Experiment Components

Storage:
Store this experiment at room temperature.

This experiment is designed for 10 groups.

Contents

A Crime Scene Simulated DNA Sample 1
B Crime Scene Simulated DNA Sample 2
C Suspect One Simulated DNA Sample 1
D Suspect One Simulated DNA Sample 2
E Suspect Two Simulated DNA Sample 1
F Suspect Two Simulated DNA Sample 2

Transfer pipets
UltraSpec-Agarose™
50x Electrophoresis Buffer
Practice Gel Loading Solution

None of the experiment components have been prepared from human sources. Simulated DNA samples are non-toxic, water-based dyes.

Experiment Requirements

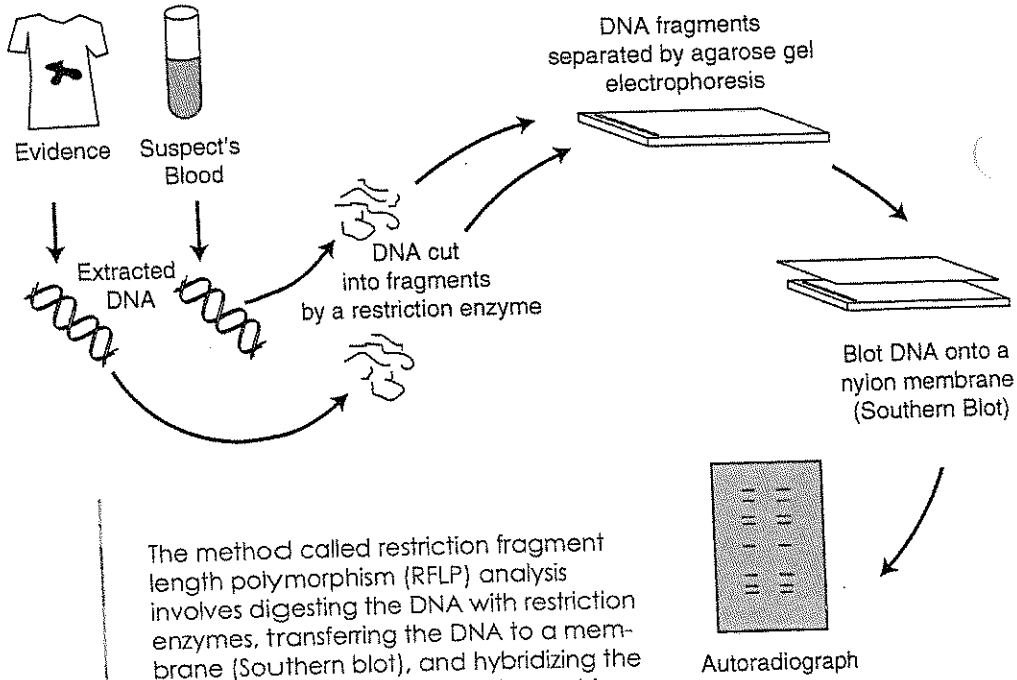
Experiment Requirements

- Electrophoresis Apparatus, M-12 or equivalent
- D.C. Power Supply
- Heat Source
- 500 ml Beaker or Flask
- Hot Gloves
- Distilled Water (used to make buffer solutions)
- Balance
- Automatic Micropipet and tips (optional)

Background and Introduction

DNA fingerprinting allows for the identification of the source of a DNA sample, which is very important in many forensic cases. DNA fingerprinting can provide positive identification with great accuracy by matching DNA obtained from a crime scene to individual suspects.

Several steps are involved in DNA fingerprinting. First, a suitable sample must be obtained. Forensic scientists use great care obtaining evidence from crime scenes so that the DNA will not be damaged. DNA is then isolated from the evidence, such as blood or hair samples. Once the DNA isolated, it is either digested with special enzymes called restriction endonucleases, or submitted to the Polymerase Chain Reaction (PCR).

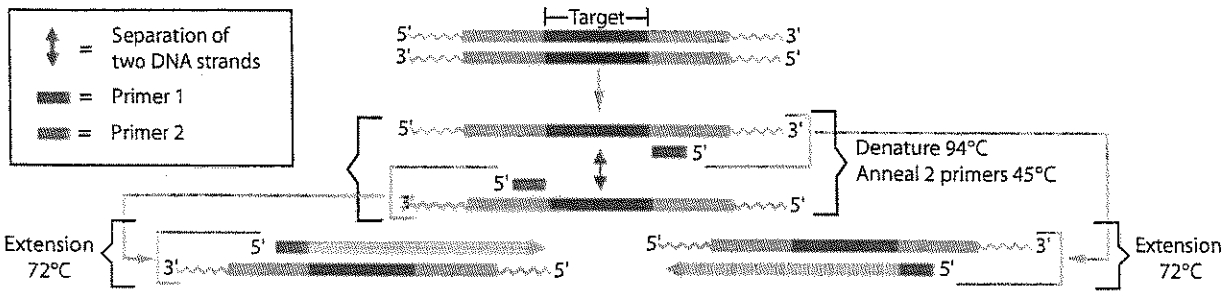


The method called restriction fragment length polymorphism (RFLP) analysis involves digesting the DNA with restriction enzymes, transferring the DNA to a membrane (Southern blot), and hybridizing the membrane with probes to polymorphic regions (autoradiography). This method requires relatively large amounts of DNA and takes several weeks. It is, however, statistically very accurate.

Background and Introduction

More recently, the Polymerase Chain Reaction (PCR) has been used in forensics to analyze DNA. This technique requires much less (500-fold) DNA than RFLP analysis and is much less time-consuming. PCR amplification uses an enzyme known as *Taq* polymerase, which was originally purified from a bacterium that inhabits hot springs. It is stable at very high (near boiling) temperatures. The PCR reaction mixture also includes two (15-30 nucleotide) synthetic oligonucleotides, known as "primers". These items are mixed with the extracted DNA, known as the "template".

The region of DNA to be amplified is known as the "target". In the first step of the PCR reaction, the template's complimentary DNA strands are separated (denatured) from each other at 94°C, while the *Taq* polymerase remains stable. In the second step, known as annealing, the sample is cooled to an intermediate temperature, usually 40°-65°C, to allow hybridization of the two primers, one to each of the two strands of the template DNA. In the third step, known as extension, the temperature is raised to 72°C and the *Taq* polymerase adds nucleotides to the primers to complete the synthesis of the new complementary strands.



These three steps - denaturation, annealing, and extension - constitute one PCR "cycle". This process is typically repeated for 20-40 cycles, amplifying the target sequence exponentially. PCR is performed in a thermal cycler, an instrument that is programmed to rapidly heat, cool and maintain samples at designated temperatures for varying amounts of time.

In this experiment, you will analyze DNAs (represented by colored dyes) using aspects of RFLP analysis. In this hypothetical case, the dyes represent DNAs obtained from a crime scene and two suspects which have been cut by restriction enzymes and the fragmentation patterns serve as the individual fingerprint. The DNA (dye) fragmentation patterns are simple enough to analyze directly in the agarose gel. The objective is to analyze and match the DNA fragmentation patterns after agarose gel electrophoresis and determine if Suspect 1 or Suspect 2 was at the crime scene.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT CONTENT OBJECTIVE

- Students will learn how restriction enzymes cut DNA molecules at specific base sequences producing DNA fragments of varying lengths.
- Students will learn how agarose gel electrophoresis separates different sizes of DNA fragments.
- Students will learn how these fragments form unique patterns for each person's DNA, which is the basis for DNA fingerprinting analysis.

WORKING HYPOTHESIS

If a DNA sample collected at the crime scene is cut with two different restriction enzymes and compared with DNA samples obtained from two suspects' DNA cut with the same two restriction enzymes, then one should be able to identify the real killer by the DNA fingerprint method.

MATERIALS FOR THE EXPERIMENT

Each Lab Group should have the following materials:

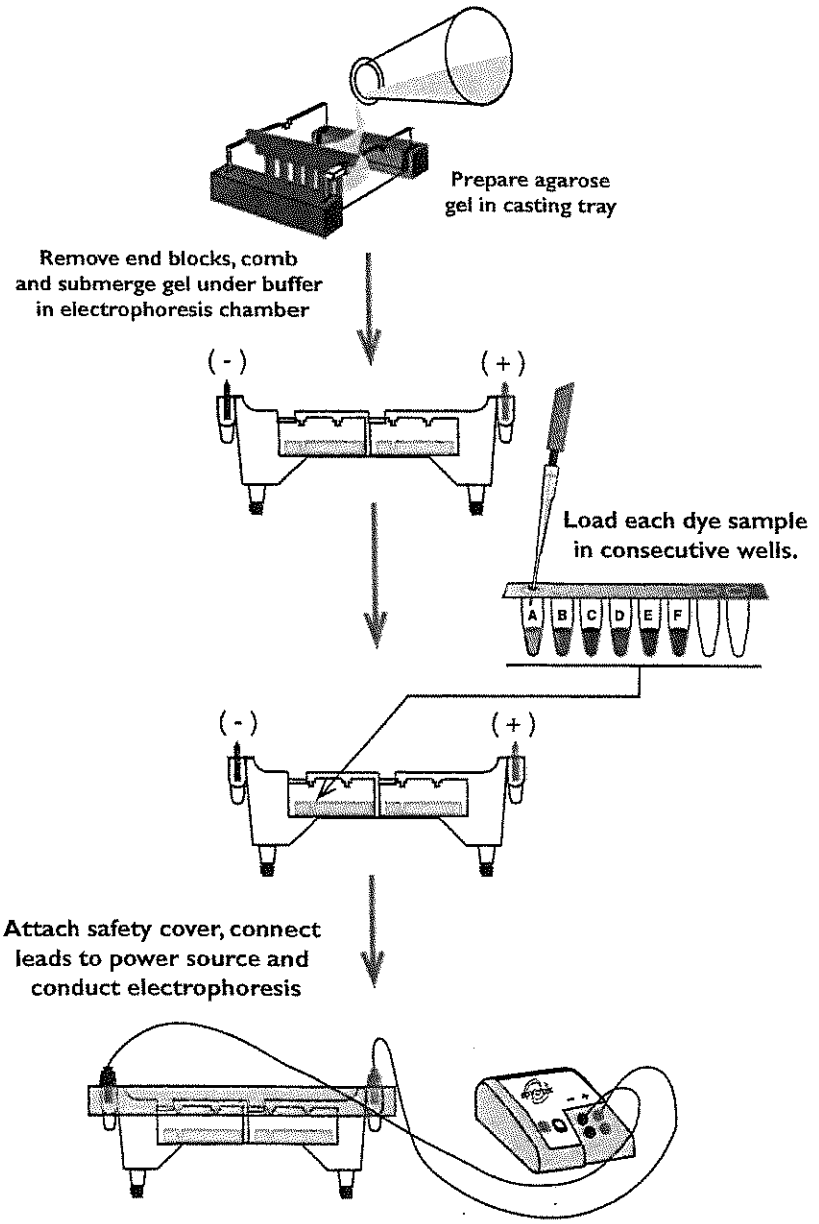
Activity One

- Electrophoresis Buffer
- Practice gel loading sample
- Sample delivery instrument
  Automatic micropipet and tips, or
  Transfer pipet and beaker of distilled water

Activity Two

- Agarose gel
- Electrophoresis apparatus
- DC power source
- Dye Samples (A - F) representing DNA
- Sample delivery instrument
  Automatic micropipet and tips, or
  Transfer pipet and beaker of distilled water

Experiment Overview

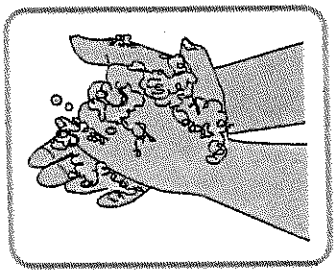


Laboratory Safety

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.


Wear gloves and safety goggles

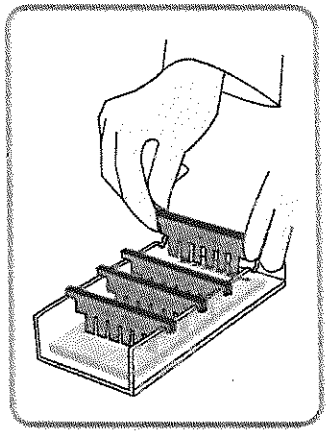
4. Exercise caution when using any electrical equipment in the laboratory.
   - Although electrical current from the power source is automatically disrupted when the cover is removed from the apparatus, first turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
   - Turn off power and unplug the equipment when not in use.
5. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.
6. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



Activity One - Practice Gel Loading

Accurate sample delivery technique ensures the best possible gel results. Pipeting 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.

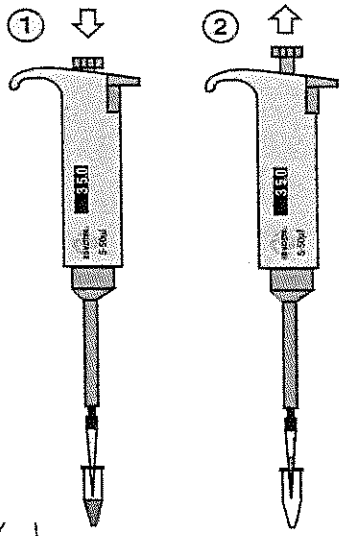
Note: The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

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: 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.

Activity One - Practice Gel Loading

SAMPLE DELIVERY WITH VARIABLE AUTOMATIC MICROPIPETS:

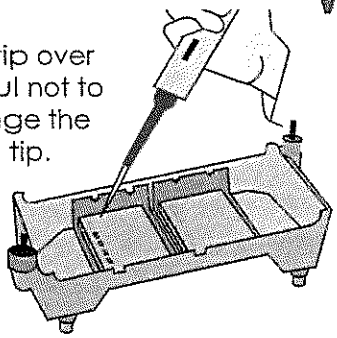


1. Set the micropipet to the appropriate volume and place a clean tip on the micropipetor.

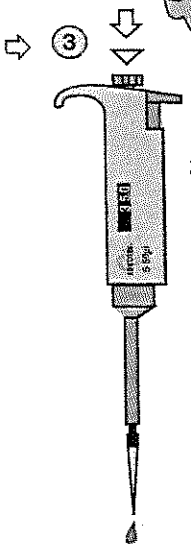
   Press the top button down to the first stop. then immerse the tip into the sample.

2. Once the tip is immersed in the sample, release the button slowly to draw sample into the tip.

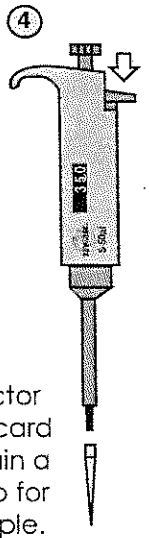
3A. Position the pipet tip over the well. Be careful not to puncture or damage the well with the pipet tip.



3B. Deliver the sample by pressing the button to the first stop - then empty the entire contents of the tip by pressing to the second stop.



3C. After delivering the sample, do not release the top button until the tip is out of the buffer.



4. Press the ejector button to discard the tip. Obtain a new clean tip for the next sample.

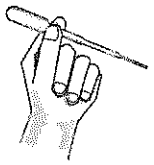
Activity One - Practice Gel Loading

SAMPLE DELIVERY WITH PLASTIC TRANSFER PIPETS:

1. Gently squeeze the pipet stem to slowly draw the sample up into the pipet. The sample should remain in the lower portion of the pipet.

   If the sample is overdrawn and becomes lodged in the bulb or on the walls, tap until the sample moves down into the lower stem of the pipet. Eject it back into the tube. Try step 1 again.

To control the delivery of small sample volumes with transfer pipets, gently squeeze the pipet stem, instead of the bulb.



2. While holding the pipet tip above the sample tube, slowly squeeze until the sample is nearly at the opening of the pipet tip.

3. Place the pipet tip in the electrophoresis buffer so it is directly above barely inside the sample well.

   Avoid placing the pipet tip all the way inside the well - this will minimize the chances of inadvertently piercing the bottom of the well.

4. MAINTAIN STEADY PRESSURE on the pipet stem to prevent buffer from being drawn in and diluting the sample.

5. Slowly squeeze to eject the sample. Stop squeezing when the well is completely full. Put any remaining sample in the pipet back into the sample tube.

6. Rinse the pipet with distilled water before obtaining the next sample for gel loading.

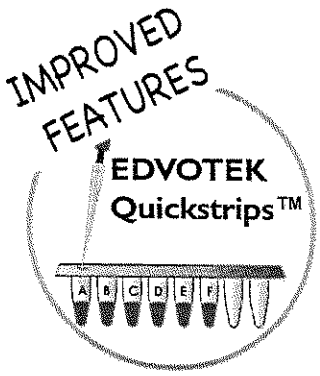
Activity Two - Conducting Agarose Gel Electrophoresis

ELECTROPHORESIS SAMPLES

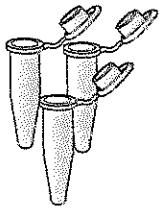
Samples in EDVOTEK Series 100 and S-series electrophoresis experiments are packaged in one of two different formats:

1. Pre-aliquoted Quickstrip™ connected tubes (new format)

   To remove samples from the Quickstrip™ tubes, simply pierce the foil top with the micropipet tip and withdraw the sample.

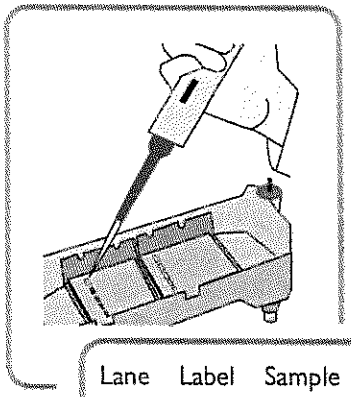


Quickstrips
patent pending



2. Individual 1.5 ml or 0.5 ml microtest tubes

   Your instructor may have aliquoted these into a set of sample tubes for each lab group. Alternatively, you may be required to withdraw the appropriate amount from the experiment stock tubes.



LOADING THE SAMPLES

1. Check the Sample Volumes

   Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the the bottom of the tubes before starting to load the gel.

   - If your samples are in Quickstrip™ connected tubes, tap the foil top of the strip so samples fall to the bottom of the tubes.
   - If your samples are in individual 1.5 ml or 0.5 ml microtest tubes, briefly centrifuge the sample tubes, or tap each tube on the tabletop to get all the sample to the bottom of the tube.

2. Load Samples

   Load each of the dye samples in tubes A - F into the wells in consecutive order. The amount of sample that should be loaded is 35-38 µl.

Lane	Label	Sample
1	A	Crime scene DNA 1
2	B	Crime scene DNA 2
3	C	Suspect 1 DNA 1
4	D	Suspect 1 DNA 2
5	E	Suspect 2 DNA 1
6	F	Suspect 2 DNA 2

Activity Two - Conducting Agarose Gel Electrophoresis

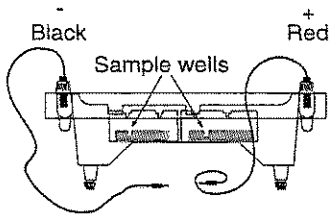
RUNNING THE GEL

3. After the samples are loaded, carefully snap the cover down onto the electrode terminals.

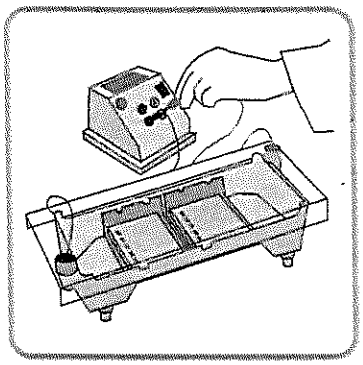
Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

Reminders:

During electrophoresis, the samples will migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



4. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).



5. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.

6. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

Table C Time and Voltage

Electrophoresis of Dyes

Volts	Recommended Time
125	20 min
70	45 min
50	1 hr 30 min

7. After approximately 10 minutes, you will begin to see separation of the colored dyes.

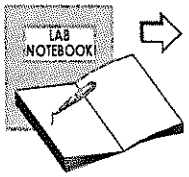
8. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

9. Document the gel results.

   A variety of documentation methods can be used, including drawing a picture of the gel, taking a photograph, or scanning an image of the gel on a flatbed scanner.

Staining is not required for Experiment # S-51, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small they will diffuse out of the gel. Therefore, the gel cannot be saved.

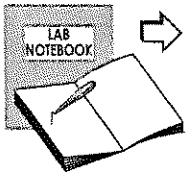
Critical Thinking and Hypothesis Development



Record the following in your Laboratory Notebook or on a separate sheet of paper:

1. Based on the evidence obtained from analysis of the gel, which suspect committed the crime? Explain.
2. What is the variable in this experiment?
3. What would you change in the experiment if you had to do it over again?
4. Write a hypothesis that would reflect these changes.

Study Questions



Record the answers to the following Study Questions in your Laboratory Notebook or on a separate sheet of paper, as instructed by your teacher:

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. Why is it important to wash the pipet between uses?
4. How will you be able to tell who committed the crime?
5. Who is the suspect that committed the crime?
6. What determines that each person has a unique pattern within their DNA?
7. Can you think of a case when two people will have identical DNA patterns?