EDVO-Kit #

225

DNA Fingerprinting II
Usage of Restriction Enzymes in DNA Fingerprinting Analysis

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this simulated forensic analysis is to develop an understanding of the use of restriction enzymes as applied to RFLP-based DNA fingerprinting.
This experiment contains reagents and materials for six groups.

All 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.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

EDVOTEK, The Biotechnology Education Company, and InstaStain are registered trademarks of EDVOTEK, Inc. Ready-to-Load and UltraSpec-Agarose are trademarks of EDVOTEK, Inc.
# Experiment Components

This experiment contains reagents for six groups.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Crime scene DNA sample, pre-cut with Restriction Enzyme 1</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>B Crime scene DNA sample, pre-cut with Restriction Enzyme 2</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>(Samples A and B are ready for electrophoresis)</td>
<td></td>
</tr>
<tr>
<td>C Suspect #1 DNA sample</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>D Suspect #2 DNA sample</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>E Standard DNA Fragments</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>F Enzyme Reaction Buffer</td>
<td>4°C Refrigerator</td>
</tr>
<tr>
<td>G Dryzymes™ Restriction Enzyme 1 (Eco RI)</td>
<td>4°C Refrigerator</td>
</tr>
<tr>
<td>H Dryzymes™ Restriction Enzyme 2 (Hind III)</td>
<td>4°C Refrigerator</td>
</tr>
<tr>
<td>I Reconstitution buffer</td>
<td>-20°C freezer</td>
</tr>
<tr>
<td>J Enzyme Grade water</td>
<td>-20°C freezer</td>
</tr>
</tbody>
</table>

- 10x Gel Loading Solution
- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- InstaStain® Blue
- 1 ml pipet
- 100 ml plastic graduated cylinder
- Microlipped Transfer Pipets

## Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Water bath (37°C)
- Balance
- Hot plate, Bunsen burner or microwave oven
- DNA visualization system (white light)
- Small plastic trays or large weigh boats (for gel destaining)
- Safety goggles and disposable laboratory gloves
- Pipet pumps
- 20 ml and 250 ml beakers or flasks
- Hot gloves
- Marking pens
- Distilled or deionized water
- Ice
DNA Fingerprinting II
Usage of Restriction Enzymes in DNA Fingerprinting Analysis

Online Ordering now available
www.edvotek.com

Visit our web site for information about EDVOTEK’s complete line of “hands-on” experiments for biotechnology and biology education.

Technical Service Department

1-800-EDVOTEK
(1-800-338-6835)

Mon - Fri
9:00 am to 6:00 pm ET

FAX: (301) 340-0582
Web: www.edvotek.com
email: edvotek@aol.com

Please have the following information ready:
• Experiment number and title
• Kit lot number on box or tube
• Literature version number (in lower right corner)
• Approximate purchase date

EDVOTEK - The Biotechnology Education Company® • 1-800-EDVOTEK • www.edvotek.com
DNA Fingerprinting

When first introduced, DNA fingerprinting (also called DNA profile analysis or DNA typing) involved the electrophoretic analysis of DNA fragment sizes generated by restriction enzymes. In contrast to more conventional methodologies, such as blood typing, which excludes suspects, traditional DNA fingerprinting provides accurate, unambiguous identification of the source of DNA samples.

Variations in DNA sequences between individuals as determined by differences in restriction enzyme cleavage patterns are known as restriction fragment length polymorphisms (RFLPs). RFLPs are a manifestation of the unique molecular genetic profile, or “fingerprint”, of an individual’s DNA.

Restriction enzymes are endonucleases that catalyze cleavage of phosphate bonds. They require Mg\(^{2+}\) for activity and generate a 5' phosphate and a 3' hydroxyl group at the point of cleavage. The distinguishing feature of restriction enzymes compared to other endonucleases is that they only cut at very specific sequences of bases. Restriction enzymes are produced by many different species of bacteria (including blue-green algae). Over 3,000 restriction enzymes have been discovered and catalogued.

Restriction enzymes are named according to the organism from which they are isolated. The first letter of the genus followed by the first two letters of the species (Fig. 1). Only certain strains or sub-strains of a particular species may produce restriction enzymes. The type of strain or substrain sometimes follows the species designation in the name. Finally, a Roman numeral is used to designate one out of several restriction enzymes produced by the same organism.

A restriction enzyme requires a specific double-stranded recognition sequence of nucleotide bases to cut DNA. Recognition sites are generally 4 to 8 base pairs in length and cleavage occurs within or near that site. Recognition sites are frequently symmetrical, i.e., both DNA strands in the site have the same base sequence when read 5' to 3' and the cleavage positions are indicated by arrows. Such sequences are called palindromes.

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgl I</td>
<td>Bactillus globigii</td>
</tr>
<tr>
<td>Bam HI</td>
<td>Bacillus amyloliquefaciens H</td>
</tr>
<tr>
<td>Eco RI</td>
<td>Escherichia coli RY13</td>
</tr>
<tr>
<td>Eco RII</td>
<td>Escherichia coli R 245</td>
</tr>
<tr>
<td>Hae III</td>
<td>Haemophilus aegyptius</td>
</tr>
<tr>
<td>Hind III</td>
<td>Haemophilus influenzae R4</td>
</tr>
</tbody>
</table>

Figure 1: Examples of restriction enzymes and the organism of origin.
## Background Information

### Consider the recognition site and cleavage pattern of Eco RI as an example. Eco RI causes staggered cleavage of its site. The resulting ends of the DNA fragments are called “sticky” or “cohesive” ends.

\[
\begin{align*}
\text{5'-GAATTC-3'} & \quad \text{5'-G AATTC-3'} \\
\text{3'-CTTAAG-5'} & \quad \text{3'-CTTAAG-5'} \\
\end{align*}
\]

In DNA forensics laboratories, the two most commonly used restriction enzymes were Hae III and Hinf I, which are 4-base and 5-base cutting enzymes.

\[
\begin{align*}
\text{Hae III} & \quad \downarrow \\
\text{5'-GGCC-3'} & \quad \text{3'-CCGG-5'} \\
\text{3'-CCGG-5'} & \quad \uparrow \\
\text{Hinf I} & \quad \downarrow \\
\text{5'-GANTC-3'} & \quad \text{3'-CTNAG-5'} \\
\text{3'-CTNAG-5'} & \quad \uparrow \\
\end{align*}
\]

The size of DNA fragments generated depends on distances between the recognition sites. In general, the longer the DNA molecule, the greater the probability that a given recognition site will occur. The DNA of an average human chromosome is very large, containing over 100 million base pairs. A restriction enzyme having a 6-base pair recognition site, such as Eco RI, would be expected to cut human DNA into approximately 750,000 different fragments.

No two individuals have exactly the same pattern of restriction enzyme recognition sites. There are several reasons for this fact that exists in a population. Alleles are alternate forms of a gene that result in alternative expressions of genetic traits that can be dominant or recessive. Chromosomes occur in matching pairs, one of maternal and the other of paternal origin. The two copies of a gene at a given chromosomal locus represent a composite of parental genes and constitute an individual’s unique genotype. It follows that alleles have differences in their base sequences which consequently creates differences in the distribution and frequencies of restriction enzyme recognition sites. Other differences in base sequences between individuals can occur because of mutations and deletions. Such changes can also create or eliminate a restriction endonuclease palindromic site. The example in Figure 2 shows how a silent mutation can eliminate a recognition site but leave a protein product unchanged.
DNA Fingerprinting

Individual variations in the distances between recognition sites in chromosomal DNA are often caused by intervening repetitive base sequences. Repetitive sequences constitute a large fraction of the mammalian genome and have no known genetic function. These sequences can occur between genes or are adjacent to them. They are also found within introns. Ten to fifteen percent of mammalian DNA consists of sets of repeated, short sequences of bases that are tandemly arranged in arrays. The length of these arrays (the amount of repeated sets) varies between individuals at different chromosomal loci.

TGTITA | TGTITA | TGTITA | ..........variable number

When these arrays are flanked by recognition sites, the length of the repeat will determine the size of the restriction enzyme fragment generated. Several types of short, repetitive sequences have been cloned and sequenced.

**AGAROSE GEL ELECTROPHORESIS**

Agarose gel electrophoresis is used to analyze DNA fragments generated by restriction enzymes. Agarose gels consist of microscopic pores that act as a molecular sieve. DNA fragments are loaded into wells made in the gel during casting. Since DNA has a negative charge at neutral pH, it migrates through the gel towards the positive electrode during electrophoresis. DNA fragments are separated by the gel according to their size, charge and shape. DNA fragments are linear and the ratio of mass to charge is the same. Therefore, only the size of the fragment affects the mobility. The smaller the fragment the faster it migrates. After electrophoresis, DNA can be visualized by staining.

Restriction enzyme cleavage of relatively small DNA molecules, such as plasmids and viral DNAs, usually results in discrete banding patterns of DNA fragments after electrophoresis. However, cleavage of large and complex DNA, such as human chromosomal DNA, generates many differently sized fragments that the resolving capacity of the gel is exceeded.

Figure 2: Silent mutation (T → C) changes the Eco RI site.
Consequently, the cleaved chromosomal DNA is visualized as a smear after staining and has no obvious banding patterns.

**SOUTHERN BLOT ANALYSIS**

RFLP analysis of genomic DNA is facilitated by Southern blot analysis. After electrophoresis, DNA fragments in the gel are denatured by soaking in an alkali solution. This causes double-stranded fragments to be converted into single-stranded form (no longer base-paired in a double helix). A replica of the electrophoretic pattern of DNA fragments in the gel is made by blotting them to a sheet of nitrocellulose or nylon membrane (Figure 3). This is done by placing the membrane on the gel after electrophoresis and transferring DNA fragments to the membrane by capillary action or electrotransfer. DNA, which is not visible, becomes permanently adsorbed to the membrane, that can then be manipulated easier than gels.

Analysis of the blotted DNA is done by hybridization with a labeled oligonucleotide DNA probe. The probe is a DNA fragment that contains base sequences that are complementary to the variable arrays of tandemly repeated sequences found in the human chromosomes. Probes can be labeled with reporter molecules that are used for detection. A solution containing the single-stranded probe is incubated with the membrane containing the blotted, single-stranded (denatured) DNA fragments. Under the proper conditions, the probe will only base pair (hybridize) to those fragments containing the complementary sequences. The membrane is then washed to remove excess probe. Only DNA fragments that
DNA Fingerprinting

are hybridized to the probe will reveal their positions on the membrane. If the probes are isotopically labeled, the hybridized fragments will appear as discrete bands (fingerprint) on the film and are in the same relative positions as they were in the agarose gel after electrophoresis. Only specific DNA fragments of the hundreds of thousands of fragments present, will hybridize with the probe because of the selective nature of the hybridization process.

In forensic analysis, DNA samples can be extracted and purified from specimens of skin, blood stains, semen, or hair roots collected at the crime scene. RFLP analyses performed on these samples is then compared to those performed on samples obtained from the suspect. If RFLP patterns match, it is beyond reasonable doubt that the suspect (or biological material from the suspect, such as blood) was at the crime scene. In forensic DNA fingerprinting, different sets of probes hybridized to different types of repetitious sequences are used in DNA profile analysis in order to satisfy certain statistical criteria for positive identification.

**DNA FINGERPRINTING USING POLYMERASE CHAIN REACTION (PCR)**

RFLP-based DNA fingerprinting analysis has been overtaken by the Polymerase Chain Reaction (PCR) because of two important advantages. The first is the sensitivity of PCR, which allows for DNA fingerprinting identification using much smaller amounts of DNA since PCR amplifies DNA. A second advantage is the speed of PCR analysis, which allows critical questions to be answered more quickly as compared to Southern Blot analysis.

PCR amplification requires the use of a thermostable DNA polymerase, such as Taq polymerase. Purified from a bacterium known as Thermus Aquaticus that inhabits hot springs, Taq polymerase is commonly used in PCR because it remains stable at near-boiling temperatures. Also included in the PCR reaction are the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides, typically 15-30 base pairs in length, known as primers. The primers are single-stranded DNA molecules that are complementary to sequences at the ends of the DNA fragments that are to be amplified.

PCR amplification involves several key steps: denaturation, annealing, and extension. In denaturation, the DNA sample is heated to about 95°C to separate the two DNA strands. In annealing, the temperature is lowered to allow the primers to hybridize to the DNA. In extension, the Taq polymerase is added and the temperature is raised to about 72°C to allow the primers to extend along the DNA strands. This process is repeated over several cycles, resulting in exponential amplification of the DNA of interest.

The amplified DNA is then separated by agarose gel electrophoresis, and the resulting DNA fragments are visualized by staining with ethidium bromide and UV light. The size and number of DNA fragments generated by the PCR reaction are used to identify and compare DNA samples.

**Figure 4: DNA Fingerprinting using PCR**

1. Collection of DNA
2. Extraction of DNA
3. DNA Amplification (PCR)
4. DNA fragments separated by agarose gel electrophoresis
5. Analysis

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1996, 1997, 1998, 2000, 2001, 2006, 2009, 2010 EDVOTEK, Inc., all rights reserved.
DNA Fingerprinting

Target Sequence

5' \underline{\text{red}} \rightarrow 3'

3' \underline{\text{blue}} \rightarrow 5'

\{ 

5' \underline{\text{green}} \rightarrow 3'

3' \underline{\text{green}} \rightarrow 5'

\}

Denature 94°C

\{ 

5' \underline{\text{red}} \rightarrow 3'

3' \underline{\text{green}} \rightarrow 5'

\}

Denature 94°C

\{ 

5' \underline{\text{blue}} \rightarrow 3'

3' \underline{\text{blue}} \rightarrow 5'

\}

Anneal 2 primers 45°C

\{ 

5' \underline{\text{green}} \rightarrow 3'

3' \underline{\text{green}} \rightarrow 5'

\}

Extension 72°C

Cycle 1

Cycle 2

Cycle 3

Figure 5: The Polymerase Chain Reaction
DNA Fingerprinting

The PCR reaction mixture (which contains the DNA polymerase, buffer, deoxynucleotides, primers, and template) is subjected to sequential heating/cooling cycles at three different temperatures (Figure 5).

• In the first step, the template is heated to near boiling (92° - 96°C.) to denature or "melt" the DNA. This step, known as "denaturation" disrupts the hydrogen bonds between the two complimentary DNA strands and causes their separation.

• In the second PCR step, the mixture is cooled to a temperature that is typically in the range of 45° - 65°. In this step, known as "annealing", the primers, present in great excess to the template, bind to the separated DNA strands.

• In the third PCR step, known as "extension", the temperature is raised to an intermediate value, usually 72°C. At this temperature the Taq polymerase is maximally active and adds nucleotides to the primers to complete the synthesis of the new complimentary strands.

DNA fingerprinting analysis has become increasingly significant in court cases involving murder, rape, physical battery, and other types of crimes. Jurors are often asked to determine the validity of DNA evidence, resulting in both acquittals and convictions of suspected criminals. To ensure greater accuracy, scientists have incorporated standardization procedures in DNA analysis. Standard DNA Fragments are used to determine the exact size of individual DNA fragments in a DNA fingerprint. It is generally accepted that DNA fingerprints are identical only in the case of identical twins.

In this experiment, emphasis is placed on concepts related to RFLP analysis. The experiment activities will focus on the identification of DNA by analyzing restriction fragmentation patterns separated by agarose gel electrophoresis.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA.
Experiment Notes
Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this simulated forensic analysis is to develop an understanding of the use of restriction enzymes as applied to RFLP-based DNA fingerprinting.

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.

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

• Write a hypothesis that reflects the experiment.
• Predict experimental outcomes.

During the Experiment:

• Record (draw) your observations, or photograph the results.

Following the Experiment:

• Formulate an explanation from the results.
• Determine what could be changed in the experiment if the experiment were repeated.
• Write a hypothesis that would reflect this change.
Crime Scene Investigation - Restriction Enzyme Digestion

In this experiment, the DNA from two suspects are each digested with two restriction enzymes in separate reactions and compared to crime scene samples after agarose gel electrophoresis. This flow chart outlines the procedure used for the restriction enzyme digestion of DNA obtained from Suspect 1. The DNA from Suspect 2 is digested in the same manner, using reaction tubes 3 and 4 (not shown).

Quick Reference:

<table>
<thead>
<tr>
<th>Dispensed Components</th>
<th>Tube Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crime scene DNA 1</td>
<td>CS 1</td>
</tr>
<tr>
<td>Crime scene DNA 2</td>
<td>CS 2</td>
</tr>
<tr>
<td>Suspect 1 DNA</td>
<td>DNA 1</td>
</tr>
<tr>
<td>Suspect 2 DNA</td>
<td>DNA 2</td>
</tr>
<tr>
<td>Standard DNA fragments</td>
<td>Markers</td>
</tr>
<tr>
<td>Enzyme Reaction Buffer</td>
<td>Rxn Buffer</td>
</tr>
<tr>
<td>Diluted Enzyme 1</td>
<td>Enzyme 1</td>
</tr>
<tr>
<td>Diluted Enzyme 2</td>
<td>Enzyme 2</td>
</tr>
</tbody>
</table>

To avoid cross-contamination, use a FRESH micropipet tip for each transfer of DNA and enzyme to the restriction enzyme reaction.
Restriction Enzyme Digestion

The enzymes used in this experiment are stored and shipped in Dryzyme™ form (lyophilized). A buffer has been added to reconstitute the enzyme to liquid form.

1. Label microtest tubes 1 through 4 for four restriction enzyme digestion reactions. Put your initials or group number on the tubes.

2. Tap all the tubes (see Quick Reference at left) on the lab bench to collect all the contents at the bottom of the tube.

3. Use an automatic micropipet to dispense 10 µl of Enzyme Reaction Buffer (Rxn Buffer) to each of four reaction tubes labeled 1 through 4.

4. Add DNA and enzyme to the reaction tubes as summarized in Chart 1. Use a FRESH micropipet tip for each transfer of DNA and enzyme.

5. Cap the reaction tubes and tap gently to mix. Then tap each tube on the lab bench to collect contents at the bottom.

6. Incubate reaction tubes in a 37°C waterbath for 30 minutes (or 60 minutes if time allows).

After the 30 or 60 minute incubation is completed:

7. Add 5 µl of 10x gel loading solution to reaction tubes 1 - 4 to stop the reactions. Cap and mix by tapping.

* 10x Gel loading solution has already been added to the crime scene samples.

**OPTIONAL STOPPING POINT**

After addition of 10x gel loading solution to stop the reaction, samples are ready for electrophoresis. Samples may be stored in the refrigerator for electrophoresis.

---

Chart 1: Summary of Restriction Enzyme Digestion Reactions

<table>
<thead>
<tr>
<th>Crime Scene Samples</th>
<th>Suspect 1</th>
<th>Suspect 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Tube</td>
<td>Reaction Buffer</td>
<td>DNA 1 (µl)</td>
</tr>
<tr>
<td>Crime Scene DNA, cut with enzyme 1 ready for electrophoresis</td>
<td>X</td>
<td>--</td>
</tr>
<tr>
<td>Crime Scene DNA, cut with enzyme 2 ready for electrophoresis</td>
<td>--</td>
<td>X</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>--</td>
</tr>
</tbody>
</table>
Electrophoresis - Agarose Gel Preparation

AGAROSE GEL REQUIREMENTS FOR THIS EXPERIMENT

- Recommended gel size: 7 x 7 cm or 7 x 14 cm
- Number of sample wells required: 7 - 8
- Well-former template (comb):
  For EDVOTEK units:
  - 7 x 14 cm gel - two standard 6-well combs in the first and middle set of notches
  - 7 x 7 cm gel - one 8-well comb in the first set of notches
- Agarose gel concentration: 0.8%

PREPARING THE GEL BED

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
   
   A. Using Rubber dams:
   - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
   
   B. Taping with labeling or masking tape:
   - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
   - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Placement of well-former template (comb):
   For EDVOTEK units:
   - 7 x 14 cm gel - two standard 6-well combs in the first and middle set of notches
   - 7 x 7 cm gel - one 8-well comb in the first set of notches
Electrophoresis - Agarose Gel Preparation

CASTING THE AGAROSE GEL(S)

3. Use a 250 ml flask or beaker to prepare the gel solution.

**IMPORTANT**

Check with your instructor regarding the concentration of the buffer you are using to prepare your gel. Use the appropriate table (A.1 or A.2) below.

**If preparing the gel with concentrated (50x) buffer, use Table A.1.**

<table>
<thead>
<tr>
<th>Size of Gel (cm)</th>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7</td>
<td>0.23</td>
<td>0.6</td>
<td>29.4</td>
<td>30</td>
</tr>
<tr>
<td>7 x 14</td>
<td>0.46</td>
<td>1.2</td>
<td>58.8</td>
<td>60</td>
</tr>
</tbody>
</table>

*0.77% UltraSpec-Agarose™ gel percentage rounded up to 0.8%

**If preparing the gel with diluted (1x) buffer, use Table A.2.**

<table>
<thead>
<tr>
<th>Size of Gel (cm)</th>
<th>Amt of Agarose (g)</th>
<th>Diluted Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7</td>
<td>0.23</td>
<td>30</td>
</tr>
<tr>
<td>7 x 14</td>
<td>0.46</td>
<td>60</td>
</tr>
</tbody>
</table>

*0.77% UltraSpec-Agarose™ gel percentage rounded up to 0.8%

4. Swirl the mixture to disperse clumps of agarose powder.

5. With a marking pen, indicate the level of the solution volume on the outside of the flask.
**Electrophoresis - Agarose Gel Preparation**

6. Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.
   
   A. Microwave method:
   - Cover the flask with plastic wrap to minimize evaporation.
   - Heat the mixture on High for 1 minute.
   - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.

   B. Hot plate method:
   - Cover the flask with aluminum foil to prevent excess evaporation.
   - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

   Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

7. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked on the flask in step 6.

After the gel is cooled to 60°C:

If you are using rubber dams, go to step 9.

If you are using tape, continue with step 8.

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.
Electrophoresis - Agarose Gel Preparation

PREPARING THE GEL FOR ELECTROPHORESIS

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed. Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.

12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer.

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.

For DNA analysis, the recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

IMPORTANT: Check with your instructor to determine if the buffer has previously been diluted. Pour the appropriate amount of 1x buffer into the electrophoresis chamber according to Table B below.

### Table B: Electrophoresis (Chamber) Buffer

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required (ml)</th>
<th>Dilution 50x Conc. Buffer (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>300</td>
<td>6</td>
<td>294</td>
</tr>
<tr>
<td>M12</td>
<td>400</td>
<td>8</td>
<td>392</td>
</tr>
<tr>
<td>M36 (blue)</td>
<td>500</td>
<td>10</td>
<td>490</td>
</tr>
<tr>
<td>M36 (clear)</td>
<td>1000</td>
<td>20</td>
<td>980</td>
</tr>
</tbody>
</table>
Electrophoresis - Conducting Electrophoresis

LOAD THE SAMPLES

1. Optional Step: Heat the samples, including the Standard DNA fragments for two minutes at 65°C. Allow the samples to cool for a few minutes.

2. Load 40 µl of each of the DNA samples in the following manner (7 x 14 cm gel):

<table>
<thead>
<tr>
<th>First Row</th>
<th>Lane</th>
<th>Tube</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Markers</td>
<td>Standard DNA Fragments</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CS 1</td>
<td>DNA from crime scene cut with Enzyme 1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CS 2</td>
<td>DNA from crime scene cut with Enzyme 2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>DNA from Suspect 1 cut with Enzyme 1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>DNA from Suspect 1 cut with Enzyme 2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second Row</th>
<th>Lane</th>
<th>Tube</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Markers</td>
<td>Standard DNA Fragments</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>DNA from Suspect 2 cut with Enzyme 1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>DNA from Suspect 2 cut with Enzyme 2</td>
<td></td>
</tr>
</tbody>
</table>

RUNNING THE GEL

1. After the DNA 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.

2. 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).
Electrophoresis - Conducting Electrophoresis

3. 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.

<table>
<thead>
<tr>
<th>Volts</th>
<th>Time and Voltage Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDVOTEK Electrophoresis Model</td>
</tr>
<tr>
<td></td>
<td>M6+</td>
</tr>
<tr>
<td></td>
<td>Minimum / Maximum</td>
</tr>
<tr>
<td>150</td>
<td>15 / 20 min</td>
</tr>
<tr>
<td>125</td>
<td>20 / 30 min</td>
</tr>
<tr>
<td>70</td>
<td>35 / 45 min</td>
</tr>
<tr>
<td>50</td>
<td>50 / 80 min</td>
</tr>
<tr>
<td></td>
<td>M12 &amp; M36</td>
</tr>
<tr>
<td></td>
<td>Minimum / Maximum</td>
</tr>
<tr>
<td>150</td>
<td>25 / 35 min</td>
</tr>
<tr>
<td>125</td>
<td>35 / 45 min</td>
</tr>
<tr>
<td>70</td>
<td>60 / 90 min</td>
</tr>
<tr>
<td>50</td>
<td>95 / 130 min</td>
</tr>
</tbody>
</table>

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

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

6. Remove the gel from the bed for staining.

ABOUT DNA GEL STAINING

After electrophoresis, the agarose gels require staining in order to visualize the separated DNA samples. This experiment features a proprietary stain called InstaStain® Blue. Two options are provided for using the InstaStain® Blue cards. Check with your instructor regarding which staining method you should use.

Method 1: One-step Staining and Destaining with InstaStain® MetBlue

Method 2: Staining with InstaStain® Blue
Electrophoresis - Staining and Visualization of DNA

**METHOD 1: ONE-STEP STAINING AND DESTAINING WITH INSTASTAIN® BLUE**

Agarose gels can be stained and destained in one easy step with InstaStain™ Blue cards. This one-step method can be completed in approximately 3 hours, or can be left overnight.

1. Remove the 7 x 14 cm agarose gel from its bed and completely submerge the gel in a small, clean tray containing 150 ml of distilled or deionized water, or used electrophoresis buffer. The agarose gel should be completely covered with liquid.

   Examples of small trays include large weigh boats, or small plastic food containers

2. Gently float two 7 x 7 cm cards of InstaStain® MetBlue with the stain side (blue) facing the liquid.

3. Let the gel soak undisturbed in the liquid for approximately 3 hours. The gel can be left in the liquid overnight (cover with plastic wrap to prevent evaporation).

4. After staining and destaining, the gel is ready for visualization and photography.

**STORAGE AND DISPOSAL OF INSTASTAIN® BLUE CARDS AND GELS**

- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.

  **DO NOT FREEZE AGAROSE GELS!**

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.

- Destaining solutions can be disposed down the drain.
Electrophoresis - Staining and Visualization of DNA

METHOD 2: STAINING WITH INSTASTAIN® BLUE CARDS

1. After electrophoresis, place the agarose gel on a flat surface covered with plastic wrap.

2. Wearing gloves, place the blue dye side of two InstaStain® Blue cards on the gel.

3. Firmly run your fingers several times over the entire surface of the InstaStain® cards to establish good contact between the InstaStain® cards and the gel.

4. To ensure continuous contact between the gel and the InstaStain® cards, place a gel casting tray and weight, such as a small empty beaker, on top of the InstaStain® cards.

5. Allow the InstaStain® Blue to sit on the gel for 5 to 10 minutes.

6. After staining, remove the InstaStain® cards. If the color of the gel appears very light, wet the gel surface with buffer or distilled water and place the InstaStain® cards back on the gel for an additional 5 minutes.

Destaining and Visualization of DNA

7. Transfer the gel to a large weigh boat or small plastic container.

8. Destain with distilled water.*
   • Add approximately 150 ml of distilled water to cover the gel.

9. Repeat destaining by changing the distilled water as needed.

   The larger DNA bands will initially be vis-

InstaStain is a registered trademark of EDVOTEK, Inc. Patents Pending.
Electrophoresis - Staining and Visualization of DNA

The gel will usually appear as dark blue bands against a lighter blue background. When the gel is completely destained, the larger DNA bands will become sharper and the smaller bands will be visible. With additional destaining, the entire background will become uniformly light blue.

10. Carefully remove the gel from the destain solution and examine the gel on a Visible Light Gel Visualization System. To optimize visibility, use the amber filter provided with EDVOTEK equipment.

11. If the gel is too light and bands are difficult to see, repeat the staining and destaining procedures.

*Destaining Notes*

- Warmed distilled water at 37°C will accelerate destaining. Destaining will take longer with room temperature water.
- DO NOT EXCEED 37°C! Warmer temperatures will soften the gel and may cause it to break.
- The volume of distilled water for destaining depends upon the size of the tray. Use the smallest tray available that will accommodate the gel. The gel should be completely submerged during destaining.
- Do not exceed 3 changes of water for destaining. Excessive destaining will cause the bands to be very light.

**STORAGE AND DISPOSAL OF INSTASTAIN® BLUE CARDS AND GELS**

- Stained gels may be stored in the refrigerator for several weeks. Place the gel in a sealable plastic bag with destaining liquid.
  
  DO NOT FREEZE AGAROSE GELS!

- Used InstaStain® cards and destained gels can be discarded in solid waste disposal.
- Destaining solutions can be disposed down the drain.
Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Which suspect’s DNA matches that found at the crime scene? Does this automatically mean that the suspect is guilty?

2. What possible experimental problems could occur to invalidate the results?

3. If only Restriction Enzyme 1 was used, would the interpretation be the same?
Instructor’s Guide

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will learn to extract chromosomal DNA, load samples and run agarose gel electrophoresis. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation. Please visit our website for specific content and skill standards for various experiments.

EDUCATIONAL RESOURCES

Electrophoresis Hints, Help and Frequently Asked Questions

EDVOTEK Electrophoresis Experiments are easy to perform and are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. The EDVOTEK web site provides several suggestions and reminders for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Laboratory Extensions and Supplemental Activities

Laboratory extensions are easy to perform using EDVOTEK experiment kits. For laboratory extension suggestions, please check the EDVOTEK website, which is updated on a continuous basis with educational activities and resources.
Notes to the Instructor:

This experiment simulates a forensic case in which DNA samples from a hypothetical crime scene and suspects are digested by six-base cutting enzymes (Eco RI and Hind III). The objective is to analyze suspect DNA fingerprint patterns and compare them with "crime scene" samples. Each DNA sample will be cleaved with two restriction enzymes in separate reactions, and pairs of fragmentation patterns will serve as the fingerprints. The DNA fragmentation patterns will be analyzed in the stained agarose gel, without the need for Southern blot analysis.

This experiment module contains biologicals and reagents for six groups. The experimental procedures consist of two major parts: 1) restriction enzyme digestion of DNA, which is followed by 2) agarose gel electrophoresis.

Each laboratory group receives two predigested, ready-for-electrophoresis "crime scene" samples and standard DNA fragments (markers). Four additional DNA samples are generated by performing restriction enzyme digestion reactions on the DNAs of two suspects.

If you have six (6) electrophoresis units, one for each of the six lab groups, electrophoresis can be performed simultaneously by all six groups. Alternatively, some lab groups can store their samples at 4°C and perform the electrophoresis at different times.

**APPROXIMATE TIME REQUIREMENTS**

1. Prelab preparation and dispensing of biologicals and reagents take approximately 1-2 hours.

2. The approximate time required for students to perform the restriction enzyme digestion and prepare samples for electrophoresis is 50 minutes. The incubation time for restriction enzyme digestion may be extended to 60 minutes.

3. **Gel preparation:** Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30-40 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

4. **Practice Gel Loading:** If your students are unfamiliar with using micropipets and sample loading techniques, a practice activity is suggested prior to conducting the experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. This activity can require anywhere from 10 minutes to an entire laboratory session, depending upon the skill level of your students.
Notes to the Instructor:

5. **Conducting Electrophoresis:**
The approximate time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Different models of electrophoresis units will separate DNA at different rates depending upon its configuration and the distance between the two electrodes. Generally, the higher the voltage applied the faster the samples migrate. However, the maximum amount of voltage significantly depends upon the design of the electrophoresis apparatus and should not exceed manufacturer’s recommendations. Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.

### Table C

<table>
<thead>
<tr>
<th>Volts</th>
<th>EDVOTEK Electrophoresis Model</th>
<th>Minimum / Maximum</th>
<th>Minimum / Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>M6+</td>
<td>15 / 20 min</td>
<td>25 / 35 min</td>
</tr>
<tr>
<td>125</td>
<td>M12 &amp; M36</td>
<td>20 / 30 min</td>
<td>35 / 45 min</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>35 / 45 min</td>
<td>60 / 90 min</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>50 / 80 min</td>
<td>95 / 130 min</td>
</tr>
</tbody>
</table>

**GEL STAINING AND DESTAINING AFTER ELECTROPHORESIS**

This experiment features InstaStain® Blue for gel staining after electrophoresis. It is a proprietary new staining method which saves time and reduces liquid waste. EDVOTEK also offers InstaStain® Ethidium Bromide (InstaStain® EtBr) and Protein InstaStain® for staining Protein polyacrylamide gels.

Two options are provided for using the InstaStain® Blue cards.

- **Method 1:** One-step Staining and Destaining with InstaStain® MetBlue
  
  Agarose gels can be stained and destained in one easy step, which can be completed in approximately 3 hours, or can be left in liquid overnight.

- **Method 2:** Staining with InstaStain® Blue
  
  Using InstaStain® Blue cards requires approximately 5-10 minutes for staining. DNA bands will become visible after destaining for approximately 20 minutes, and will become sharper with additional destaining. For the best photographic results, allow the gel to destain for several hours to overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background.
Pre-Lab Preparations For Restriction Enzyme Digestion

PREPARATION OF BIOLOGICALS AND REAGENTS

1. Thaw all DNAs. Tap tubes on a table to get all the sample to the bottom of the tube.

2. Two tubes, components A and B, contain crime scene samples. These DNA samples have been cut with restriction enzymes and are ready for electrophoresis. Sample A represents “crime scene” DNA cut with Restriction Enzyme 1. Sample B represents “crime scene” DNA cut with Restriction Enzyme 2.
   - Label six tubes “CS 1” for the crime scene sample #1 (A)
   - Label six tubes “CS 2” for the crime scene sample #2 (B)
   - Dispense 45 µl of each crime scene sample in the appropriate tubes for each of the six lab groups.

   - Label six tubes “Markers”.
   - Dispense 85 µl of Standard DNA fragments to each tube for each of the six groups.

4. Component F is the Enzyme Reaction buffer.
   - Label six tubes “Rxn Buffer”.
   - Dispense 45 µl of Enzyme Reaction buffer to each tube for each of the six groups.

PREPARATION OF SUSPECT DNA

5. Using an automatic micropipet, dispense the two Suspect DNAs (C, D) for each of the six lab groups.
   - For each of 6 groups, label two tubes: “DNA 1”, & “DNA 2”.
   - Dispense 35 µl of each Suspect DNA to the appropriate...

Quick Reference:
Components for Restriction Enzyme Digestion

A  Crime scene DNA sample, pre-cut with Restriction Enzyme 1
B  Crime scene DNA sample, pre-cut with Restriction Enzyme 2
   (Samples A and B are ready for electrophoresis)
C  Suspect #1 DNA sample
D  Suspect #2 DNA sample
E  Standard DNA Fragments
F  Enzyme Reaction Buffer
G  Restriction Enzyme 1
H  Restriction Enzyme 2
Pre-Lab Preparations For Restriction Enzyme Digestion

**PREPARATION OF DRYZYME™ RESTRICTION ENZYMES**

Prepare restriction digests within 30 minutes of reconstituting Dryzymes™.

1. Make sure that the solid material is at the bottom of the tubes. If not, centrifuge the tubes in a microcentrifuge at full speed for 20 seconds or tap the tube on the lab bench.

2. Add 150 µl Reconstitution Buffer (I) to the solid at the bottom of each tube containing Dryzymes™

3. Allow the samples to hydrate for 1 minute.

4. Mix the samples vigorously by flicking the tubes with your finger or by vortexing for 30 seconds until the solid appears to be completely dissolved.

5. Add 150 µl Enzyme Grade Water (J) to each of the tubes of rehydrated Dryzymes™.

6. Mix or vortex the samples and then centrifuge for 20 seconds or tap the tube on the lab bench.

   **After the rehydration, check that no undissolved particulate matter remains. If not completely dissolved, repeat mixing or vortexing.**

7. Label six tubes “Enzyme 1” and six tubes “Enzyme 2”

8. Transfer 35 µl of diluted Restriction Enzyme 1 to each tube labeled “Enzyme 1”. Cap the tubes and immediately put on ice.

9. Transfer 35 µl of diluted Restriction Enzyme 2 to each tube labeled “Enzyme 2”. Cap the tubes and immediately put on ice.
Pre-Lab Preparations For Restriction Enzyme Digestion

GENERAL PREPARATIONS

1. Allow ample time to equilibrate a water bath at 37°C on the day of the experiment.

2. Each student group can perform 4 restriction enzyme reactions. Each student group should receive the following materials:
   - Reagents and biologicals summarized in Table 1
   - Automatic micropipet and tips
   - 4 microtest tubes with attached caps
   - Marking pen

Table 1: Summary of Biologicals and Reagents required for each of six groups

<table>
<thead>
<tr>
<th>Component</th>
<th>Label 6 tubes each</th>
<th>Dispense for each tube*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Crime scene DNA 1</td>
<td>CS 1</td>
<td>45 µl</td>
</tr>
<tr>
<td>B Crime scene DNA 2</td>
<td>CS 2</td>
<td>45 µl</td>
</tr>
<tr>
<td>C Suspect 1 DNA</td>
<td>DNA 1</td>
<td>35 µl</td>
</tr>
<tr>
<td>D Suspect 2 DNA</td>
<td>DNA 2</td>
<td>35 µl</td>
</tr>
<tr>
<td>E Standard DNA fragments</td>
<td>Markers</td>
<td>85 µl</td>
</tr>
<tr>
<td>F Reaction Buffer</td>
<td>Rxn Buffer</td>
<td>45 µl</td>
</tr>
<tr>
<td>I, J, G Diluted Enzyme 1</td>
<td>Enzyme 1</td>
<td>35 µl on ice</td>
</tr>
<tr>
<td>I, J, H Diluted Enzyme 2</td>
<td>Enzyme 2</td>
<td>35 µl on ice</td>
</tr>
</tbody>
</table>

* Recommended dispensing volumes include a small amount of "excess", which is 5 µl more than the total volume required for the experiment.
Pre-Lab Preparations - Electrophoresis

PREPARING AGAROSE GELS

Preparing gels for electrophoresis
There are several options for preparing agarose gels for the electrophoresis experiments:

1. Individual Gel Casting: Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. Batch Gel Preparation: A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

3. 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 store gels at -20°C. 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 hot, molten agarose before placing the gels into the apparatus for electrophoresis. This will prevent the gels from sliding around in the trays and the chambers.
Quantity Preparations for Agarose Gel Electrophoresis

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 Preparation of Electrophoresis Buffer**

<table>
<thead>
<tr>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2,940</td>
<td>3000 (3 L)</td>
</tr>
</tbody>
</table>

**Table D**

**Batch Preparation of 0.8% UltraSpec-Agarose™**

<table>
<thead>
<tr>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>7.5</td>
<td>382.5</td>
<td>390</td>
</tr>
</tbody>
</table>

Note: The UltraSpec-Agarose™ kit component is often labeled with the amount it contains. In many cases, the entire contents of the bottle is 3.0 grams. 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.

**BULK ELECTROPHORESIS BUFFER**

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

**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.
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.
Experiment Results and Analysis

**GEL ELECTROPHORESIS RESULTS**

The idealized schematic shows relative positions of DNA fragments. Actual results will yield broader bands of varying intensities. Smaller fragments will stain less efficiently and will appear as fainter bands. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.

![Diagram of gel electrophoresis results](image)

<table>
<thead>
<tr>
<th>First Row</th>
<th>Lane</th>
<th>Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Markers</td>
<td>Standard DNA Fragments</td>
</tr>
<tr>
<td>2</td>
<td>CS 1</td>
<td>DNA from crime scene cut with Enzyme 1</td>
</tr>
<tr>
<td>3</td>
<td>CS 2</td>
<td>DNA from crime scene cut with Enzyme 2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>DNA from Suspect 1 cut with Enzyme 1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>DNA from Suspect 1 cut with Enzyme 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second Row</th>
<th>Lane</th>
<th>Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Markers</td>
<td>Standard DNA Fragments</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>DNA from Suspect 2 cut with Enzyme 1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>DNA from Suspect 2 cut with Enzyme 2</td>
</tr>
</tbody>
</table>
Study Questions and Answers

1. Which suspect DNA matches that found at the crime scene? Does this automatically mean that the suspect is guilty?

The DNA profile for Suspect 2 matches the DNA obtained at the crime scene. The results do not automatically mean that the suspect is guilty (see answers to questions 2 and 3).

2. What possible experimental problems could occur to invalidate the results?

Experimental problems which could invalidate the results include contamination of DNA samples or incomplete cleavage by the restriction enzymes.

3. If only Restriction Enzyme 1 was used, would the interpretation be the same?

The interpretation would not be the same if only one enzyme were used. For instance, both suspects have the same fragment pattern with Restriction Enzyme 1. The results would be inconclusive. As covered in the background information, in practice, several different probes containing different types of repetitious sequences are used in DNA profile analysis in order to satisfy certain statistical criteria for positive identification. The use of different restriction enzymes allow for accuracies in positive identifications of greater than one in 100 million.
Material Safety Data Sheet

IDENTITY (As Used on Label and List)

EDVOTEK, Inc.

EDVOTEK, Inc.

14676 Rockbridge Drive
Rockville, MD 20850

Section I - Emergency Telephone Numbers

Emergency Telephone Numbers

(301) 251-5990

Telephone Number for information

(301) 251-5990

Date Prepared

10/05/06

Signature of Preparer (optional)


Section II - Hazardous Ingredients/Information

Chemical Identity; Common Name(s)

Agarose

Other Limits

N.D.

Other Limits

N.D.

Section III - Physical/Chemical Characteristics

Boiling Point

No data

Vapor Pressure (mm Hg.)

No data

Vapor Density (Air = 1)

No data

Section IV - Physical/Chemical Characteristics

Mass Density (g/ml)

No data

Specific Gravity

1

Inflammability

N.D.

Flammable Limits

UEL: N.D.

EL: N.D.

Flammable Range

N.D.

Unusual Fire and Explosion Hazards

May produce toxic gases

Section V - Reactivity Data

Stability

Unstable

Incompatibility

Strong oxidizing agents

Hazardous Decomposition or Byproducts

Carbon monoxide, Carbon dioxide

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Ingestion? Skin?

Yes   Yes            Yes

Hazardous Components

May cause irritation to skin/eye, mucous membranes and upper respiratory tract.

Signs and Symptoms of Exposure

Irritation to upper respiratory tract, skin, eyes

Section VII - Control Measures

Respiratory Protection (Specify Type)

Chemical cartridge respirator with full facepiece.

Ventilation Local Exhaust Special

Yes

Waste Disposal Method

Mix with vermiculite and dry caustic, wrap in paper and burn in a chemical incinerator equipped with normal solid waste disposal equipment.

Waste Disposal Method

Normal solid waste disposal

Section VIII - Control Measures

Precautions to be Taken in Handling and Storing

Wear protective gear to avoid skin/eye contact

Other Procedures

None
**Material Safety Data Sheet**

**Material:**
Methylene Blue, Methylene Blue Phosphate

**Basis:**

**Section I - Identification Information**

<table>
<thead>
<tr>
<th>Material Safety Data Sheet</th>
<th>EDVOTEK, Inc.</th>
<th>14676 Rothgeb Drive</th>
<th>Rockville, MD 20850</th>
</tr>
</thead>
</table>

**Date Prepared:**
14676 Rothgeb Drive

**Telephone Number for Information:**
(301) 251-5990

**Address  (Number, Street, City, State, Zip Code):**
14676 Rothgeb Drive

**Signature of Preparer (optional):**

**Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.**

**This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.**

**Chemical Identity; Common Name(s):**

<table>
<thead>
<tr>
<th>Chemical Identity; Common Name(s)</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene Blue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Section II - Hazardous Ingredients/Identity Information**

<table>
<thead>
<tr>
<th>Chemical Identity; Common Name(s)</th>
<th>OSHA PEL</th>
<th>ACGIH TLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene Blue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Section III - Physical/Chemical Characteristics**

<table>
<thead>
<tr>
<th>Physical/Chemical Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling Point</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td></td>
</tr>
<tr>
<td>Vapor Density</td>
<td></td>
</tr>
<tr>
<td>Magnetic susceptibility</td>
<td></td>
</tr>
<tr>
<td>Solubility in Water</td>
<td></td>
</tr>
<tr>
<td>Vapor Pressure</td>
<td></td>
</tr>
<tr>
<td>Explosion Index</td>
<td></td>
</tr>
<tr>
<td>Stability in Water</td>
<td></td>
</tr>
<tr>
<td>Extinguishing Media</td>
<td></td>
</tr>
<tr>
<td>Appearance and Odor</td>
<td></td>
</tr>
</tbody>
</table>

**Section IV - Physical/Chemical Characteristics**

<table>
<thead>
<tr>
<th>Physical/Chemical Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling Point</td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td></td>
</tr>
<tr>
<td>Vapor Density</td>
<td></td>
</tr>
<tr>
<td>Magnetic susceptibility</td>
<td></td>
</tr>
<tr>
<td>Stability in Water</td>
<td></td>
</tr>
<tr>
<td>Extinguishing Media</td>
<td></td>
</tr>
<tr>
<td>Appearance and Odor</td>
<td></td>
</tr>
</tbody>
</table>

**Section V - Reactivity Data**

<table>
<thead>
<tr>
<th>Reactivity Data</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity</td>
<td></td>
</tr>
<tr>
<td>Inflammability</td>
<td>Strong oxidizing agents</td>
</tr>
</tbody>
</table>

**Section VI - Health Hazard Data**

<table>
<thead>
<tr>
<th>Health Hazard Data</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Hazard (Acute and Chronic)</td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td></td>
</tr>
<tr>
<td>Genotoxicity</td>
<td></td>
</tr>
<tr>
<td>Damaging to Reproductive Organs</td>
<td></td>
</tr>
<tr>
<td>Occupational</td>
<td></td>
</tr>
<tr>
<td>Cancerous</td>
<td></td>
</tr>
<tr>
<td>Other Physical Hazard</td>
<td></td>
</tr>
</tbody>
</table>

**Section VII - Precautions for Safe Handling and Use**

<table>
<thead>
<tr>
<th>Precautions for Safe Handling and Use</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steps to be Taken in Case Material is Released for Spill</td>
<td></td>
</tr>
<tr>
<td>Emergency First Aid Procedures</td>
<td></td>
</tr>
<tr>
<td>Emergency and Fire Fighting Procedures</td>
<td></td>
</tr>
<tr>
<td>Emergency and Fire Fighting Procedures</td>
<td></td>
</tr>
<tr>
<td>Emergency and Fire Fighting Procedures</td>
<td></td>
</tr>
<tr>
<td>Emergency and Fire Fighting Procedures</td>
<td></td>
</tr>
</tbody>
</table>

**Section VIII - Control Measures**

<table>
<thead>
<tr>
<th>Control Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory Protection</td>
<td></td>
</tr>
<tr>
<td>Protective Glasses</td>
<td></td>
</tr>
<tr>
<td>Other Protective Clothing or Equipment</td>
<td></td>
</tr>
<tr>
<td>Workstation Practices</td>
<td></td>
</tr>
</tbody>
</table>

**Section IX - Other Information**

<table>
<thead>
<tr>
<th>Other Information</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste Treatment Method</td>
<td></td>
</tr>
<tr>
<td>Waste Disposal Method</td>
<td></td>
</tr>
</tbody>
</table>

**Section X - Miscellaneous Information**

<table>
<thead>
<tr>
<th>Miscellaneous Information</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material Safety Data Sheet</td>
<td></td>
</tr>
<tr>
<td>Material Safety Data Sheet</td>
<td></td>
</tr>
<tr>
<td>Material Safety Data Sheet</td>
<td></td>
</tr>
</tbody>
</table>

**Section XI - Certification**

<table>
<thead>
<tr>
<th>Certification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certification</td>
<td></td>
</tr>
</tbody>
</table>

**Section XII - Appendices**

<table>
<thead>
<tr>
<th>Appendices</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendices</td>
<td></td>
</tr>
</tbody>
</table>

**Section XIII - References**

<table>
<thead>
<tr>
<th>References</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td></td>
</tr>
</tbody>
</table>

**Section XIV -译文**

<table>
<thead>
<tr>
<th>译文</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>译文</td>
<td></td>
</tr>
</tbody>
</table>