Biotechnology Explorer™ Forensic DNA Fingerprinting Kit

Instruction Manual

Catalog #166-0077EDU
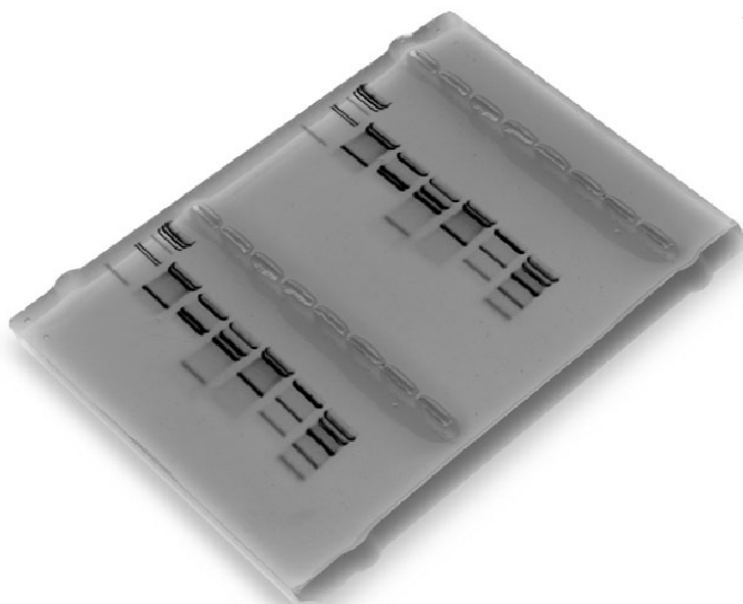
BIO·RAD

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 Fast Blast™ DNA stain. 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!

You can download this complete instruction manual on the Internet. Visit us on the Web at explorer.bio-rad.com or call us in the US at 1-800-424-6723.

This curriculum was developed in collaboration with Len Poli and Russ Janigian of the S.F. Base Biotechnology Program in San Francisco, California, and Peggy Skinner of the Bush School in Seattle, Washington. We'd like to thank them for their invaluable guidance and contributions to this curriculum.

Create context. Reinforce learning. Stay current.
New scientific discoveries and technologies
create more content for you to teach,
but not more time. Biotechnology
Explorer kits help you teach more
effectively by integrating multiple
core content subjects into a
single lab. Connect concepts
with techniques and put
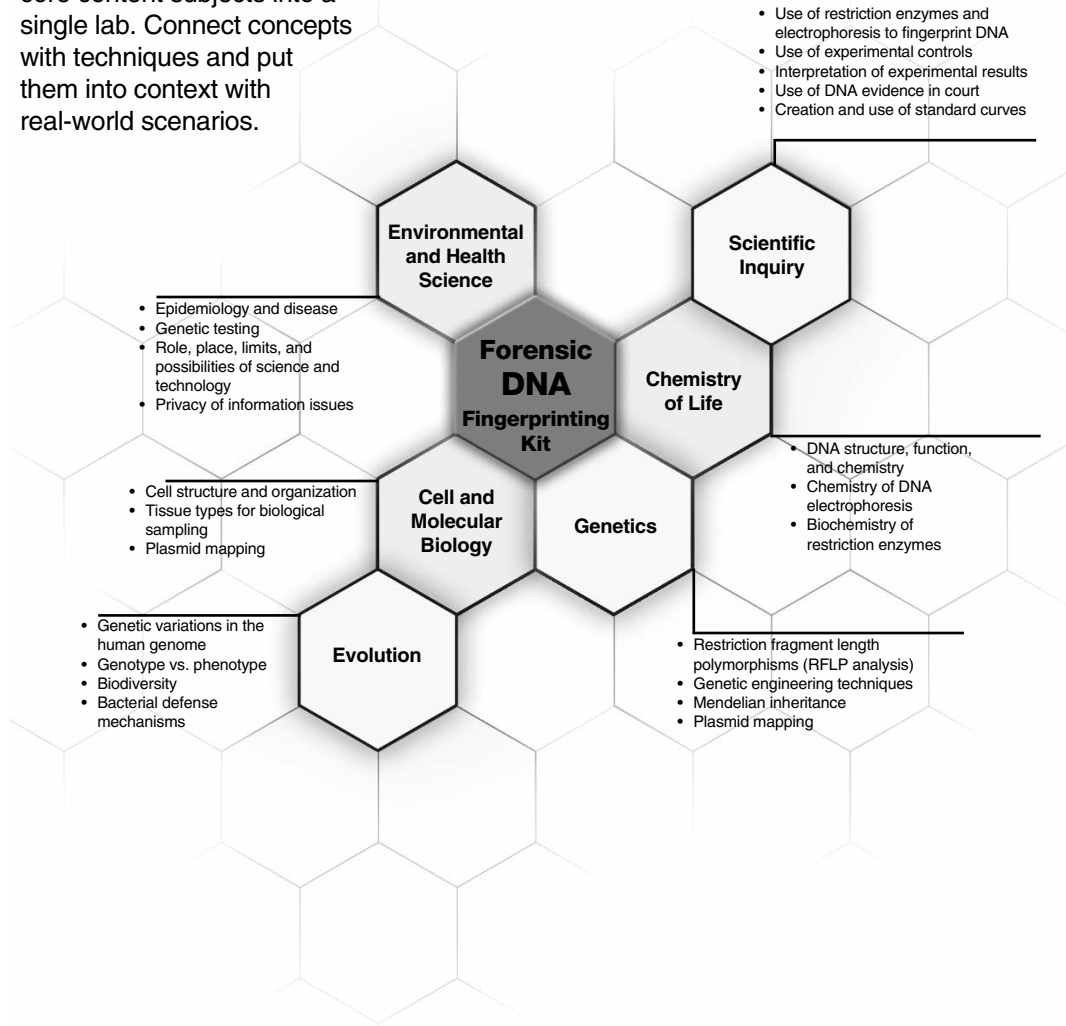them into context with
real-world scenarios.

- Use of restriction enzymes and
  electrophoresis to fingerprint DNA
- Use of experimental controls
- Interpretation of experimental results
- Use of DNA evidence in court
- Creation and use of standard curves

Environmental and Health Science

Scientific Inquiry

- Epidemiology and disease
- Genetic testing
- Role, place, limits, and possibilities of science and technology
- Privacy of information issues

Forensic DNA Fingerprinting Kit

Chemistry of Life

Cell and Molecular Biology

Genetics

- Cell structure and organization
- Tissue types for biological sampling
- Plasmid mapping

- DNA structure, function, and chemistry
- Chemistry of DNA electrophoresis
- Biochemistry of restriction enzymes

Evolution

- Genetic variations in the human genome
- Genotype vs. phenotype
- Biodiversity
- Bacterial defense mechanisms

- Restriction fragment length polymorphisms (RFLP analysis)
- Genetic engineering techniques
- Mendelian inheritance
- Plasmid mapping

Table of Contents

Overview for the Instructor

Intended Audience

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

Student Objectives

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 and analysis of plasmid mapping.

3) Weigh evidence and be able to analyze and interpret the data that are 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.

6) Use critical thinking to solve problems.

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 level 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.

Safety Issues

Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Wearing protective eyewear and gloves is strongly recommended. Students should wash their hands with soap before and after this exercise. If any of the solutions gets into a student's eyes, flush with water for 15 minutes. Although Fast Blast DNA stain is not toxic, latex or vinyl gloves should be worn while handling the stain to keep hands from becoming stained. Lab coats or other protective clothing should be worn to avoid staining clothes.

Storage Temperatures

The kit is shipped at room temperature. Open immediately upon arrival and store the reagent bag at –20°C within 3 weeks of receipt.

Kit Inventory Checklist

This section lists the equipment and reagents necessary to conduct the Forensic DNA Fingerprinting laboratory. We recommend that students be teamed up – two to four students per workstation.

Kit Components	Number/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. EcoRI/PstI, restriction enzyme mix, lyophilized, 3,000 units	1 vial	❏
8. Sterile water, 2.5 ml	1 vial	❏
9. HindIII lambda digest (DNA size standard), 0.2 µg/µl, 100 µl	1 vial	❏
10. DNA sample loading dye	1 vial	❏
11. Fast Blast DNA stain, 500x, 100 ml	1 vial	❏
12. Colored microcentrifuge tubes, 2.0 ml	60	❏
13. Clear microcentrifuge tubes, 1.5 ml	30	❏
14. Agarose powder, 5 g	1	❏
15. Electrophoresis buffer, 50x TAE, 100 ml	1	❏
16. Foam micro test tube holders	8	❏

Required Accessories	Number/Kit	(✔)
Adjustable micropipet, 2–20 µl, (catalog #166-0506EDU)	1–8	❏
Pipet tips, 2–200 µl 5 racks of 200, (catalog #223-9347EDU)	1	❏
Horizontal electrophoresis chamber, (catalog #166-4000EDU)	1–8	❏
Power supply, (catalog #164-5050EDU)	1–4	❏
Adjustable micropipet, 20–200 µl, (catalog #166-0507EDU, 166-0552EDU)	1	❏
Adjustable micropipet, 100–1,000 µl, (catalog #166-0508EDU, 166-0553EDU)	1	❏
Pipet tips, 100–1,000 µl, 5 racks of 200, (catalog #223-9350EDU)	1	❏
Permanent markers	1	❏
Microwave oven or hot plate	1	❏
Distilled water (4 L)	1	❏
500 ml Erlenmeyer flask for microwaving agarose	1	❏
500 ml flask or beaker for diluting DNA stain	1	❏
Ice bucket with ice	1	❏
Laboratory tape (not regular sticky tape)	1	❏
Gel Staining Trays (catalog #166-477EDU)	8	❏

Optional Accessories	Number/Kit	(✔)

37°C water bath (catalog #166-0504EDU), or
    dry bath (catalog #166-0562EDU, or
    mini incubation oven (catalog #166-0501EDU)
	1	❏

Microcentrifuge tube racks (catalog #166-0481EDU)	1	❏
Gel support film (50 sheets) (catalog #170-2984EDU)	1	❏
Microcentrifuge (catalog #166-0602EDU) or mini centrifuge (catalog #166-0603ED)	1	❏
Rocking platform (catalog #166-0709EDU, catalog #166-0710EDU)	1	❏
Vernier White Digital Bioimaging system (catalog #WHT-DBS)	1	❏

Refills Available Separately

Catalog #	Product Description
166-0027EDU	**DNA fingerprinting kit refill package** (contains crime scene and suspect DNA samples, EcoRI/PstI restriction enzyme mix, sample loading dye, DNA standards, sterile water)
166-0047EDU	**EcoRI/PstI restriction enzyme mix**
166-0450EDU	**Small DNA Electrophoresis Reagent Pack** (to pour, run & stain 48 1% or 16 3% 7x10 cm agarose gels)
166-0455EDU	**Medium DNA Electrophoresis Reagent Pack** (to pour, run & stain 270 1% or 90 3% 7x10 cm agarose gels)
166-0460EDU	**Large DNA Electrophoresis Reagent Pack** (to pour, run & stain 1080 1% or 360 3% 7x10 cm agarose gels)
166-0479EDU	**Jellyfish Foam Floating Racks**, 8 racks with 12 microcentrifuge tube wells
166-0477EDU	**Gel staining trays**, 4
166-0481EDU	**Green Racks**, set of 5 racks
161-0743EDU	**TAE**, 50x, 1 L
161-0773EDU	**TAE**, 50x, 5 L cube
161-0767EDU	**Sample Loading Dye**, 5x, 10 ml
161-3100EDU	**Certified™ Molecular Biology Agarose**, 25 g
161-3101EDU	**Certified Molecular Biology Agarose**, 125 g
161-3102EDU	**Certified Molecular Biology Agarose**, 500 g
166-0420EDU	**Fast Blast DNA Stain**, 500x, 100 ml
166-0450EDU	**Small Fast Blast DNA Electrophoresis Reagent Pack**
166-0455EDU	**Medium Fast Blast DNA Electrophoresis Reagent Pack**
166-0460EDU	**Large Fast Blast DNA Electrophoresis Reagent Pack**
166-0474EDU	**Disposable Plastic Transfer Pipets**, sterile, 500
166-0480EDU	**Disposable Plastic Transfer Pipets**, nonsterile, 500
166-0473EDU	**Colored 1.5 ml Microcentrifuge Tubes**, 6 colors, 600
223-9480EDU	**EZ Micro™ Test Tubes,** 1.5 ml, natural, 500
223-9430EDU	**EZ Micro Test Tubes**, 2.0 ml, natural, 500

Instructor's Manual Background

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. Restriction Fragment Length Polymorphism (RFLP) has been the workhorse of forensic DNA profiling for many years. First described by English geneticist Alec Jeffries in 1985, RFLP analysis provides a unique banding pattern based on the restriction sites present in an individual's DNA sequence. Currently, DNA profiling involves polymerase chain reaction (PCR) amplification which allows the analysis of minute quantities of DNA in a much shorter time. In this lab activity, students will compare band patterns produced by restriction enzyme cleavage of DNA samples when separated on an agarose gel (RFLP). 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

Scientists have benefited from a natural, bacterial defense mechanism: the restriction enzyme. A restriction enzyme acts like molecular scissors, making cuts at specific sequence of base pairs that it recognizes. These enzymes destroy DNA from invading viruses, or bacteriophages (phages). Phages are viruses that infect and destroy bacteria. Bacterial restriction enzymes recognize very specific DNA sequences within the phage DNA and then cut the DNA at that site. Although the bacteria's own DNA may also contain these sites the bacteria protect their own restriction sites by adding a methyl group. Once purified in the laboratory the fragmented phage DNA no longer poses a threat to the bacteria. These restriction endonucleases (endo = within, nuclease = enzyme that cuts nucleic acids) are named for the bacteria from which they were isolated. For example, EcoRI was isolated from _Escherichia coli_. Restriction enzymes can be used to cut DNA isolated from any source. Restriction enzymes were named before scientists understood how they functioned because they would limit (or restrict) the growth of phages. A restriction enzyme sits on a DNA molecule and slides along the helix until it recognizes specific sequences of base pairs that signal the enzyme to stop sliding. The enzyme then cuts or chemically separates the DNA molecule at that site—called a restriction site.

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 linear piece of DNA is cut with a restriction enzyme whose specific recognition site is found at two different locations on the DNA molecule, the result will be three fragments of different lengths. If the piece of DNA is circular and is cut with a restriction enzyme whose specific recognition site 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_. The resulting fragments can then be used to create a plasmid map – see page 47.

Agarose Gel Electrophoresis

Agarose gel electrophoresis separates DNA fragments by size. DNA fragments are loaded into an agarose gel slab, which is placed into a chamber filled with a conductive buffer solution. A direct current is passed between wire electrodes at each end of the chamber. Since DNA fragments are negatively charged, they will be drawn toward the positive pole (anode) when placed in an electric field. The matrix of the agarose gel acts as a molecular sieve through which smaller DNA fragments can move more easily than larger ones. Therefore, the rate at which a DNA fragment migrates through the gel is inversely proportional to its size in base pairs. Over a period of time, smaller DNA fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in single bands of DNA. These bands will be seen in the gel after the DNA is stained.

Consider this analogy. Imagine that all the desks and chairs in the classroom have been randomly pushed together. An individual student can wind his/her way through the maze quickly and with little difficulty, whereas a string of four students would require more time and have difficulty working their way through the maze.

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 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.

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. 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.

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* **recognition sequence** (four- or six- base pair (bp)) on a segment of DNA, it cuts the DNA molecule at that point. The recognition sequences for two commonly used enzymes, **EcoRI** and **PstI**, are shown below. The place on the DNA backbones where the DNA is actually cut is shown with a (✂) symbol:

For the enzyme **EcoRI**

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

For the enzyme **PstI**

C T G C A G
G A C G T C
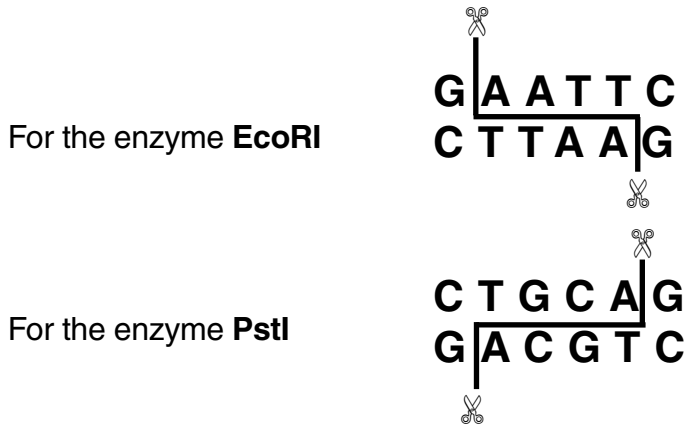
Like all enzymes, restriction enzymes function best under specific buffer and temperature conditions. The proper restriction enzyme buffer has been included with the DNA sample in this kit, so that when the rehydrated DNA and enzymes are mixed, the ideal conditions are created for the enzymes to function 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 EcoRI and PstI enzymes to function.

Making DNA Visible

DNA is colorless so DNA fragments in the gel cannot be seen during electrophoresis. A sample loading buffer containing two bluish dyes is added to the DNA samples. The loading dye does not stain the DNA itself but makes it easier to load the samples and monitor the progress of the DNA electrophoresis. The dye fronts migrate toward the positive end of the gel, just like the DNA fragments. Bromophenol blue, the "faster" dye, comigrates with DNA fragments of approximately 500 bp in a 1% agarose gel, while the xylene cyanol, the "slower" dye, comigrates with DNA fragments of approximately 4,000 bp in a 1% agarose gel.

Staining the DNA pinpoints its location on the gel. When the gel is immersed in Fast Blast DNA stain, the stain molecules attach to the DNA trapped in the agarose gel. When the bands are visible, your students can compare the DNA restriction patterns of the different samples of DNA.

The gel on page 7 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 lambda digest (DNA size standards). 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.

S	CS	S1	S2	S3	S4	S5	
1	2	3	4	5	6	7	8
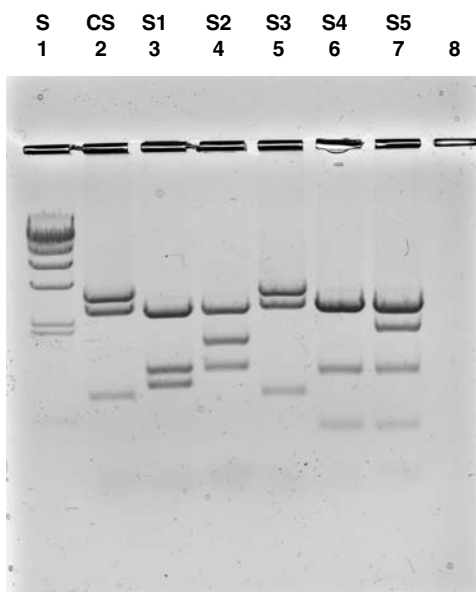
It is easy to see that the DNA taken from the crime scene and the DNA from S3 are identical. You may want to point out how "strong" or "weak" this evidence is in convicting a suspect. The DNA evidence may place the suspect at the scene, but other evidence may be needed to prove him or her guilty!

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.

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. In general one can assume that any two humans are 99.9% identical DNA sequence. Thus they will differ by only 1 bp in 1,000. It is necessary to examine areas that differ to create a useful DNA fingerprint.

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 have the same pattern as that taken from a crime scene: 1 in 1,000,000? 1 in 10,000? Or, 1 in 10?"

Plasmid Mapping Extension

The information contained in this kit can be used to perform an optional plasmid mapping activity extension. Plasmid mapping is a technique that allows molecular biologists to quickly evaluate the success of cloning experiments and to easily identify plasmids and associated traits in different organisms. The crime scene and suspect DNA samples in this kit do not contain human DNA but are constructed using plasmid DNA isolated from bacteria.

Plasmids are circular, non-chromosomal pieces of DNA that can replicate in and are commonly found in bacteria and simple eukaryotic organisms such as yeast. They typically carry accessory genes separate from the the organism's genomic DNA. In nature, bacteria evolved plasmids containing genes that enabled them to grow in the presence of antibiotics produced by other micro-organisms in the environment. This antibiotic resistance gave the bacteria harboring these plasmids a selective advantage over their competitors. Bacteria were able to pass the beneficial plasmid DNA to other bacteria via conjugation.

Scientists routinely take advantage of plasmid DNA because its small size makes it easy to purify, and once a genetically-engineered DNA sequence has been added it can be reintroduced into bacterial cells using a procedure called transformation. Plasmids cut with a restriction enzyme 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 perpetuated. Every hybrid plasmid now contains a 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". During this hybrid plasmid construction process, it is necessary to confirm that the foreign DNA has been successfully inserted into the host plasmid. While it is possible to have the complete DNA sequence of each construct determined, it is easier and quicker to use a restriction digestion to create a plasmid map.

Plasmids can be mapped (described) in terms of the location of restriction sites, using simple experiments and the use of logic. The general procedure is to digest a plasmid with two restriction enzymes separately (two single digests) and then together (a double digest). Sizes of fragments are then estimated by comparison with known standards. The sizes of the fragments from the single digests and the double digest are determined then logic is used to assess the relative location of restriction sites.

Instructor's Advance Preparation Guide

Implementation Timeline

There are four activities and two optional extension activities in this fingerprinting curriculum. All activities are designed to be carried out in consecutive 50 minute periods. Activities include:

- A series of prelab considerations for students

- An active student investigation

- Questions for analysis and interpretation of lab results

Student Schedule

Pre-Lab Activity: **Introduction to DNA Fingerprinting**

Activity
Lecture and discussion
Prelab considerations 1 and 2

Lesson 1 **Restriction Digest of DNA Samples**

Activity
Pour gels; perform the restriction digests
Complete preliminary analysis and review questions

Lesson 2 **Electrophoresis of DNA Samples**

Activity
Load and run gels; stain gels
Do analysis and review questions

Post-Lab Activity **Analysis and Interpretation of Results**

Activity
Do analysis questions
Generate standard curve
Discuss results and weigh evidence

Extension Activities

Activity 1 **Plasmid Mapping**

Work through questions

Activity 2 **Constructing a Plasmid**

Work through questions

Instructor's Advance Preparation

This sections outlines the recommended schedule for advanced preparation on the part of the instructor. A detailed Advance Preparation Guide is provided on pages 13–19.

Activity	When	Time required
Read 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 Fast Blast DNA stain, Prepare *Hin*dIII standard, Aliquot loading dye and prepared standard	Prior to Lesson 3	20 minutes
Set up workstations	The day of student labs	10 minutes/day

Workstation Checklist

Student Workstations. Materials and supplies that should be present at each student workstation prior to beginning each laboratory experiment are listed below. The components provided in this kit are sufficient for 8 student workstations (we recommend 2–4 students per workstation).

Teacher's (Common) Workstation. A list of materials, supplies, and equipment that should be present at a common location, which 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 and equipment, or whether the teacher should aliquot solutions and operate equipment. To avoid the potential for contamination and spills, you may choose to aliquot stock solutions of DNA and enzymes for the students. All other reagents should be kept at the front of the room for student teams to access as they need them.

Lesson 1 Restriction Digestion of DNA Samples

Student Workstation

Material	Quantity	(✔)
Agarose gel electrophoresis system (electrophoresis chamber, casting tray, 8-well comb)	1	❑
EcoRI/PstI enzyme mix	1 tube (80 µl)	❑
Pipet tips, 2–200 µl	15 tips	❑
Adjustable micropipet, 2–20 µl	1	❑
Colored microcentrifuge tubes: green, blue, orange, violet, pink, yellow	1	❑
Microcentrifuge tube rack	1	❑
Permanent marker	1	❑
Waste container	1	❑
Foam micro test tube holder	1	❑
Laboratory tape (not 3M Scotch brand or similar tape)	1	❑

Common Workstation

Material		(✔)
Crime scene (CS) DNA with buffer, rehydrated	1 vial	❑
Suspect 1 (S1) DNA with buffer, rehydrated	1 vial	❑
Suspect 2 (S2) DNA with buffer, rehydrated	1 vial	❑
Suspect 3 (S3) DNA with buffer, rehydrated	1 vial	❑
Suspect 4 (S4) DNA with buffer, rehydrated	1 vial	❑
Suspect 5 (S5) DNA with buffer, rehydrated	1 vial	❑
Molten 1% agarose in 1x TAE(See Advance Prep page 14)	40–50 ml per gel	❑
37°C water bath, dry bath, or incubator (optional)	1 per class	❑
Microcentrifuge	1 per class	❑
or mini centrifuge (optional)	4 per class	❑

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 2 Electrophoresis of DNA Samples

Student Workstation

Material	Quantity	(✔)
Agarose gel electrophoresis system	1	❑
Agarose gel	1	❑
Digested DNA samples	6	❑
Microcentrifuge tube rack	1	❑
HindIII lambda digest (DNA standard)	1	❑
DNA sample loading dye	1	❑
Permanent marker	1	❑
Pipet tips, 2–20 µl	13	❑
Adjustable micropipet, 2–20 µl	1	❑
Waste container	1	❑
Gel support film (if applicable)*	1	❑
Fast Blast DNA stain, 1x or 100x*	120 ml per 2 stations	❑
Large containers for destaining (if applicable)*	1–3 per 2 stations	❑
Foam micro test tube holder	1	❑
Power supply	1	❑
Gel staining tray	1 per 2 stations	❑
Electrophoresis buffer (1x TAE)	275 ml per station	❑

Common Workstation

Material		
Microcentrifuge	1	❑
or mini centrifuge (optional)	4	❑
Rocking platform (optional)	1	❑

Post-Lab Activity: Analysis of Results

Student Workstation

Material	Quantity	(✔)
Millimeter ruler	1	❑
Semilog graph paper	1	❑
Gel support film (if applicable)*	1	❑

Common Workstation

None required

* Depending on whether the quick or overnight staining will be followed.

Instructor's Advance 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 1 Restriction Digestion of DNA Samples

Advance Preparation

Objectives: Rehydrate DNA/buffer samples and restriction enzymes
Aliquot restriction enzymes
Pour agarose gels to prepare for lesson 2. **If you prefer to have your students pour their own gels during the lab, prepare the molten agarose ahead of time. If prepared in advance, molten agarose should be kept in a water bath set at 50–55°C until gels are poured.**
Set temperature of 37°C for water bath or incubator
Set up student and instructor workstations

Time required: Thirty minutes to 1 hour, depending on how you choose to prepare agarose gels

What's required: Horizontal electrophoresis gel chamber, casting trays, and combs
Electrophoresis buffer (50x TAE)
Agarose powder
8 clear microtubes
3 liters distilled water

Procedures

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 EcoRI/PstI enzyme mix is in an amber vial.

1. **Rehydrate DNA samples**

   A. To rehydrate DNA samples, carefully remove the stopper and add 200 μl of sterile water to each lyophilized DNA vial. Replace the stopper and vigorously shake the vial. It is critical to dissolve all the powder, some of which may be stuck to the stopper. Allow DNA/buffer samples to rehydrate at room temperature for 15 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 EcoRI and PstI enzymes to function.

2. **Rehydrate lyophilized EcoRI/PstI enzyme mix.** To rehydrate EcoRI/PstI 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**.

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

4. **Prepare agarose gels.*** The recommended agarose concentration for gels in this classroom application is 1% agarose. This concentration of agarose provides good resolution and minimizes run time required for electrophoretic separation of DNA fragments. The recommended thickness for the gel is 0.75–1.0 cm for easy sample loading and gel handling. **Be sure to use electrophoresis buffer, not water, to prepare agarose gels.** (See Appendix D for alternative Fast Gel Protocol)

   a. **Electrophoresis buffer preparation.** TAE (Tris-acetate-EDTA) electrophoresis buffer is provided 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 prepare 8 agarose gels. To make 3 L of 1x TAE from 50x TAE concentrate, add 60 ml of 50x concentrate to 2.94 L of distilled water.

   b. **Agarose preparation**. 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.

      i. To make a 1% agarose solution, use 1 gram of agarose for each 100 ml of 1x TAE electrophoresis buffer. Be sure to use electrophoresis buffer, not water.

         If electrophoresis chambers 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 ml	100 ml
4	160 ml	200 ml
8	320 ml	400 ml

   ii. 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 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. Heated agarose can quickly boil over so watch the process carefully.

Caution: Always wear protective gloves, goggles, and lab coat while preparing and casting agarose gels. Boiling molten agarose or the flasks 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 transparent agarose particles are dissolved. Set aside and cool to 55–60°C before pouring.

* Convenient precast agarose gels (catalog #161-3057EDU) are available from Bio-Rad. These are 2 x 8-well, 1% TAE gels and fit into Bio-Rad's Mini-Sub Cell GT cell or any horizontal gel electrophoresis system that fits 7 x 10 cm gels.

Magnetic hot plate method. Add a stirbar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Bubbles or foam should be disrupted before rising to the neck of the flask.

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

c. **Procedure for Casting Gels**

This laboratory activity requires that each gel has at least 7 wells. Follow the instructions above to prepare the agarose and to determine what volume of 1% agarose will be needed for your class(es). 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. Solidified gels can be stored in sealable bags at room temperature for 1 day or in the refrigerator for up to 1 week before using. 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. This section outlines the tape-the-tray method for casting gels. Other methods are detailed in the Sub-Cell GT cell (electrophoresis chamber) instruction manual.

   i.   Seal the ends of the gel tray securely with strips of standard laboratory tape (not Scotch tape or similar). Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.

   ii.   Level the gel tray on a leveling table or workbench using the leveling bubble provided with the chamber.

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

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

   v.   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 1/2 inch of the end of the gel casting tray if a single-well, 7 x 7 cm gel is cast. To pour a double-well gel using a 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. The combs will form the wells into which the samples will be loaded.

   vi.   Allow the gel to solidify at room temperature for 10 to 20 minutes. It will appear cloudy, or opaque, when ready to use.

   vii.   Carefully remove the comb from the solidified gel.

   viii. Remove the tape from the edges of the gel tray.

   ix.   You have two options:

Option 1: If you do not have sufficient time to proceed to Lesson 2, store gels in a sealable plastic bag with 1–2 ml of 1x TAE running buffer at room temperature for 1 day or in the refrigerator (4°C) for up to 1 week before using. Have your students label their plastic bags.

Option 2: If there is sufficient time to proceed to Lesson 2, place the tray onto the leveled DNA electrophoresis chamber so that the sample wells are at the black (cathode) end of the base. DNA samples will migrate towards the red (anode) end of the chamber during electrophoresis.

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 floats, 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.

Practice Using Micropipets (Optional)

We recommend that you familiarize your students with proper pipeting techniques prior to Lesson 1. Have your students learn how to transfer different volumes of a solution from one tube into another with a micropipet. Students may practice by using either sample loading dye or food coloring mixed with either a dense saturated sugar or glycerol solution. Here is a quick summary on how to use micropipets:

1. Look at the micropipet to determine the volume range.

2. Twist the dial on the micropipet to set the desired volume.

3. Attach a clean pipet tip.

4. Press the micropipet plunger to the **first** (soft) stop.

5. Insert the pipet tip into the solution to be transferred.

6. **Slowly** release the plunger to retrieve the liquid.

7. Insert the pipet tip into the desired tube.

8. Press the plunger past the first stop to the **second** (hard) stop to transfer the liquid. Make sure to keep the plunger pressed when lifting the pipet tip out of the tube.

9. Eject the pipet tip.

Lesson 2 Agarose Gel Electrophoresis and Visualization of DNA Fragments

Advance Preparation

Objectives	Prepare HindIII lambda digest (DNA standard) and aliquot (optional)
	Aliquot sample DNA loading dye (optional)
	Prepare the electrophoresis chamber
	Dilute Fast Blast DNA strain to 1x (for overnight staining)
	or 100x concentration (for quick staining)
	Set up student and teacher workstations
Time required	45 minutes
What is required	HindIII lambda digest (DNA standard)
	Sample loading dye
	Electrophoresis chambers, casting trays, and combs
	Electrophoresis buffer (1x TAE)*
	Fast Blast DNA stain, 500x

Procedures

1. **Prepare HindIII lambda digest (DNA standard) and aliquot (optional).** Add 20 µl of DNA sample loading dye to the stock tube containing the HindIII lambda digest DNA standard. Heat the standard to 65°C for 5 minutes, then chill on ice — this results in better separation of the standard bands. Label clear microcentrifuge tubes "S". Aliquot 15 µl of the DNA standards containing loading dye to 8 clear microcentrifuge tubes labeled "**S**".

2. **Aliquot DNA sample loading dye.** Label eight clean microcentrifuge tubes "**LD**" for loading dye and aliquot 50 µl of sample loading dye into each tube. Distribute one tube to each team.

3. **Prepare the electrophoresis chamber.** When the agarose gel has solidified, sample loading and electrophoresis can begin.

    a. When placing the gel tray into the electrophoresis chamber, make sure that the sample wells are at the black cathode end. DNA samples will migrate toward the red anode end during electrophoresis. Make sure the tray is fully seated in the chamber.

    b. Prepare the required volume of 1x TAE buffer, if you have not prepared it already.

    c. Submerge the gel under about 2 mm of 1x TAE buffer.

    d. Prepare samples for gel loading. See laboratory protocol in the student section.

Note: Power requirements vary depending on gel thickness, length, and concentration, and on type of electrophoresis buffer used. For this exercise we recommend using a constant voltage of 100 V for 30 min. See Appendix D for a faster electrophoresis protocol which allows the gel to be run in 20 min.

4. **Prepare Fast Blast DNA stain**

    a. To prepare 100x stain (for quick staining), dilute 100 ml of 500x Fast Blast with 400 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

    b. To prepare 1x stain (for overnight staining), dilute 1 ml of 500x Fast Blast with 499 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

* 0.25 x TAE buffer is used for fast gel electrophoresis. Refer to Appendix D for detailed information.

Making DNA Visible

Fast Blast DNA stain is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA in agarose gels following electrophoresis. Fast Blast contains a cationic compound that is in the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA molecules. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results and detects as little as 50 nanograms of DNA.

Fast Blast DNA stain is provided as a 500x concentrate that must be diluted prior to use. The stain can be used as a quick stain when diluted to a **100x** concentration to allow the visualization of DNA within 12–15 minutes or used as an overnight stain when diluted to **1x** concentration. When the agarose gel is immersed in Fast Blast DNA stain, the dye molecules attach to the DNA molecules trapped in the agarose gel. When the DNA bands are visible, your students can compare the DNA restriction patterns of the different samples of DNA.

Detailed instructions on using Fast Blast are included in the student manual.

WARNING

Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or a 70% alcohol solution to remove Fast Blast from most surfaces. Verify that these solutions do not harm the surface prior to use.

Note:

- We recommend using 120 ml of diluted Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in each staining tray (if using catalog #166-0477EDU, staining trays. You may want to notch gel corners for identification). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

- Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand.

- Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another during the destaining steps involved with the quick staining protocol.

- Destaining (when performing the quick staining protocol) requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

- 100x Fast Blast can be reused at least seven times.

- No washing or destaining is required when using the overnight staining protocol.

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, take a photograph with a digital camera, or digital gel imaging system (Bio-Rad's Gel Doc™ EZ imaging system with white light sample tray, catalog #170-8270EDU and 170-8272EDU, Gel Doc XR+ imaging system, catalog #170-8195EDU, or Vernier's White BioImaging System, catalog #WHT-DBS), photocopy the gel or scan the gel.

Drying the Agarose Gel as a Permanent Record of the Experiment

Note: Drying agarose gels requires the use of Bio-Rad's specially formulated high-strength analytical grade agarose. Other gel media may not be appropriate for this purpose.

We recommend using Bio-Rad's exclusive **gel support film** (catalog #170-2984EDU) to dry agarose gels. Remove the stained agarose gel from its staining tray and trim away any unloaded lanes with a knife or razor blade. 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 and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry, making sure to avoid direct exposure to light. As the gel dries, it will bond to the film but 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.
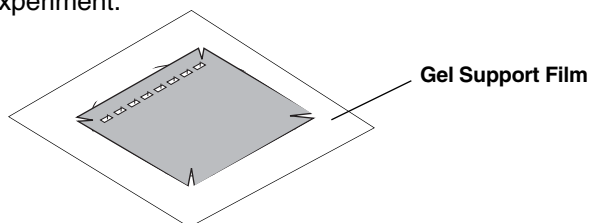
Gel Support Film

Note: Avoid extended exposure of dried gels to direct light to prevent band fading. However, DNA bands will reappear if the dried gels are stored in the dark for 2–3 weeks after fading.

Graphing the Data

Many of your students may not be familiar with logarithms and semilog graph paper. It is suggested that you prepare a short lesson to demonstrate the proper way to label the coordinates and plot the points. You might also choose to discuss the advantage of using semilog vs. standard graph paper in this instance. A math extension here can also provide an opportunity to explore linear and exponential (arithmetic and geometric) sequences of numbers. We have included semilog graph paper on page 44 of this manual.

Forensic DNA Fingerprinting Kit Quick Guide

Lesson 1 Restriction Digestion

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

2. Label one of each colored microcentrifuge tubes as follows:

 | | |
   |---|---|
   | green tube | **CS** (crime scene) |
   | blue tube | **S1** (suspect 1) |
   | orange tube | **S2** (suspect 2) |
   | violet tube | **S3** (suspect 3) |
   | pink tube | **S4** (suspect 4) |
   | yellow tube | **S5** (suspect 5) |

   Label the tubes with your name, date, and lab period. Place the tubes in your microcentrifuge tube rack.
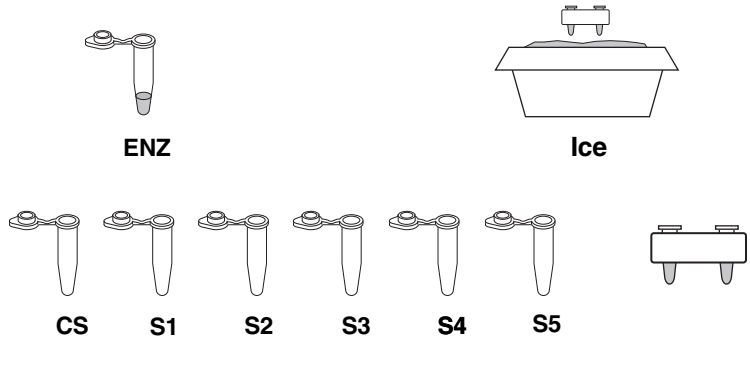
3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored microcentrifuge tubes. Make sure the sample is transferred to the bottom of the tubes.

4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well.
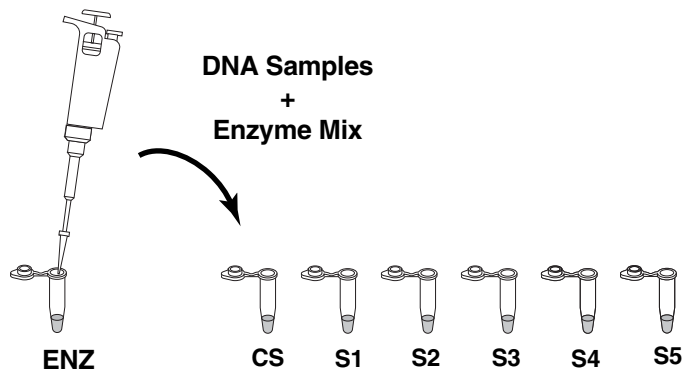
5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse-spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.
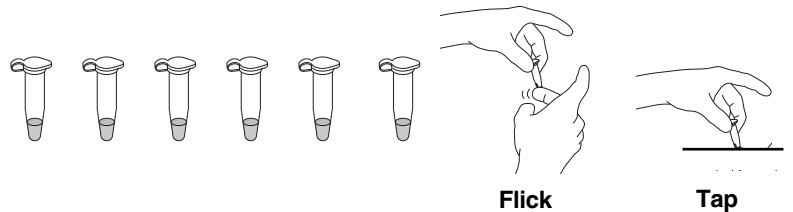
6. Incubate the tubes for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.
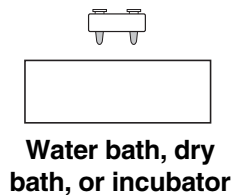
7. If required, follow the instructors directions to pour a 1% agarose gel.

8. After the incubation period place the tubes in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to step 2 of Lesson 2.

ENZ

Ice

CS   S1   S2   S3   S4   S5

DNA Samples + Enzyme Mix

ENZ   CS   S1   S2   S3   S4   S5

Flick   Tap

Water bath, dry bath, or incubator

20
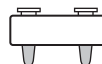
Lesson 2 Agarose Gel Electrophoresis

1. Remove the digested DNA samples from the refrigerator (if applicable).
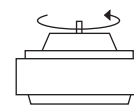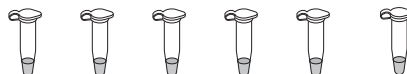
Centrifuge

2. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.

3. 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. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.

DNA Loading Dye

4. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap.

5. Place the agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer* to cover the gel, using approximately 275 ml of buffer for a Bio-Rad Mini-Sub Cell, horizontal electrophoresis chamber.

(−)   (+)
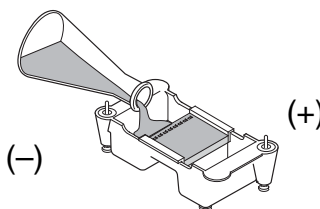
6. Check that the wells of the agarose gels are near the black (−) electrode and the bottom edge of the gel is near the red (+) electrode.

7. 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:   **S**, DNA size standard, 10 µl
    Lane 2:   **CS**, green tube, 20 µl
    Lane 3:   **S1**, blue tube, 20 µl
    Lane 4:   **S2**, orange tube, 20 µl
    Lane 5:   **S3**, violet tube, 20 µl
    Lane 6:   **S4**, red tube, 20 µl
    Lane 7:   **S5**, yellow tube, 20 µl
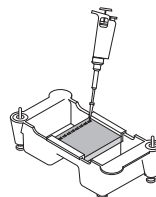
8. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid of the horizontal electrophoresis chambers will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.
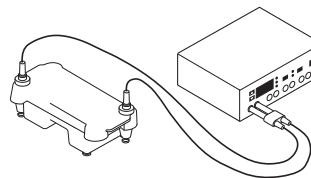
9. Turn on the power and electrophorese your samples at 100 V for 30 minutes.

* or 0.25x TAE if using the Fast Gel Protocol

Student Manual

Visualization of DNA Fragments

1. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.
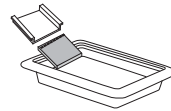
2. You have two options for staining your gel:

   Quick staining (requires 12–15 minutes)

   a. Add 120 ml of **100x** Fast Blast DNA stain into a staining tray (2 gels per tray).

   b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.

   c. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.

   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.

   e. Record results.

   f. Trim away any unloaded lanes.

   g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.
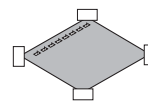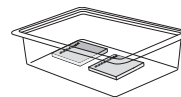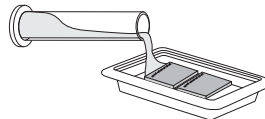
   Overnight staining

   a. Add 120 ml of **1x** Fast Blast DNA stain to a staining tray (2 gels per tray).

   b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.

   c. Pour off the stain into a waste beaker.

   d. Record results.

   e. Trim away any unloaded lanes.

   f. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.
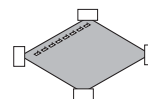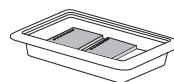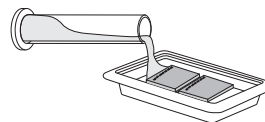
Student Manual

Pre-Lab 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.

DNA consists of a series of nitrogenous base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar-phosphate backbone. The four nitrogenous bases are **adenine**, **thymine**, **guanine**, and **cytosine** (**A**, **T**, **G**, and **C**). Remember the base-pairing rule is **A** - **T** and **G** - **C**. Refer to the figure below of a DNA molecule.

The Structure of DNA

| DNA MOLECULE #1 | DNA MOLECULE #2 | DNA MOLECULE #3 |
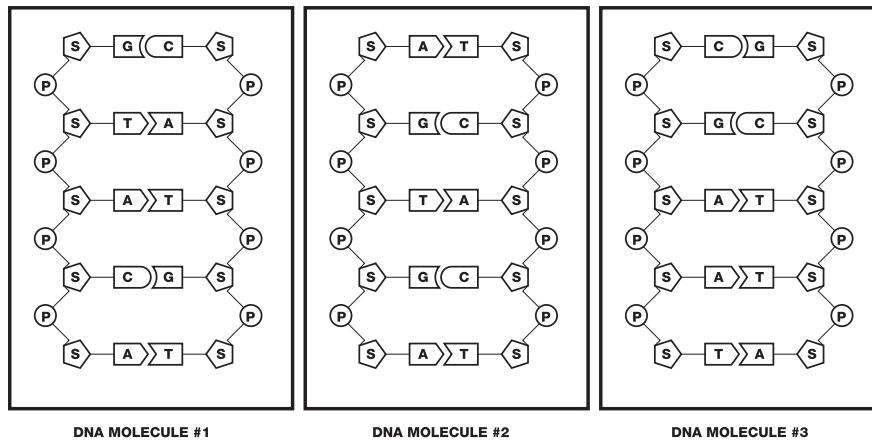
The schematics above represent a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows:

Backbone:

S = Five carbon sugar molecule known as deoxyribose

P = Phosphate group

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.

Pre-Lab Focus Questions: Introduction to DNA Fingerprinting

Consideration 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 1 Restriction Digestion of DNA Samples

Consideration 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 historically important discoveries **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**.

Two common restriction enzymes (endonucleases) are EcoRI and PstI which will be provided to you in this lab procedure. To better understand how EcoRI and PstI may help you in performing your DNA fingerprinting experiment, first you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA:

A T GA A T T C T C A A T T A C C T
T A C T T A AG A G T T A A T G G A

The line through the base pairs represents the sites where bonds will break if the restriction endonuclease EcoRI 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 the DNA fragments on both the left and right side of the "cut".

    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 the restriction enzyme EcoRI [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 1 Restriction Digestion of DNA Samples

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 you have learned 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 Checklist

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

Student Workstation

Material	Quantity	(✔)
Agarose gel electrophoresis system (electrophoresis chamber, casting tray, 8-well comb)	1	❑
EcoRI/PstI enzyme mix	1 tube (80 µl)	❑
Pipet tips, 2–200 µl	15 tips	❑
Micropipet, 2–20 µl	1	❑
Colored microcentrifuge tubes:		
green, blue, orange, violet, pink, yellow	1	❑
Permanent marker	1	❑
Waste container	1	❑
Microcentrifuge tube rack	1	❑
Foam micro test tube holder	1	❑
Laboratory tape (not regular sticky tape)	1	❑

### Common Workstation

Material	Quantity	(✔)
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	❑
Molten 1% agarose in 1x TAE (See Advance Prep)	40–50 ml per gel	❑
37°C water bath, dry bath, or incubator (optional)	1 per class	❑
Microcentrifuge	1 per class	❑
or mini centrifuge (optional)	4 per class	❑

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 a new pipet tip for each sample, pipet 10 µl of the enzyme mix "ENZ" to each reaction tube as shown below. Pipet up and down carefully to mix well.

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.

ENZ   CS   S1   S2   S3   S4   S5
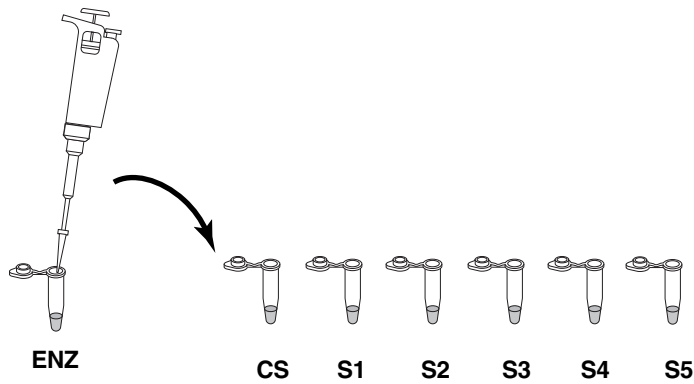
Now your DNA samples should contain:

| Total DNA Samples (10 µl each)	EcoRI/PstI Enzyme Mix	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 tube contents.

   Tightly cap on each tube. 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.

   CS   **S1**   **S2**   **S3**   **S4**   **S5**   **Flick**   **Tap**
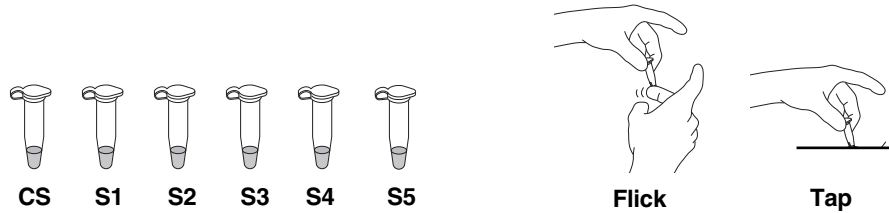
6. Incubate the samples.

   Incubate the tubes 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, or proceed directly to step 2 of Lesson 2 if instructed by your teacher.
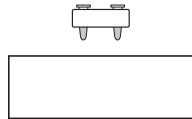
   **Water bath, dry
   bath, or incubator**

   Note: While you are waiting, this is a good time to cast your agarose gel, unless they have already been prepared for you. Check with your teacher for the proper procedure.

Lesson 1 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 EcoRI/PstI? 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 the restriction digest)

   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 2 Agarose Gel Electrophoresis (Laboratory Procedure)

Student Workstation

Material	Quantity	(✔)
Agarose gel electrophoresis system	1	❑
Agarose gel	1	❑
Digested DNA samples	6	❑
HindIII lambda digest (DNA standards)	1	❑
DNA sample loading dye	1	❑
Permanent marker	1	❑
Pipet tips, 2–20 µl	13	❑
Micropipet, 2–20 µl	1	❑
Waste container	1	❑
Gel support film (if applicable)*	1	❑
Fast Blast DNA stain, 1x or 100x*	120 ml per 2 stations	❑
Large containers for destaining (if applicable)*	1–3 per 2 stations	❑
Microcentrifuge tube rack	1	❑
Power supply	1	❑
Gel staining tray	1 per 2 stations	❑
Foam micro test tube holder	1	❑
Electrophoresis buffer (1x TAE)**	275 ml per station	❑

Common Workstation

Material	Quantity	(✔)
Microcentrifuge	1	❑
or mini centrifuge (optional)	4	❑
Rocking platform (optional)	1	❑

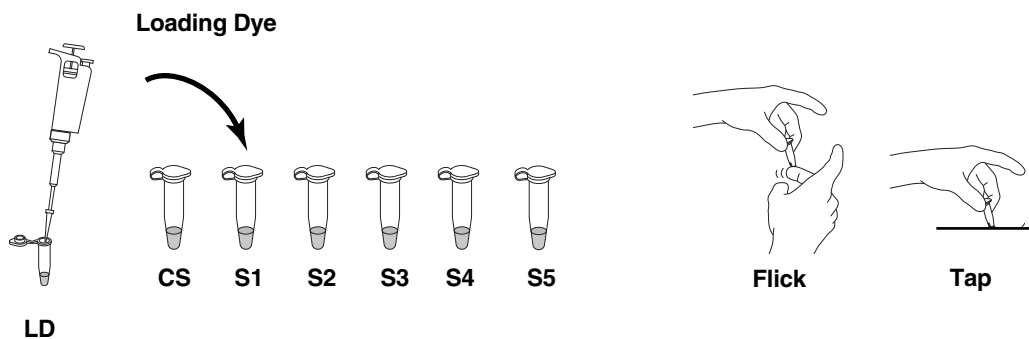*If performing the quick staining procedure.

** 0.25 x TAE buffer is used for fast gel electrophoresis. Refer to Appendix D for detailed information.

Lesson 2 Agarose Gel Electrophoresis (Laboratory Procedure)

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

Loading Dye

CS   S1   S2   S3   S4   S5   Flick   Tap

LD

   Tightly cap each tube. 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, gently tap the tubes on the 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 of the gel by 1–2 mm.
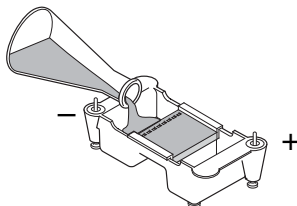

5. Obtain the tube of *Hin*dIII lambda digest (DNA standard). The loading dye should already have been added by your instructor.

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6. Using a separate pipet tip for each sample, load your digested DNA samples into the gel. Gels are read from left to right. The first sample is loaded in the well at the left hand corner of the gel.

    Lane 1:   HindIII lambda digest (DNA standards), clear tube, 10 µl

    Lane 2:   CS, green tube, 20 µl

    Lane 3:   S1, blue tube, 20 µl

    Lane 4:   S2, orange tube, 20 µl

    Lane 5:   S3, violet tube, 20 µl

    Lane 6:   S4, red tube, 20 µl

    Lane 7:   S5, yellow tube, 20 µl
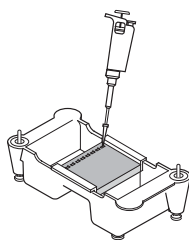
7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect electrical leads to the power supply.

8. Turn on the power supply. Set it for 100 V and electrophorese the samples for at least 30 min. The gel can be run for up to 40 min to improve resolution if the time is available. The Fast Gel Protocol in Appendix D allows the gel to be run in 20 min at 200 V.
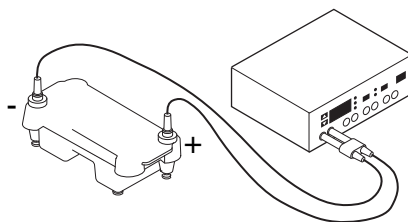
While you are waiting for the gel to run, you may begin the review questions on the following page.

9. When the electrophoresis is complete, turn off the power supply and remove the lid from the gel box. Carefully remove the gel tray and the gel from the electrophoresis chamber. Be careful, the gel is very slippery! Proceed to pg 35 for detailed instructions on staining your gel.

Lesson 2 Agarose Gel Electrophoresis

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.

Staining DNA with Fast Blast DNA Stain (Laboratory Procedure)

Consideration: Are any of the DNA samples from the suspects the same as that of the 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

Since DNA is naturally colorless, it is not immediately visible in the gel. Unaided visual examination of the gel after electrophoresis 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 stain called Fast Blast DNA stain. The blue stain molecules are positively charged and have a high affinity for the DNA. These blue stain molecules strongly bind to the DNA fragments and allow DNA to become visible. These visible bands of DNA may then be traced, photographed, sketched, or retained as a permanently dried gel for analysis. Detained instruction on staining your gel are found on the following pages.

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 endonuclease.

With reference to the numbered lanes, analyze the bands in the gel drawing below, then answer the questions on page 40. Note that this picture is an example and it may not correspond to the pattern of bands that you will see in the lab.
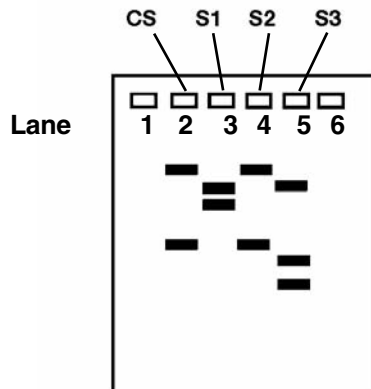
Staining DNA with Fast Blast DNA Stain (Laboratory Procedure)

There are two protocols for using Fast Blast DNA stain in the classroom. Use option 1 for quick staining of gels to visualize DNA bands in 12–15 minutes, and option 2 for overnight staining. Depending on the amount of time available, your teacher will decide which protocol to use. Two student teams will stain the gels per staining tray (you may want to notch gel corners for identification). Mark staining trays with initials and class period before beginning this activity.

WARNING
Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes.

Protocol 1: Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

This protocol allows quick visualization of DNA bands in agarose gels within 15 minutes. For quick staining, Fast Blast DNA stain (500x) should be diluted to a **100x** concentration. We recommend using 120 ml of 100x Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in each staining tray (if using catalog #166-0477EDU, staining trays). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand. Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another. Destaining requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

1. Label a staining trays with your initials and class period. You will stain 2 gels per tray.

2. **Stain gels**
   Remove each gel from the gel tray and carefully slide it into the staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2–3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100x stain into a storage bottle and save it for future use. **The stain can be reused at least 7 times**.
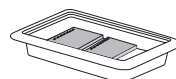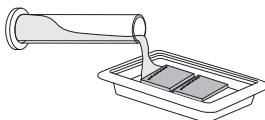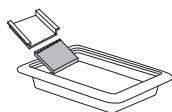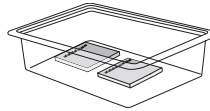

2–3 minutes

3. **Rinse gels**
   Transfer the gels into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gel in the water for ~10 seconds to rinse.
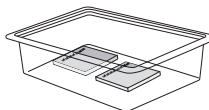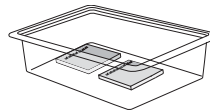

10 seconds

4. Wash gels

Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gel on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.

5 minutes

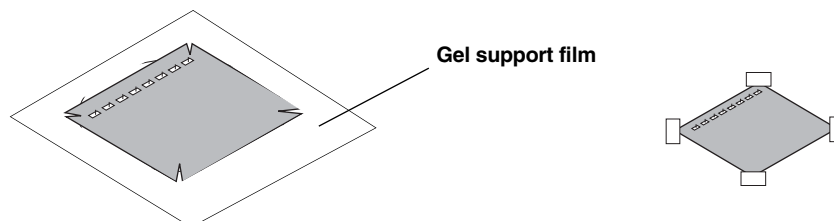## 5. Wash gels

Perform a second wash as in step 4.

5 minutes

6. Record results

Pour off the water and examine the stained gels for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 minutes after the second wash. This is due to Fast Blast stain molecules migrating into the gel and binding more tightly to the DNA.

To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gel in water overnight. If you cannot complete the destaining in the allocated time, you may transfer the gel to 1x Fast Blast stain for overnight staining. **See Protocol 2**.

a. Place your gel on a light background and record your results by making a diagram as follows. Place a clear sheet of plastic sheet or acetate over the gel. With a permanent marker, trace the wells and band patterns onto the plastic sheet to make a replica picture of your gel. Remove the plastic sheet for later analysis. Alternatively, gels can be photocopied on a yellow piece of transparent film for optimal contrast.

b. Dry the agarose gel as a permanent record of the experiment.

i. Trim away any unloaded lanes with a knife or razor blade. Cut your gel from top to bottom to remove the lanes that you did not load samples into, leaving only lanes 1–4.

ii. Place the gel directly upon the hydrophilic size of a piece of gel support film. (Water will form beads on the hydrophobic side of a piece of gel support film.) Center the gel on the film and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry in a well-ventilated area, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but 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 for the experiment.

Gel support film

## Protocol 2: Overnight Staining of Agarose Gels in 1x Fast Blast DNA Stain

For overnight staining, Fast Blast DNA stain (500x) should be diluted to a **1x** concentration. We recommend using 120 ml of 1x Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in each staining tray (if using catalog #166-0477EDU, staining trays). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

Following DNA electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand. Because the gel is fragile, special attention must be given when handling it.

1. Label the staining tray with your initials and class period. You will stain 2 gels per tray.

2. Stain gels (overnight)*

Pour **1x** stain into a gel staining tray. Remove the gel from the gel tray and carefully slide it into the staining tray containing the stain. If necessary, add more 1x staining solution to completely submerge the gels. Place the staining tray on a rocking platform and agitate overnight. If no rocking platform is available, agitate the gels staining tray a few times during the staining period. You should begin to see DNA bands after 2 hours, but at least 8 hours of staining is recommended for complete visibility of stained bands.
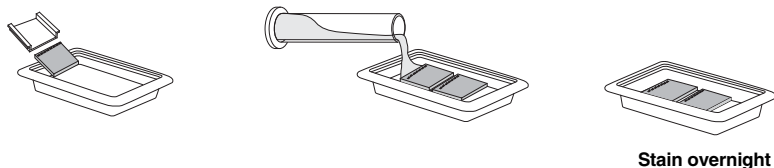
Stain overnight

* It is crucial that you shake gels gently and intermittently while performing the overnight staining in 1x Fast Blast stain since smaller fragments tend to diffuse without shaking.

3.   Record results

No destaining is required after staining with 1x Fast Blast. The gels can be analyzed immediately after staining.

a. Place your gel on a light background and record your results by making a diagram as follows. Place a clear sheet of plastic sheet or acetate over the gel. With a permanent marker, trace the wells and band patterns onto the plastic sheet to make a replica picture of your gel. Remove the plastic sheet for later analysis. Alternatively, gels can be photocopied on a yellow piece of transparent film for optimal contrast.

b. Dry the agarose gel as a permanent record of the experiment.

i.   Trim away any unloaded lanes with a knife or razor blade. Cut your gel from top to bottom to remove the lanes that you did not load samples into, leaving only lanes 1–4.

ii. Place the gel directly upon the hydrophilic size of a piece of gel support film. (Water will form beads on the hydrophobic side of a piece of gel support film.) Center the gel on the film on a paper towel and let the gel dry in a well-ventilated area, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but 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 for the experiment.

Gel support film
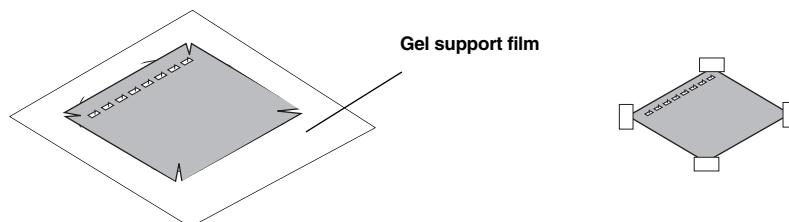
Note: Avoid extended exposure of dried gels to direct light to prevent band fading. However, DNA bands will reappear if the dried gels are stored in the dark for 2–3 weeks after fading.

Post-Lab: Thought 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. From the gel drawing on page 35, which DNA samples appear to have been "cut" into the same number and size of fragments?

9. Based on your analysis of the example gel drawing on page 35, what is your conclusion about the DNA samples in the drawing? Do any of the samples seem to be from the same source? If so, which ones? Describe the evidence that supports your conclusion.

Post-Lab: Analysis of Results

If the overnight staining protocol was used to stain gels, record your results and dry gels as described in the gel staining procedures in Lesson 2 page 38.

Attach the plastic sheet tracing of the banding patterns from the DNA electrophoresis below.
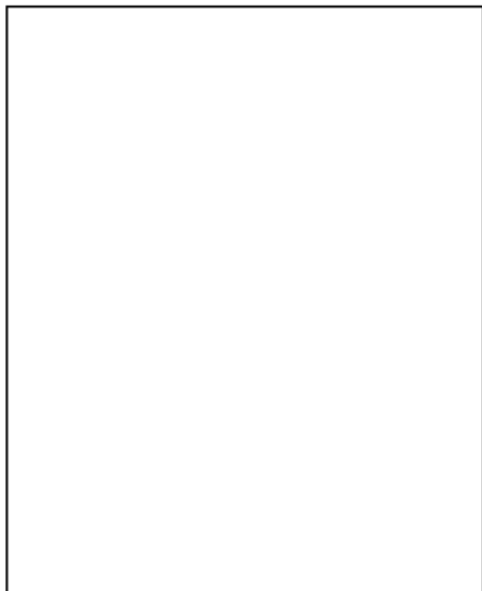
Tracing of electrophoresis gel

Attach the dried gel showing the banding patterns from the DNA electrophoresis below.
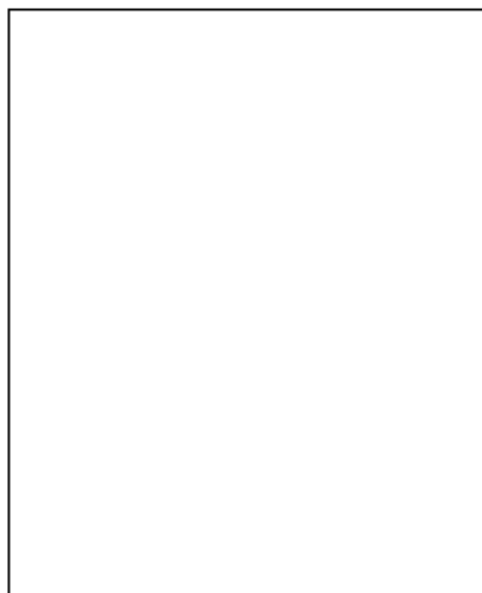
Dried electrophoresis gel

Quantitative Analysis of DNA Fragment Sizes

If you were on trial or were trying to identify an endangered species, 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 completed. This is described below:

1. Using a ruler, measure the distance (in mm) that each of your DNA fragments or bands traveled from the well. Measure the distance from the bottom of the well to the 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 suspect DNA samples, a standard curve is created using the distance (x-axis) and fragment size (y-axis) data from the known HindIII lambda digest (DNA standard). Using both linear and semilog graph paper, plot distance versus size for bands 2–6. On each graph, draw a line of best fit through 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 semilog, 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.
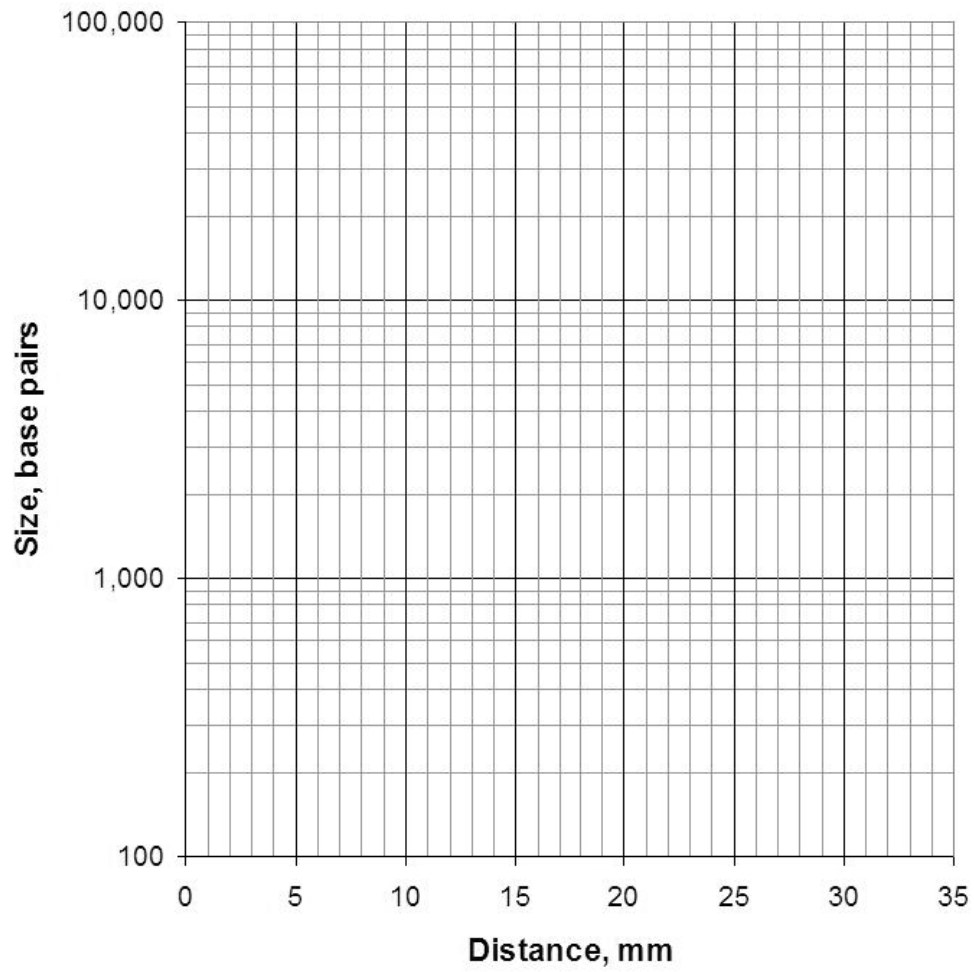
   Is there a suspect that matches the crime scene?

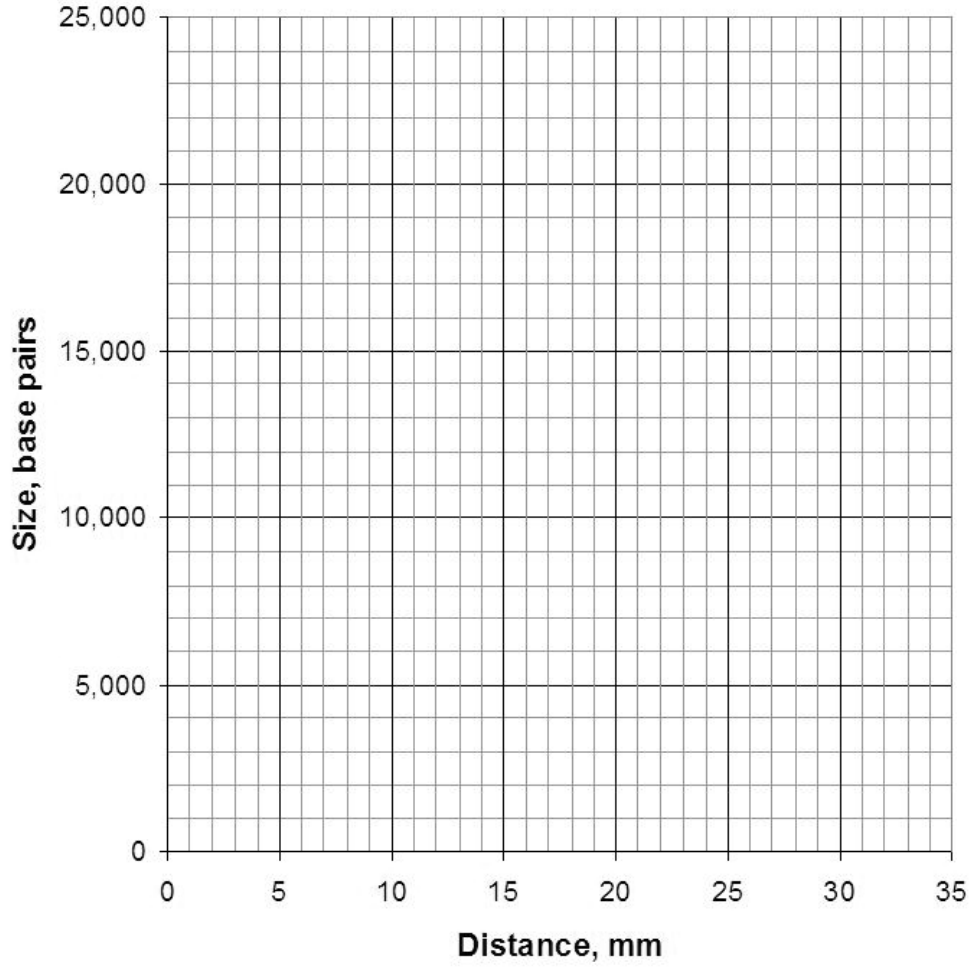   How sure are you that this is a match?

Electrophoresis data: Measure the distance (in millimeters) that each fragment traveled from the well and record it in the table. Estimate its size, in base pairs, by comparing its position to the HindIII lambda DNA standards. **Remember: some lanes will have fewer than 6 fragments.**

Band	Lambda/HindIII size standard		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5	
	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												

Semilog Graph Paper

Size, base pairs (y-axis, logarithmic scale from 100 to 100,000)
Distance, mm (x-axis, from 0 to 35)

Graph Paper

Student Manual

Post Lab: Interpretation of Results

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 EcoRI or PstI 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.

Extension Activity 1: Plasmid Mapping

Plasmids and Restriction Enzymes

This lesson will demonstrate the principles of plasmid mapping by examining restriction digestion patterns of plasmids used in the laboratory section of the kit and determining the position of restriction enzyme recognition sites in the plasmids by use of logic. Plasmid mapping has revolutionized molecular biology and paved the way for the biotechnology industry. This technique allows molecular biologists to quickly evaluate the success of cloning experiments as well as to easily identify plasmids and associated traits in different organisms. Although real-world DNA fingerprinting is performed on genomic DNA, this activity utilizes plasmid DNA to simulate how real DNA fingerprints are analyzed. Plasmids are circular, non-chromosomal pieces of DNA that can replicate in bacteria. Plasmids often carry genes encoding resistance to antibiotics which gives bacteria a selective advantage over their competitors.

Scientists routinely take advantage of plasmid DNA and a natural bacterial defense mechanism, the restriction enzyme, as the basis for much of biotechnology. Restriction enzymes allow bacteria to destroy DNA from invading bacteriophages (phages) which are viruses that infect and destroy bacteria. Restriction enzymes recognize specific DNA sequences within the phage DNA and then cut the DNA at that site. Fragmented DNA no longer poses a threat to bacterial survival. Purified restriction enzymes can be used in the laboratory to cut DNA isolated from any organism not just phage DNA.

After plasmids are cut with a restriction enzyme, they can be joined (or ligated) to a piece of DNA from any organism (foreign DNA) that has been cut with the same enzyme. The resulting hybrid DNA plasmid can be put into (transformed) bacterial cells. A hybrid plasmid can replicate itself in bacteria similar to the original plasmid, except that the foreign DNA that was incorporated is also perpetuated. Every hybrid plasmid now contains a 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 carries it is called a "vector".

The crime scene and suspect DNA samples in this kit were created by inserting lambda phage DNA that had been digested with the PstI restriction enzyme into PstI-digested plasmid pTZ18U. Recombinant plasmids were selected that gave distinct, striking banding patterns, or restriction fragment length polymorphisms (RFLP), when digested with restriction enzymes PstI and EcoRI and analyzed on an agarose gel. Restriction maps of some of the crime scene and suspect plasmids are included on page 48.
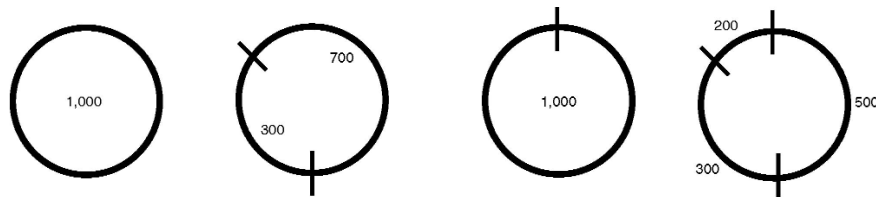
Plasmids can be mapped or described in terms of location of restriction sites using simple experiments and logic. The general procedure is to cut (digest) a plasmid with two restriction enzymes separately (two single digests) and the together (a double digest). Sizes of the resulting DNA fragments are determined then one uses logic to determine the relative location of the restriction sites. In the forensic DNA fingerprinting lab two restriction enzymes, PstI and EcoRI, were used together in a double digest. The resulting fragments were run on a gel to solve the "who done it". The background material can be used to construct a plasmid map

Since the plasmids are circular, the number of fragments represents the number of cuts or restriction sites. To visualize this, take a rubber band and cut it once. How many fragments are there? Cut the same rubber band again. How many fragments are there now? The most informative part of plasmid mapping comes from using logic to overlap the information from two single digests with information obtained from a double digest. How do the cuts from one restriction enzyme overlay with cuts from a second restriction enzyme? There are clues to see how to overlap them: Do any of the first fragments remain uncut

with the second enzyme? Do the sizes of any of fragments from the double digest add up the size of a fragment from a single digest? Do any of the fragments seem to remain the same size after being cut by the second enzyme? A simple example is shown below:

1000 bp plasmid example

DNA size standard	Undigested	Digested with Enzyme 1	Digested with Enzyme 2	Digested with Enzyme 1 & 2
1000 bp	1000bp		1000 bp	
700 bp		700 bp		
500 bp				500 bp
300 bp		300 bp		300 bp
200 bp				200 bp

Note that the two 1000 bp fragments (undigested sample and sample digested with enzyme 2) might not run at exactly the same distance on an agarose gel. There is a small difference in migration if a fragment of the same size is uncut (circular), cut (linear) or uncut and twisted (supercoiled). Also fragments that are very similar in size may migrate together in an agarose gel and it may not be possible to distinguish between them. In addition, fragments that are very small, may not be detected by the DNA stain or may run off the end of the gel.
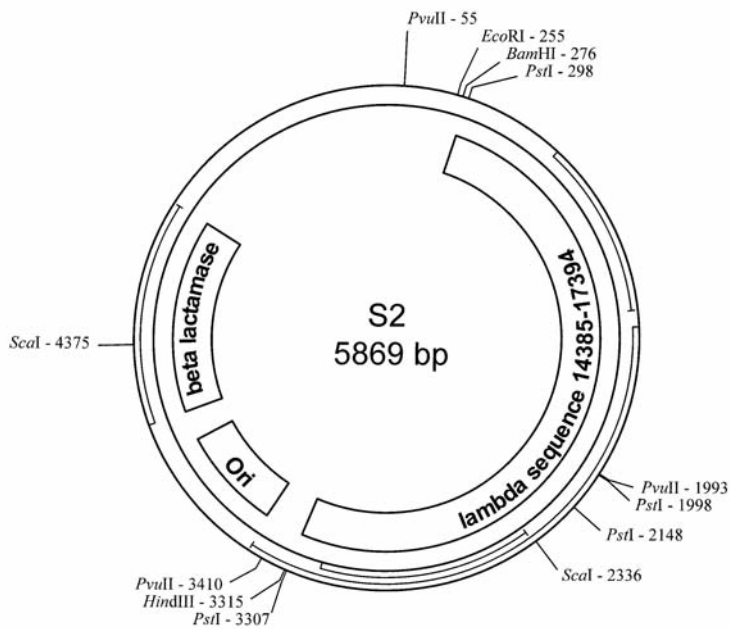
Reading a Plasmid Map

A plasmid map includes information on the size of the plasmid, the genes present, the origin of replication site, and restriction sites for restriction enzymes. All five of the plasmids used in the DNA fingerprinting activity were constructed from the same pTZ18U plasmid parent but had different foreign fragments of DNA inserted into them. In the DNA fingerprinting exercise, only two restriction enzymes were used, but other enzymes could also have been used to cut these plasmids. The restriction sites are marked on the map with a number that indicates the location of the site. Since the plasmid is circular, there is an arbitrary zero point. All of the restriction sites are indicated with a number between zero and the total base pairs in the plasmid. Fragment sizes can then be calculated by simple subtraction (and in some cases addition) between points on the plasmid.
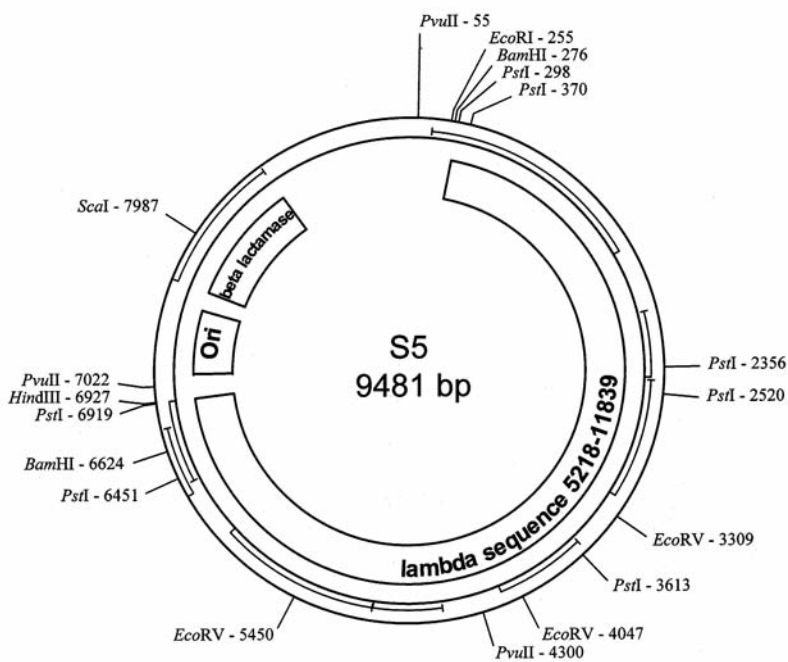
Plasmids Used In This Lesson

Plasmid maps show the positions (numbered by DNA base pairs) of sites where the plasmid may be cut by particular restriction enzymes. The name of the plasmid and its size of DNA in bp is shown inside the circle. In addition it shows the Origin of Replication (Ori), the gene encoding beta lactamase (the enzyme that gives the bacteria resistance to the antibiotic ampicillin) and the location where foreign DNA from the lambda bacteriophage was inserted. Refer to the plasmid maps on page 49 for more detail.

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Sample Plasmid Maps

Plasmid S2

*Pvu*II - 55
*Eco*RI - 255
*Bam*HI - 276
*Pst*I - 298

S2
5869 bp

beta lactamase

Ori

lambda sequence 14385-17394

*Sca*I - 4375

*Pvu*II - 1993
*Pst*I - 1998
*Pst*I - 2148
*Sca*I - 2336

*Pvu*II - 3410
*Hin*dIII - 3315
*Pst*I - 3307

Plasmid S5

*Pvu*II - 55
*Eco*RI - 255
*Bam*HI - 276
*Pst*I - 298
*Pst*I - 370

S5
9481 bp

beta lactamase

Ori

lambda sequence 5218-11839

*Sca*I - 7987

*Pst*I - 2356
*Pst*I - 2520

*Pvu*II - 7022
*Hin*dIII - 6927
*Pst*I - 6919

*Bam*HI - 6624

*Pst*I - 6451

*Eco*RV - 3309

*Pst*I - 3613

*Eco*RV - 5450

*Eco*RV - 4047
*Pvu*II - 4300

Reading a Plasmid Map Questions

1. From the map of plasmid S2 list all the restriction enzymes that would cut this plasmid.

2. Which plasmid, S2 or S5, is the biggest and what is its size?

3. Using plasmid S2 as an example, find the restriction sites for the enzyme PvuII. How many sites are there? What is their location? If PvuII was used to cut (digest) this plasmid, how many fragments would it make?

4. Next determine the size of the fragments created when plasmid S2 is cut by PvuII. DNA fragment size is calculated by subtracting the site locations from each other. (Note: if a fragment contains the 0 point of the plasmid, it is not just a simple subtraction!). How big are the fragments from plasmid S2 that is cut with PvuII? The fragment sizes should add up to the total for that plasmid (5869 bp).

5. If the fragments from the plasmid S2 digested with PvuII were run on an agarose gel, what would they look like? Draw the gel and label the fragments and their sizes.

6. Now you can determine the fragment sizes of the plasmids when cut with the two enzymes, EcoRI and PstI. Indicate the sizes of the fragments that would be generated if the plasmid were a digest by PstI alone, EcoRI alone or by both PstI and EcoRI.

Plasmid S2 (5869 bp)			
Enzymes	EcoRI	PstI	Both
Fragments			
Total			

7. If plasmid S2 was digested and run on an agarose gel, what would the gel look like? Draw a gel and the fragment sizes if digested by EcoRI alone, PstI alone and by EcoRI and PstI together.

8. How does your diagram in question 7 compare to what was observed in your gel after the experiment? Indicate a reason for why your data in question 7 might be different from the actual experimental data seen from lesson 2.

Mapping the Plasmid

The first step in mapping a plasmid is to determine how many times a restriction site is found on that plasmid. First examine the results for plasmid S5 as an example. The data given in the following table are for the double digest using both EcoI and PstI. Also given are the data for single digests by the individual enzymes. The numbers in the columns under each enzyme represent the sizes of all of the fragments that are formed when each enzyme is used for a single digest.

The charts below show the sizes of fragments that will be generated when plasmid S5 has been digested with the indicated restriction enzyme.
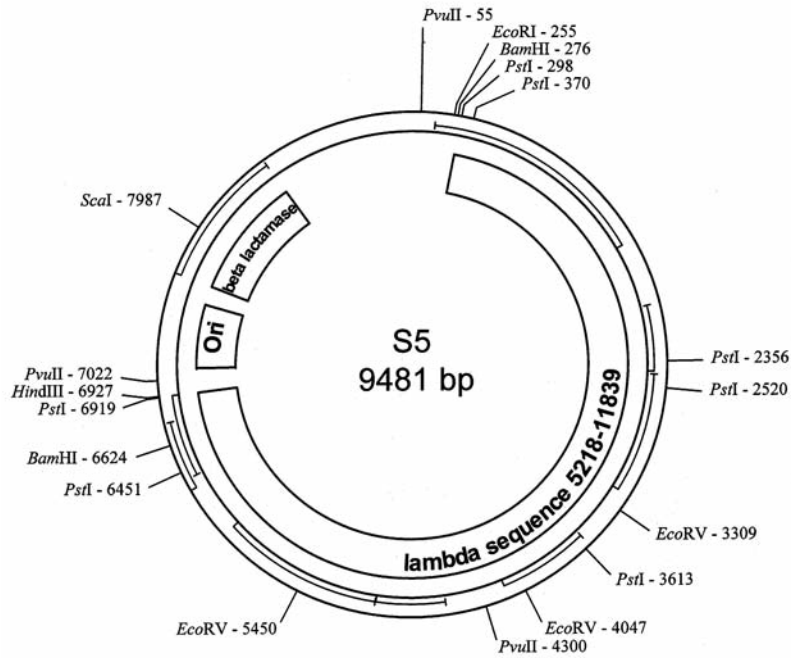
Plasmid S5

Plasmid S5 (9481 bp)			
Enzymes	EcoRI	PstI	Both
Fragments	9481	2860	2838
		2838	2817
		1986	1986
		1093	1093
		468	468
		164	164
		72	72
			43
Total			

Mapping the Plasmid Questions

1. How big is plasmid S5? Add the fragments in each column. The total should add up to the size of the plasmid. Why?

2. Look at the data from the EcoRI digest of plasmid S5. How many fragments are there? Did the enzyme cut the plasmid, or did it remain as a circle? How could you tell?

3. Compare the data from the PstI digest of plasmid S5 with that of the EcoRI digest. How many fragments are there? How many restriction sites are there for PstI?

4. How many fragments are there when EcoRI and PstI are used to digest plasmid S5? Does that answer the question of whether or not EcoRI cut the plasmid? Why?

5. Which fragment of PstI digested plasmid S5 was shortened by a cut with EcoRI? How do you know this?

6. Draw the PstI fragment that is cut with EcoRI in plasmid S5 to demonstrate how the fragment was cut with EcoRI.

Plasmid S3 (7367 bp)			
Enzymes	EcoRI	PstI	Both
Fragments	6504 863	4507 2860	3687 2817 820 1093 43
Total			

7. Restriction mapping is an exercise in critical thinking and logic. Plasmid S5 is difficult to completely map because of the numerous PstI restriction sites. With the data, it would be very difficult to place all the restriction sites in order. It is easier to map plasmid S3.

   Shown above is the data generated from digestion of plasmid S3 with EcoRI and PstI. How many times did EcoRI cut plasmid S3? What are the fragment sizes?

8. The data from the EcoRI digest of plasmid S3 indicate that the fragments are not equal. Draw a possible map and label the EcoRI sites and the sizes of the fragments.

9. Now draw an approximate map of the PstI sites on plasmid S3 and label the PstI sites and the sizes of the fragments.

10. Draw a circular map of plasmid S3 digested with both PstI and EcoRI. Mark sizes of each fragment and name the restriction sites on your figure.

11. Is there another possible order of restriction sites on plasmid S3 digested with both PstI and EcoRI? How might you resolve these possibilities?

12. When the gels were run for this experiment, there were only three bands for plasmid S3. Which band is missing from your gel? Why?

## Extension Activity 2: Constructing a Plasmid

Some plasmids replicate when the bacterial genomic DNA replicates, but others replicate independently producing hundreds of copies of the plasmid within one cell. A self-replicating plasmid contains its own origin of replication (Ori). Many plasmids also contain genes that give antibiotic resistance to bacteria.

Plasmids are made of DNA just like the bacterial genome. Since DNA is universal in all living things, plasmids with inserted foreign DNA may be put into a bacteria (transforming them), causing the bacteria to transcribe and translate that message into a protein product. The biotech industry is in part based on this principle. Bacteria can be given human genes via a plasmid, and once transformed can produce a human protein product.

This exercise is based on plasmids used in the forensic DNA fingerprinting lab. Five plasmids were constructed from a parent plasmid, and then were cut (digested) to make fragments of different sizes for a gel analysis. How were the plasmids constructed? Could one have made the plasmids differently? Can one predict fragment sizes using plasmid maps constructed from the lambda bacteriophage genome? In this activity you will design a new plasmid.

The parent plasmid pTZ18U used for plasmid construction is 2860 bp in size. It has numerous restriction sites (see diagram on pages 56–57). The five plasmids constructed for the DNA fingerprinting lab were all based on this plasmid.

The pTZ18U plasmid was digested with the PstI restriction enzyme. Lambda phage DNA was also digested with the PstI restriction enzyme and the resulting fragments were then inserted into the PstI – digested pTZ18U plasmid. As you look at the different plasmids, notice that each plasmid contains different fragments of lambda phage DNA. For example, plasmid S1 contains lambda sequence 20285–22425.
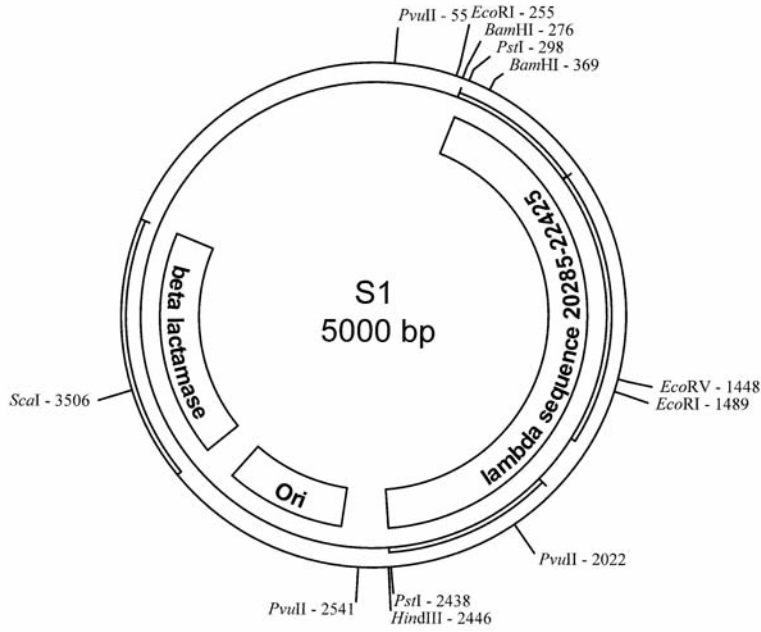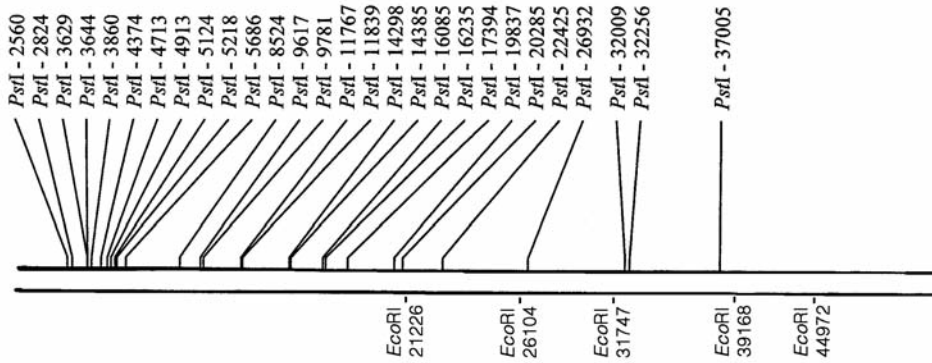
Plasmid S1
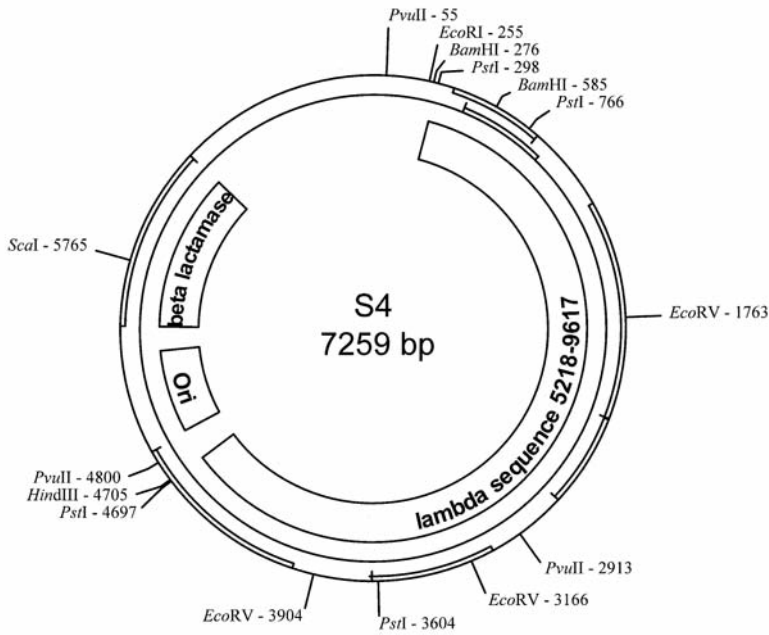
Plasmid S4
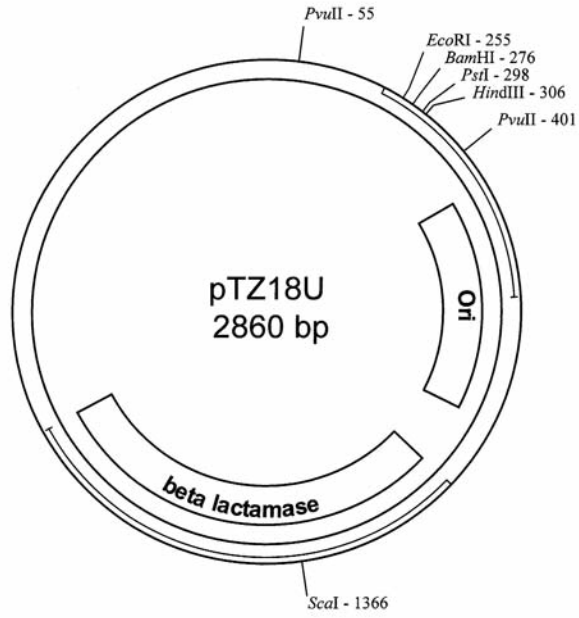
Parent Plasmid pTZ18U

lambda bacteriophage genome
48502 bp

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Constructing a Plasmid Questions

1. Where is the PstI site on the pTZ18U plasmid?

2. Look at plasmid S4. What segment of the lambda bacteriophage has been inserted?

3. After looking at the plasmid map and also the lambda phage map, can you determine how many PstI restriction sites were added to the plasmid because of the inserted lambda phage DNA fragment? Note that it is possible for these extra PstI sites to have been added if the original restriction digestion was done for a short time so that not all PstI sites would have been completely cut in every piece of lambda phage DNA.

4. Look at plasmid S1. What segment of lambda was added to that plasmid? Were any PstI restriction sites added to the plasmid with the inserted fragment of lambda DNA?

5. Now let us create a different plasmid from the parent plasmid. You will use EcoRI for the construction and need to refer to the lambda bacteriophage genome map that includes the EcoRI sites. You must make a plasmid that is at least 5,000 base pairs but not more than 10,000 base pairs in size. Remember that the parent plasmid is 2860 bp in size. Where is the EcoRI site on the parent pTZ18U plasmid?

6. Choose a segment of lambda bacteriophage genome that could be cut out by the EcoRI enzyme. Which segment will you use?

7. Draw your new plasmid with the insert of your choice. Be sure to include the restriction sites for PstI and EcoRI in your drawing. How big is your new plasmid? Give the positions of the restriction sites in your new plasmid a number indicating the location. Remember that the first EcoRI site will still be position 255 as it is in the parent pTZ18U plasmid map.

8. How many restriction sites are there now for PstI in your new plasmid? Predict what fragments you would generate if you were to digest your plasmid with:

   i.   EcoRI alone

   ii. PstI alone

   iii. EcoRI and PstI together (a double digest)

9. Draw an agarose gel for each of these digests and label the fragment sizes.

10. The lambda phage fragment can be inserted into the host plasmid in either orientation – forwards or backwards. How could you use plasmid mapping to determine in which orientation your fragment was inserted? Use a diagram in your explanation.

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Appendix A

Alternative DNA Fingerprinting Scenarios

DNA typing, DNA profiling, and DNA fingerprinting are all names for the same process, a process that 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 (or impurity) of ground beef 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 1/3 of all sexual assault suspects are freed as a result of DNA testing.

3. **Identifying 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 was compared to that of known descendants and it was determined that the bones do belong to the czar and his family ("Identification of the remains of the Romanov family by DNA analysis" Nature Genetics vol 6, 130, 1994.)

4. **Determining relatedness of humans.**

DNA typing has shown that the 5,000 year old "Iceman" 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, has helped 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, vol. 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, vol. 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 nitrogenous base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar–phosphate backbone. The four different nitrogenous bases are **adenine**, **thymine**, **guanine** and **cytosine**. (**A**, **T**, **G**, and **C**: Remember the base-pairing rule is A-T and G-C). Refer to the figures below to review the structure of a DNA molecule.

Fig. 1. The Structure of DNA

DNA MOLECULE #1   DNA MOLECULE #2   DNA MOLECULE #3
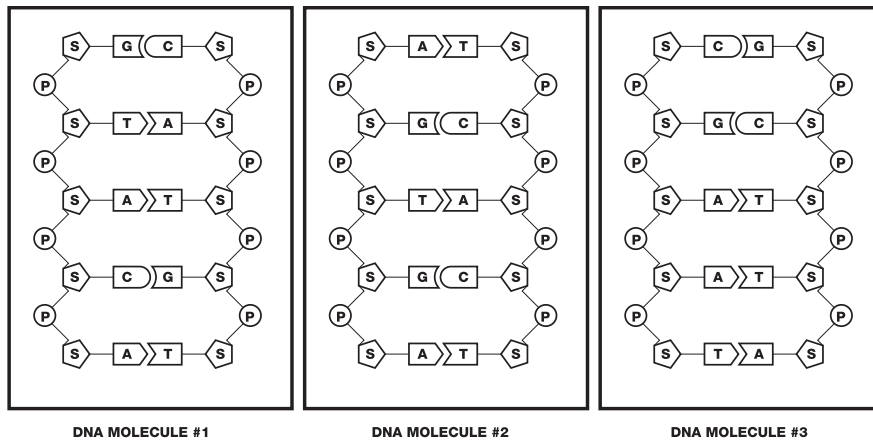
If a **segment** of DNA is diagrammed without the sugars and phosphates, the base-pair sequence might appear as:

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:

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

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

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

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You may have discovered that the sequence of base pairs is seemingly random and that the two strands are complementary to each other: **A**s are paired with **T**s and **C**s are paired with **G**s. You may have also noticed that a portion of the top strand **GAATTC** (read toward the right), has a counterpart in the lower strand, **CTTAAG** (read toward the left). Example sequences are:

GAATTC	**AAGCTT**	**CTGCAG**
CTTAAG	**TTCGAA**	**GACGTC**

When such a sequence is looked at together with its complementary sequence the group reads the same in both directions. These sequences, called palindromes, are fairly common along the DNA molecule.

Restriction Enzymes — Molecular Scissors

Viruses called bacteriophages frequently infect bacteria. These viruses inject their own DNA into bacteria and force the bacteria to multiply the DNA. Bacteria have evolved restriction enzymes, to cut up and destroy the invading viral DNA. Bacteria prevent destruction of their own DNA by modifying certain DNA bases within the specific enzyme recognition sequence, which allows them to protect their own DNA while cutting up viral DNA. This could be considered a very primitive immune system. Restriction enzymes search the viral DNA for specific palindromic sequences of base pairs, such as **GAATTC**, and cut the DNA at these sites. The actual sequence of DNA is called a **restriction site**. Some restriction enzymes may leave a short length of unpaired nucleotide bases, called a "sticky" end, at the DNA site where they cut, whereas other restriction enzymes make a cut across both strands at the same place creating double stranded DNA fragments with "blunt" ends.

Look at the DNA sequence below:
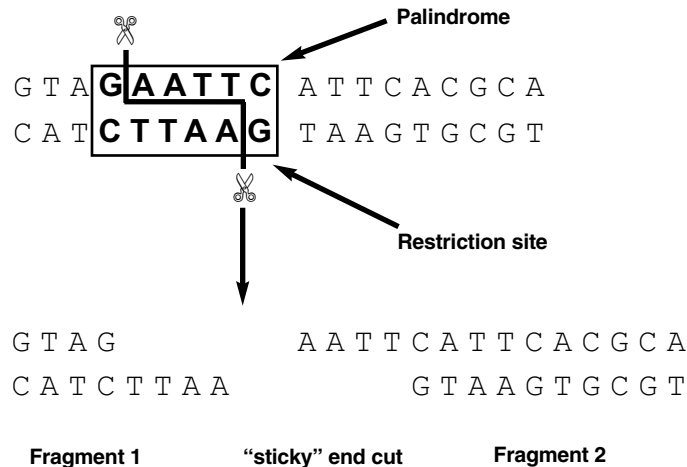
A restriction enzyme cut the DNA between the **G** and the **A** in a **GAATTC** palindrome.

4. How many bases are still paired to the left of the "cut"?

5. How many bases are still paired to the right of the "cut"?

6. Counting the number of paired bases, is the right fragment the same size as the left fragment?

7. How could you describe the size of each fragment in terms of the number of base pairs in the fragment?

An important feature of restriction enzymes is that each enzyme only recognizes a specific palindrome and cuts the DNA only at that specific sequence of bases. A palindromic sequence can be repeated a number of times on a strand of DNA, and the specific restriction enzyme **will cut all those palindromes**, no matter what species the DNA comes from.

8. If the **GAATTC** palindrome is repeated four times on the same piece of linear DNA, and the restriction enzyme that recognizes that base sequence is present and digests the DNA, how many DNA fragments will be produced?

9. If the **GAATTC** palindrome repeats are randomly found along the DNA strand, then what can you say about the sizes of the fragments that will be produced when the DNA is digested with a restriction enzyme that recognizes that sequence?

The table below shows palindromic sequences that are recognized by the enzymes that are used to digest the DNA you will be analyzing in this activity.

Palindromic sequence	Name of restriction enzyme that recognizes the palindrome
GAATTC CTTAAG	EcoRI
AAGCTT TTCGAA	HindIII
CTGCAG GACGTC	PstI

Below is the summary of what we have learned so far:

- A sequence on one strand of DNA and its complementary sequence on the other strand can form a palindrome   i.e., GAATTC
                                                                                                                CTTAAG

- Palindromes can be detected by restriction enzymes

- Restriction enzymes cut the palindromes at restriction sites

- Restriction enzymes recognize specific palindromes

- Cutting DNA at restriction sites will produce DNA fragments

- Fragment size can be described by the number of base pairs a fragment contains

Applying What You Have Learned

A linear DNA molecule is represented below. The DNA is represented by one line, although in actuality, DNA has two strands.

1. If the DNA molecule has two restriction sites, for restriction enzyme A, how many fragments would be produced if the DNA is cut by that enzyme?

2. Number each fragment.

3. Which fragment is the largest?

4. Which fragment is the smallest?

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

6. Label each fragment.

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

A   B

↓   ↓
_____

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

8. Explain why only two fragments would be produced.

Prelab Activity 2 A Review of Electrophoresis

Agarose gel electrophoresis is a procedure used to separate DNA fragments based on their sizes. DNA is an acid and has many negative electrical charges. Scientists have used this fact to design a method that can be used to separate pieces of DNA. A solution containing a mixture of DNA fragments of variable sizes is placed into a small well formed in an agarose gel that has a texture similar to gelatin. An electric current causes the negatively-charged DNA molecules to move towards the positive electrode.

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 the electrical current, the DNA fragments will sort themselves out by size. Fragments that are the same size will tend to move together through the gel and form bands.

A piece of DNA is cut into four fragments as shown in the diagram. A solution containing the four 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.

Negative   Well

Positive

Agarose gel

A   B   C   D
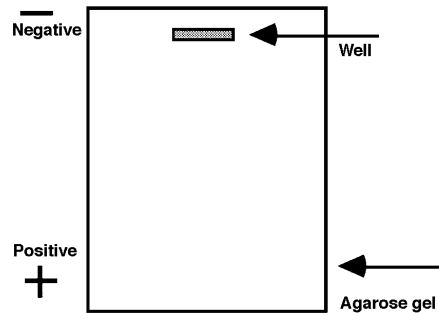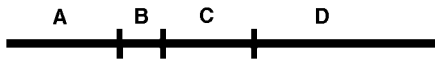
Have your teacher check your diagram before you proceed.

1. 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?

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

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

4. Complete this rule for the movement of DNA fragments through an agarose gel.

   The larger the DNA fragment, the …

APPENDIX B

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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.

23,130   2,027   2,322   9,416   6,557   4,361
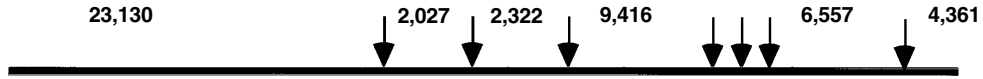
5. How many fragments were produced by the restriction enzyme *Hin*dIII?

6. On the gel diagram, show how you believe these fragments will sort out during electrophoresis. The two fragments with no length indicated will be too small to be visualized on the gel.

—
Negative

Well

Positive
+

Agarose gel
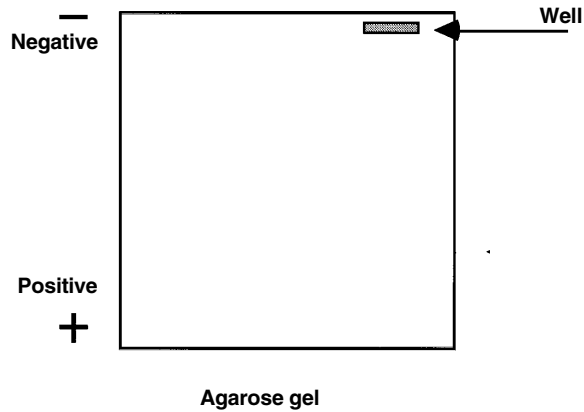
7. Label each fragment with its correct number of base pairs.

Appendix C
Instructor's Answer Guide

Pre-Lab Focus Questions: 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 non-identical?

   The sequence of base pairs in each individual sample.

Lesson 1 Restriction Digests of DNA Samples

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

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

    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__

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: __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 1 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 1 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 + EcoRI/PstI 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 EcoRI/PstI? Explain.

   No. No visible change is 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 is 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 2 Agarose Gel Electrophoresis

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?

   The negative pole (or cathode) is black. The positive pole (or anode) is red.

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.

Post-Lab 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. Assuming a circular piece of DNA (plasmid) was used as starting material, how many restriction sites were there in lane three? Please note that the starting material was a circular piece of DNA.

    Two sites that cut the sample into two fragments.

8. From the gel drawing on page 35, 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 example gel drawing on page 35, 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.

Band	Lambda/HindIII size standard		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4***		Suspect 5***	
	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	2,817**	21.0	2,817**	19.0	3,679	21.0	2,817**	21.0	2,817**
2	13.0	9,416	20.5	2,817**	23.5	1,191	25.0	1,700	20.5	2,817**	24.0	1,986	24.0	1,986
3	15.0	6,557	32.0	820	30.5	949	28.5	1,159	32.0	820	29.5	1,093	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 standards were not heated to 65 °C. Lambda HindIII 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. The 2,817 bp bands in plasmids S4 and S5 are especially intense because they are actually two individual bands (2,817 bp and 2,838 bp) that are too close to be visibly separated.

***S4 and S5 DNA lanes may also contain a very faint band of 468 bp.

DNA Standard Band Migration
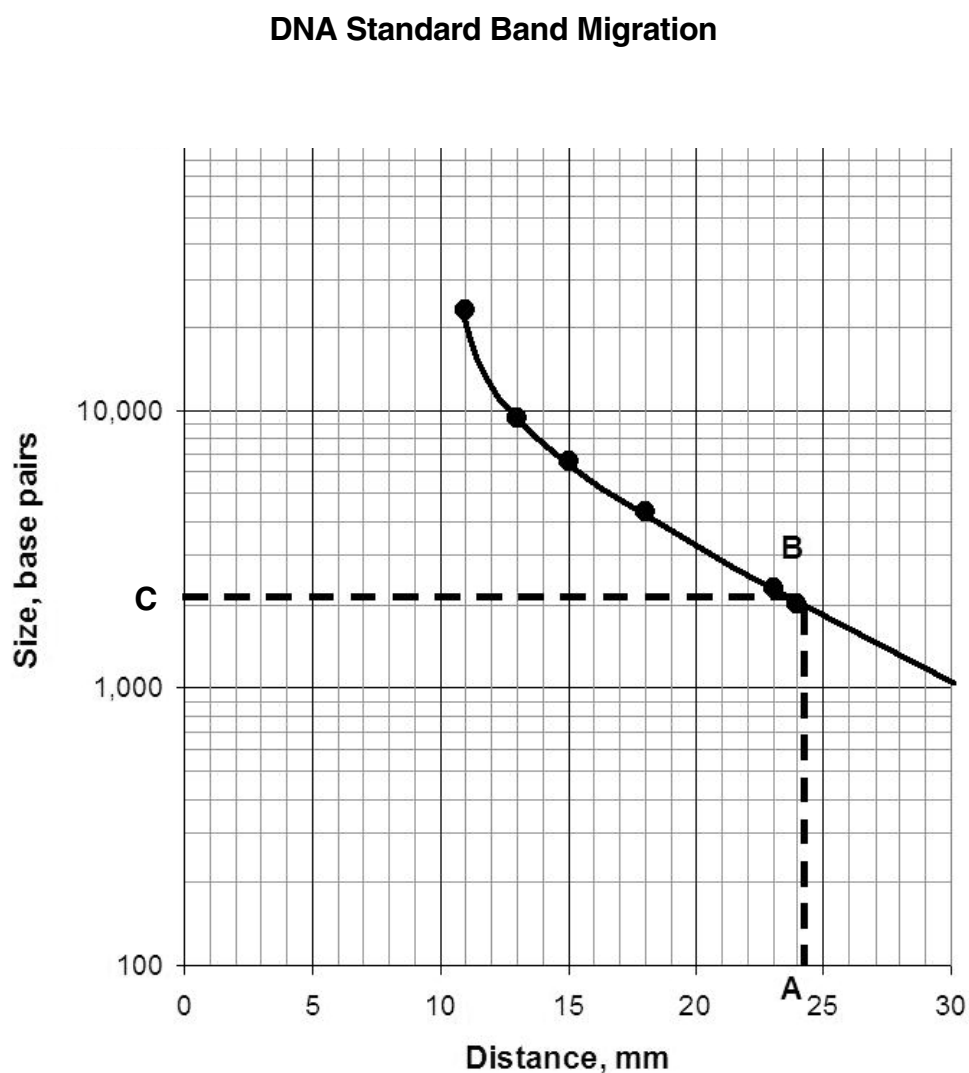
To estimate the size of any unknown crime scene or suspect fragment, you first need to determine the distances the specific fragment travelled. Locate the distance on the x-axis of your standard graph. For example, suspect 5, band 2 migrated 24 mm (A). From the 24 mm mark on the x-axis, read up to the 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). Therefore, suspect 5, band 2 is approximately 2,000 bp. Repeat this procedure for the crime scene and all suspects' fragments. As you determine the approximate fragment sizes, fill in the data in the data table.

Fingerprinting Standard Curve: Linear
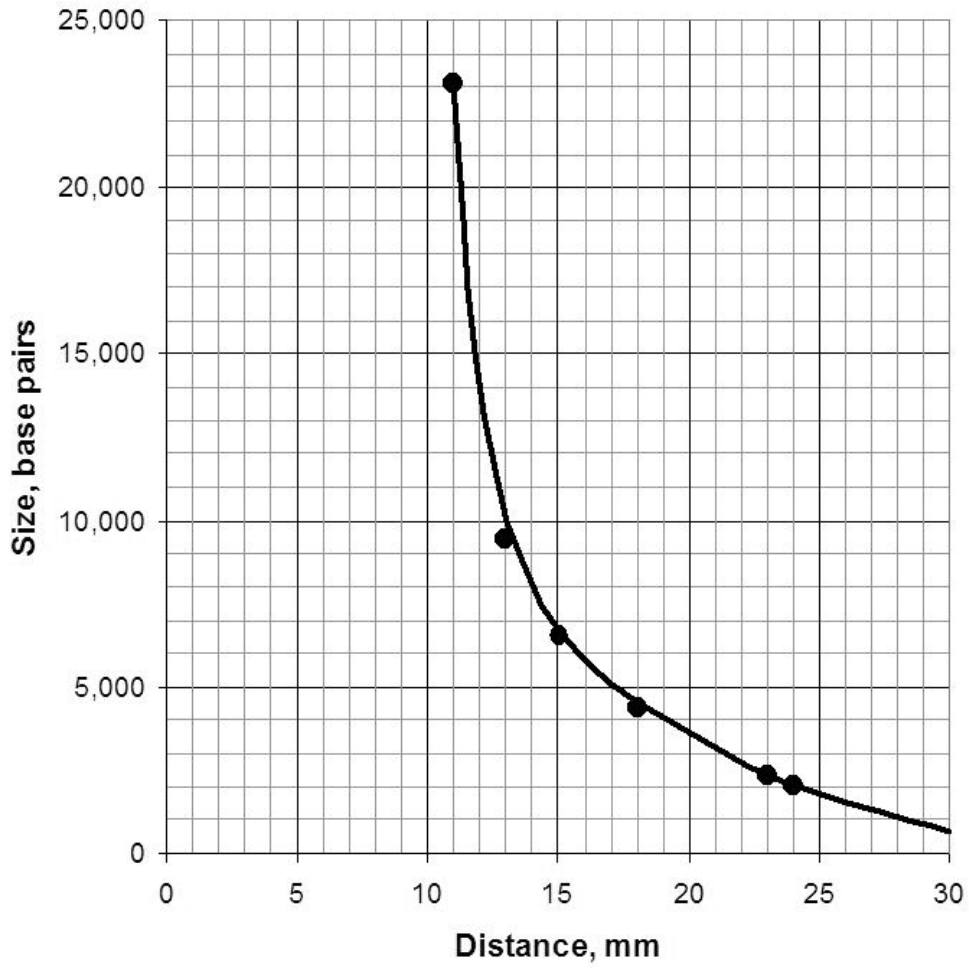
Post Lab Activity: Interpretation of Results

1. What are we trying to determine? Restate 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 EcoRI or PstI 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.

Extension Activity 1: Plasmid Mapping

Reading a plasmid map

1. From the map of plasmid S2 list all the restriction enzymes would cut this plasmid.

    PvuII, EcoRI, BamHI, PstI, EcoRV, HindIII, ScaI

2. Which plasmid, S2 or S5, is the biggest and what is its size?

    S5 is the largest (9481 bp)

3. Using plasmid S2 as an example, find the restriction sites for the enzyme PvuII. How many sites are there? What is their location? If PvuII was used to cut (digest) this plasmid, how many fragments would it make?

    There are three sites for PvuII on plasmid S2. They are at position 55, 1993 and 3410. Three fragments would be created if plasmid S2 was digested with PvuII.

4. Next determine the size of the fragments created when plasmid S2 is cut by PvuII. Size is calculated by subtracting the site locations from each other. (Note: if a fragment contains the 0 point of the plasmid, it is not just a simple subtraction!). How big are the fragments from plasmid S2 that is cut with PvuII? The fragment sizes should add up to the total for that plasmid (5869 base pairs).

    The fragment sizes are 1417, 1938 and 2514 bp.

5. If the fragments from the plasmid S2 digested with PvuII were run on an agarose gel, what would they look like? Draw the gel and label the fragments and their sizes.

    —— **2514**

    —— **1938**

    —— **1417**

6. Now you can determine the fragment sizes of the plasmids when cut with the two enzymes, EcoRI and PstI. Indicate the sizes of the fragments that would be generated if the plasmid were a digest by PstI alone, EcoRI alone or by both PstI and EcoRI.

Plasmid S2 (5869 bp)			
Enzymes	EcoRI	PstI	Both
Fragments	5869	2860 1700 1159 150	2817 1700 1159 150 43
Total			

7. If plasmid S2 was digested and run on an agarose gel, what would the gel look like? Draw a gel and the fragment sizes if digested by EcoRI alone, PstI alone and by EcoRI and PstI together.

EcoRI	PstI	Both Enzymes
___ 5809		
	___ 2860	___ 2817
	___ 1700	___ 1700
	___ 1159	___ 1159
	150	150
	43	43

8. How does your diagram in question 7 compare to what was observed in your gel after the experiment? Indicate a reason for why your data in question 7 might be different from the actual experimental data seen from lesson 2.

   They should be similar but the bands at 150 and 43 bp are too small to be observed on the gel.

Mapping the Plasmid Questions

1. How big is plasmid S5? Add the fragments in each column. The total should add up to the size of the plasmid. Why?

   The plasmid is 9481 bp in size. The fragments are cut from the plasmid and all the pieces together should be equal to the size of the original plasmid.

2. Look at the data from the EcoRI digest of plasmid S5. How many fragments are there? Did the enzyme cut the plasmid, or did it remain as a circle? How could you tell?

   There should be only one fragment. At first glance, it is not possible to tell if the plasmid was cut although sometimes a circular (and possibly twisted) plasmid does not run through an agarose gel at the same place as a linear piece of DNA of the same size.

3. Compare the data from the PstI digest of plasmid S5 with that of the EcoRI digest. How many fragments are there? How many restriction sites are there for PstI?

   **There should be 7 fragments and therefore 7 restriction sites.**

4. How many fragments are there when EcoRI and PstI are used to digest plasmid S5? Does that answer the question of whether or not EcoRI cut the plasmid? Why?

   There should be 8 fragments when the plasmid is cut by both enzymes. The additional fragment shows that EcoRI also cut the plasmid one time.

5. Which fragment of PstI digested plasmid S5 was shortened by an EcoRI cut?

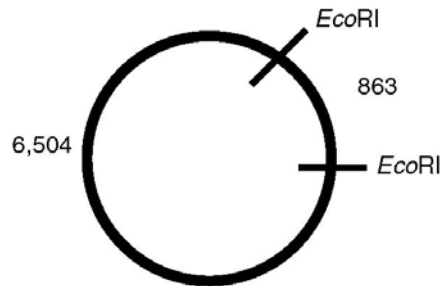   **The 2860 bp fragment was cut into two fragments (2817 bp and 43 bp)**

6. Draw the PstI fragment that is cut with EcoRI in plasmid S5 to demonstrate how the fragment was cut with EcoRI.

2,860

___ 43   2,817

*Eco*RI

81
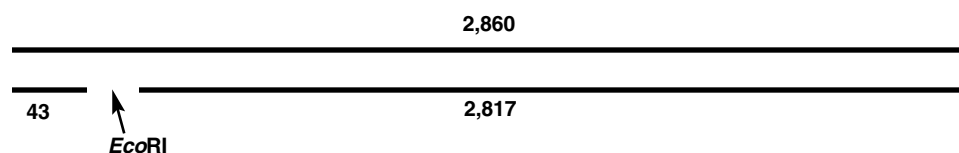
7. Restriction mapping is an exercise in critical thinking and logic. Plasmid S5 is difficult to completely map because of the numerous PstI restriction sites. With the data, it would be very difficult to place all the restriction sites in order. It is easier to map plasmid S3. How many times did EcoRI cut plasmid S3? What are the fragment sizes?

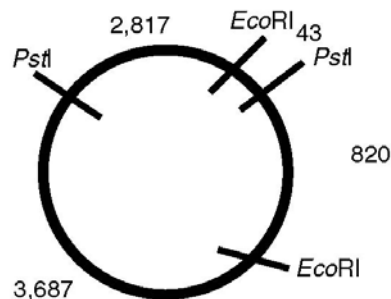   **EcoR1 cut plasmid S3 twice. The fragments are 863 and 6505 bp in size.**

8. The data from the EcoRI digest of plasmid S3 indicate that the fragments are not equal. Draw a possible map and label the EcoRI sites and the sizes of the fragments.

9. Now draw an approximate map of the PstI sites on plasmid S3 and label the PstI sites and the sizes of the fragments.
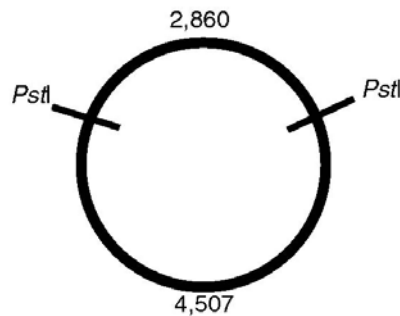

10. Draw a circular map of plasmid S3 digested with both PstI and EcoRI? Mark sizes of each fragment and name the restriction sites on your figure.

11. Is there another possible order of restriction sites on plasmid S3 digested with both PstI and EcoRI? How might you resolve these possibilities?

Using the data from the double digest there are two possibilities (see diagram). However the single digest with PstI would have fragments of 3687 and 3637 bp (not 4507 and 2860 bp as was seen).
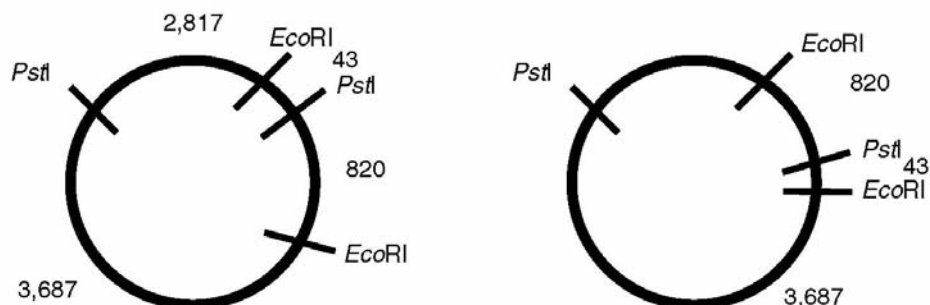
12. When the gels were run for this experiment, there were only three bands for plasmid S3. Which band is missing from your gel? Why?

The 43 bp band is so small that it does not bind enough Fastblast stain to be visible or it may have run off the gel.

Extension Activity 2: Constructing a Plasmid Questions

1. Where is the PstI site on the pTZ18U plasmid?

    Position 298

2. Look at plasmid S4. What segment of the lambda bacteriophage has been inserted?

    Lambda fragment 5,218–9,617 was added.

3. After looking at the plasmid map and also the lambda phage map, can you determine how many PstI restriction sites were added to the plasmid because of the inserted lambda phage DNA fragment? Note that it is possible for these extra PstI sites to have been added if the original restriction digestion was done for a short time so that not all PstI sites would have been completely cut in every piece of lambda phage DNA.

    Two restriction sites for PstI were added: site 766 and site 3,604.

4. Look at plasmid S1. What segment of lambda was added to that plasmid? Were any PstI restriction sites added to the plasmid with the inserted fragment of lambda DNA?

    Lambda fragment 20,285–22,425 was added to plasmid S1. No additional PstI sites were added.

5. Now let us create a different plasmid from the parent plasmid. You will use EcoRI for the construction and need to refer to the lambda bacteriophage genome map that includes the EcoRI sites. You must make a plasmid that is at least 5,000 base pairs but not more than 10,000 base pairs in size. Remember that the parent plasmid is 2,860 bp in size. Where is the EcoRI site on the parent pTZ18U plasmid?

    The EcoRI site is at 255 bp.

6. Choose a segment of lambda bacteriophage genome that could be cut out by the EcoRI enzyme. Which segment will you use?

    There are three possibilities: lambda 21,226–26,104; lambda 26,104–31,747; lambda 39,168–44,972. All other options would make the plasmid larger than 10,000 bp.

7. Draw your new plasmid with the insert of your choice. Be sure to include the restriction sites for PstI and EcoRI in your drawing. How big is your new plasmid? Give the positions of the restriction sites in your new plasmid a number indicating the location. Remember that the first EcoRI site will still be position 255 as it is in the parent pTZ18U plasmid map.

POSSIBILITY 1	POSSIBILITY 2	POSSIBILITY 3
Lambda 21,226–26,104	**Lambda 26,104–31,747**	**Lambda 39,168–44,972**
4,878 bp insert	**5,643 bp insert**	**5,804 bp insert**
7,738 bp total	**8,503 bp total**	**8664 bp total**
EcoEI 255, 5,133	**EcoRI 255, 5,898**	**EcoRI 255, 5,059**
PstI 1,454, 5,176	**PstI 1,083, 5,943**	**PstI 6,102**
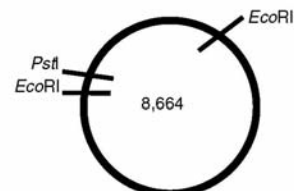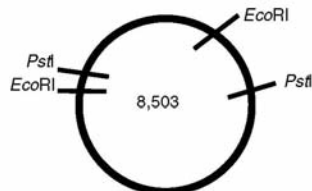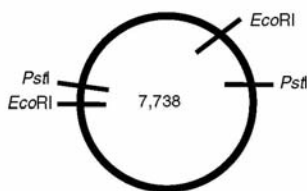
8. How many restriction sites are there now for PstI in your new plasmid? Predict what fragments you would generate if you were to digest your plasmid with:

i. EcoRI alone

    Two fragments in each case since there are two EcoRI sites.

POSSIBILITY 1	**POSSIBILITY 2**	**POSSIBILITY 3**
Lambda	**Lambda**	**Lambda**
21,226-26,104	**26,104-31,747**	**39,168-44,972**
4878	**5643**	**5804**
2860	**2860**	**2860**

ii. PstI alone

    Two fragments for possibility 1 & 2 and one fragment with possibility 3.

POSSIBILITY 1	**POSSIBILITY 2**	**POSSIBILITY 3**
Lambda	**Lambda**	**Lambda**
21,226-26,104	**26,104-31,747**	**39,168-44,972**
4016	**4858**	**8664**
3722	**3645**	

iii. EcoRI and PstI together (a double digest)

    Four fragments for possibility 1 and 2 and three fragments with possibility 3.

POSSIBILITY 1	**POSSIBILITY 2**	**POSSIBILITY 3**
Lambda	**Lambda**	**Lambda**
21,226–26,104	**26,104–31,747**	**39,168–44,972**
3679	**4815**	**5804**
2817	**2817**	**2817**
1199	**828**	**43**
43	**43**	

9. Draw an agarose gel for each of these digests and label the fragment sizes.

    POSSIBILITY 1

EcoRI	**PstI**	**Both**
4878		
	3722	**3679**
2860		**2817**
		1199

    POSSIBILITY 2

EcoRI	**PstI**	**Both**
5643		
	4858	**4815**
2860		**2817**
		828

    POSSIBILITY 3

EcoRI	**PstI**	**Both**
	8664	
5804		**5804**
	2860	**2817**

    The band at 43 bp is too small to be seen.

10. The lambda phage fragment can be inserted into the host plasmid in either orientation – forwards or backwards. How could you use plasmid mapping to determine in which orientation your fragment was inserted? Use a diagram in your explanation.

    Using possibility 1 from questions 8 and 9 above. The double digests will show the same bands with either orientation but the PstI single digest will be different. In the first orientation it will give two fragments of 4016 bp and 3722 bp. In the second orientation it will give two bands at 6492 bp and 1242 bp.

Prelab Activity 1 A Review of Restriction Enzymes (from Appendix B)

1. 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.

2. Compare the bases in the upper DNA strand to those in the lower strand. Describe any relationship you can see.

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

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

   CTTAAG.

4. How many base pairs are there to the left of the cut?

   4

5. How many base pairs are there to the right of the cut?

   10

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

   No, it is larger.

7. How could you describe the size of each fragment in terms of the number of base pairs in the fragment?

   Fragment 1 is a 4-base-pair fragment.

   Fragment 2 is a 10-base-pair fragment.

8. If the **GAATTC** palindrome is repeated four times on the same piece of linear DNA, and the restriction enzyme that recognizes that base sequence is present and digests the DNA, how many DNA fragments will be produced?

   5

9. 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 when the DNA is digested with a restriction enzyme that recognizes that sequence?

   Random sized fragments will be produced.

Applying What You Have Learned

1. If a DNA molecule has two restriction sites, for restriction enzyme A, how many fragments would be produced, if the DNA is cut by enzyme A?

   3

   A   A

   ↓   ↓
   _____

2. Number each fragment.

   1 A 2 A   3
   _____
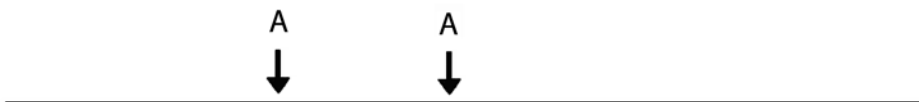
3. Which fragment would be the largest?

   Fragment 3.

4. Which fragment would be the smallest?

   Fragment 2.

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

   6

6. Label each fragment

   Answers will vary.

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

   Answers will vary.

   A   B

   ↓   ↓
   _____

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

8. Explain why only two fragments would be produced.

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

Prelab Activity 2 A Review of Electrophoresis (from Appendix B)

A piece of DNA is cut into four fragments as shown in the diagram. A solution of the four fragments is placed in a well in an agarose gel. Using the information given above, draw on the diagram how you think the fragments might be separated. Label each fragment with its corresponding letter.

Negative

Well

D ——
A ——
C ——
B ——

Positive

Agarose gel

A   B   C   D
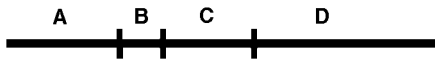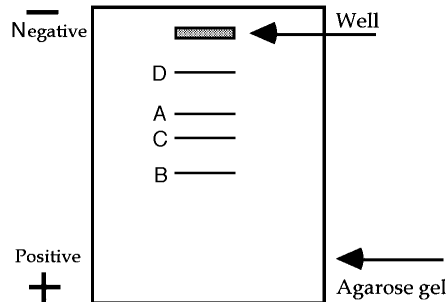
Have your teacher check your diagram before you proceed.

1. 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?

    The large fragments would be towards the top of the gel because it is more difficult for the larger pieces to be strained through the gel.

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

    There would still be only 4 bands present.

3. 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.

4. 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 restriction sites pointed to by the arrows. The numbers represent the number of base pairs in each fragment.

23,130   2,027   2,322   9,416   6,557   4,361

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

     8, however only 6 will be large enough to appear on the gel.

6.   On the gel diagram, show how you believe these fragments will sort out during electrophoresis. The two fragments with no length indicated will be too small to be visualized on the gel.

Negative

23,130	▨	◄ Well
9,416	―	
6,557	―	
4,361	―	
2,322		
2,027	―	
	―	◄ Agarose gel

Positive
+

Agarose gel
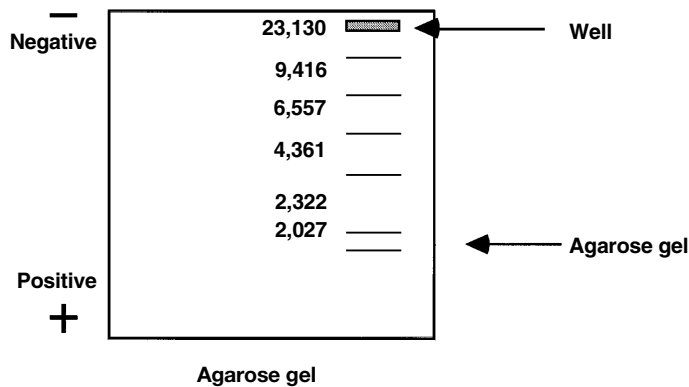
7.   Label each fragment with its correct number of base pairs.

     See gel above.

Appendix D
Fast Gel Protocol

The DNA Fingerprinting gels can be run in under 20 minutes by using a reduced concentration of running buffer (0.25x TAE), the same concentration of buffer in the gel itself (1x TAE) and higher voltage (200 volts). Resolution is excellent and the gel can be run 33% faster.

The provided 50x TAE buffer is mixed with distilled water to yield the necessary concentrations for making agarose gels and electrophoresis running buffer.

Use 1x TAE to make agarose gels:
Half a liter of 1x TAE is sufficient to make eight 7 x 10 cm agarose gels. To make 500 ml of 1x TAE from a 50x TAE concentrate, add 10 ml of concentrated TAE to 490 ml of distilled water. For added convenience, precast 1% agarose gels made with 1x TAE are available from Bio-Rad (catalog #161-3057EDU)

Use 0.25x TAE to make electrophoresis running buffer:
A 2.5 liter volume of 0.25x TAE buffer is required to run eight 7 x 10 agarose gels. To make 2.5 L of 0.25x TAE from a 1x TAE solution, add 625 ml of 1x TAE to 1,875 ml of distilled water.

Note: Do not use 0.25x TAE to make agarose gels; doing so can lead to a loss of DNA resolution.

To run gels:
Place the gel in an electrophoresis chamber and cover it with 0.25x TAE; ensure the gel is submerged. Run gels at 200 V for no more than 20 min. Monitor the movement of gel loading dye to get a relative idea of electrophoresis progress.

Appendix E
Digital Imaging and Analysis of Gels Stained with Fast Blast™ DNA Stain

Fast Blast DNA stain is a convenient, safe, and nontoxic substitute to ethidium bromide, a traditional DNA stain. DNA fragments stained with Fast Blast stain appear deep blue against a light blue background and are visible to the naked eye; they may be documented and analyzed manually as described in Lesson 4.

Alternate Method of Documentation and Analysis

A gel stained with Fast Blast DNA stain can be illuminated with Vernier's White Light Transilluminator and the image captured digitally with a ProScope HR digital USB camera. Then the image can be analyzed with the Gel Analysis function of Vernier's Logger *Pro* 3 software.

Obtaining Gel Images with Gel Analysis

The Gel Analysis function of Logger *Pro* 3 software can be used to analyze DNA banding patterns and calculate base pair values from stored digital images or from images taken directly with a USB camera through the software.

The gel's image can be taken directly with a ProScope HR or ProScope HR2 USB camera through the Gel Analysis function of Logger *Pro*. The gel image is focused with the camera lens as the gel sits on the White Light Transilluminator adjacent to the built-in ruler. To capture an image from within Logger *Pro* software, select the following:

Connect ProScope>Start Logger *Pro*>Insert Menu>Gel Analysis>Take Photo

Adjust and focus image for clarity and sharpness, then take the photo. Brightness and saturation can be regulated from the Camera Settings to provide the best image.

Gel analysis can also be performed in Logger *Pro* using a digital gel image file stored on the computer desktop. The procedure is as follows:

Start Logger Pro>Insert Menu>Gel Analysis>From File>Navigate to gel image

Using Gel Analysis for Standard Curve and Gel Banding Values

The Gel Analysis function of Logger *Pro* software can assist the user in developing a standard curve and then offer base pair values for DNA fragment bands in each experimental lane.

Figure 1 is an example of a Forensic DNA Fingerprinting gel stained with Fast Blast DNA stain, illuminated on a White Light Transilluminator, and documented and analyzed using the Gel Analysis function of Logger *Pro* software.
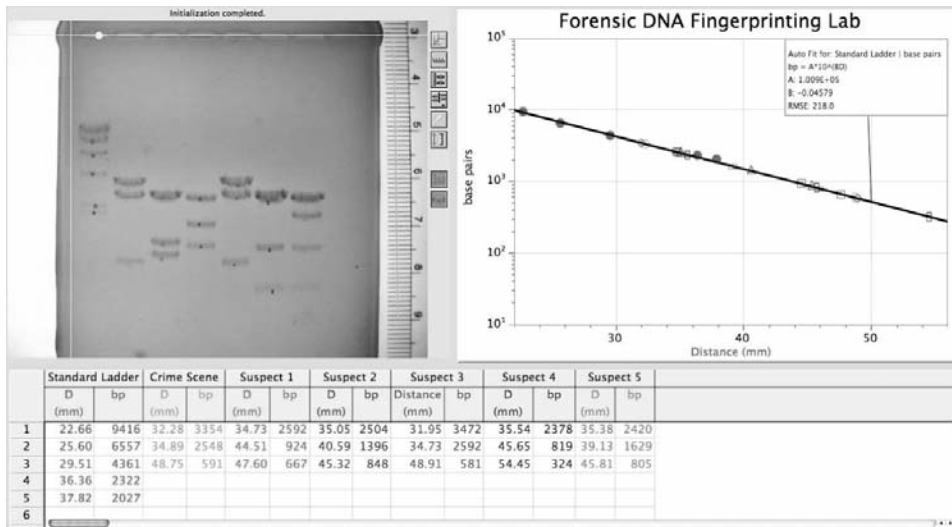
Fig. 1: Forensic DNA Fingerprinting gel examined with Gel Analysis. Image was captured using a ProScope HR USB digital camera.

For detailed directions on imaging, analyzing, and ordering, please contact Vernier.

Vernier ordering information:

White Digital Bioimaging system (catalog # WHT-DBS) includes:
White Light Transilluminator (catalog # WHT-TRANS)
Imaging Hood (catalog # HOOD)
ProScope HR digital USB camera (catalog # BD-BODY)
ProScope 1x–10x Lens (catalog # BD-10x)
ProScope Stand (catalog # BD-STAND)
Items sold collectively or separately

Logger *Pro* 3 software (catalog # LP)

Vernier Software and Technology
Toll free: 888-837-6437
Web: store.vernier.com
Email: orders@vernier.com

Appendix F
Suspect Plasmid Maps
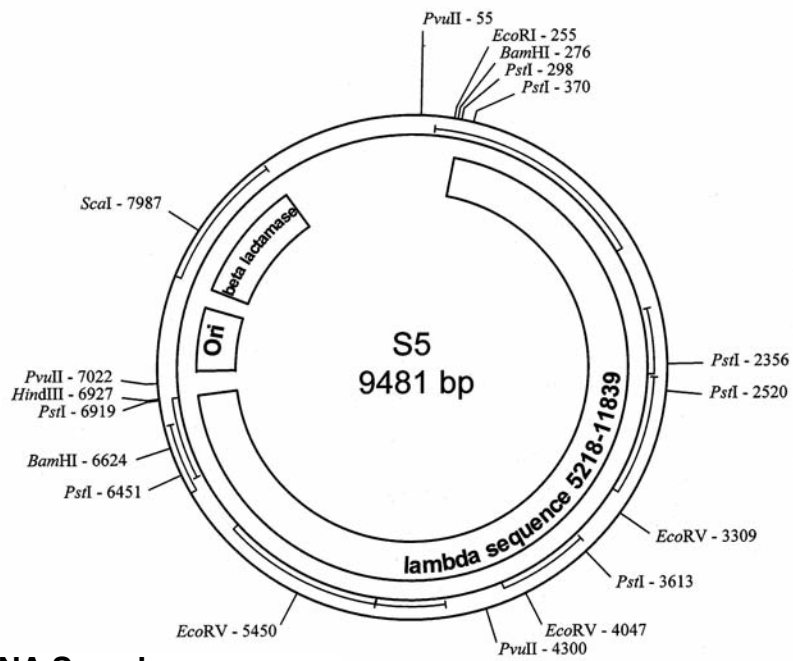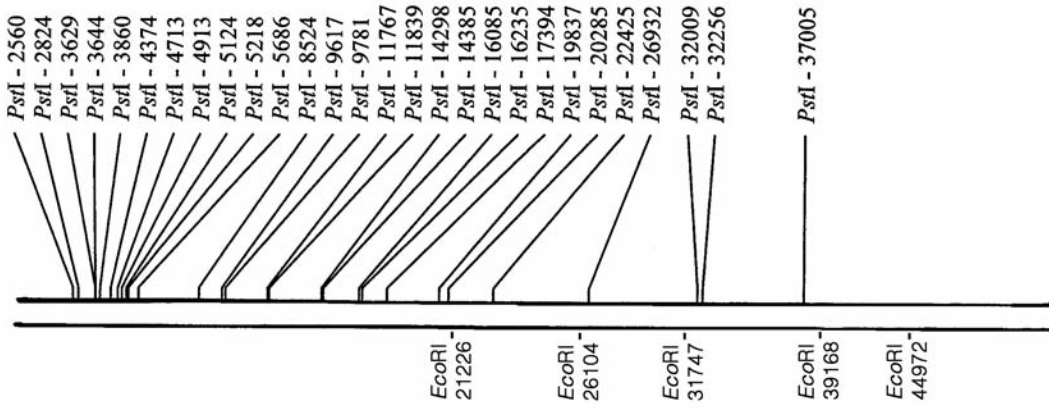
Plasmid Maps
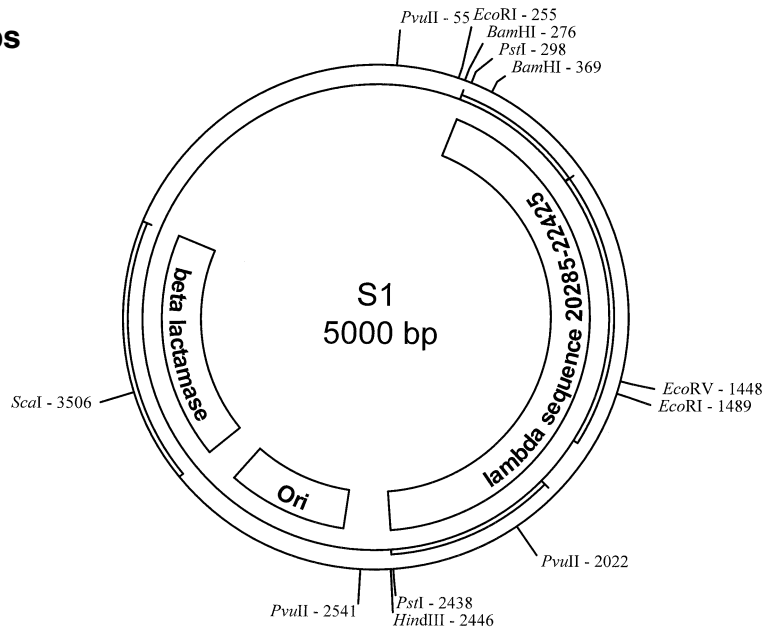
Suspect 1 DNA Sample
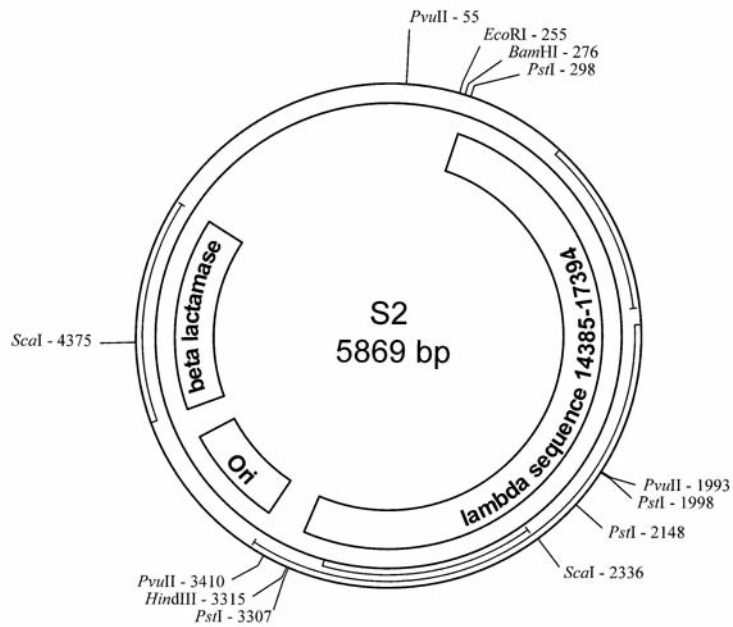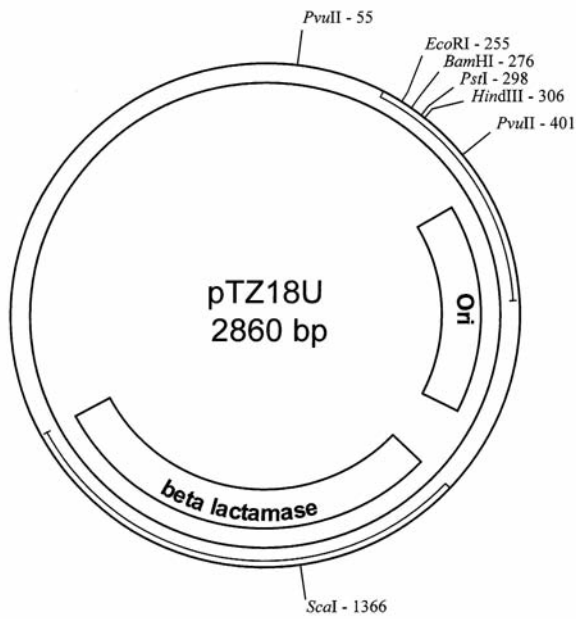

Suspect 2 DNA Sample

93
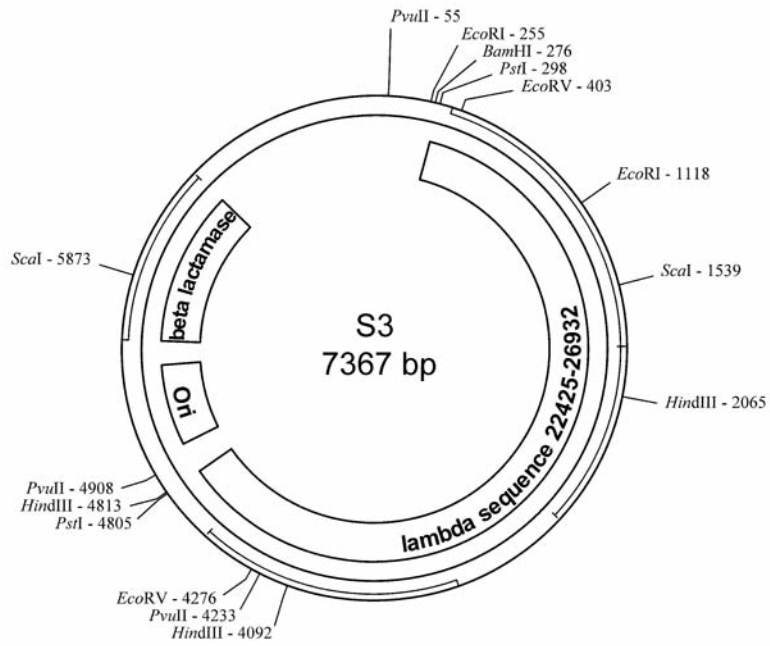
Crime Scene/Suspect 3 DNA Sample
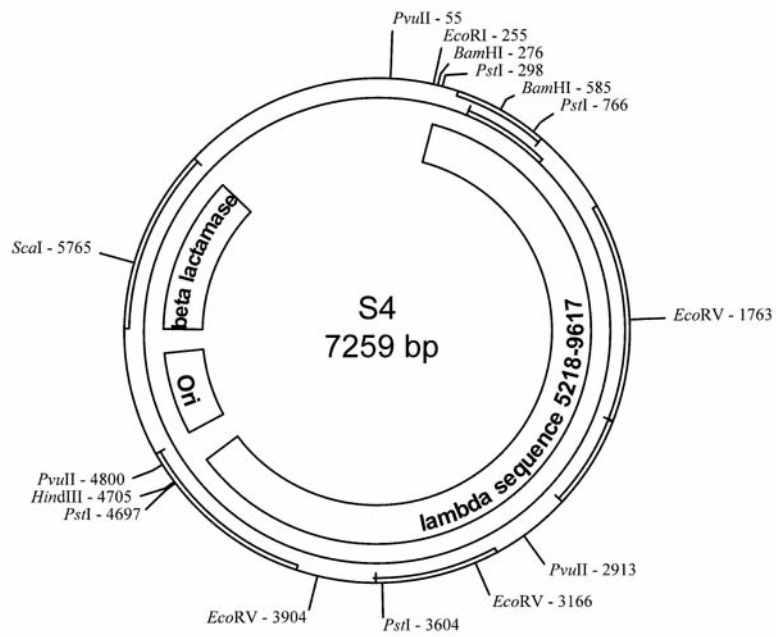
Suspect 4 DNA Sample

Suspect 5 DNA Sample

lambda bacteriophage genome
48502 bp

PstI - 2560
PstI - 2824
PstI - 3629
PstI - 3644
PstI - 3860
PstI - 4374
PstI - 4713
PstI - 4913
PstI - 5124
PstI - 5218
PstI - 5686
PstI - 8524
PstI - 9617
PstI - 9781
PstI - 11767
PstI - 11839
PstI - 14298
PstI - 14385
PstI - 16085
PstI - 16235
PstI - 17394
PstI - 19837
PstI - 20285
PstI - 22425
PstI - 26932
PstI - 32009
PstI - 32256
PstI - 37005

EcoRI 21226
EcoRI 26104
EcoRI 31747
EcoRI 39168
EcoRI 44972

PvuII - 55
EcoRI - 255
BamHI - 276
PstI - 298
HindIII - 306
PvuII - 401
Ori
pTZ18U
2860 bp
beta lactamase
ScaI - 1366

Plasmid Parent Vector

Appendix G
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.

7. Using Restriction Mapping to Teach Basic Skills in the Molecular Biology Lab, Lauren Walsh, Elizabeth Shakir, and Elizabeth A De Stasio, *Biochemistry and Molecular Biology Education*, Vol 35, No 3, pp 199–205, 2007.

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