EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-43

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DNA DuraGel™

Experiment Objective:

In this simulation, students explore agarose gel electrophoresis using simulated polymer gels. First, students learn how to load an agarose gel using a micropipette or a transfer pipet. Next, students will use gel images to simulate separation of DNA fragments by electrophoresis. Finally, students calculate the size of DNA fragments using a linear standard curve.

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Experiment Components

Contents	Check (√)
• DNA DuraGels™	❑
• Practice Gel Loading Buffer	❑
• Plastic pipets	❑
• FlashBlue™ and InstaStain® Ethidium Bromide Gel Pictures	❑

Experiment #S-43 is designed for 10 groups.

Storage:
Store entire experiment in the refrigerator.

Requirements

- Automatic Micropipets with tips ❑
- Weigh boat or small plastic tray ❑
- Distilled or deionized water ❑
- Optional white light visualization system ❑

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

PRINCIPLES OF GEL ELECTROPHORESIS

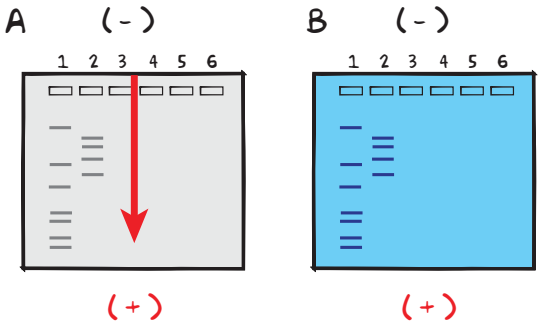


Figure 1:
Overview of Agarose Gel Electrophoresis

Gel electrophoresis is widely used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins. This technique possesses great resolving power, yet is relatively simple and straightforward to perform.

Agarose, a polysaccharide derived from seaweed, is commonly used to form the separation matrix used for gel electrophoresis. To make a gel, solid agarose powder is added to buffer and melted by boiling. The buffer controls the pH of the solution throughout the electrophoresis process, which is important to the charge and stability of biological molecules. Once the solution has cooled to approximately 60° C, it is poured into a gel tray to solidify. A special comb is used to form depressions in the gel called loading wells. Once solidified, the gel is placed in a horizontal electrophoresis chamber and covered with a pH-balanced buffer. Electrodes placed at each end of the electrophoresis chamber generate current when connected to a direct current power supply. The buffer contains ions necessary to conduct the electrical current.

Samples are prepared for electrophoresis by mixing them with glycerol or sucrose, which makes them denser than the electrophoresis buffer. When the samples are loaded into the wells, the dense samples sink through the buffer and remain in the wells. An electrical current is passed through the gel to drive molecules through the gel (Figure 1). Generally, the higher the applied voltage, the faster the samples are separated by electrophoresis. Once the current is applied, the biomolecules in the sample are pulled into the gel matrix. At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the molecules can pass. These pores act as a molecular sieve that affects the rate at which a molecule can migrate through the gel.

Factors such as the molecular charge, size and shape, together with buffer conditions, gel concentrations and voltage, can affect the mobility of molecules in a gel. For example, small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. Given two molecules of the same molecular weight and shape, like dyes, the one with the greater amount of charge will migrate faster. Molecules with a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Furthermore, molecules may have the same molecular weight and charge but different shapes. Molecules having a more compact shape, like a sphere, would move through the pores more quickly than molecules with a looser conformation.

Background Information

ACCURACY AND PRECISION IN BIOTECHNOLOGY

In biotechnology, small differences in volumes can make a big difference in the results of our experiments. For example, differences in primer or template volumes will change the concentration of these reagents, which can make a big difference in the results of PCR experiments. To address this problem, scientists use carefully calibrated micropipettes to measure small volumes in the laboratory. These micropipettes are very accurate, ensuring that the experiments are both successful and reproducible.

Micropipettes draw liquid into a disposable plastic tip by creating a vacuum in the barrel above the tip. The liquid is dispensed when the vacuum is released. The plastic tip is changed between samples to prevent cross-contamination. There are two types of micropipettes that are used in the classroom laboratory: fixed-volume and adjustable volume. Fixed-volume micropipettes are preset to a specific volume so that the same amount of sample is dispensed each time. In contrast, adjustable-volume micropipettes let you "dial in" the amount of sample to be measured and dispensed in one-microliter increments. These tools are manufactured to deliver samples in various ranges (common sizes include 0.5-10 µl, 5-50 µl, 200-1000 µl).

In a standard DNA electrophoresis experiment, 35 microliters (µl) of the DNA/Loading Dye mixture are loaded into each well. To ensure the success of the experiment, students need to accurately measure the volume of the sample to be loaded, and to be sure to dispense the entire the sample into the well. Students should practice pipetting and/or loading an agarose gel prior to conducting the experiment, especially if they are unfamiliar with the technique. EDVOTEK® DuraGels™ are reusable polymer gel models that allows students to practice sample delivery techniques before performing agarose gel electrophoresis (Figure 2). The use of DuraGels™ eliminates the preparation time, expense, and waste of pouring actual agarose practice gels.

Although scientists prefer micropipettes to load agarose gels, disposable plastic transfer pipets can also be used to transfer the DNA sample into the well. However, their volumes cannot be accurately controlled, meaning that there may be significant sample-to-sample variation in volume. When using transfer pipets for sample delivery, slowly load each sample well until it is full. To help control the delivery of the sample, gently squeeze the pipet stem instead of the bulb. Clean the transfer pipet between uses by thoroughly flushing it with distilled water several times.

Figure 2 - Loading a DNA DuraGel™

Background Information

SIZE DETERMINATION OF DNA FRAGMENTS

One of the most significant discoveries of molecular biology is a class of enzymes known as restriction endonucleases. These endonucleases (also known as restriction enzymes) are produced by many species of bacteria to protect themselves from invading viral DNA. Restriction enzymes act like molecular scissors, cutting double-stranded DNA at specific sequences. The utility of restriction enzymes has made molecular cloning, DNA mapping, sequencing and various genome-wide studies possible, launching the era of biotechnology.

After digesting DNA with restriction enzymes, the samples are analyzed using agarose gel electrophoresis. This technique separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the log10 of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard" (Figure 3). We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve".

The unknown DNA fragments will migrate through the gel according to their respective sizes. After measuring the migration distance of each band, the standard curve can be used to calculate the size of the unknown DNA fragments. If you are unfamiliar with sizing DNA fragments, EDVOTEK® DuraGels™ serve as a dry exercise for estimating the size of unknown DNA fragments from agarose gel before conducting the actual experiment. In this activity, students will learn how to accurately size DNA fragments using the EDVOTEK® DuraGels™ and simulated gel imprints. DuraGels™ contain grids imprinted with ruler for sizing DNA fragment.

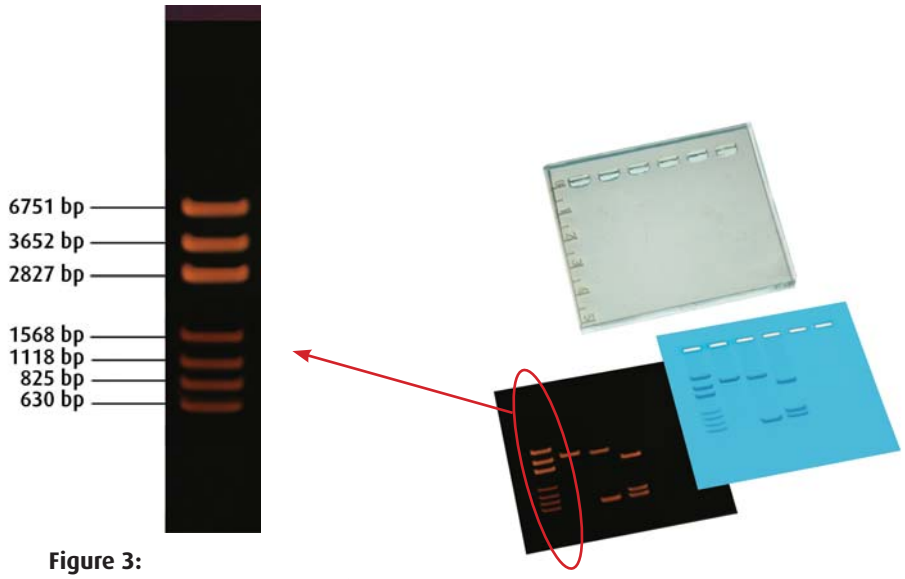


Figure 3:
DNA Standard markers (Lane 1)

Size Determination of DNA Fragments Using DuraGels™ and Gel Images

SIZE DETERMINATION OF DNA FRAGMENTS

1. Measure and Record Migration Distances

Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

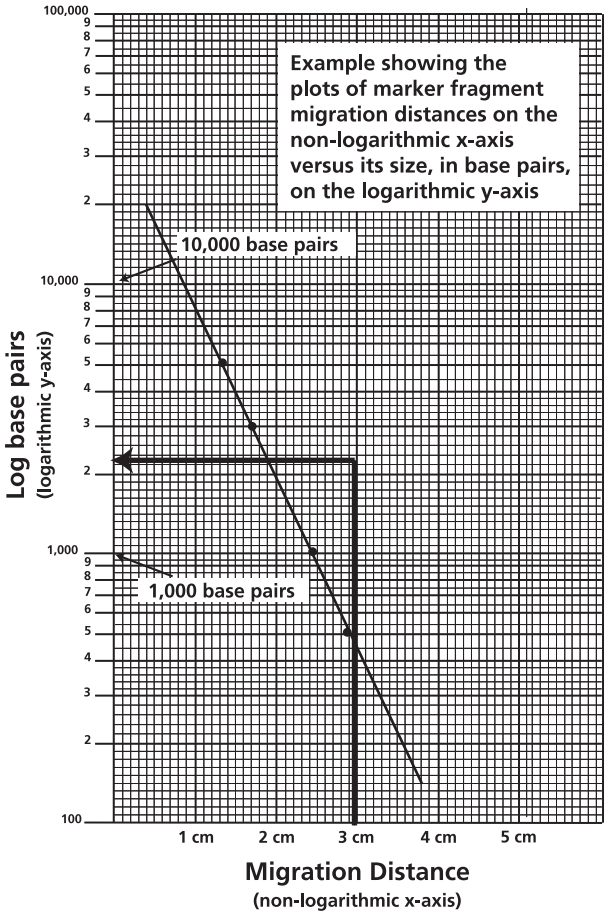
Figure 4: Measure distance migrated from the lower edge of the well to the lower edge of each band.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the $\log_{10}$ of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 5 for an example).

Figure 5: Semilog graph example

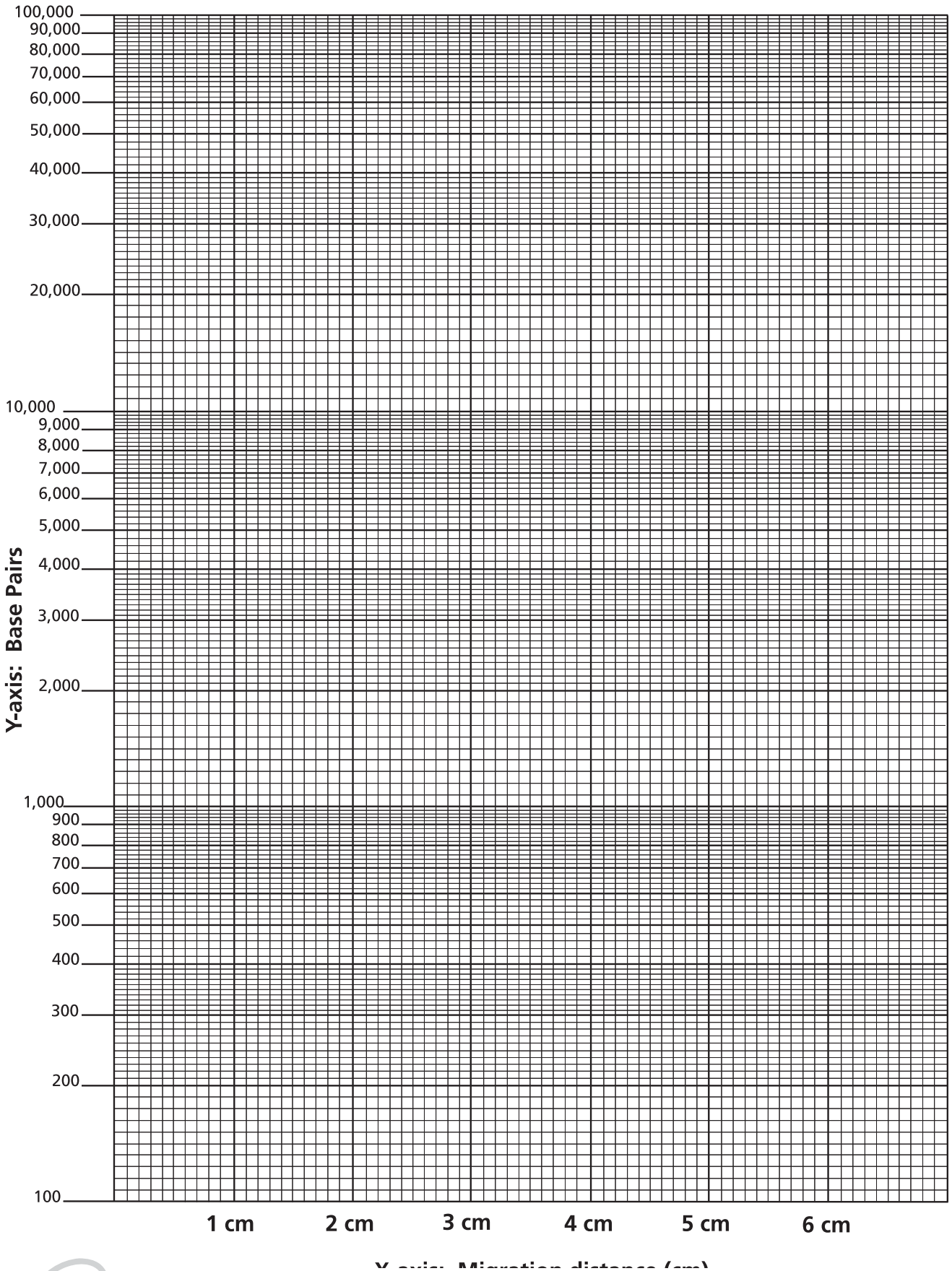


3. Determine the length of each unknown fragment.

a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 5 for an example). Make note of this in your lab notebook.

c. Repeat for each fragment in your unknown sample.



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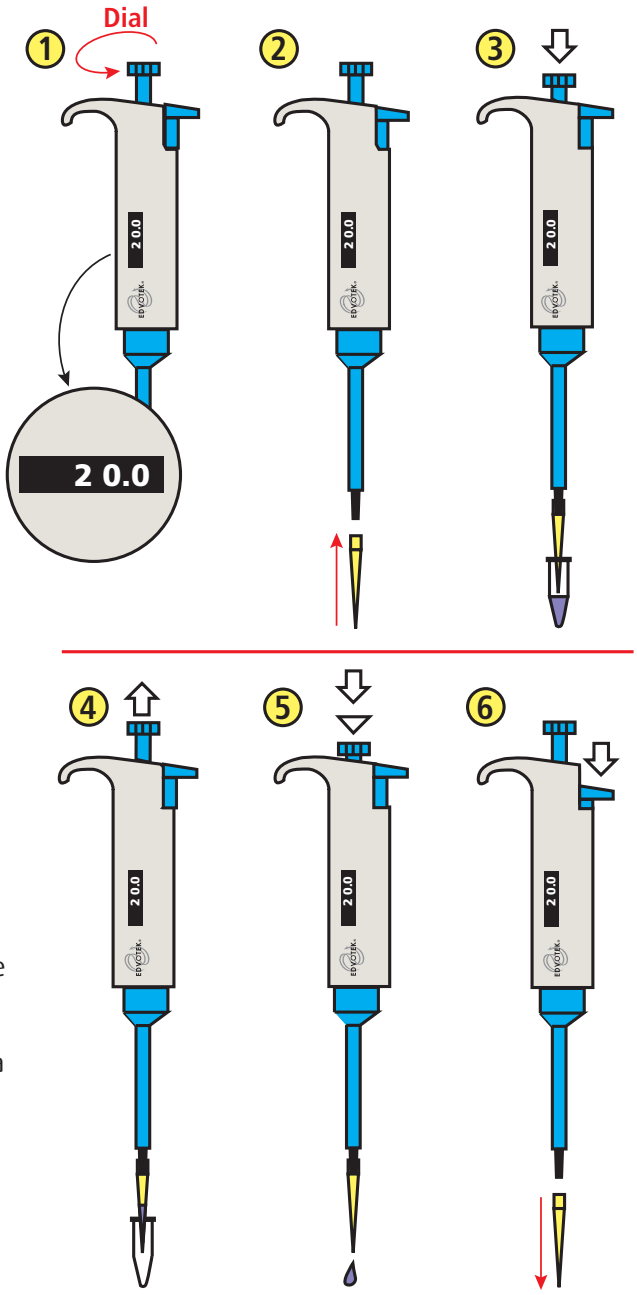
Measuring Liquids with a Variable Automatic Micropipet

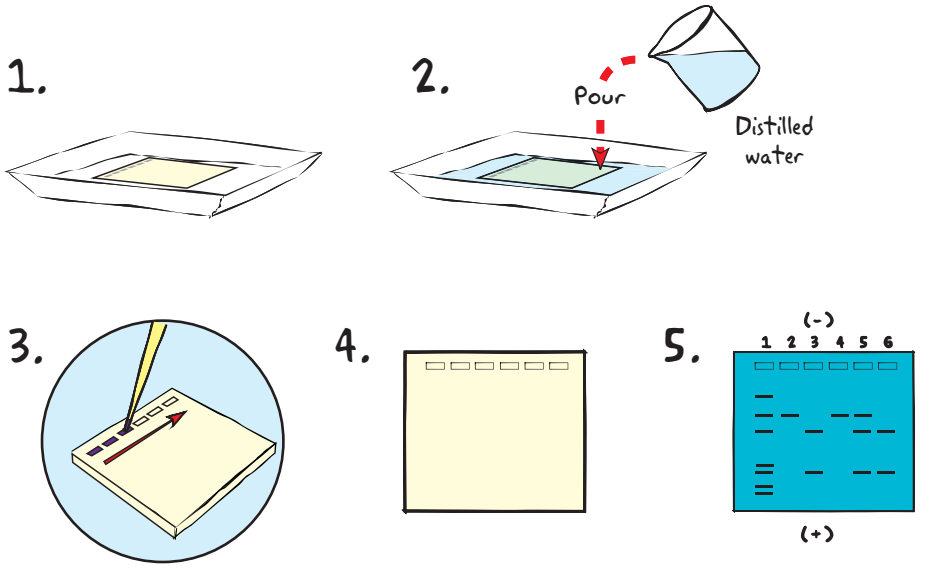
To measure microliter volumes, a special instrument called a micropipet is used. The variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample. These instruments are manufactured to deliver samples in various ranges (e.g., 0.5-10 µl, 5-50 µl, 200-1000 µl, etc.) and usually can be adjusted in one-microliter increments. Typically, these instruments have an ejector button for releasing the tip after sample delivery. Variable automatic micropipets can also be multi-channeled, designed to uniformly deliver several samples at the sample time. However, for this experiment, only one sample will be delivered at a time.



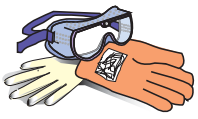
1. **SET** the micropipet to the appropriate volume by adjusting the dial.
2. **PLACE** a clean tip on the micropipet.
3. **PRESS** the plunger down to the first stop. **HOLD** the plunger down while placing the tip beneath the surface of the liquid.
4. Slowly **RELEASE** the plunger to draw sample into the pipette tip.
5. **DELIVER** the sample by slowly pressing the plunger to the first stop. Depress the plunger to the second stop to expel any remaining sample. **DO NOT RELEASE** the plunger until the tip is out of the sample container.
6. **DISCARD** the tip by pressing the ejector button. Use a new tip for the next sample.

NOTE:
Although the variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample, other less expensive equipment alternatives such as fixed volume micropipets or disposable transfer pipets can be used effectively.

Using the DNA DuraGel™



1. **PLACE** the DuraGel™ into a clear or white shallow dish.
2. Completely **COVER** the gel with distilled or deionized water.
3. **LOAD** 35 µL of the Practice Gel Loading Buffer into consecutive wells in the gel.
4. **REMOVE** the DuraGel™ from the dish and dry thoroughly.
5. **PLACE** the DuraGel™ on top of one of the gel images and **ANALYZE** your results.



Wear gloves and safety goggles

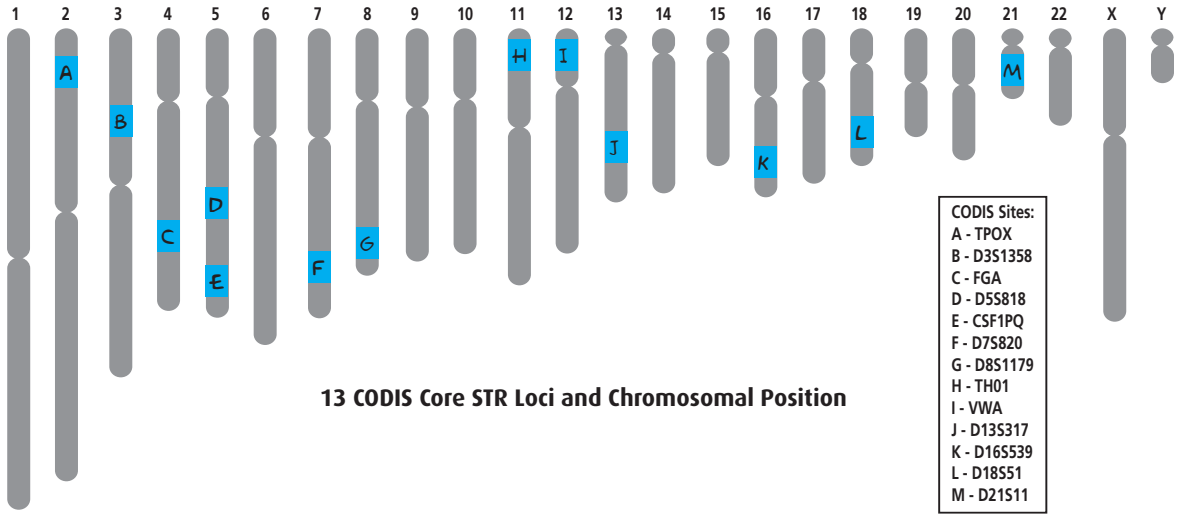
LESSON A: DNA Fingerprinting by PCR Amplification

In humans, DNA is packaged into 23 pairs of chromosomes that are inherited from an individual's biological parents. Although most of this genetic material is identical in every person, small differences, or "polymorphisms", in the DNA sequence occur throughout the genome. For example, short repetitive stretches of DNA at specific locations in the genome can vary in number to produce STRs (Short Tandem Repeats) and VNTRs (Variable Number of Tandem Repeats). The polymerase chain reaction (PCR) can be used to analyze VNTRs and STRs at several loci within the human genome. Since an individual's genome contains a different combination of polymorphisms, we can generate a unique "DNA fingerprint" for that person.

The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are utilized to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. After the crime scene sample is analyzed, it is compared to DNA fingerprints from the suspects or those stored in CODIS (**CO**mbined **D**NA **I**ndex **S**ystem), a database of DNA fingerprints from convicted offenders, other crime scenes, and missing persons. Each DNA fingerprint analyzes thirteen separate loci, making the odds of an exact match less than one in a trillion. This evidence is then used in court to link a suspect to a crime scene.

Lane	Sample
1	DNA Standard marker
2	Crime scene PCR reaction
3	Suspect 1 PCR reaction
4	Suspect 2 PCR reaction
5	Suspect 3 PCR reaction

In this forensic DNA fingerprinting experiment, students will analyze one locus of crime scene and suspect samples using agarose gel electrophoresis. A match provides strong evidence that the suspect was present at the crime scene.



13 CODIS Core STR Loci and Chromosomal Position

LESSON A: STUDY QUESTIONS

1. What is a polymorphism? How are they used for identification purposes?

2. Compare the crime scene DNA sample to the three suspects. Who was at the crime scene?

LESSON B: DNA Mapping

Many restriction enzymes recognize palindromic stretches of DNA, generally 4-8 base pairs in length. The probability that a given enzyme will cut, or "digest", a piece of DNA is directly proportional to the length of its recognition site. Statistically, an enzyme will average one cut for every 4^n base pairs, where n is the length of the recognition site. For instance, an enzyme that recognizes a six base pairs long site (e.g., *Eco*RI) will cut once every 4096 (or 4^6) base pairs. Therefore, the longer a DNA molecule is, the greater the probability is that it contains one or more restriction sites. For example, if *Eco*RI is used to digest human chromosomal DNA containing 3 billion base pairs and a plasmid containing 5,000 base pairs, it will cut the chromosomal DNA over 700,000 times (3 billion base pairs, cut every 4096 base pairs), but may only cut the plasmid once (5,000 base pairs, cut every 4096 base pairs).

Digestion of plasmid DNA by a restriction enzyme will produce fragments of varying lengths depending on the locations of the recognition sites. This technique performed in the biotechnology laboratory to create a "map" of the molecule. If we digest a plasmid with several different restriction enzymes, we can determine the relative distance between the enzyme sites. This information is very important for downstream applications like creating recombinant DNA constructs.

For example, consider a 5000 base pair, circular plasmid DNA containing single recognition sites for three separate enzymes - A, B, and C. Each enzyme cuts the plasmid once. Different combinations of these enzymes will produce the following DNA fragments (in base pairs):

A+B	A+C	B+C	A+B+C
4500	3000	3500	3000
500	2000	1500	1500
			500

Looking at this data, we see that the combination of enzymes A and B generated the smallest fragment (500 bp) out of all of the double digests. This suggests that the cleavage site of B is the closest to that of A. Next, examining the other two double digests, we can determine that the restriction sites for enzymes A and C are 2000 base pairs apart, and that the restriction sites between enzymes B and C are 1500 base pairs apart. (Figure 6)

Now that we have determined the shortest relative distance between each of the restriction sites, we can begin to assign their positions in the plasmid. As a starting point, the cleavage site for enzyme A is assigned to position zero, and the cleavage site for enzyme B is assigned to position 500. We also know that the restriction site for enzyme C cannot between A and B, because the 500 base pair fragment remains after the triple digest (A+B+C).

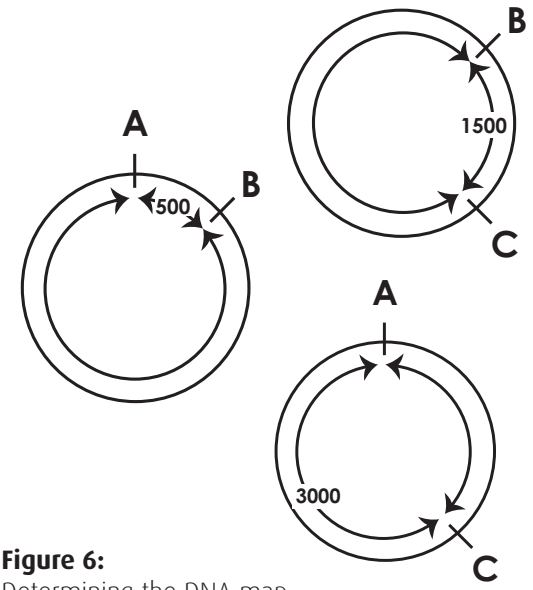


Figure 6:
Determining the DNA map based on experimental DNA fragments obtained.

LESSON B: DNA Mapping

Next, the data from the triple digest shows that the 2000 base pair fragment found in the A+C digest is cleaved into 1500 and 500 base pair fragments. This suggests that the restriction site for enzyme B is located between the sites for enzyme A and C. This kind of logic enables the construction of a map from DNA fragment sizes.

Going in a counter-clockwise direction from A, the distances between A and B is 500 bp, B and C is 1500 bp, and C and A is 3000 bp (Figure 7). Note that the data from this experiment cannot tell us the absolute orientation of the cleavage sites since it can lead to an alternative map as shown in Figure 8. However, the relative positions between the cut sites remain the same (B is between A and C).

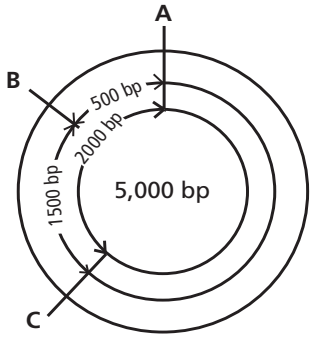


Figure 7:
Construction of a plasmid map using three enzymes.

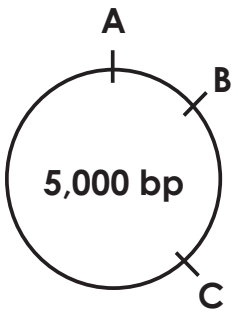


Figure 8:
Plasmid with single restriction enzyme sites for three enzymes - A, B, & C.

LESSON B: STUDY QUESTIONS

1. What is a restriction enzyme?

2. Describe DNA mapping and list some important uses for this technology.

3. When plotting the sizes of DNA fragments, which axis is used to plot the migration distances of the known and unknown fragments? Which axis is used to plot the sizes of the known and unknown fragments?

4. A plasmid DNA was cut with several restriction enzymes and the following fragment sizes were determined by comparing the unknown fragments to a standard DNA marker:

Enzyme 1	3000
Enzyme 2	3000
Enzyme 3	1800 & 1200
Enzymes 1 & 2	1450 & 1550
Enzymes 2& 3	1800, 650, & 550
Enzymes 1 & 3	1200, 1000, & 800

Draw a restriction map based on the data.

Instructor's Guide

CARE AND MAINTENANCE OF DURAGELS™

- If the DuraGel™ gets dirty over time, gently rinse it with distilled water and let air dry.
- Do not use abrasive detergents to clean the DuraGels™ since this will result in unwanted scratches on the surface of the gel. Instead, use a mild detergent to clean the gel. Be sure to completely rinse the detergent off of the gel with distilled wiater.
- Allow the DuraGels™ to completely dry before storing them for future use. The DuraGels and the gel image should be stored in separate plastic bags.

Lesson A: Experiment Results

The DNA standards in Lane 1 make it possible to measure the DNA bands obtained from the PCR reactions. The results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA and Suspect 2 match. In criminal investigations, several known variable regions in DNA are analyzed to match crime scene and suspect DNAs.

Lane	Tube	Sample	Molecular Weights (in bp)
1	A	DNA Standard Markers	--------
2	B	Crime scene PCR Reaction	3000, 1282
3	C	Suspect #1 PCR Reaction	3000
4	D	Suspect #2 PCR Reatcion	3000, 1282
5	E	Suspect #3 PCR Reaction	3652, 630

Answers to Study Questions

LESSON A

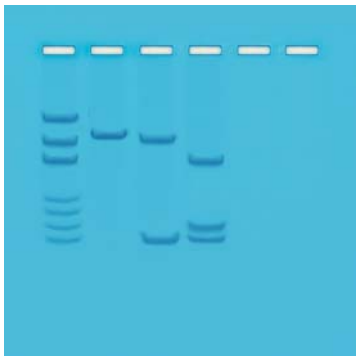
1. What is a polymorphism? How are they used for identification purposes?

Polymorphic DNA refers to chromosomal regions that vary widely from person to person. This variation is usually in the length of a specific DNA region. By analyzing a number of these regions, one can obtain a "DNA fingerprint" of a person that will not match the DNA fingerprint of any other individual. "DNA fingerprinting" is used for identification of missing persons, human remains, and matching criminal suspects to crime scenes.

2. Compare the crime scene DNA sample to the three suspects. Who was at the crime scene?

The DNA sample from suspect #2 matches the crime scene DNA.

Lesson B: Experiment Results



Lane	Tube	Sample	Molecular Weights (in bp)
1	A	DNA Standard Markers	--------
2	B	Enzyme 1	4300
3	C	Enzyme 2	3650, 650
4	D	Enzyme 1 & 2	2810, 840, 650

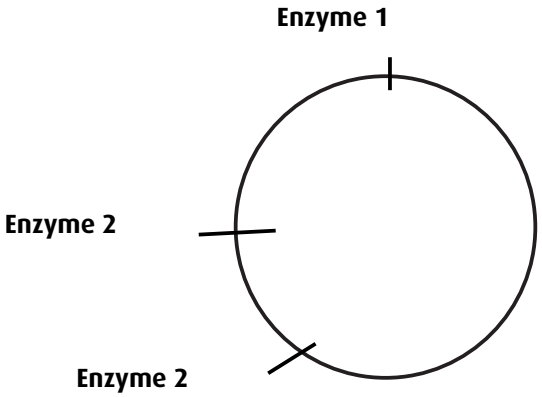


Figure A

or

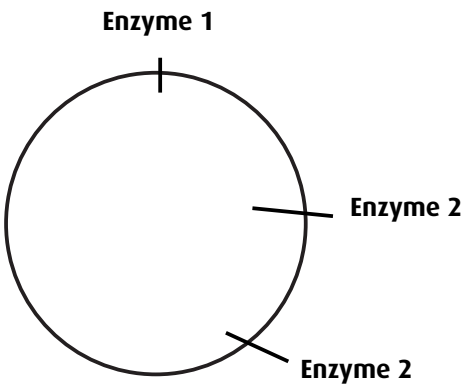


Figure B

Referring to Figure B, going in a clockwise direction, the approximate distance, in base pairs between:

Enzyme 1 and nearest Enzyme 2: 840

Enzyme 2 and Enzyme 2: 650

Enzyme 1 and farthest Enzyme 2: 1490

Please refer to the kit insert for the Answers to Study Questions