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Revised  
and  
Updated

Edvo-Kit #371

## DNA Fingerprinting Using PCR

### Experiment Objective:

In this experiment, students will conduct a DNA fingerprinting exercise on simulated samples from a crime scene and four different suspects. Polymorphic regions of DNA are amplified using PCR. Students will then analyze the amplified DNA segments by agarose gel electrophoresis.

See page 3 for storage instructions.

Edvo-Kit #

# 371



### NOTE:

PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.

# Table of Contents

|  | Page |
|--|------|
| Experiment Components                                    | 3    |
| Experiment Requirements                                  | 4    |
| Background Information                                   | 5    |
| Experiment Procedures                                    |      |
| Experiment Overview                                      | 9    |
| Module I: PCR Amplification of Crime Scene & Suspect DNA | 10   |
| Module II: Separation of PCR Products by Electrophoresis | 11   |
| Module III: Staining Agarose Gels                        | 13   |
| Study Questions  | 15   |
| Instructor's Guidelines                                  | 16   |
| Pre-Lab Preparations                                     | 17   |
| Experiment Results and Analysis                          | 20   |
| Study Questions and Answers                              | 21   |
| Appendices   | 22   |

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## Experiment Components

| Components  | Storage          | Check (✓)                |
|---|------------------|--------------------------|
| A Vial containing PCR EdvoBeads™<br><i>Each PCR EdvoBead™ contains:</i><br>• dNTP Mixture<br>• Taq DNA Polymerase<br>• MgCl <sub>2</sub><br>• Reaction Buffer | Room Temperature | <input type="checkbox"/> |
| B Primer mix concentrate  | -20°C Freezer    | <input type="checkbox"/> |
| C EdvoQuick™ DNA Ladder   | -20°C Freezer    | <input type="checkbox"/> |
| D DNA Template #1   | -20°C Freezer    | <input type="checkbox"/> |
| E DNA Template #2   | -20°C Freezer    | <input type="checkbox"/> |
| F DNA Template #3   | -20°C Freezer    | <input type="checkbox"/> |
| G DNA Template #4   | -20°C Freezer    | <input type="checkbox"/> |
| H TE Buffer   | -20°C Freezer    | <input type="checkbox"/> |

**NOTE: Component B is now supplied in concentrated form.**

### Reagents & Supplies *(Included with this experiment)*

Store all components below at room temperature.

| Component  | Check (✓)                |
|--|--------------------------|
| • UltraSpec-Agarose™                                 | <input type="checkbox"/> |
| • Electrophoresis Buffer (50x)                       | <input type="checkbox"/> |
| • 10x Gel Loading Solution                           | <input type="checkbox"/> |
| • InstaStain® Ethidium Bromide                       | <input type="checkbox"/> |
| • FlashBlue™ Liquid Stain                            | <input type="checkbox"/> |
| • Microcentrifuge Tubes                              | <input type="checkbox"/> |
| • 0.2 ml PCR tubes                                   | <input type="checkbox"/> |
| • Wax beads (for thermal cyclers without heated lid) | <input type="checkbox"/> |

Experiment #371 contains enough reagents to amplify 25 DNA samples using the Polymerase Chain Reaction. This represents five complete sets of reactions.

Sample volumes are very small. For liquid samples, it is important to quickly spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.

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.

## Requirements *(NOT included with this experiment)*

---

- Thermal cycler (EDVOTEK Cat. # 541 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- UV Transilluminator or UV Photodocumentation system (*use if staining with InstaStain® Ethidium Bromide*)
- UV safety goggles
- White light visualization system (*optional - use if staining with FlashBlue™*)
- Automatic micropipets (5-50 µl) with tips
- Microwave or hot plate
- 250 ml flasks or beakers
- Hot gloves
- Disposable laboratory gloves



## Background Information

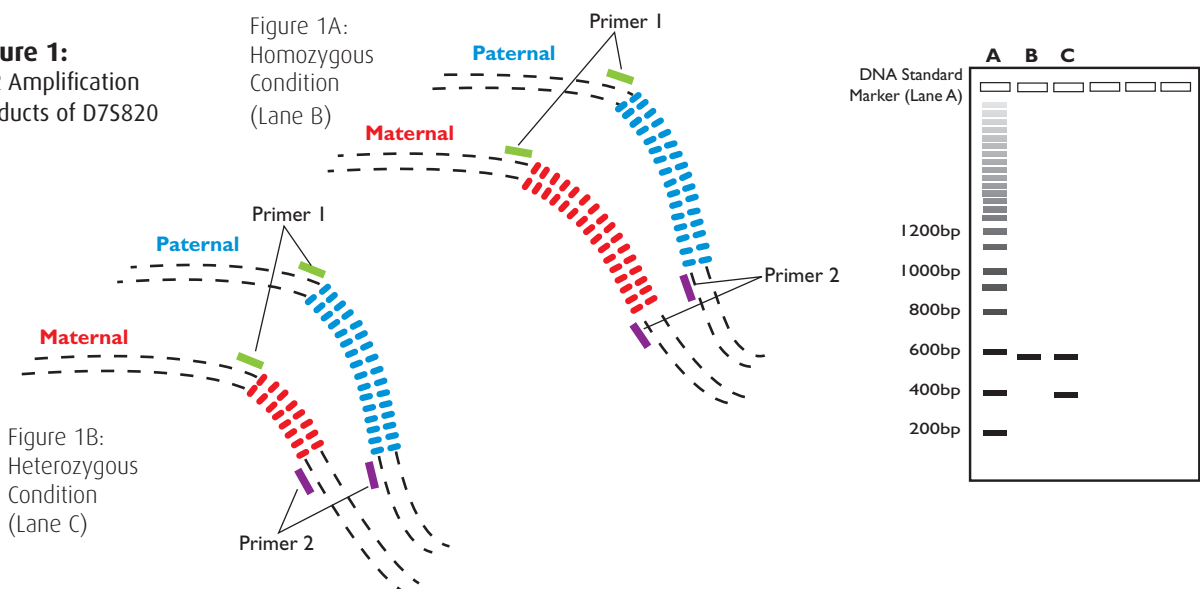
### VARIABILITY IN THE HUMAN GENOME ALLOWS FOR THE CREATION OF DNA FINGERPRINTS

The human genome is 99.9% identical between individuals. However, there are regions of variation within our genome called polymorphisms. Each of us inherits a unique combination of polymorphisms from our parents. By focusing on several polymorphic regions scientist can uncover a person's "DNA fingerprint". Like a regular fingerprint, a DNA fingerprint can be used to identify and distinguish between individuals. Because polymorphisms are inherited, DNA fingerprints can also be used to determine family relationships. DNA fingerprinting is used for the identification of missing persons and human remains, the determination of parentage, and the matching of criminal suspects to crime scenes.

DNA fingerprinting is used extensively in the field of forensic science. It establishes the probability that Sample X from the crime scene came from Person Y. Sir Alex Jeffreys first developed this method in 1984 at the University of Leicester, England. In 1986 analysis by Jeffreys led to the acquittal of Richard Buckland from murder charges. In 1987 Colin Pitchfork was the first criminal caught and convicted using DNA fingerprinting. Since then DNA analysis has been used in thousands of convictions. Additionally, hundreds of convicted prison inmates have been exonerated from their crimes.

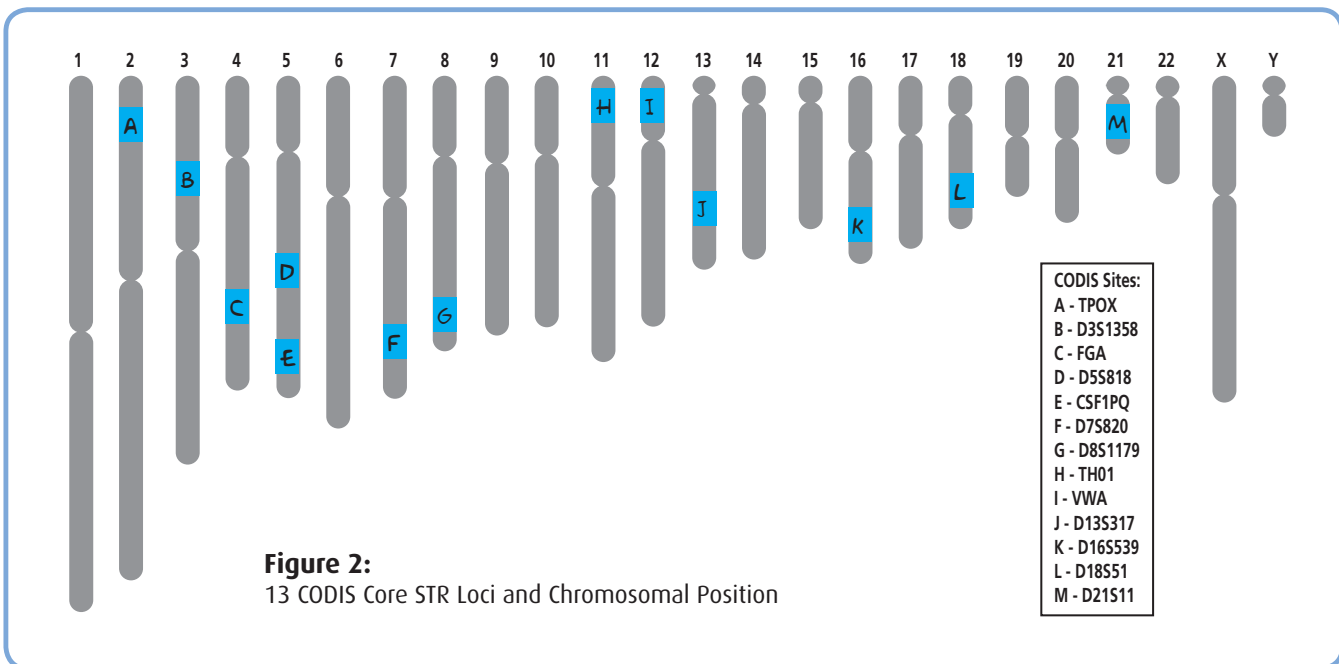
The goal of DNA fingerprinting is to analyze the genetic sample in a way that shows the tiny differences in the DNA of different people. In the early days of DNA fingerprinting analysis, this was done using restriction fragment length polymorphisms. Restriction enzymes recognize and cut DNA at specific nucleotide sequences. When added to a sample, restriction enzymes create fragments of DNA that differ in length based on the presence or absence of these sequences. These differences are visualized using gel electrophoresis followed by southern blotting and hybridization to a labeled probe. An RFLP is demonstrated whenever the Southern blot pattern obtained with one individual is different from the one obtained with another individual. This method is accurate but requires a considerable amount of starting material.

**Figure 1:**  
PCR Amplification  
Products of D7S820



Today, DNA fingerprinting is based on short repeat regions that can be analyzed even when they come from a small amount of degraded DNA sample. Individuals possess regions within their chromosomal DNA that are non-coding and that contain repeating units of nucleotides. These are called VNTRs (Variable Number of Tandem Repeats) when the nucleotide repeats are 15 to 70 bp long and STR (Short Tandem Repeats) when the nucleotide repeats are 2 to 6 bp long. VNTRs and STRs can repeat anywhere between three times and a hundred times. Each repeat forms an allele of a specific size. An example of an STR is D7S820, found on chromosome 7, which contains between 5 and 16 repeats of "GATA". Humans have two copies of this genetic locus – one from our biological mother and one from our biological father. This means we have two alleles at this site. If both alleles are the same the individual is homozygous and if the alleles are different the individual is heterozygous at D7S820 (Figure 1). Because D7S820 can be many different lengths, there are hundreds of different allele combinations that can occur at this location.

The power of STR analysis comes from looking at multiple STR loci simultaneously. Thirteen independent loci have been established as the standard for human identification - CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11. The probability of a random match when all 13 loci are analyzed is 1 in 3 trillion. In 1990, the Federal Bureau of Investigation (FBI) established the Combined DNA Index System (CODIS), based on these thirteen STRs (Figure 2). CODIS is a database system which allows comparison of crime scene DNA to DNA profiles in a convicted offender and a forensic (crime scene) index. A match of crime scene DNA to a profile in the convicted offender index indicates a suspect for the crime, whereas a match of crime scene DNA to the forensic index (a different crime scene) indicates a serial offender. CODIS has now been used to solve dozens of cases where authorities had not been able to identify a suspect for the crime under investigation.



## CREATING A DNA FINGERPRINT FROM A CRIME SCENE SAMPLE

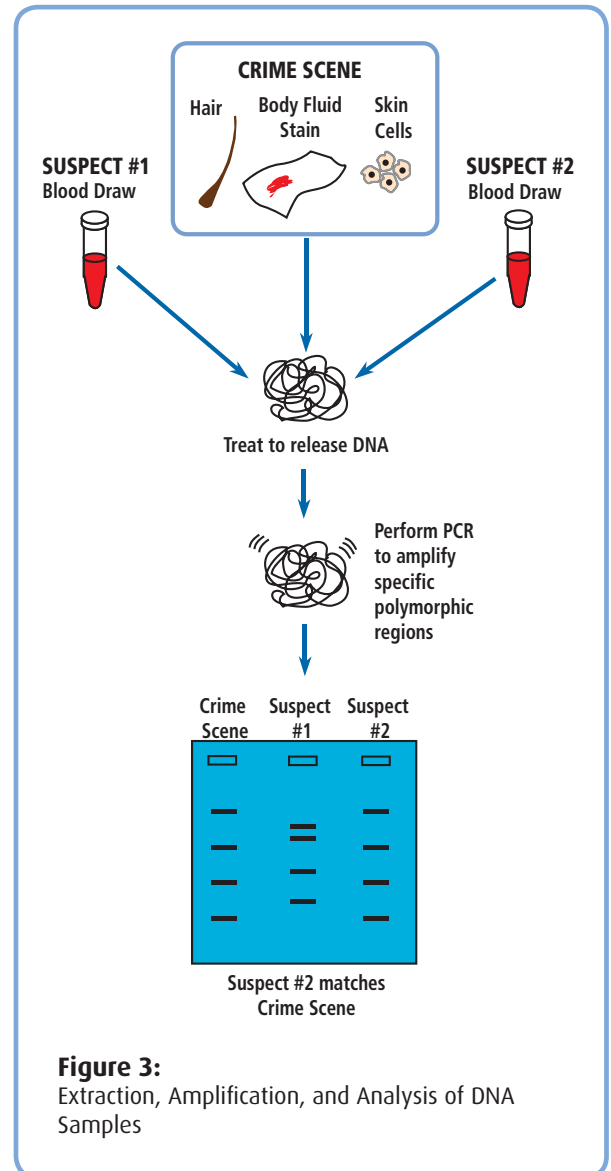
Forensic DNA fingerprinting begins with the legal collection of biological evidence (often present as a stain) from the crime scene or victim. The sample is treated with a detergent to rupture (lyse) cell membranes, and the cellular DNA is extracted for further analysis (Figure 3). After DNA is extracted from the sample, forensic scientists can develop 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.

In today's forensics laboratories, the polymerase chain reaction (or PCR) is used to create a DNA fingerprint. During PCR, DNA is copied in vitro much like it is during replication in a dividing cell. While many enzymes are involved in replication, PCR uses one enzyme and instead changes the temperature to initiate different steps. The first step for both PCR and replication is for two complementary DNA strands to separate from one another. In PCR, this is accomplished by heating the sample to 94°C, which breaks the hydrogen bonds between the base pairs. This step is known as denaturing.

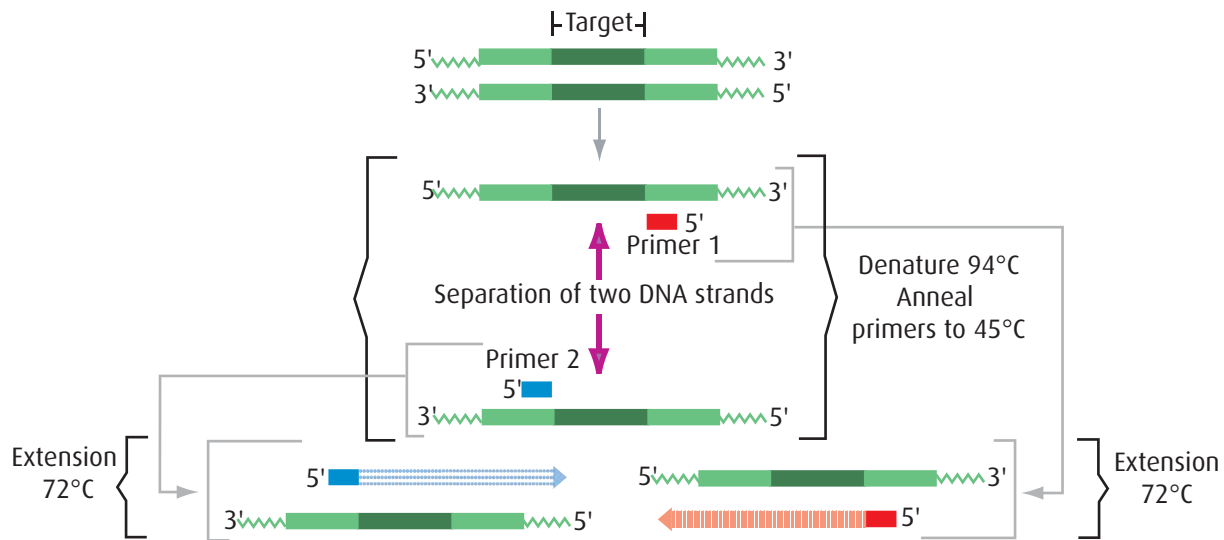
In the second step of DNA replication, the enzyme Primase places small RNA primers on the single stranded DNA to target the genome for replication by polymerase. To replicate this in vitro, scientists create short, synthetic pieces of DNA that target a specific part of the genome. Primers have sequences unique to the regions above and below the sequence of interest (Figure 1). These primers bind to their specific DNA match and in turn serve as an attachment site for DNA polymerase. In PCR, the sample is cooled to an intermediate temperature, usually 40°C to 65°C, to allow binding of the two primers to the target DNA in a process called annealing.

In the third step of replication and PCR, an enzyme called polymerase duplicates the DNA molecule by reading the template DNA strand (starting from the primer) and assembling the appropriate nucleotides into a complementary strand. In PCR, this is accomplished by raising the temperature to 72°C, which is the optimal temperature for polymerase to synthesize the new complementary strand. An important difference between PCR and replication is that in PCR a small section of the genome is amplified (as dictated by the primers) rather than the whole genome.

These three steps - denaturation, annealing, and extension - constitute one cycle of PCR. Each cycle doubles the amount of targeted DNA (see Figure 4), exponentially increasing the amount of DNA in the sample. These three distinct temperature steps are repeated many times. When these cycles are repeated 20-40 times, the amount of target sequence increases to the point that it can be easily examined. 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 3:**  
Extraction, Amplification, and Analysis of DNA Samples

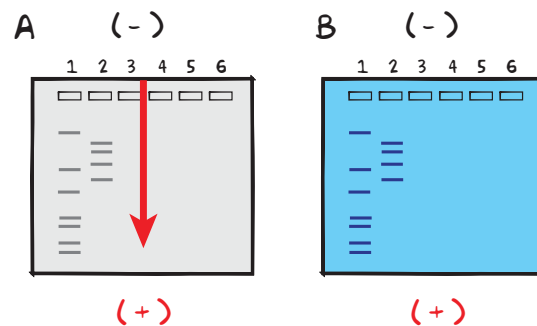


**Figure 4:** Polymerase Chain Reaction

Because PCR occurs in a test tube, scientists must provide the key ingredients of nucleotides, primers, the initial DNA template, and DNA polymerase. For PCR to be successful, this polymerase must be stable at high temperatures so that it is not denatured each time the PCR sample is raised to 94°C. To combat this problem, the thermostable DNA polymerase “*Taq*” was isolated from the bacterium *Thermus aquaticus*.

Following amplification, the PCR products are visualized and sized using gel electrophoresis. In gel electrophoresis the amplified product is added into depressions (or “wells”) within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete “bands” within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA (Figure 5). The loci’s length is then calculated by comparing the distance the band traveled to a DNA ladder made up of several known lengths. In forensic analysis this process of amplification, visualization, and measurement is repeated for several loci.

In this experiment, students and teachers will design their own crime scene and develop a plan to test their crime-solving skills using PCR and gel electrophoresis.



**Figure 5:**  
Overview of Agarose Gel Electrophoresis

## Experiment Overview

### EXPERIMENT OBJECTIVE:

In this experiment, students will conduct a DNA fingerprinting exercise on simulated samples from a crime scene and four different suspects. Polymorphic regions of DNA are amplified using PCR. Students will then analyze the amplified DNA segments by agarose gel electrophoresis.

### 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 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:

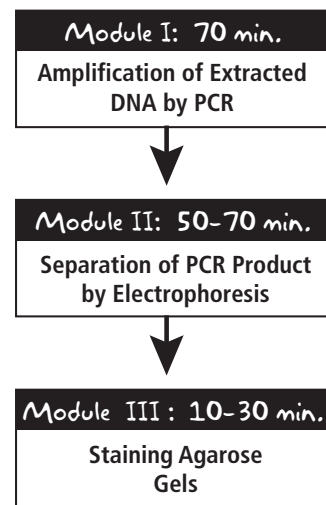
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

#### During the Experiment:

- Record your observations.

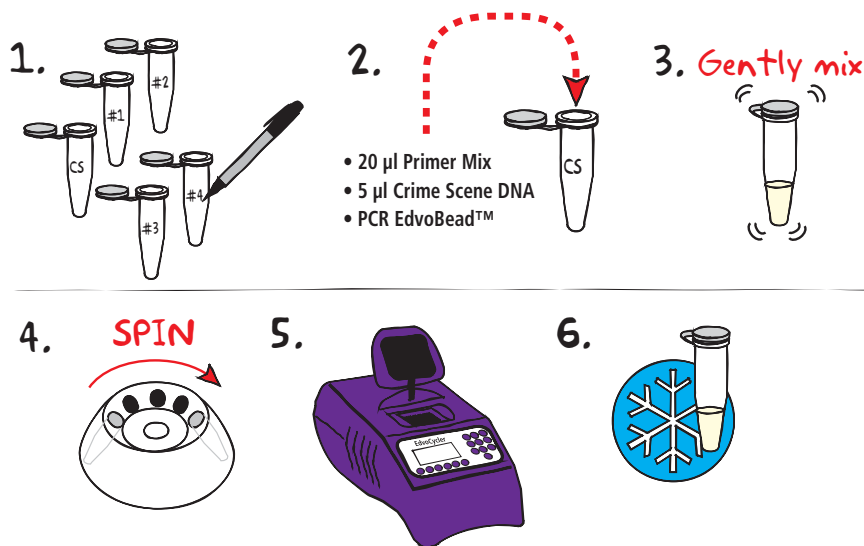
#### After the Experiment:

- 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.



NOTE: Experimental times are approximate.

## Module I: PCR Amplification of Crime Scene and Suspect DNA



### NOTES AND REMINDERS:

This kit includes enough DNA to set up five sets of PCR samples.

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax to prevent evaporation. See Appendix B for guidelines.

1. **LABEL** 5 PCR tubes for: Crime scene PCR, Suspect #1 PCR, Suspect #2 PCR, Suspect #3 PCR, and Suspect #4 PCR. Put your initials or group number on the tubes.
2. Each individual PCR reaction should be prepared as follows: **ADD** 20 µL primer mix, 5 µL Crime Scene DNA and one PCR EdvoBead to the appropriately labeled 0.2 ml PCR tube. **REPEAT** for Suspect #1 through #4 DNA.
3. **MIX** each PCR sample. Make sure the PCR EdvoBeads are completely dissolved.
4. **CENTRIFUGE** to collect the samples at the bottom of the tubes.
5. **AMPLIFY** DNA using PCR:

#### PCR cycling conditions:

Initial denaturation 94°C for 3 minutes  
 94°C for 30 seconds  
 55°C for 65 seconds } 30 cycles  
 72°C for 30 seconds  
 Final Extension 72°C for 4 minutes

### NOTE:

PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.



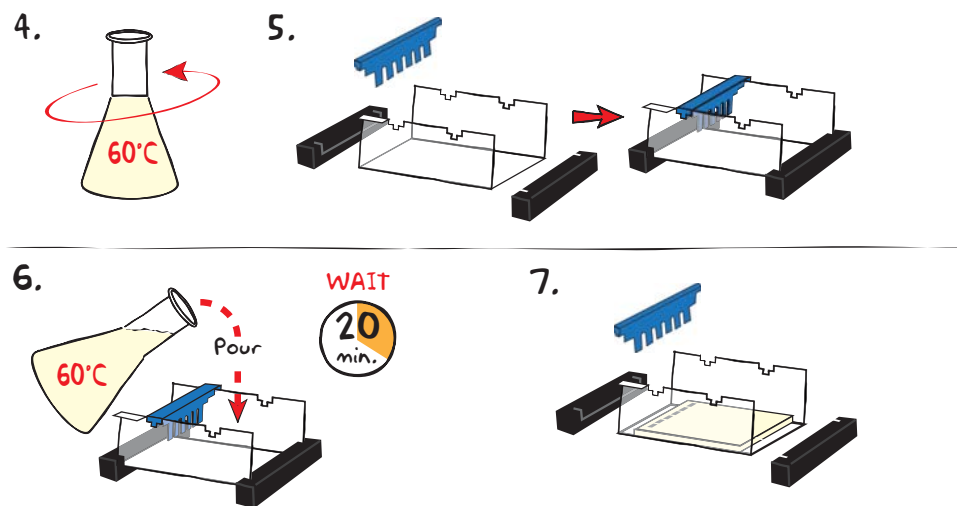
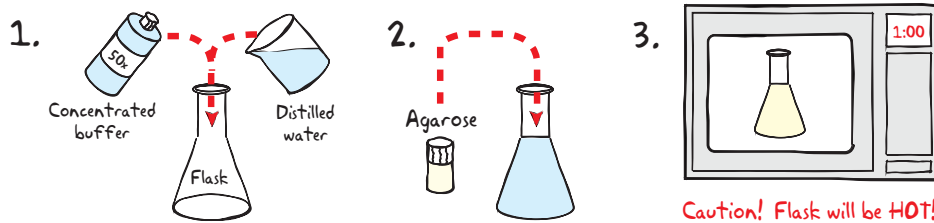
6. **ADD** 5 µL 10x gel loading solution to each tube. **PROCEED** to Module II: Electrophoresis of PCR product.



### OPTIONAL STOPPING POINT

The PCR samples may be stored at -20°C for electrophoresis at a later time.

## Module II: Separation of PCR Products by Electrophoresis



### NOTES:

7 x 14 cm gels are recommended. Each gel will be used by 1 group to analyze 1 crime scene scenario. Place well-former template (comb) in the first set of notches.

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at [www.edvotek.com](http://www.edvotek.com)

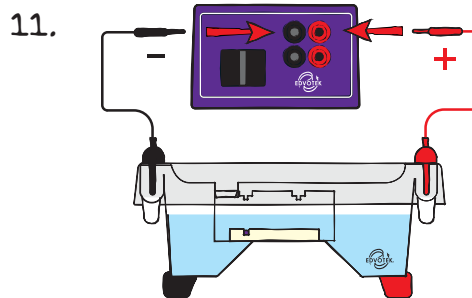
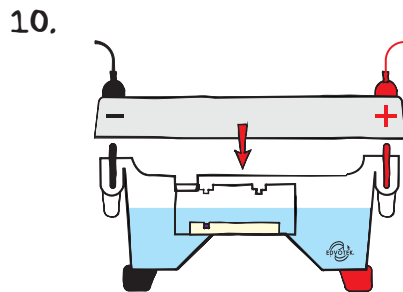
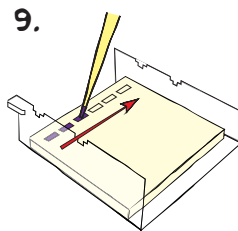
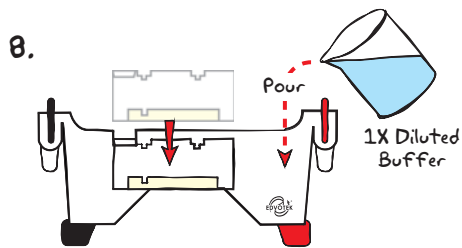
- DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
- MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
- DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from 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).
- COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table  
**A**

Individual 1.0% UltraSpec-Agarose™ Gel

| Size of Gel Casting tray | Concentrated Buffer (50x) | + Distilled Water | + Amt of Agarose | = TOTAL Volume |
|--------------------------|---------------------------|-------------------|------------------|----------------|
| 7 x 7 cm                 | 0.5 ml                    | 24.5 ml           | 0.25g            | 25 ml          |
| 7 x 14 cm                | 1.0 ml                    | 49.0 ml           | 0.50 g           | 50 ml          |

## Module II: Separation of PCR Products by Electrophoresis



### Reminder:

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. **LOAD** the entire sample (20-25  $\mu\text{L}$ ) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA 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 proceed to Module III: **STAINING** Agarose Gels.

Table 1: Gel Loading

| Lane | Sample          |
|------|-----------------|
| 1    | 200 bp Ladder   |
| 2    | Crime scene PCR |
| 3    | Suspect #1 PCR  |
| 4    | Suspect #2 PCR  |
| 5    | Suspect #3 PCR  |
| 6    | Suspect #4 PCR  |

Table B  
1x Electrophoresis Buffer (Chamber Buffer)

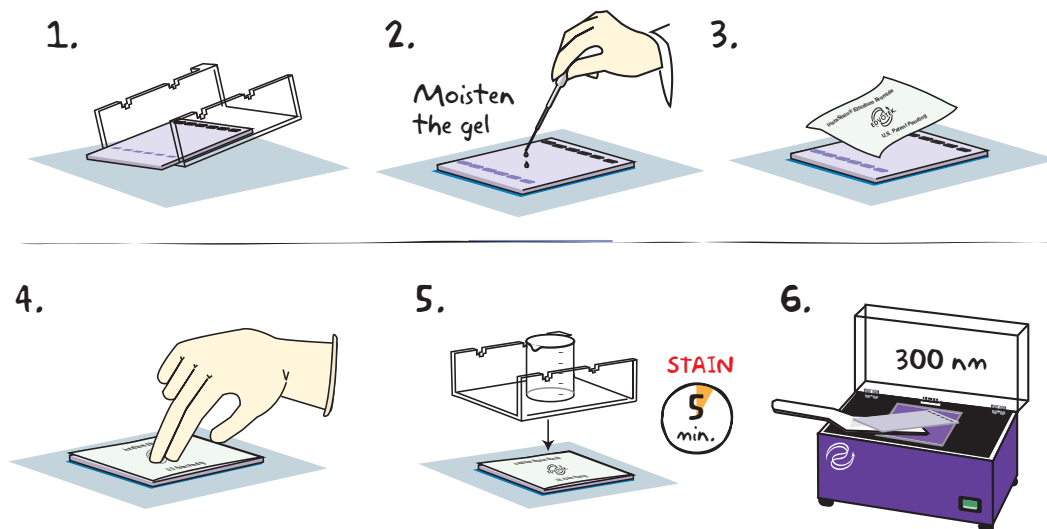
| EDVOTEK Model # | Total Volume Required | Dilution         |                   |
|-----------------|-----------------------|------------------|-------------------|
|                 |                       | 50x Conc. Buffer | + Distilled Water |
| M6+             | 300 ml                | 6 ml   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

Table C  
Time and Voltage Guidelines  
(1.0% - 7 x 14 cm Agarose Gel)

| Volts | Recommended Time |                |
|-------|------------------|----------------|
|       | Minimum          | Maximum        |
| 125   | 55 min.          | 1 hour 15 min. |
| 70    | 2 hours 15 min.  | 3 hours        |
| 50    | 3 hours 25 min.  | 5 hours        |

## Module III-A: Staining Agarose Gels Using InstaStain® Ethidium Bromide

Preferred Method

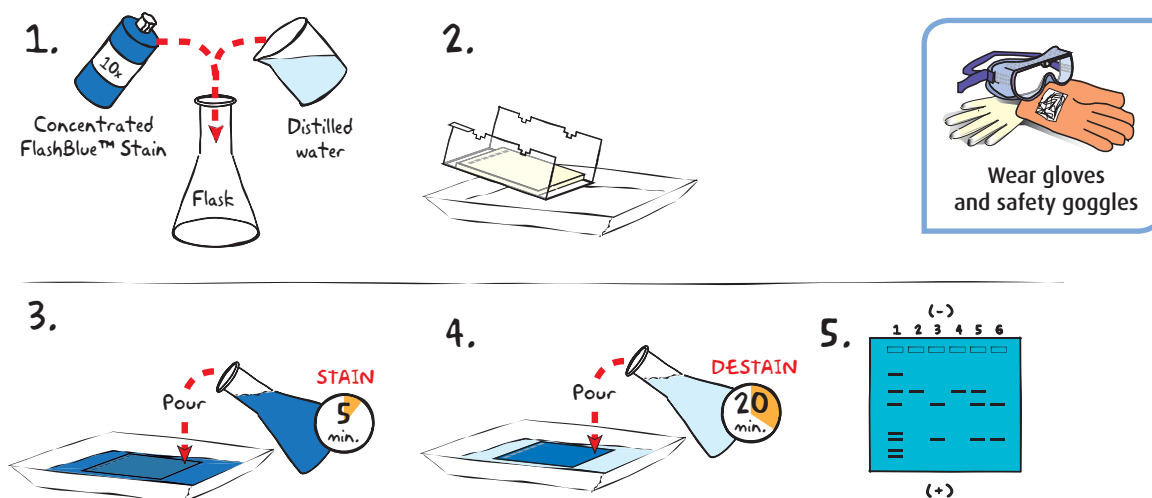


- Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.  
**DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**
- MOISTEN** the gel with a few drops of electrophoresis buffer.
- Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.
- With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
- PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 minutes.
- REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a long wavelength ultra-violet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

**BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**



## Module III-B: Staining Agarose Gels Using FlashBlue™



- DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
- REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
- COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
- TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
- Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

### Alternate Protocol:

- DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH<sub>2</sub>O.
- COVER** the gel with diluted FlashBlue™ stain.
- SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

## Study Questions

---

1. What is polymorphic DNA? How is it used for identification purposes?
2. What is an STR? Why is it used in law enforcement?
3. What are the three steps in a PCR cycle and what does each step accomplish?

# Instructor's Guide

## ADVANCE PREPARATION:

| Preparation For:  | What to do:   | When:  | Time Required: |
|---|---|--|----------------|
| <b>Module I:<br/>PCR Amplification of<br/>Crime Scene and<br/>Suspect DNA</b> | Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.) | One day to 30 min. before performing the experiment. | 30 min.        |
|   | Program Thermal Cycler  | One hour before performing the experiment.           | 15 min.        |
| <b>Module II:<br/>Separation of PCR<br/>Product by<br/>Electrophoresis</b>    | Prepare diluted TAE buffer (optional)                                     | Up to one day before performing the experiment.      | 45 min.        |
|   | Prepare molten agarose and pour gel (optional)                            |  |                |
| <b>Module III:<br/>Staining Agarose Gels</b>                                  | Prepare staining components   | Any time before the class period.                    | 10 min.        |

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## Pre-Lab Preparations: Module I

There are four different DNA samples provided. Choose one sample to represent the crime scene sample. (The designated CS sample can be different for different groups.) Make sure that one of the suspect DNAs is repeated as the crime scene sample for the respective groups. Alternatively, students can design their own crime scene scenario where they designate the DNAs for the crime scene and suspects.

In our scenario, the DNA sample for suspect #2 is repeated as the crime scene sample (see pg. 21).

### Preparation of the DNA Template

- Label 25 1.5 mL snap-top microcentrifuge tubes as follows:
  - 5 - Crime Scene (CS)
  - 5 - Suspect #1 (S1)
  - 5 - Suspect #2 (S2)
  - 5 - Suspect #3 (S3)
  - 5 - Suspect #4 (S4)
- Dispense 7  $\mu$ L of the DNA template into the appropriately labeled tube. Remember to use one of the suspect samples as the crime scene sample.

### Preparation of the Primer

- Thaw the Primer Mix Concentrate (B) on ice.
- Add 1 ml of TE Buffer (H) to the tube of Primer Mix Concentrate (B). Cap tube and mix.
- Aliquot 120  $\mu$ L of the diluted Primer Mix into 5 labeled microcentrifuge tubes.
- Distribute one tube of diluted Primer Mix to each student group.

### Additional Materials

- Dispense 30  $\mu$ L of 10x Gel Loading Solution to each student group.

### Programming the Thermal Cycler

The Thermal cycler should be programmed as outlined in Module II in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation. See Appendix B for instructions.

#### NOTE:

There is enough material to perform 25 PCR reactions and 5 gels. Students can be divided into groups of five students per group and samples from each group can be run on a gel. There is enough template DNA (Components D-G) for each group to perform a unique forensic crime scene scenario.

#### FOR MODULE II Each Student Group should receive:

- 5 PCR tubes
- 5 PCR EdvoBeads™
- 7  $\mu$ L Crime Scene DNA
- 7  $\mu$ L Suspect #1 DNA
- 7  $\mu$ L Suspect #2 DNA
- 7  $\mu$ L Suspect #3 DNA
- 7  $\mu$ L Suspect #4 DNA
- 120  $\mu$ L Diluted Primer Mix
- 30  $\mu$ L 10x Gel Loading Solution

#### NOTE:

PCR Cycling Conditions have changed. Please review your PCR program before performing the experiment.



## Pre-Lab Preparations: Module II

### AGAROSE GEL ELECTROPHORESIS

This experiment requires one 1.0% agarose gel per student group. For best results, we recommend using a 7 x 14 cm gel. 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 Module II in the Student's Experimental Procedure. Students will need 50x concentrated buffer, 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. See Appendix C.

#### 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  $-20^{\circ}\text{C}$  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.

#### Additional Materials:

Each 1.0% gel should be loaded with the EdvoQuick™ DNA ladder and the PCR samples from Module I.

- Aliquot 30  $\mu\text{l}$  of the EdvoQuick™ DNA ladder (C) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.

#### NOTE:

Accurate pipetting is critical for maximizing successful experiment results. EDVOTEK PCR experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommend performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

#### FOR MODULE II Each Student Group should receive:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- PCR Samples from Module I
- 30  $\mu\text{l}$  EdvoQuick™ DNA ladder



QuickGuide instructions and guidelines for casting various agarose gels can be found on our website.

[www.edvotek.com/Quick-Guides](http://www.edvotek.com/Quick-Guides)



## Pre-Lab Preparations: Module III

### MODULE III-A: STAINING WITH INSTASTAIN® ETHIDIUM BROMIDE

*Preferred Method*

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm<sup>2</sup> of gel (7 x 7 cm). You will need 2 cards to stain a 7 x 14 cm gel.

Use a mid-range ultraviolet transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

- Standard DNA markers should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain card for an additional 3-5 min. If markers are not visible, troubleshoot for problems with electrophoretic separation (see Appendix A).
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

#### FOR MODULE III-A Each Student Group should receive:

- 2 InstaStain® cards per 7 x 14 cm gel



Wear gloves  
and safety goggles

### MODULE III-B: STAINING AGAROSE GELS WITH FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid 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. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

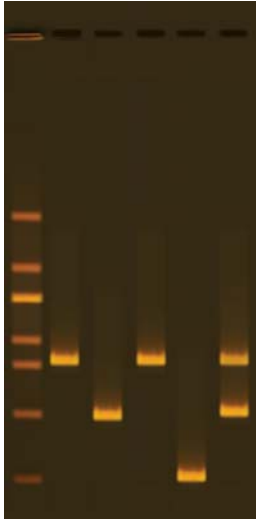
#### FOR MODULE III-B Each Student Group should receive:

- 10 ml 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

### MODULE III: PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

## Experiment Results and Analysis



### Includes EDVOTEK's All-NEW EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:  
2640, 1400, 1100, 700, 600, 400, 200



| Lane | Sample                | Size     |
|------|-----------------------|----------|
| 1    | EdvoQuick™ DNA Ladder |          |
| 2    | Crime Scene DNA       | 625      |
| 3    | Suspect #1 DNA        | 400      |
| 4    | Suspect #2 DNA        | 625      |
| 5    | Suspect #3 DNA        | 200      |
| 6    | Suspect #4 DNA        | 625, 400 |

### ANALYSIS:

The above is an example outcome of this exercise, in which we chose suspect #2 to match the crime scene DNA. Your results for the crime scene DNA will vary depending on which suspect was selected.

In criminal investigations, several known variable regions in DNA are analyzed to match crime scene and suspect DNAs. In this exercise, we are analyzing one of these variable regions. A match between the crime scene DNA and a suspect provides strong evidence that the suspect was present at the crime scene. After analyzing the samples, a match was made between the crime scene DNA in lane 2 and Suspect #2 in lane 4. This is strong evidence that suspect #2 was at the crime scene, but it does not prove that this suspect committed the crime.

The EdvoQuick™ DNA Ladder in lane 1 makes it possible to measure the size of the amplicons produced by the PCR reactions in lanes 2-6.

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

# Appendices

- A EDVOTEK® Troubleshooting Guide
- B Preparation and Handling of PCR Samples With Wax
- C Bulk Preparation of Agarose Gels

Material Safety Data Sheets:

Now available for your convenient download on [www.edvotek.com](http://www.edvotek.com).

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## Appendix A

### EDVOTEK® Troubleshooting Guides

#### PCR AND ELECTROPHORESIS

| PROBLEM:   | CAUSE:  | ANSWER:  |
|--|---|--|
| There is very little liquid left in tube after PCR                     | Sample has evaporated   | Make sure the heated lid reaches the appropriate temperature.  |
|  |   | If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)  |
|  | Make sure students close the lid of the PCR tube properly.  |  |
| The ladder, control DNA, and PCR products are not visible on the gel.  | Pipetting error   | Make sure students pipet 20 $\mu$ L primer mix and 5 $\mu$ L DNA into the 0.2 mL tubes.  |
|  | The gel was not prepared properly.  | Ensure that the electrophoresis buffer was correctly diluted.  |
|  |   | Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels. |
| The gel was not stained properly.                                      | Repeat staining.  |  |
| After staining the gel, the DNA bands are faint.                       | Malfunctioning electrophoresis unit or power source.  | Contact the manufacturer of the electrophoresis unit or power source.  |
|  |   | Repeat staining protocol.  |
| After staining, the ladder is visible but no PCR products are present. | PCR amplification was unsuccessful.   | Repeat PCR with fresh PCR EdvoBeads™ and primers.  |
|  |   | Ensure that the thermal cycler has been properly programmed. See Module I for guidelines   |
| DNA bands were not resolved.   | Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation. | Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).  |
| DNA bands fade when gels are kept at 4°C.                              | DNA stained with FlashBlue™ may fade with time  | Re-stain the gel with FlashBlue™   |

## Appendix B

### Preparation & Handling of PCR Samples with Wax

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#### **ONLY For Thermal Cyclers WITHOUT Heated Lids**

Using a wax overlay on reaction components prevents evaporation during the PCR process.

#### **HOW TO PREPARE A WAX OVERLAY**

1. Add PCR components to the 0.2 ml PCR Tube as outlined in Module I.
2. Centrifuge at full speed for five seconds to collect sample at bottom of the tube.
3. Using clean forceps, add one wax bead to the PCR tube.
4. Place samples in PCR machine and proceed with Module I.

#### **PREPARING PCR SAMPLES FOR ELECTROPHORESIS**

1. After PCR is completed, melt the wax overlay by heating the sample at 94° C for three minutes or until the wax melts.
2. Using a clean pipette, remove as much overlay wax as possible.
3. Allow the remaining wax to solidify.
4. Use a pipette tip to puncture the thin layer of remaining wax. Using a fresh pipette tip, remove the PCR product and transfer to a new tube.
5. Add 5 µL of 10x Gel Loading Buffer to the sample. Proceed to Module II to perform electrophoresis.

## Appendix C

### Bulk Preparation of Agarose Gels

To save time, 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 can be remelted.

#### BULK ELECTROPHORESIS BUFFER

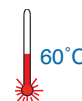
Bulk preparation of 1X electrophoresis buffer is outlined in Table D.

| Table D<br>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 (1.0%)

Bulk preparation of 1.0% agarose gel is outlined in Table E.

- Use a 500 ml flask to prepare the diluted gel buffer
- Pour the measured UltraSpec-Agarose™ into the prepared buffer. Refer to **Table E** for the mass. Swirl to disperse clumps.
- With a marking pen, indicate the level of solution volume on the outside of the flask.
- 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.
- 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.
- Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 ml for each 7 x 7 cm gel, 50 ml for each 7 x 14 cm gel. **For this experiment, 7 x 14 cm gels are recommended.**
- 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.



| Table E<br>Batch Preparation of 1.0% UltraSpec-Agarose™ |   |                  |   |                 |   |                     |
|---|---|------------------|---|-----------------|---|---------------------|
| Amt of Agarose  | + | 50x Conc. Buffer | + | Distilled Water | = | Diluted Buffer (1x) |
| 3.0 g   |   | 6.0 ml           |   | 294 ml          |   | 300 ml              |
| 4.0 g   |   | 8.0 ml           |   | 392 ml          |   | 400 ml              |

#### 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.

#### NOTE:

QuickGuide instructions and guidelines for casting various agarose gels can be found on our website. [www.edvotek.com/quick-guides](http://www.edvotek.com/quick-guides)