rehydrate DNA/buffer samples, add 200 µl of sterile water to each lyophilized DNA vial and swirl to resuspend. Allow DNA/buffer samples to rehydrate at room temperature for 5 minutes or until dissolved. Gentle heating at 37 °C for 10 minutes may be necessary. You may choose to transfer the rehydrated DNA/buffer samples to color-coded, labeled 1.5 ml microtubes to make pipetting easier for your students.

   The rehydrated DNA samples are now at a concentration of 0.3 µg/µl in 100 mM Tris, 200 mM NaCl, 20 mM $MgCl_2$, 2 mM DTT, pH 8.0. Once the DNA in buffer is added to the enzyme, the final concentration of buffer will be 50 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 8.0, which is the ideal condition for *Eco*RI and *Pst*I enzymes to function.

   B. To rehydrate *Eco*RI/*Pst*I enzyme mix, add 750 µl sterile water and swirl to resuspend the enzymes. Allow enzymes to rehydrate on ice for 5 minutes. It is critical that the enzyme mix is kept **on ice**, but not frozen, once it has been rehydrated. The rehydrated enzymes should be used within 12 hours.

2. Aliquot enzyme mix: Transfer 80 µl of the rehydrated enzyme mix into each of eight, 1.5 ml microtubes labeled **ENZ**.

3. Prepare electrophoresis buffer. TAE (Tris, acetate, EDTA) electrophoresis buffer is available as a 50x concentrated solution. In addition to the 1x TAE buffer needed to make the agarose gels, approximately 275 ml is also required for each electrophoresis chamber. Three liters of 1x TAE buffer will be sufficient to run 8 electrophoresis chambers and pour 8 agarose gels. To make 3 liters of 1x TAE from a 50x TAE concentrate add 60 ml of 50x concentrate to 2.94 liters of distilled water.

4. **Prepare agarose**. These procedures may be carried out 1 to 2 days ahead of time by the teacher or done during class by the individual student teams.

   A. The recommended gel concentration for this classroom application is 1% agarose. This concentration of agarose provides excellent resolution and minimizes run time required for electrophoretic separation of DNA fragments. To make a 1% solution, add 1 gram of agarose to 100 ml of 1x TAE electrophoresis buffer. The agarose must be made using electrophoresis buffer, not water.

   If gel boxes are limiting, you can use a 7 x 10 cm tray and two 8-well combs to pour a gel that can be used to run two sets of student digests.

   Use this table as a guide for gel volume requirements when casting single or multiple gels.

   Volume of 1% agarose for:

Number of gels	7 x 7 cm tray	7 x 10 cm tray
1	40 ml	50 ml
2	80	100
4	160	200
8	320	400

   B. Add the agarose powder to a suitable container (*e.g.* 500-ml Erlenmeyer flask for 200 ml or less). Add the appropriate amount of 1x TAE electrophoresis buffer and swirl to suspend the agarose powder in the buffer. If using an Erlenmeyer flask, invert a 25-ml Erlenmeyer flask into the open end of the 500 ml Erlenmeyer flask containing the agarose. The small flask acts as a reflux chamber, thus allowing long or vigorous boiling without much evaporation. The agarose can be melted for gel casting by boiling until agarose has melted completely on a magnetic hot plate, hot water bath, or in a microwave oven.

Caution: Always wear protective gloves, goggles, and lab coat while preparing and casting agarose gels. Boiling molten agarose or the vessels containing hot agarose can cause severe burns if allowed to contact skin.

Microwave Oven Method. This technique is the fastest and safest way to dissolve agarose. Place the gel solution in an appropriate bottle or flask into the microwave. LOOSEN THE CAP IF YOU ARE USING A BOTTLE. Use a medium setting and set to 3 minutes. Stop the microwave oven every 30 seconds and swirl the flask to suspend any undissolved agarose. Boil and swirl the solution until all of the small translucent agarose particles are dissolved. Set aside to cool to 55-60 °C before pouring.

Magnetic Hot Plate Method. Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Bubbles or foam should disrupt before rising to the neck of the flask.

Boil the solution until all of the small translucent agarose particles are dissolved. Set aside to cool to 55-60 °C before pouring gels.

If you choose, you can melt the agarose several hours in advance and keep it in a waterbath at 55-60 °C until you or your students are ready to pour the gels.

5. **Pour agarose gels**. This lab activity requires that each gel has at least 8 sample loading wells. Follow the above instructions to prepare the agarose and determine what volume of 1% agarose will be needed. Pour enough agarose to cover the gel comb teeth or to a depth of 0.5–0.75 cm. Do not move or handle the gel tray until the gel has solidified. When solidified, gels can be stored in sealable bags at room temperature or in the refrigerator until use on the next day. Have students label their plastic bags. The time needed to pour gels by an entire class is approximately 30 minutes. If possible, pour one or two extra gels for back-up.

6. **Restriction Digests.** A 45 minute incubation at 37 °C is the optimum digestion condition. If a 37 °C heating block, water bath, or incubator is not available, samples can be digested by placing tubes in foam racks, floating them in a large volume (1 liter or more) of 37 °C water, and allowing them to incubate overnight as the water cools to room temperature.

Procedure for casting gels

Using Bio-Rad's Mini Sub-Cell® GT system, gels can be cast directly in the gel box by using the casting gates with the gel tray.

This section outlines the conventional tape-the-tray method for casting gels. Other methods are detailed in Bio-Rad's Sub-Cell GT instruction manual.

Step 1. Seal the ends of the gel tray securely with strips of standard laboratory tape. Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.

Step 2. Level the gel tray on a leveling table or workbench using the leveling bubble provided with the instrument.

Step 3. Prepare the desired concentration and amount of agarose in 1x TAE electrophoresis buffer.

Step 4. Cool the agarose to at least 60 °C before pouring.

Step 5. While the agarose is cooling to 60 °C, place the comb into the appropriate slot of the gel tray. Gel combs should be placed within 3/4 of an inch of the end of the gel casting tray (not in the middle of the gel).

Step 6. Allow the gel to solidify at room temperature for 10 to 20 minutes—it will appear cloudy, or opaque, when ready to use.

Step 7. Carefully remove the comb from the solidified gel.

Step 8. Remove the tape from the edges of the gel tray.

Step 9. Place the tray onto the leveled DNA electrophoresis cell so that the sample wells are at the cathode (black) end of the base. DNA samples will migrate towards the anode (red) end of the base during electrophoresis.

To pour a double gel using the 7 x 10 cm tray and two 8-well combs, place one comb at one end of the tray and the other comb in the middle of the tray.

Lesson 3 (Lab) Electrophoresis and Staining of DNA Samples

Advance Preparation

Objective:	Aliquot DNA sample loading dye Prepare Lambda *Hin*dIII size markers Prepare 1x Bio-Safe DNA staining solution Set up student and instructor workstations	
Time required:	Twenty minutes	
What's required:	Stock solution:	DNA sample loading dye
	Stock solution:	Bio-Safe DNA staining solution
	Stock solution:	DNA size marker (Lambda *Hin*dIII digest)

1. Aliquot loading dye.

   A. Label 8 clear microtubes "**LD**" for Loading Dye. Aliquot 35 µl of loading dye into 8 clear microtubes that are labeled "**LD**". Distribute to student workstations.

   B. Add 20 µl of loading dye to the stock tube containing the *Hin*dIII DNA Size Markers. If possible, heat the markers to 65 °C for 5 minutes, then chill on ice—this results in better separation of the marker bands. Label clear microtubes "M". Aliquot 15 µl of the DNA markers containing loading dye to the 8 clear microtubes labeled "M". Distribute to student workstations.

2. Prepare Bio-Safe DNA staining solution.

   Dilute the 1 ml volume of 500x DNA stain in 499 ml of distilled water in an appropriate sized flask. Cover the flask and store at room temperature until ready to use.

3. Electrophoresis of samples.

   Suggested running time is 30 minutes. If your laboratory schedule allows, increasing running time to 40 minutes will enhance the resolution.

DNA Staining Procedure—Bio-Safe DNA Staining Solution

The volume of 1x Bio-Safe solution needed to stain one 7 x 7 or 7 x 10 cm gel is approximately 60 ml. Gels should be removed from the gel tray before staining. This is easily accomplished by holding the base of the gel in one hand, and gently pushing out the gel with the thumb of the other hand. Special attention must be given to supporting the well portion of the gel since it can crack along the well line. Pour enough stain into the tray to cover the gel(s) completely.

For best results, shake gels while staining overnight. If you have a rocking platform, multiple gels can be stained in one large container if they are marked to distinguish different student groups' gels, by cutting off different corners, for example. If the provided staining trays are used, each gel should be stained in an individual staining tray.

Stain the gels overnight in 1x Bio-Safe stain. The next day, rinse the stained gel with water and destain at least 10 minutes. To produce maximum contrast, the gels can be destained overnight with water. This stain is nontoxic; however, you should use latex or vinyl gloves while handling gels to keep your hands from being stained.

Lesson 4 Drying Gels and Analyzing the DNA Patterns

Advance Preparation

Objective: Set up workstations

Time required: 10 minutes

Procedures: There are no reagents to make or aliquot for this laboratory.

To obtain a permanent record of the gel, before it is dried, either trace the gel outline, including wells and DNA bands on a piece of paper or acetate, or take a photograph using standard cameras and film (Bio-Rad's standard Polaroid gel documentation system).

Dry the Agarose Gel as a Permanent Record of the Experiment

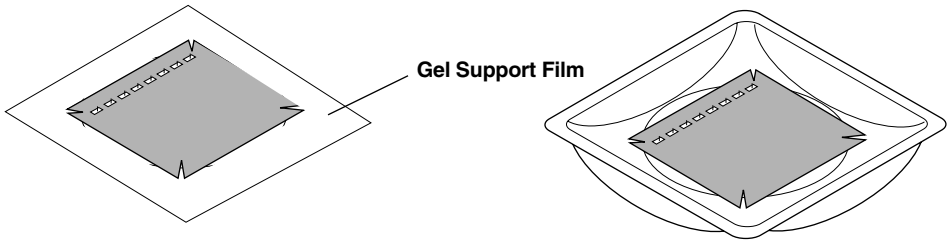
Note: Drying agarose gels requires the use Bio-Rad's specially formulated high strength analytical grade agarose. Other gel media may not be appropriate for this purpose. There are two methods that can be used to dry destained agarose gels.

Method 1

Method 1 is the preferred method and requires the use of Bio-Rad's exclusive **gel support film** (catalog number 170-2984-EDU). Simply remove the destained agarose gel from its staining tray and trim away any unloaded lanes with a knife or razorblade. Place the gel directly upon the hydrophilic side of a piece of gel support film. Water will form beads on the hydrophobic side but will spread flat on the hydrophilic side of the film. Center the gel on the film. Place the film on a sheet of paper towel and dry, avoiding direct exposure to light. As the gel dries it will bond to the film and will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2–3 days. The result will be a flat, transparent and durable record of the experiment.

Method 2

After staining and destaining the gel, leave the gel in the plastic staining tray. Let it air dry for 2–3 days. As the gel dries it will shrink considerably, but proportionately. If left undisturbed in the tray, the gel should remain relatively flat but may wrinkle as it dries.



Method 1 **Method 2**

Note: Avoid extended exposure of dried gels to direct light to prevent band fading.

Graphing the Data

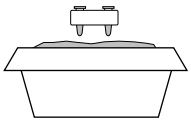
Many of your students may not be familiar with logarithms and semi-log graph paper. It is suggested that you prepare a short lesson presented on the overhead or computer to demonstrate the proper way to label coordinates and plot points. You may choose to include a lesson on the different uses of semi-log vs. standard graph paper in this instance. A math extension implemented here may provide a perfect opportunity to explore linear and exponential (arithmetic and geometric) sequences of numbers. We have included both semi-log and standard graph paper on pages 38 and 39 of this manual.

Quick Guide for DNA Fingerprinting Kit

Day 1 Preparing the DNA Samples

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.

ENZ

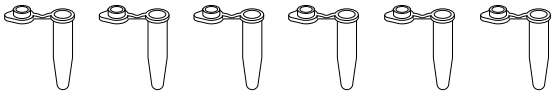


Ice

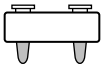
2. Label one of each colored microtube as follows:

green	CS = crime scene
blue	S1 = suspect 1
orange	S2 = suspect 2
violet	S3 = suspect 3
red	S4 = suspect 4
yellow	S5 = suspect 5

Label the tubes with your name, date, and lab period. Place the tubes in the foam microtube rack.

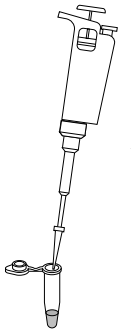


CS S1 S2 S3 S4 S5



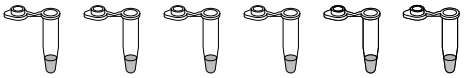
3. Pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored microtubes. Use a separate tip for each DNA sample. Make sure the sample is transferred to the bottom of the tubes.

4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a separate tip for each ENZ sample.



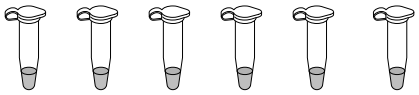
Stock

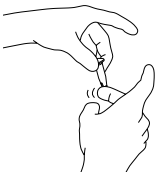
DNA Samples
+
Enzyme Mix



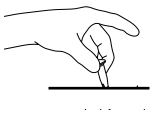
CS S1 S2 S3 S4 S5

5. Cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, tap the tube on a table top.



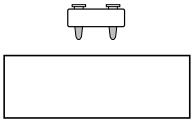


Flick



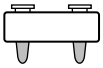
Tap

6. Place the tubes in the floating rack and incubate 45 min at 37 °C or overnight at room temperature in a large volume of water heated to 37 °C.



Water bath

7. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period.

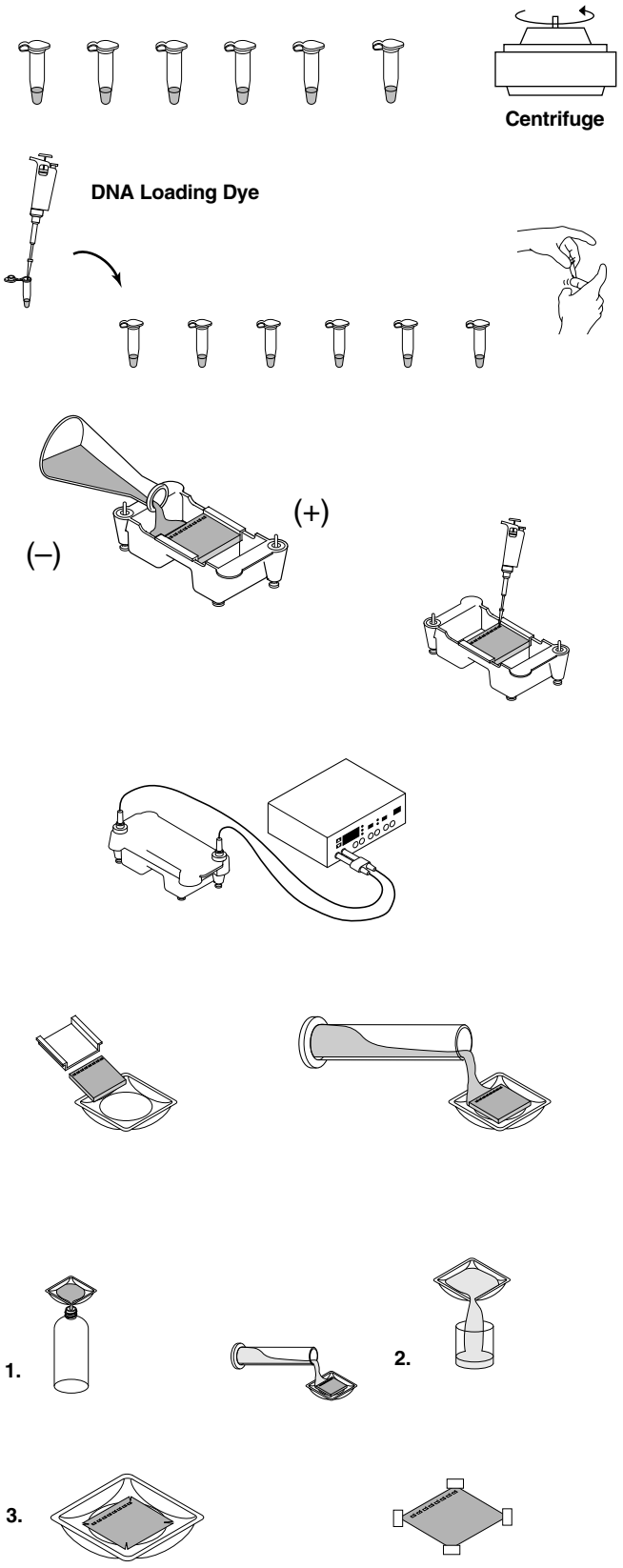


Day 2 Gel Electrophoresis

1. Remove your digested DNA samples from the refrigerator. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube.
2. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger.
3. Place an agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer.
4. Check that the wells of the agarose gels are near the black (-) electrode and the base of the gel is near the red (+) electrode.
5. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:
   - Lane 1: M, DNA size marker, 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
6. Place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid will match with the red and black jacks on the base. Plug the electrodes into the power supply.
7. Turn on the power and electrophorese your samples at 100 V for 30 minutes.
8. When the electrophoresis is complete, turn off the power and remove the top of the gel box. Carefully remove the gel and tray from the gel box. Be careful—the gel is very slippery! Slide the gel into the staining tray.
9. Add 60 ml of DNA stain to the tray. Cover the tray with plastic wrap. Let the gel stain overnight, with shaking for best results.

Day 3 Analysis of the Gel

1. Pour off the DNA stain into a bottle. Add 60 ml of water to the gel and let the gel destain 15 minutes.
2. Pour off the water into a waste beaker. Analyze the results with the help of your teacher.
3. Let the gel dry on gel support film or on your lab bench until completely dry. When the gel is dry, tape into your lab notebook for a permanent record.



DNA Fingerprinting

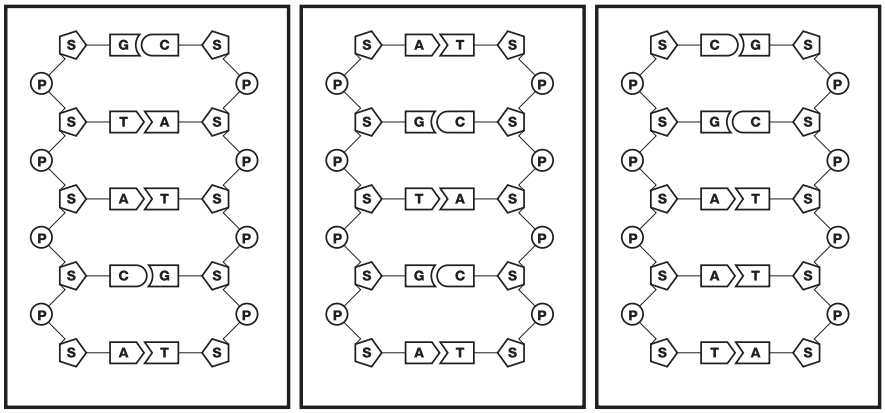
Student Manual

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)

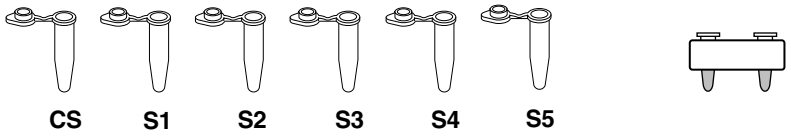
   Orange S2 (suspect 2)

   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.

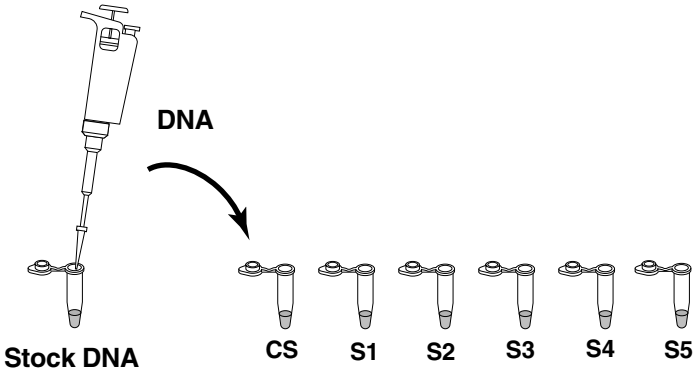


2. Locate the clear microtube that contains the restriction enzyme mix, labeled "ENZ".

   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).

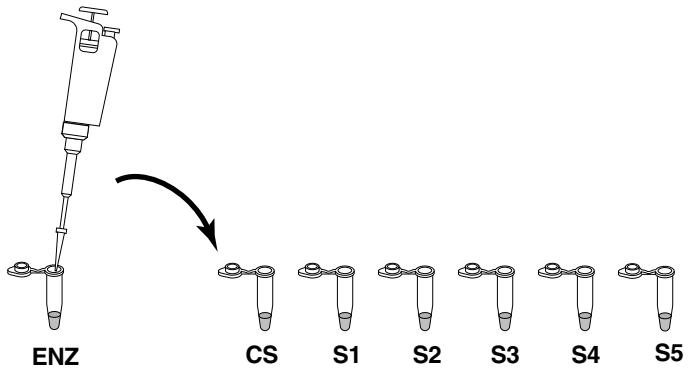
2) Is there any observable difference between the samples of DNA?

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.

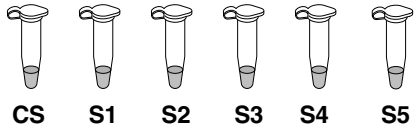


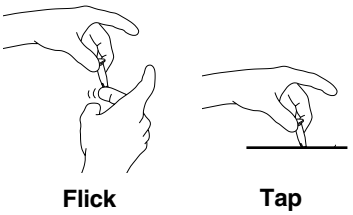
Now your DNA samples should contain:

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.

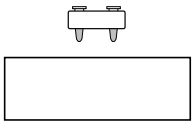
   Close the caps on all the tubes. Mix the components by gently flicking the tubes with your finger. If there is a centrifuge available, pulse the tubes for two seconds to force the liquid into the bottom of the tube to mix and combine reactants. (Be sure the tubes are in a **BALANCED** arrangement in 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	❑

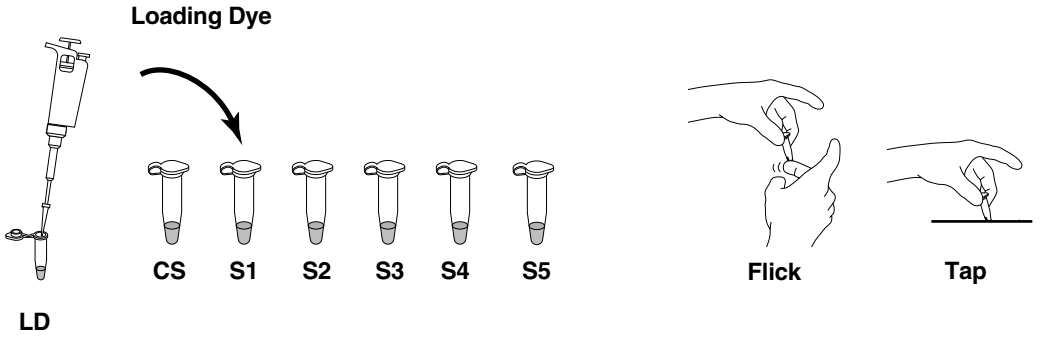
Lesson 3 Laboratory

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.

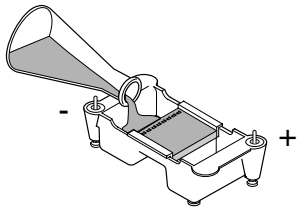
   Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube:

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

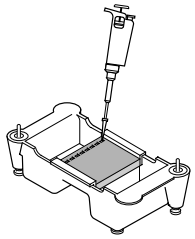
   Lane 3: S1, blue, 20 µl

   Lane 4: S2, orange, 20 µl

   Lane 5: S3, violet, 20 µl

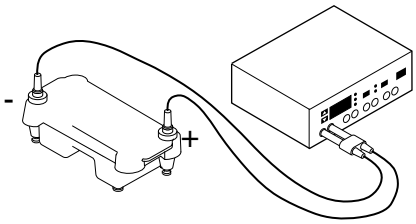
   Lane 6: S4, red, 20 µl

   Lane 7: S5, yellow, 20 µl



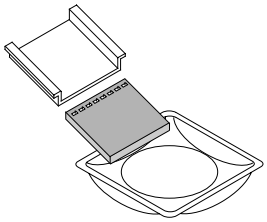
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect electrical leads to the power supply.

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

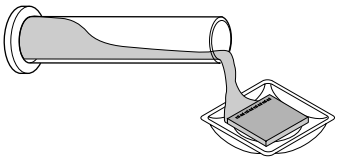


While you are waiting for the gel to run, you may begin the review questions on the following page.

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

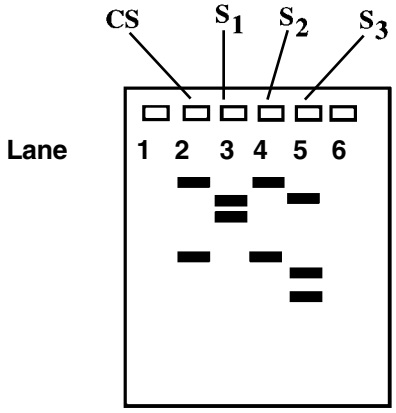
Unaided visual examination of gels indicates only the positions of the loading dyes and not the positions of the DNA fragments. DNA fragments are visualized by staining the gel with a blue dye. The blue dye molecules have a high affinity for the DNA and strongly bind to the DNA fragments, which makes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

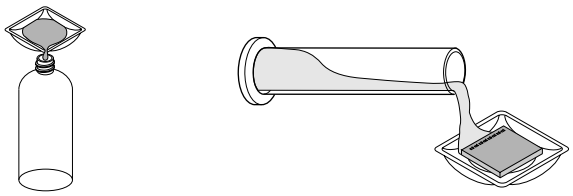
Student Workstations	Number	(✔)
Water for destaining gels	60 ml	❑
Millimeter ruler	1	❑
Linear graph paper	1	❑
Semi-log graph paper	1	❑

Instructor's Workstation

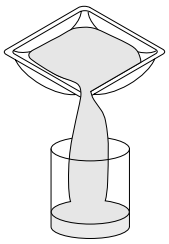
None required

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.

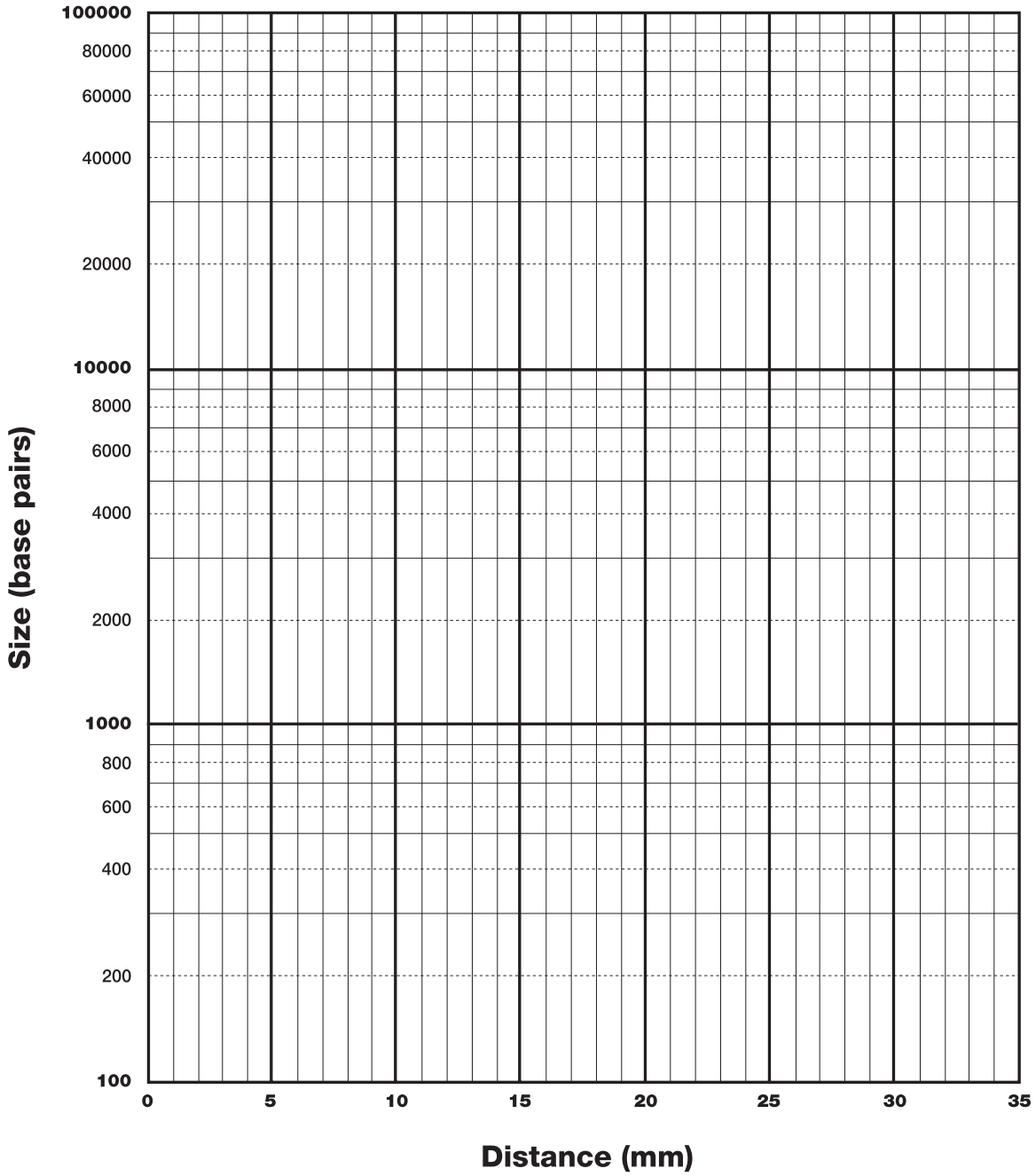
5. Compare the fragment sizes of the suspects and the crime scene.

   Is there a suspect that matches the crime scene?

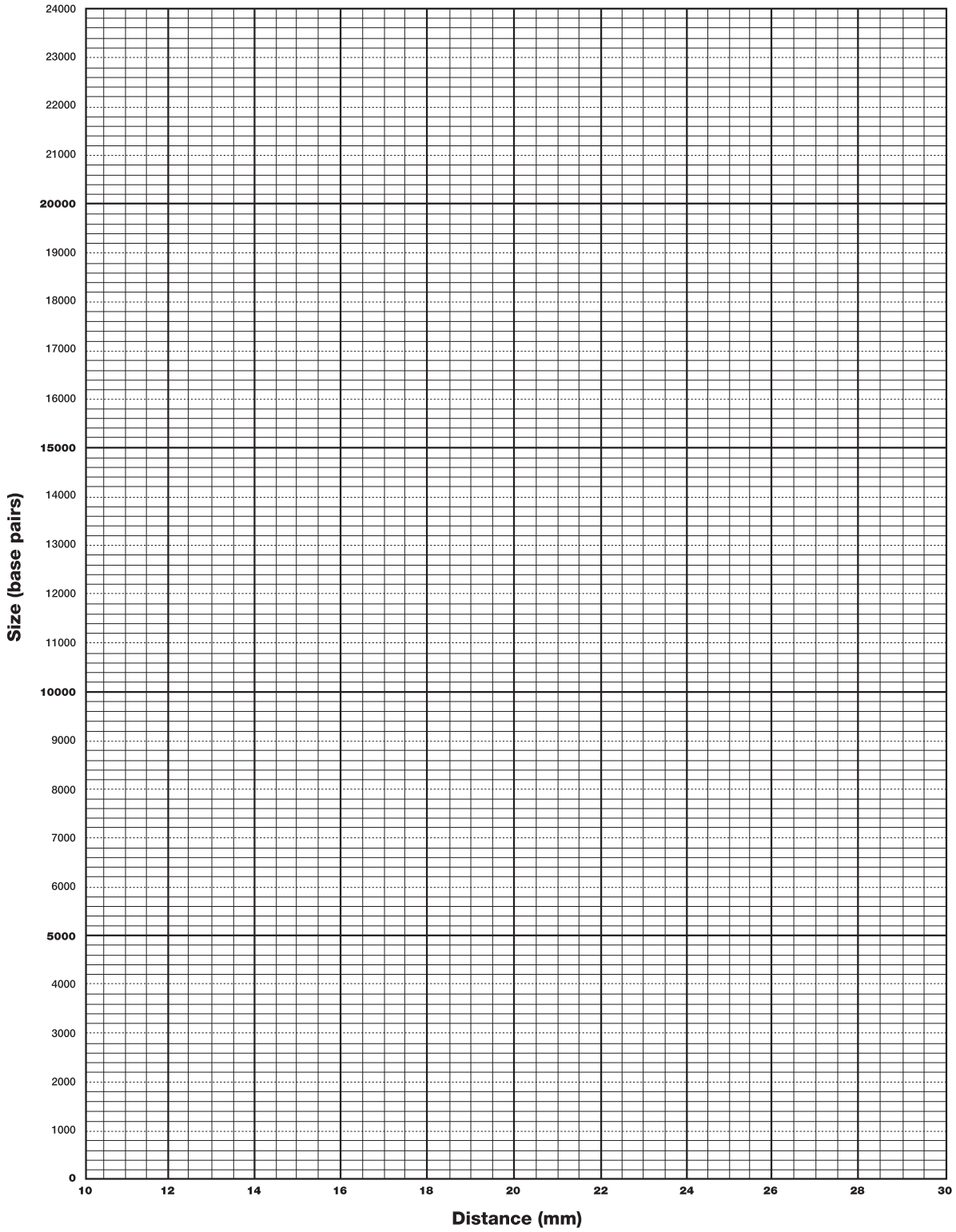
   How sure are you that this is a match?

	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.

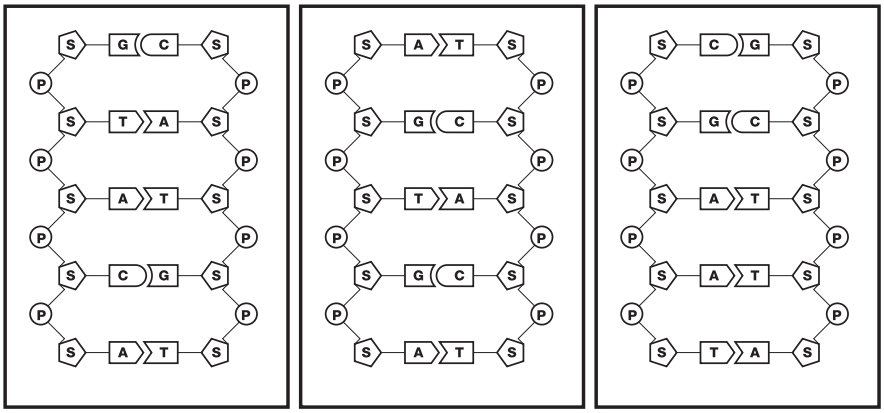
Two small seed pods caught in the bed of his pick-up truck put an accused murderer at the murder scene. Genetic testing showed that DNA in the seed pod exactly matched the DNA of a plant found at the scene of the murder. The accused had admitted he had given the victim a ride, but he denied ever having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

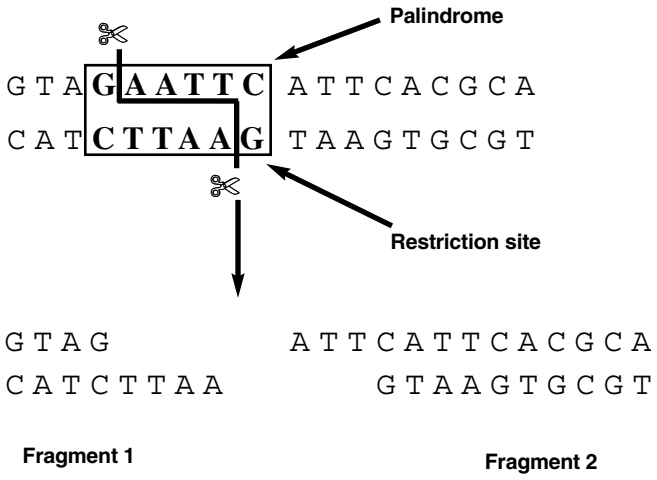
- Compare the bases in the upper portion of the molecule to those in the lower portion. Describe any relationship you can see.

- 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?

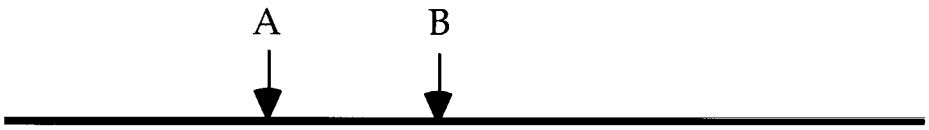
- If the **GAATTC** palindrome repeats are randomly spaced along the DNA strand, then what can you say about the size of the fragments that will be produced?

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.

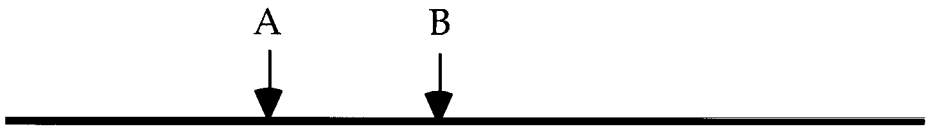
- Which fragment would be the largest?

- 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.

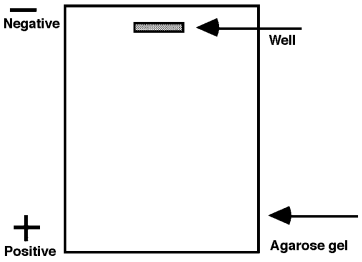
- Explain why only two fragments would be produced.

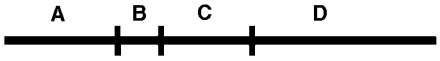
Prelab activity 2 Review of Electrophoresis

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?

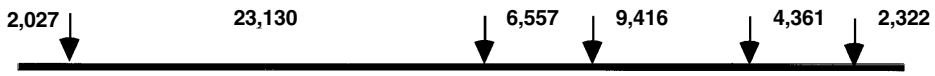
- Suppose you had 500 pieces of each of the four fragments, how would the gel appear?

- 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 ...

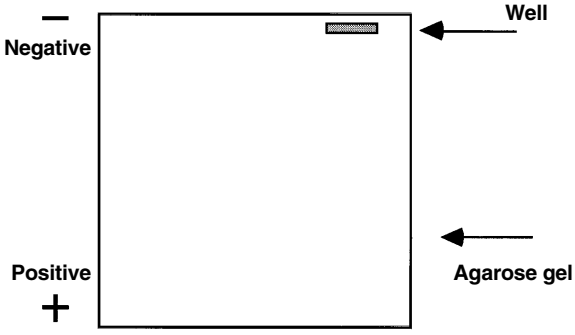
This diagram represents a piece of DNA cut with *Hin*dIII at each of the restriction sites pointed to by the arrows. The numbers represent the number of base pairs in each fragment.



- 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 C
Teacher's Answer Guide

Lesson 1 Introduction to DNA Fingerprinting

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

   The arrangement is identical for all three samples.

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

   All samples contain the same bases: adenine, thymine, guanine and cytosine.

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

   The adenine is always bonded with thymine and the cytosine is always bonded with the guanine.

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?

   The sugar phosphate arrangement is the same for all samples and so are the kind of bases; what is different is the arrangement of bases among the three samples.

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

   The sequence of base pairs in each individual sample.

Lesson 2 Restriction Digests of DNA Samples

1. How many pieces of DNA would result from this cut ? __2__

2. Write the **sequence** of the DNA fragments.

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

3. What differences are there in the two pieces?

   Each fragment is a different size.

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].

   One fragment is short and one is long; also some bases are unpaired.

   a) The smaller fragment is__3__ base pairs (bp).

   b) What is the length of the longer fragment ? __11__

   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:__2__ # of fragments:__2__

   List fragment size in ascending order: largest ——> smallest

   Sample # 1 Sample # 2

   17 bp fragment **23 bp fragment**

   11 bp fragment **5 bp fragment**

Lesson 2 Restriction Digestion of DNA Samples

Observation Questions:

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

   The DNA samples are clear, colorless liquid samples.

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

   No. All samples appear similar.

3. Describe the appearance of the restriction endonuclease mix.

   The restriction enzymes appear to be clear, colorless liquids.

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 combined with the restriction enzymes.

   DNA + *Eco*RI/*Pst*I enzyme mix:

   No visible change apparent in the tubes.

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.

   No. No visible change apparent in the tubes.

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

   Yes. They may be chemically changed but the changes may not be visible. Enzymes may have cut the DNA.

4. **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.

   No. No visible change apparent in the tubes but the enzymes may have cut the DNA. The reactions are at the molecular level and too small to be seen.

Lesson 3 Electrophoresis of your DNA samples

Review Questions:

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

   Positive.

2. What color represents the negative pole?

   Black.

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

   Smaller. There is less resistance to their movement through the gel matrix.

4. Which fragments are expected to travel the shortest distance [remain closest to the well]? Explain.

   Larger. There is more resistance to their movement through the gel matrix.

Lesson 4 Thought Questions

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

   DNA fragments.

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

   One.

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

   The DNA must have been cut into fragments by restriction enzymes.

4. What probably caused the DNA to become fragmented?

   The chemical action of the restriction enzymes cutting at specific base sequences.

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

   Lanes 2, 3, and 4 (CS, S1, and S2).

6. Which sample has the smallest DNA fragment?

   The sample in lane 5 (S3).

7. How many restriction sites were there in lane three?

   Two sites that cut the sample into two fragments.

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

   Lanes 2 and 4 (CS and S2).

9. Based on your analysis of the photograph, 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.

   The DNA samples in lanes 2 and 4 (CS and S2) are from the same individual because they have identical restrictions sites that yield identical fragments.

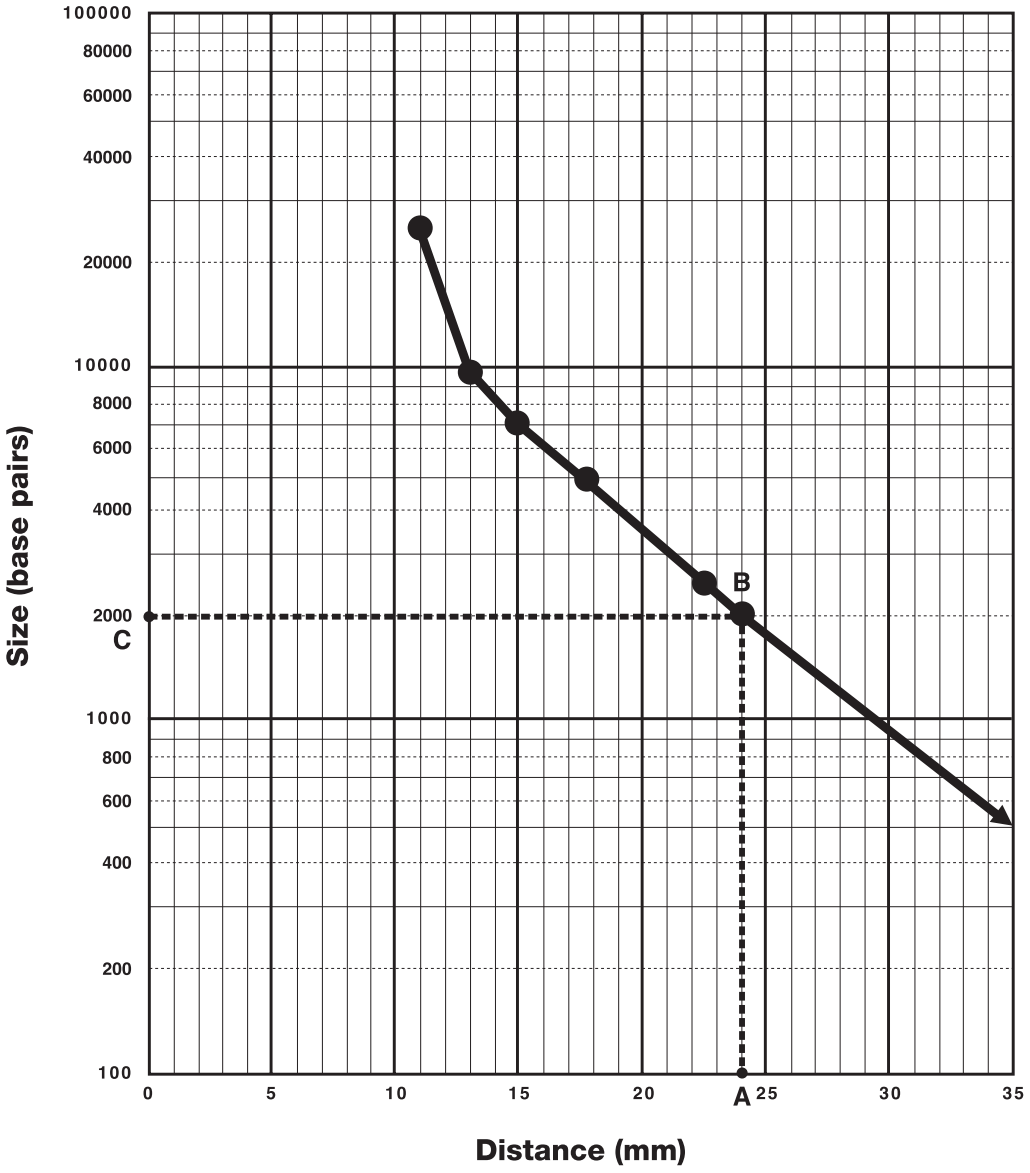
	Lambda/*Hin*dIII size marker		**Crime Scene**		**Suspect 1**		**Suspect 2**		**Suspect 3**		**Suspect 4**		**Suspect 5*** **	
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)
1	11.0	23,130	19.0	3,679	21.0	2,860**	21.0	2,860**	19.0	3,679	21.0	2,860**	21.0	2,860**
2	13.0	9,416	20.5	2,860**	23.5	1,199	25.0	1,700	20.5	2,860**	29.5	1,093	24.0	1,986
3	15.0	6,557	32.0	828	30.5	941	28.5	1,159	32.0	828			29.5	1,093
4	18.0	4,361*												
5	23.0	2,322												
6	24.0	2,027												

*This fragment may appear faint if the markers were not heated to 65 °C. Lamba *Hin*dIII digestion also generates bands of 564 and 125 bp that are usually too faint to see on a gel.

**The measured migration distance for these bands varies depending upon the thickness of the bands. See Appendix D to understand why the bands are so intense in S4 and S5.

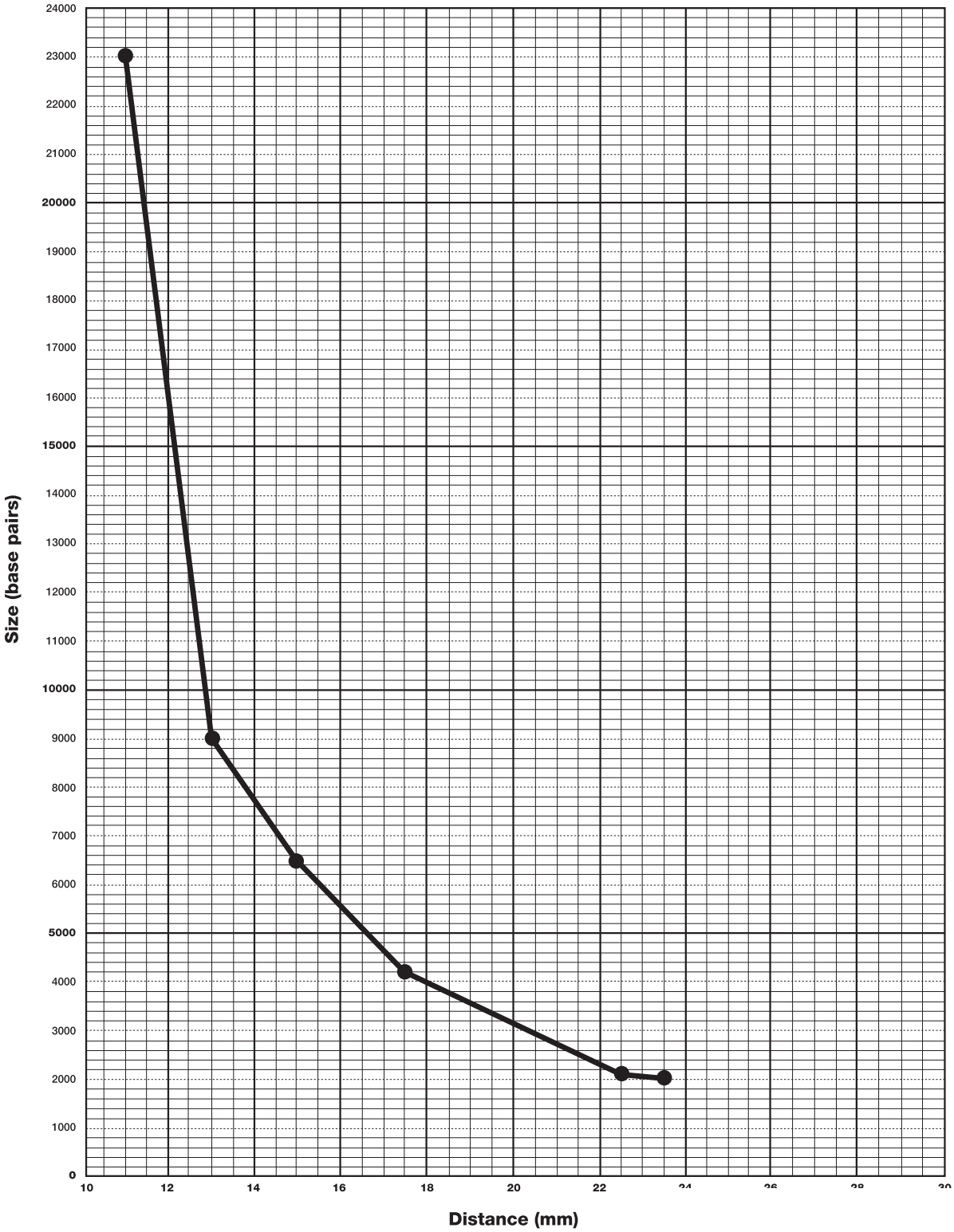
***S4 and S5 DNA lanes may also contain a very faint band of 500 bp.

DNA Standard Band Migration



To estimate the size of any unknown crime scene or suspect fragment, you first need to determine the distances the specific fragment travelled. Locate the distance on the X-axis of your standard graph. For example, Suspect 5, Band 2 migrated 24 mm (A). From the 24 mm mark on the X-axis, read up to the standard line; when you intersect your standard curve, mark the spot with a shaded circle (B). Follow the intersect point over to the Y-axis and determine where the graph line meets the Y-axis this is the approximate size of the fragment (C). Suspect 5, Band 2 is approximately 2000 bp. Repeat this procedure for the Crime Scene and all Suspects fragments. As you determine the approximate the approximate fragment sizes, fill the data into the data table.

Fingerprinting Standard Curve: Linear



Lesson 4 Analyzing DNA Patterns

Review Questions:

1. What are we trying to determine? Re-state the central question.

   We are trying to determine if samples of DNA that we were provided with are from the same individual or from different individuals.

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

   The number of fragmented samples will vary. They will have one band on the gel if the DNA was not cut.

3. What caused the DNA to become fragmented?

   The addition of restriction enzymes.

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

   A special sequence of bases on the DNA called restriction sites.

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

   The restriction sites are identical.

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?

   The samples in lanes 2 and 5 match (CS and S3).

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.

   The CS and S3 samples appear to be identical. They both produce similar banding patterns on the gel.

Prelab Activity 1 (from Appendix B)

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.

  There is no specific type of pattern associated with 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:

  A always pairs with T; G always pairs with C.

- 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?

  GAATTC.

  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"?

  4

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

  10

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

  No, it is larger.

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

  Fragment 1 is a 4 base pair fragment.

  Fragment 2 is a 10 base pair fragment.

  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?

  5

- 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?

  Random sized fragments will be produced.

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

  3

- Number each fragment.

- Which fragment would be the largest?

  Fragment 3.

- Which fragment would be the smallest?

  Fragment 2.

- 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?

  Answers will vary.

- Label each fragment

  Answers will vary.

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

  Answers will vary.

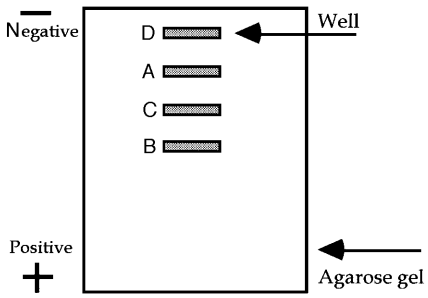
In this diagram. A and B are different palindrome sequences on a DNA strand. Only the restriction enzyme that recognizes site B is present.

- Explain why only two fragments would be produced.

  The enzyme would cut at site B, producing 2 DNA fragments.

Prelab Activity 2 Review of Electrophoresis

A 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 be separated. 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; towards the top of the gel or the bottom? Why?

  The large fragments would be toward the top of the gel because it is more difficult for the larger pieces to strain through the gel.
- Suppose you had 500 pieces of each of the four fragments, how would the gel appear?

  There would still be only 4 bands present.
- If it were possible to weigh each of the fragments, which one would be the heaviest? Why?

  Fragment D would be heaviest because it is the largest piece of DNA and would thus have the greatest mass.
- Complete this rule for the movement of DNA fragments through an agarose gel:

  The larger the DNA fragment, the slower it migrates through an agarose gel.

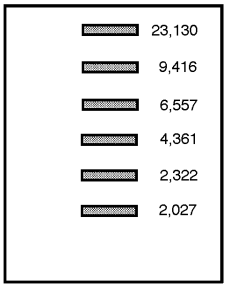
This diagram represents a piece of DNA cut with *Hin*dIII at each of the restrictions sites pointed to by the arrows. The numbers represent the number of base pairs in each fragment.

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

  6

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.

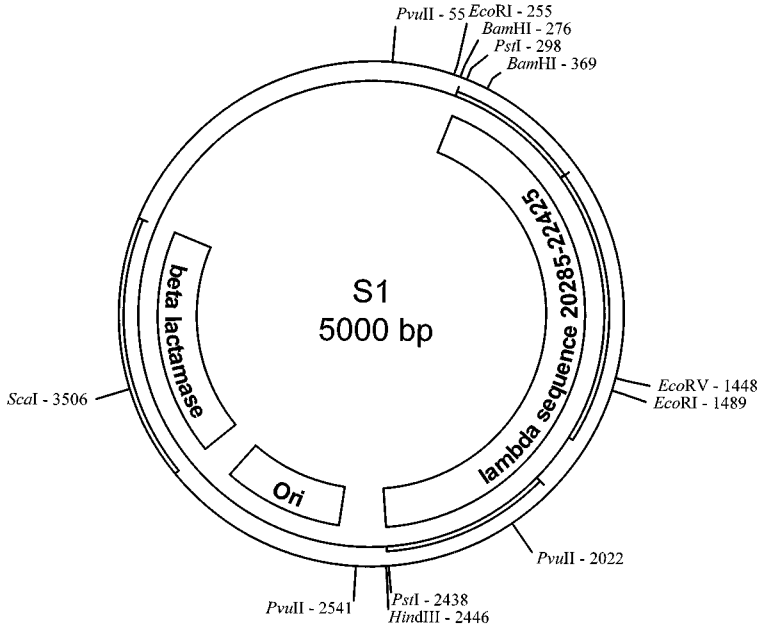
Scientists have taken advantage of plasmid DNA because its small size makes it easy to purify, and it can be reintroduced into bacterial cells using a procedure called transformation. Scientists have also benefited from another natural, bacterial defense mechanism: the restriction enzyme. Bacteria evolved 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.

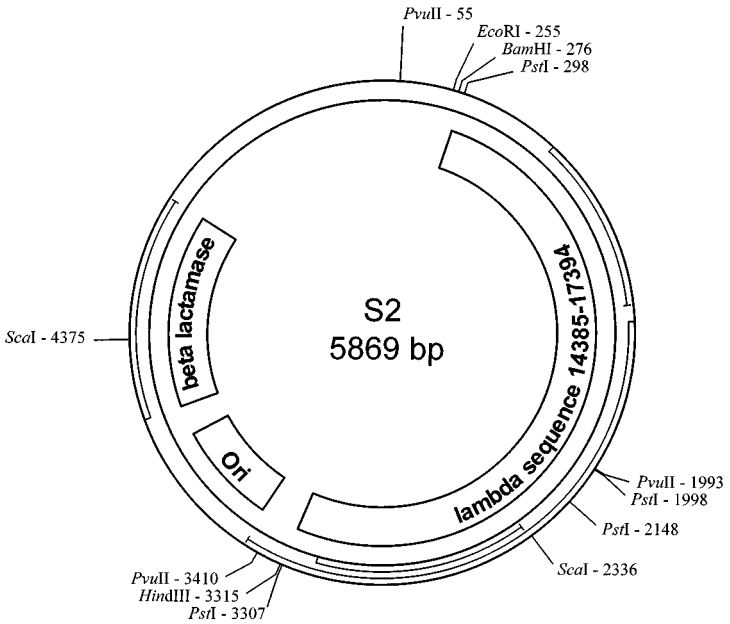
The Crime Scene and Suspect DNA samples in this kit were created by joining *Pst*I-digested bacteriophage lambda DNA with *Pst*I-digested plasmid vector pTZ18U. Recombinant plasmids were selected that gave distinct, striking banding patterns, or restriction fragment length polymorphisms (RFLPs again!),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

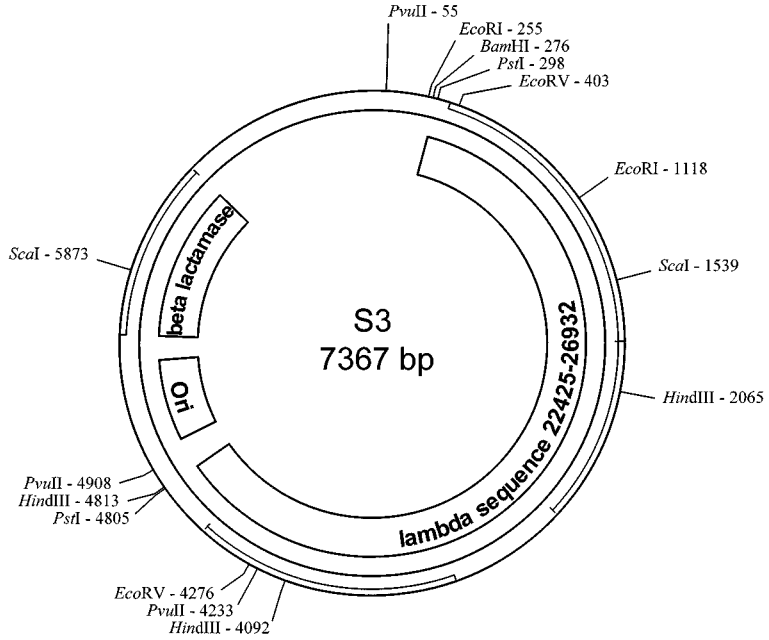
Plasmid Maps



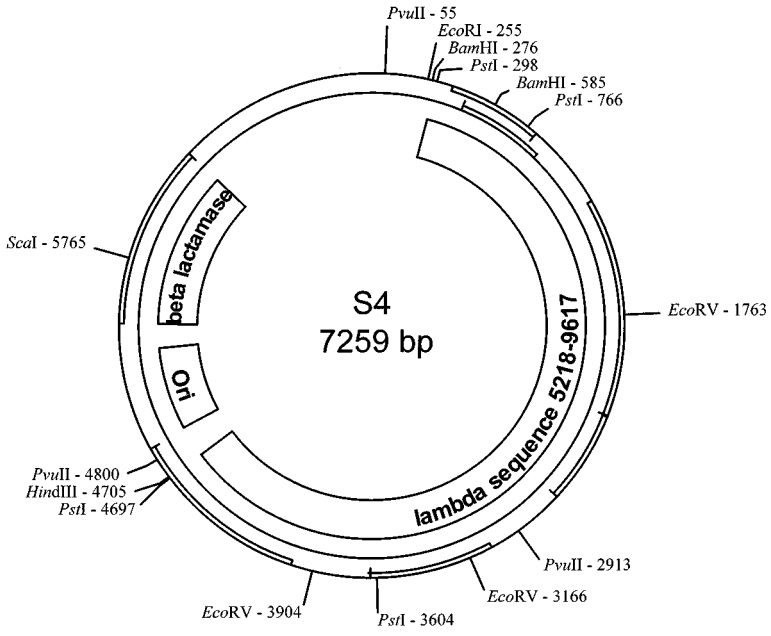
Suspect 1 DNA Sample



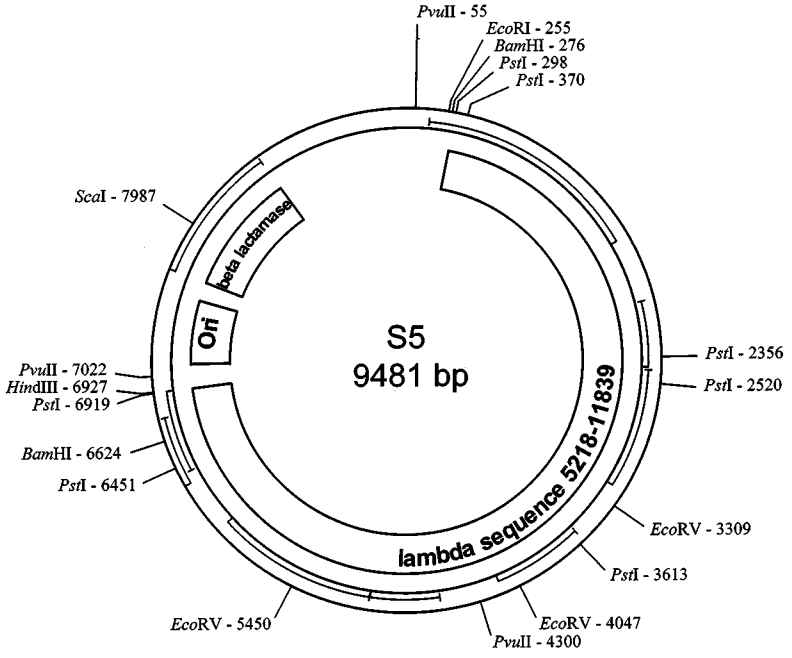
Suspect 2 DNA Sample



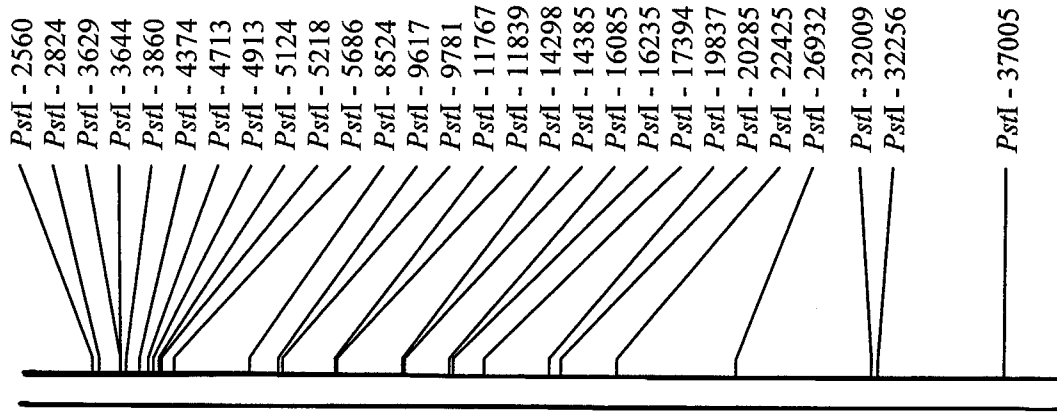
Crime Scene/Suspect 3 DNA Sample



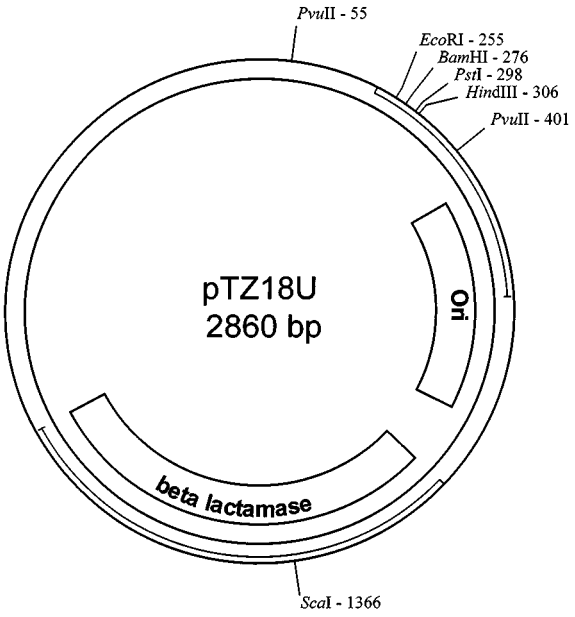
Suspect 4 DNA Sample



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*
Also in: ***Australia*** *Ph. 02 9914 2800, Fx. 02 9914 2889* ***Austria*** *Ph. (01) 877 89 01, Fx. (01) 876 56 29* ***Belgium*** *Ph. 09-385 55 11, Fx. 09-385 65 54*
Canada *Ph. (905) 712-2771, Fx. (905) 712-2990* ***China*** *Ph. 86-10-62051850/51, Fx. 86-10-62051876* ***Denmark*** *Ph. 45 39 17 99 47, Fx. 45 39 27 16 98*
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*
Hong Kong *Ph. 852-2789-3300, Fx. 852-2789-1257* ***India*** *Ph. (91-11) 461-0103, Fx. (91-11) 461-0765* ***Israel*** *Ph. 03 951 4127, Fx. 03 951 4129*
Italy *Ph. 39-02-216091, Fx.39-02-21609-399* ***Japan*** *Ph. 03-5811-6270, Fx. 03-5811-6272* ***Korea*** *Ph. 82-2-3473-4460, Fx. 82-2-3472-7003*
Latin America *Ph. 305-894-5950, Fx. 305-894-5960* ***Mexico*** *Ph. 514-2210, Fx. 514-2209* ***The Netherlands*** *Ph. 0318-540666, Fx. 0318-542216*
New Zealand *Ph. 64-9-4152280, Fx. 64-9-4152284* ***Norway*** *Ph. 22-74-18-70, Fx. 22-74-18-71* ***Russia*** *Ph. 7 095 979 98 00, Fx. 7 095 979 98 56*
Singapore *Ph. 65-2729877, Fx. 65-2734835* ***Spain*** *Ph. 34-91-661-7085, Fx. 34-91-661-9698* ***Sweden*** *Ph. 46 (0)8-55 51 27 00, Fx. 46 (0)8-55 51 27 80*
Switzerland *Ph. 01-809 55 55, Fx. 01-809 55 00* ***United Kingdom*** *Ph. 0800-181134, Fx. 01442-259118*

Sig 031799

4006096 Rev E