EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-51

Edvo-Kit #S-51

Whose DNA Was Left Behind?

Experiment Objective:

This experiment explores the principles of DNA fingerprinting for the analysis of crime scene DNA. After performing agarose gel electrophoresis with colorful dyes, students will determine which suspect was at the crime scene.

See page 3 for storage instructions.

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

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store QuickStrip™ samples in the refrigerator immediately upon receipt.
All other components can be stored at room temperature.

Components (in QuickStrip™ format)		Check (√)
A	Crime Scene Simulated DNA Digest 1	❑
B	Crime Scene Simulated DNA Digest 2	❑
C	Suspect #1 Simulated DNA Digest 1	❑
D	Suspect #1 Simulated DNA Digest 2	❑
E	Suspect #2 Simulated DNA Digest 1	❑
F	Suspect #2 Simulated DNA Digest 2	❑

Reagents & Supplies

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Gel Loading Solution ❑
- 1 ml pipet ❑
- Microtipped Transfer Pipets ❑

Experiment #S-51 is designed for 10 gels.

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips (optional)
- Balance
- Microwave, hot plate or burner
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Visualization system (white light box)
- Distilled or deionized water

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

In humans, DNA is packaged into 23 pairs of chromosomes. Although most of this DNA is identical between individuals, small sequence differences, or "polymorphisms", occur at specific locations throughout the genome. These polymorphisms include single base pair changes and repetitive DNA elements. Analyzing several polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another.

The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are used to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. First, a suitable sample must be found. Forensic scientists use great care collecting evidence from crime scenes so that the DNA will not be damaged. After DNA is extracted from these samples, forensic scientists analyze the sample to create a DNA fingerprint. The DNA fingerprint from a crime scene can then be compared to the DNA fingerprints of different suspects. A match provides strong evidence that the suspect was present at the crime scene.

Early fingerprinting analysis involved treating the isolated DNA with special enzymes called restriction endonucleases, which act like molecular scissors to cut DNA at specific sites. Based on the distances between recognition sites, digestion of DNA by a restriction enzyme will produce DNA fragments of varying lengths. After electrophoresis of the digested sample, the DNA is transferred to a nylon membrane during a process known as Southern blotting. Sequence-specific DNA probes are used to visualize the membrane-bound DNA. If the DNA is not digested by the restriction enzyme, the probes will only hybridize to a single DNA segment. If a restriction site occurs within this sequence, the probe will hybridize with multiple bands of DNA. This technique, called Restriction Fragment Length Polymorphism (RFLP) analysis, was first used in a criminal investigation in the mid-1980s (summarized in Figure 1).

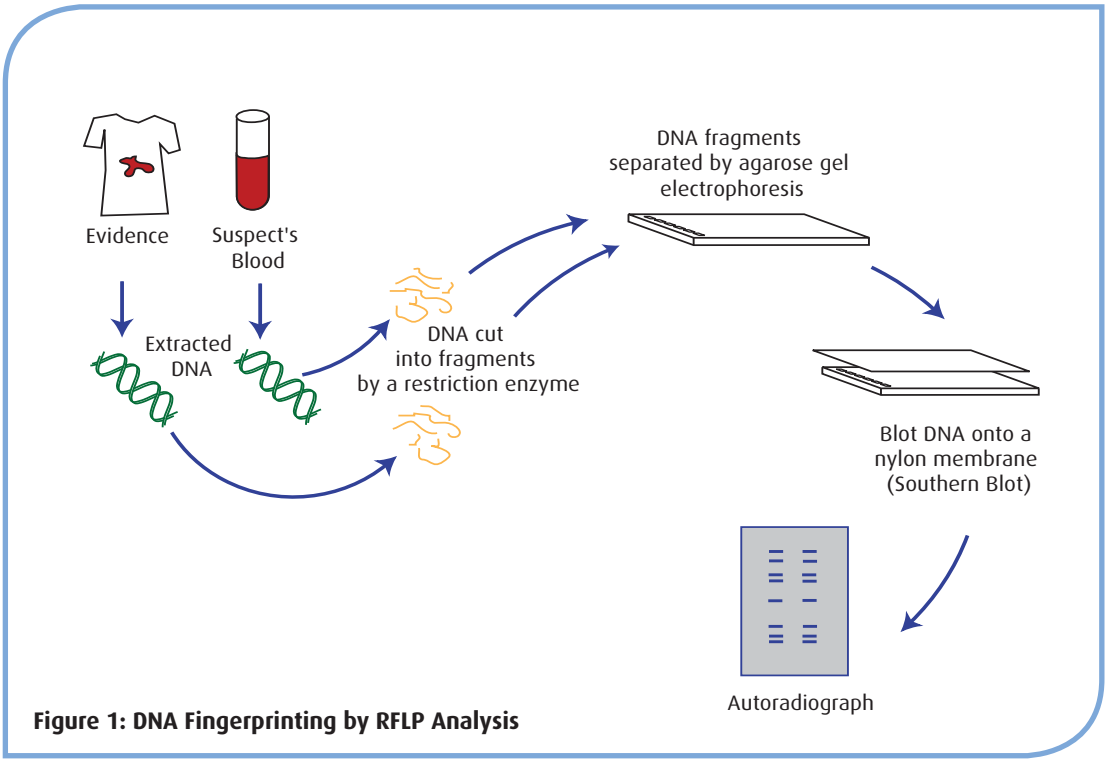


Figure 1: DNA Fingerprinting by RFLP Analysis

Background Information

Although RFLP analysis is very precise, it is time-consuming and requires large amounts of DNA. To address these problems, forensic scientists use the polymerase chain reaction (PCR) to produce DNA fingerprints. PCR allows researchers to quickly create many copies of a specific region of DNA *in vitro*. This technique requires 500-fold less DNA than traditional RFLP analysis and it can be performed in an afternoon.

To perform PCR, purified double-stranded DNA is mixed with primers (short synthetic DNA molecules that target DNA for amplification), a thermostable DNA polymerase (*Taq*) and nucleotides. The mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (Figure 2). To produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to rapidly heat and cool the samples.

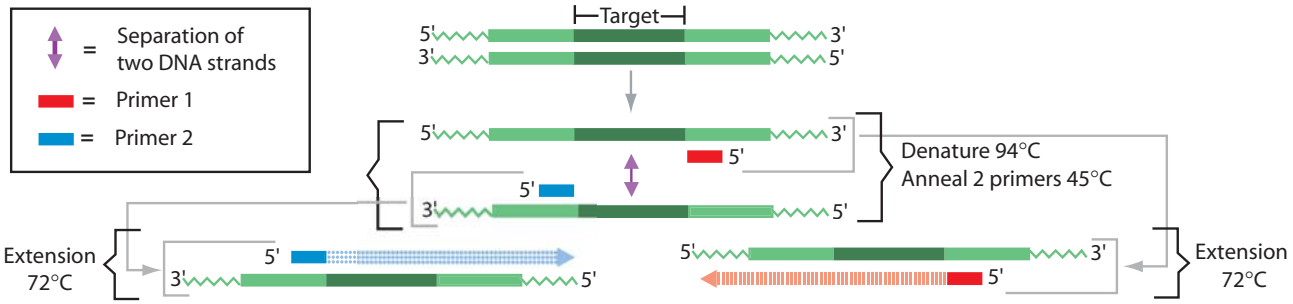


Figure 2: Amplification of DNA by PCR

After the crime scene sample is analyzed using electrophoresis, it is compared to DNA fingerprints from the suspects or those stored in CODIS (COmbined DNA Index System), 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.

In this forensic DNA fingerprinting experiment, students will perform RFLP analysis to link a suspect to a crime scene. First, a biological sample was collected at the crime scene. Next, samples were collected from two suspects. The DNA was extracted and digested using two different restriction enzymes before being separated using agarose gel electrophoresis. Each pair of restriction digests will create an individual's unique fingerprint. The goal is to analyze the DNA fingerprint patterns after agarose gel electrophoresis and to decide if Suspect 1 or Suspect 2 was at the crime scene. The DNA fragmentation patterns are simple enough to analyze directly in the agarose gel. In this experiment, the DNA fragments produced by restriction digest are represented by various dyes. The purple, blue, and orange bands represent DNA fragments of different sizes. By using brightly colored dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time.

Experiment Overview

EXPERIMENT OBJECTIVE:

This experiment explores the principles of DNA fingerprinting for the analysis of crime scene DNA. After performing agarose gel electrophoresis with colorful dyes, students will determine which suspect was at the crime scene.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

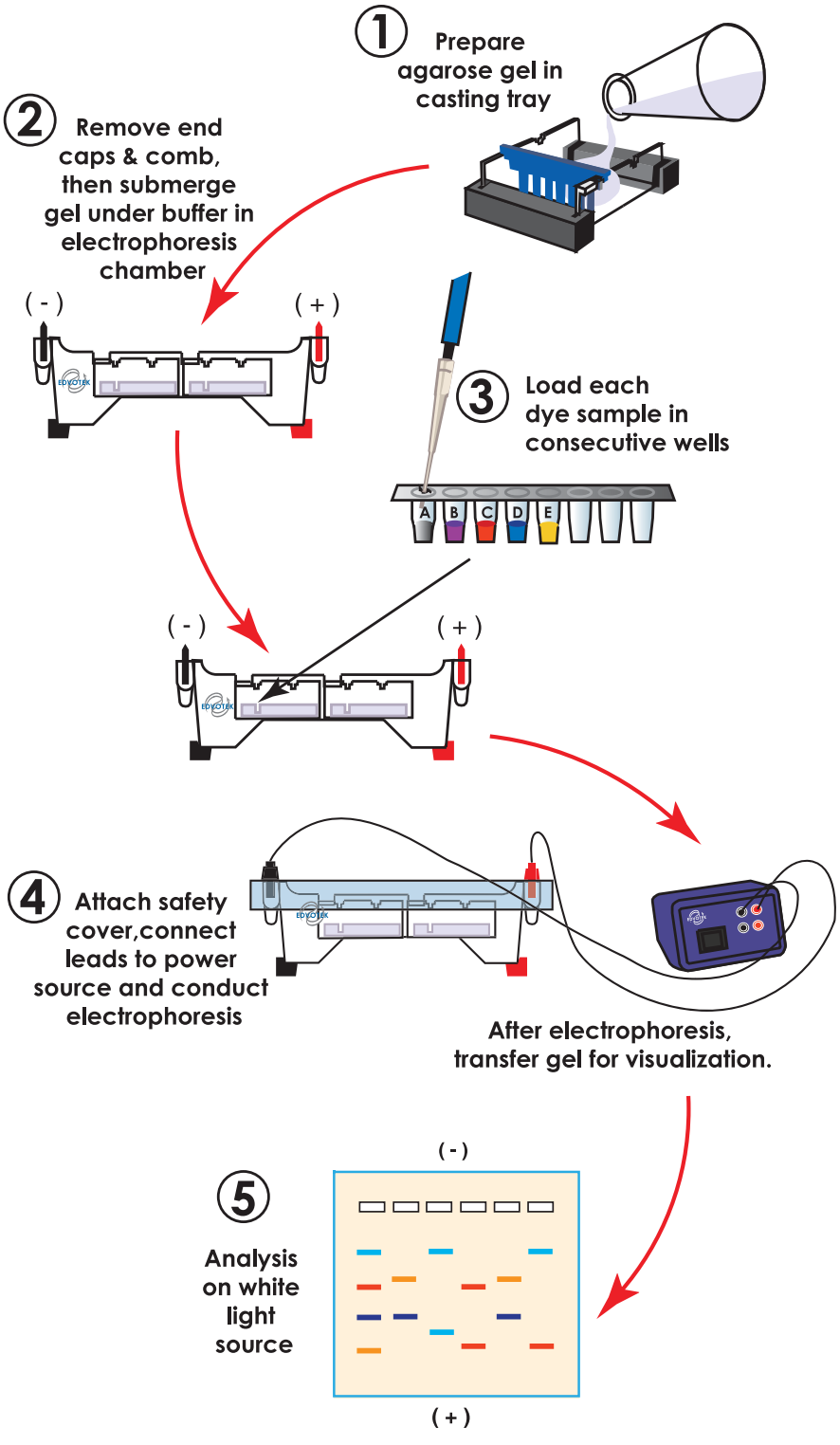
During the Experiment:

- Record your observations.

After the Experiment:

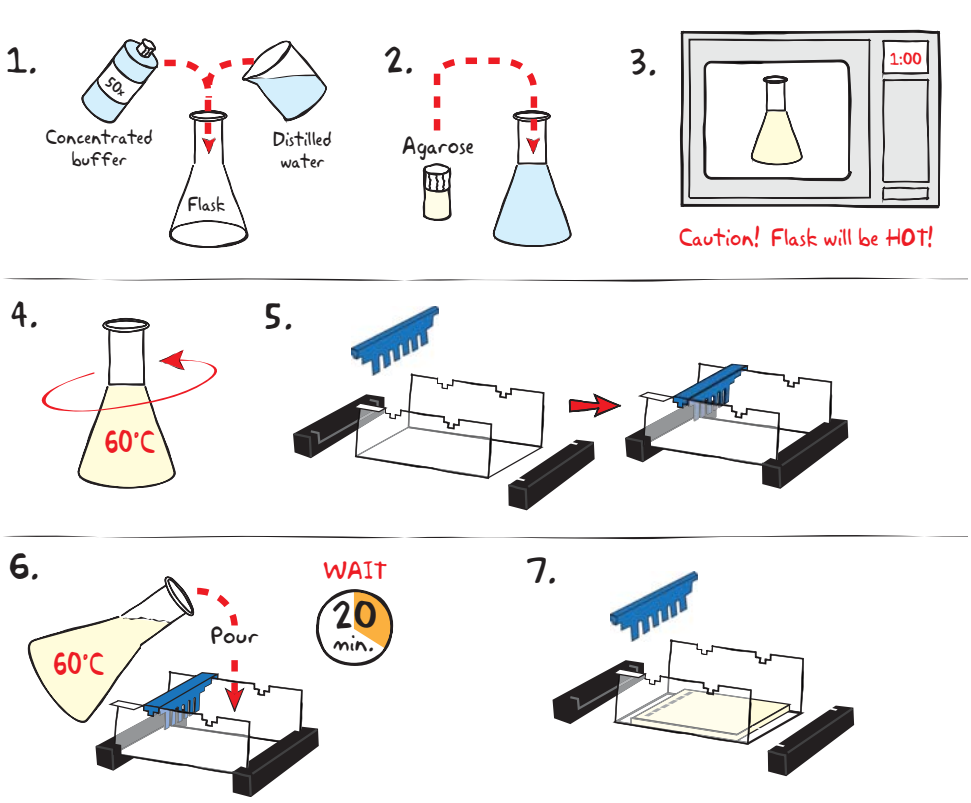
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview



Gel pattern will vary depending upon the experiment.

Agarose Gel Electrophoresis



IMPORTANT:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

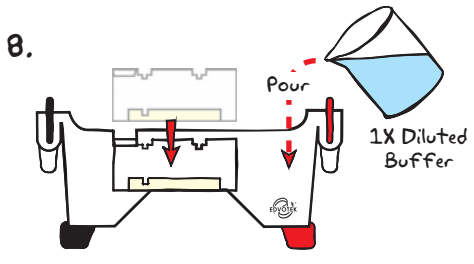
Wear gloves and safety goggles

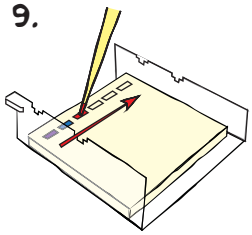
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

Agarose Gel Electrophoresis

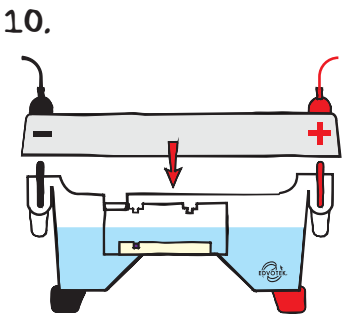


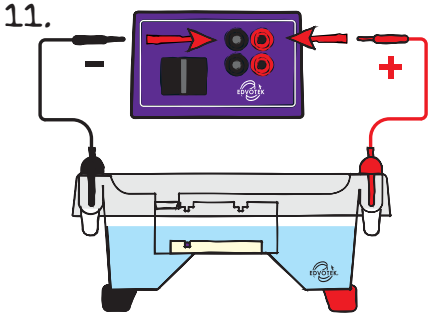


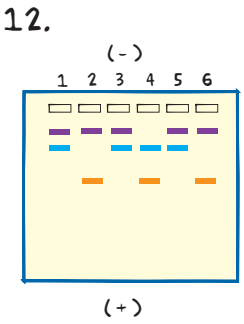
Reminders:

If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.







8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38 µL) into the well in consecutive order. The identity of each sample is provided in Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the dye samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Crime Scene Simulated DNA Digest 1
2	Tube B	Crime Scene Simulated DNA Digest 2
3	Tube C	Suspect 1 DNA Digest 1
4	Tube D	Suspect 1 DNA Digest 2
5	Tube E	Suspect 2 DNA Digest 1
6	Tube F	Suspect 2 DNA Digest 2

Table B
1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C
Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model M6+	Electrophoresis Model M12 & M36
Volts	Min. / Max.	Min. / Max.
150	15/20 min.	25 / 35 min.
125	20/30 min.	35 / 45 min.
75	35 / 45 min.	60 / 90 min.
50	50 / 80 min.	95 / 130 min.

Study Questions

1. Why is it important to position the sample wells near the negative electrode?
2. What kind of evidence would you look for at a crime scene to obtain DNA?
3. How will you be able to tell who committed the crime?
4. Who is the suspect that committed the crime?
5. What determines that each person has a unique pattern within their DNA?
6. Can you think of a case when two people will have identical DNA patterns?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

What to do:	When:	Time Required:
Prepare QuickStrips™	Up to one day before performing the experiment.	40 min.
Prepare diluted Electrophoresis Buffer		
Prepare molten agarose and pour gel		

Pre-Lab Preparations:

SEPARATION OF PCR PRODUCTS BY AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See the Student's Experimental Procedure. Students will need Electrophoresis Buffer (50x), distilled water and agarose powder.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20° C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results.

If students are unfamiliar with using micropipets, we recommend performing the optional activity found in Appendix C, Practice Gel Loading, prior to conducting the experiment.

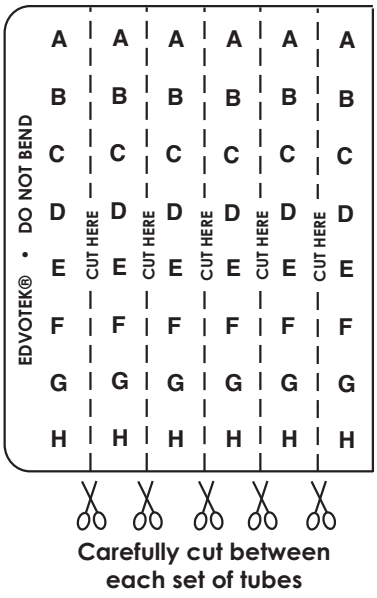
Each Student Group should receive:
- Electrophoresis Buffer (50x)
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

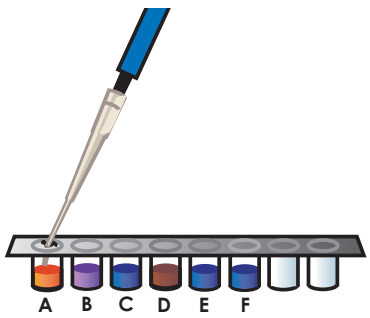
QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted dyes.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

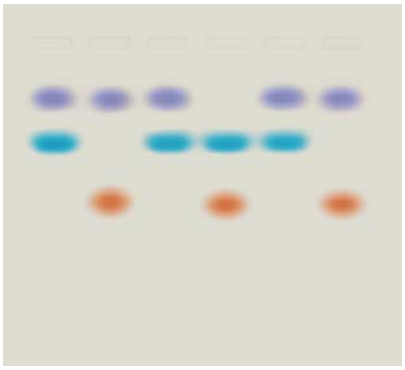
Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.

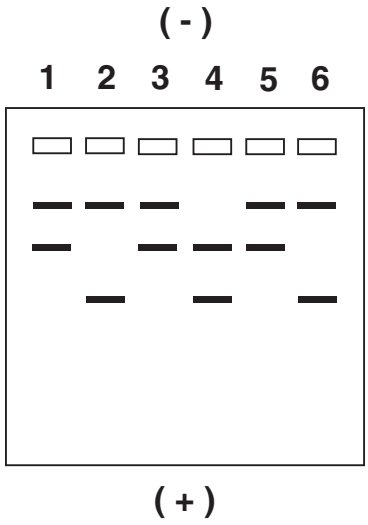


Carefully cut between each set of tubes



Experiment Results and Analysis





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5	Tube E	Suspect 2 DNA Digest 1
6	Tube F	Suspect 2 DNA Digest 2

Idealized results are shown in the figure above. Actual results will yield bands of varying intensity. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.

ANALYSIS

- Lanes 1 and 2 (set one) represent the crime scene DNA digested by two different restriction enzymes, which yield distinctly different DNA banding patterns.
- Lanes 3 and 4 (set two) represent DNA from Suspect 1. The suspect's DNA has been digested with the same two restriction enzymes as in Lanes 1 and 2.
- Lanes 5 and 6 (set three) represent DNA from Suspect 2. It also has been digested with the same two enzymes as the crime scene DNA (Lanes 1 and 2) and DNA from Suspect 1 (Lanes 3 and 4).
- The match between the crime scene DNA and Suspect 2 provides strong evidence that the suspect was present at the crime scene.

DISCUSSION QUESTION:

Could these DNA samples have been distinguished from one another if only Enzyme 1 had been used?

Questions and Answers to Study Questions

1. **Why is it important to position the sample wells near the negative electrode?**

   The DNA will travel toward the positive electrode.

2. **What kind of evidence would you look for at a crime scene to obtain DNA?**

   Hair, blood, skin

3. **How will you be able to tell who committed the crime?**

   The criminal's DNA pattern will match the pattern found at the crime scene.

4. **Who is the suspect that committed the crime?**

   The sample from Suspect Two and the crime scene samples match.

5. **What determines that each person has a unique pattern within their DNA?**

   Variations in DNA sequences among individuals will result in different cleavage patterns.

6. **Can you think of a case when two people will have identical DNA patterns?**

   Identical twins will have the same DNA pattern since they have identical DNA

Appendices

A EDVOTEK® Troubleshooting Guide
B Bulk Preparation of Agarose Gels
C Practice Gel Loading

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

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands not visible on the gel	The electrophoresis buffer was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The dyes ran off of the gel because the polarity of the leads was reversed.	Ensure that leads are attached in the correct orientation.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
Very light colored band seen after electrophoresis	Pipetting error.	Make sure students pipet 35 µl of dye sample per well.
Poor separation of bands	Gel was not prepared properly.	Make sure to prepare a 0.8% gel.
Dye bands disappear when the gels are kept at 4° C.	The dye molecules are small and will diffuse out of the gel.	The results must be analyzed upon the completion of electrophoresis

Appendix B
Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

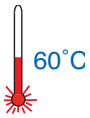
Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

Note:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

Appendix C
Practice Gel Loading

Accurate sample delivery technique ensures the best possible gel results. Pipetting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel.

If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

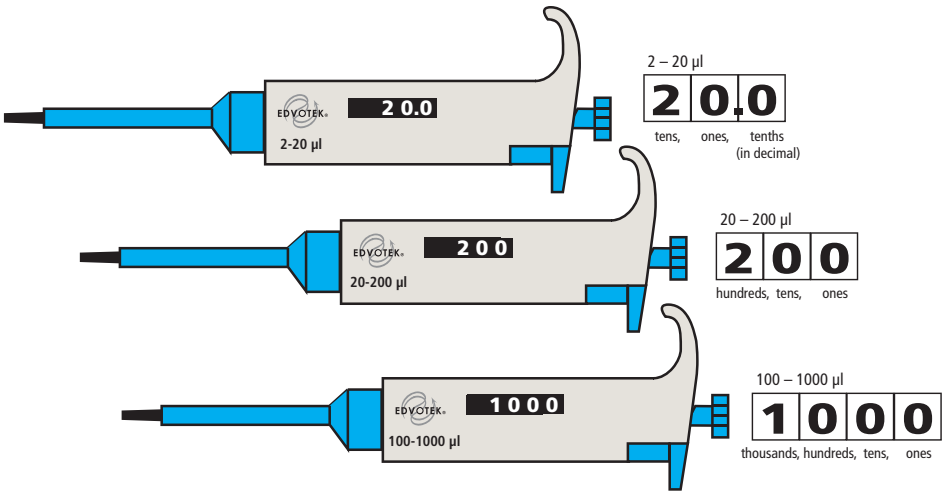
1. Cast a gel with the maximum number of wells possible.
2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

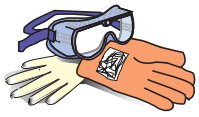
   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge it under buffer or water.
3. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.
   - For electrophoresis of dyes, load the sample well with 35-38 microliters of sample.
   - If using transfer pipets for sample delivery, load each sample well until it is full.
4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.
5. Replace the practice gel with a fresh gel for the actual experiment.

Note:
The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

Note: If practicing gel loading in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. It will not interfere with the experiment, so it is not necessary to prepare fresh buffer.

Appendix C
Practice Gel Loading





Wear gloves and safety goggles

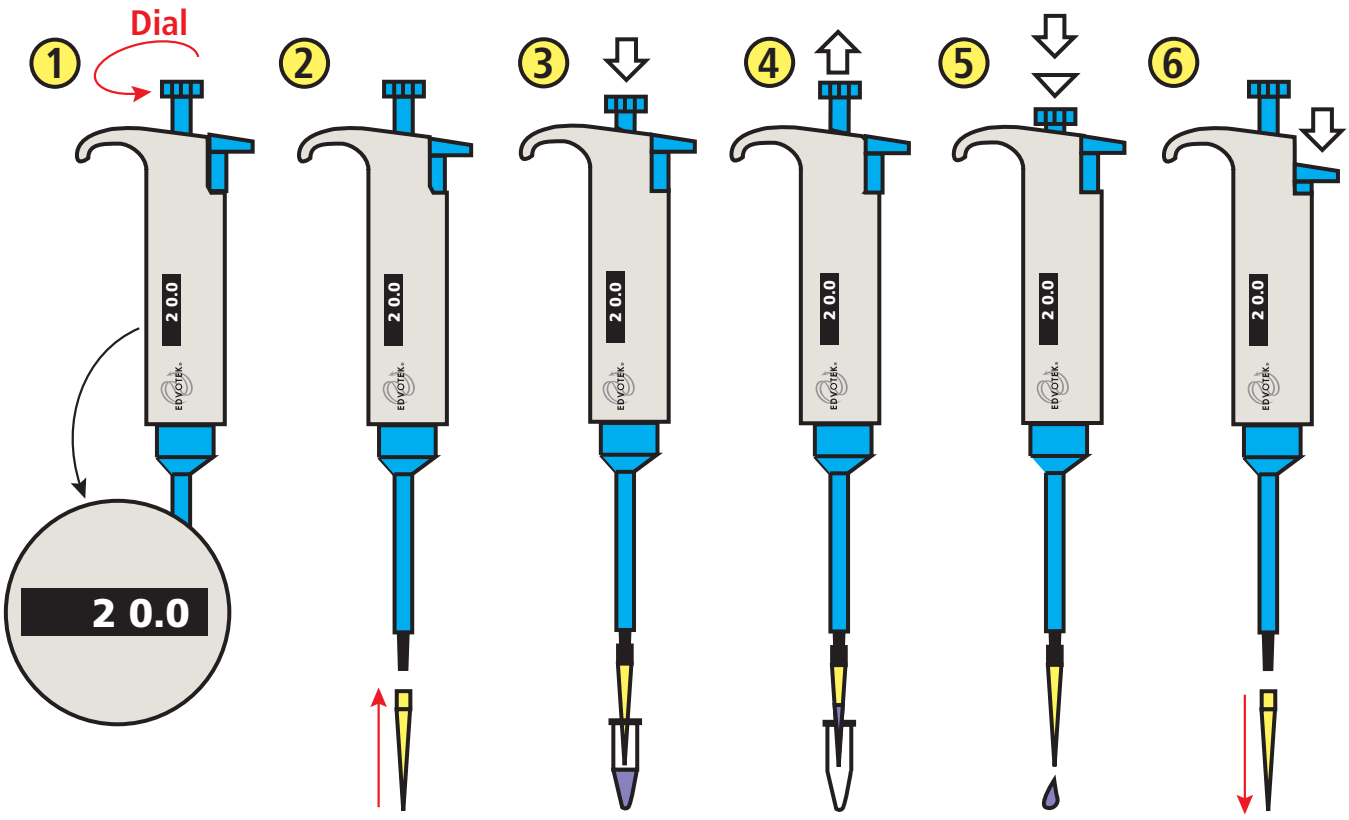
SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

1. **CHOOSE** the correct micropipet for the volume you are measuring. Make sure that the volume to be measured **DOES NOT EXCEED** the upper or lower volume setting of the micropipet.

2. **DETERMINE** the units measured by the micropipet by looking at the volume setting. The setting will appear in the window on the side of the micropipet. Note that the different micropipets use different scales for their measurements. Some micropipets are accurate to a tenth of a microliter, while others are accurate to one microliter.

3. **SET** the volume by twisting the top of the plunger. In general, twisting the plunger clockwise reduces the volume, and twisting the plunger counter clockwise increases the volume.

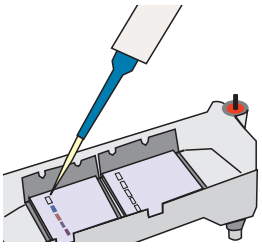
Appendix C
Practice Gel Loading



MEASURING LIQUIDS WITH A MICROPIPET


Wear gloves and safety goggles

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. Position the pipet tip over the well. Be careful not to puncture or damage the well with the pipet 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 buffer.
6. **DISCARD** the tip by pressing the ejector button. Use a new clean tip for the next sample.