WORKSHOP

# Forensic Escape Room: Design Your Own Biotech Adventure

EDVOTEK®

Designed for the Classroom

SINCE 1987

## Introduction

Explore the world of forensic science with these fun and exciting escape room activities! Try forensic blood detection and agarose gel electrophoresis experiments, decipher clues, and solve puzzles. Learn to design your own escape room to have students unravel the evidence and free the innocent.

## Background Information

*Excerpts from EDVO-Kits 191 & 130*

An abundance of material evidence can be left behind at the scene of a crime. This evidence can include blood on clothing, walls or floors, or even on the potential murder weapon. In some cases a few cells caught under the victim's nails during a struggle can provide a wealth of information. Evidence can be obtained based on microscopic examination and biotechnological analysis, and then compared to a sample obtained from a person of interest who may have been at the site of the crime. Advances in molecular biology and genetics over the past 30 years have produced a variety of applications that have forever changed forensic science. Human tissue is made up of cells that contain DNA, which can be collected from evidence. When combined with the polymerase chain reaction (PCR) and DNA fingerprinting a very small amount of DNA from a biological sample can be analyzed. In many cases the crime can only be solved, and the criminals brought to justice, through the meticulous work of forensic scientists.

**DETECTION OF BLOOD SPATTERS: Presumptive & Confirmatory Tests**

The most common presumptive forensic blood test is the Kastle-Meyer test (Box 1). The Kastle-Meyer test uses a compound known as phenolphthalein (pr. fee-nawl-thal-een), which reacts with the iron carried by hemoglobin. First, presumptive blood is gathered on a cotton-tipped swab. The cellular membranes of cells on the swabs are then broken open (lysed) by applying a few drops of 95% ethanol. Phenolphthalin solution is then applied, followed quickly by hydrogen peroxide. If the cotton swab turns pink, it means that there was likely hemoglobin in the sample.

**BOX 1:** Chemistry of the Kastle-Meyer Test

The phenolphthalein ($C_20H_{16}O_4$) used in the Kastle-Meyer test has been reduced, i.e. it has gained electrons, and is actually called phenolphthalin ($C_20H_{14}O_4$). The reaction in the Kastle-Meyer test is based on the reaction between the iron in hemoglobin and hydrogen peroxide ($H_2O_2$). The iron in hemoglobin reduces (supplies electrons to) the $H_2O_2$, creating water ($H_2O$). This reaction depletes the hemoglobin of electrons, which are in turn supplied by phenolphthalin. The oxidation, i.e. the release of electrons, of phenolphthalin turns it back into phenolphthalein, which has a characteristic pink color.

$$Fe^{4+} + C_20H_{14}O_4 + H_2O_2 \rightarrow C_20H_{16}O_4 + H_2O + Fe^{3+}$$

Presumptive tests, such as the Kastle-Meyer, must be confirmed using a test that definitively detects blood. These are known as confirmatory tests. Confirmatory tests are often much more expensive and can take more time than presumptive tests. The most common confirmatory test for blood is the Rapid Stain Identification of Human Blood (RSID). The RSID works similarly to a pregnancy test. The sample is applied to the test strip, and antibodies that recognize blood proteins specifically bind to the sample. If the antibodies bind and the sample is positive for blood, a visible line is shown in the viewing window. Another confirmatory test for blood is ABO blood type testing. Testing for blood groups relies on the precipitation of an antigen-antibody complex, called agglutination. Only blood will produce this agglutination, which is why it is classified as a confirmatory blood test.

In addition to being a confirmatory test, ABO blood typing is also a faster and more affordable identity test than other analysis techniques such as DNA fingerprinting. Indeed, forensic blood typing serves both as a confirmatory test and provides information about the suspect in the form of their blood type. Even though blood typing cannot point to a specific person as the criminal, it can point to a group of people that share the same blood type or eliminate suspects whose blood type does not match.

**BLOOD TYPING**

Blood typing is an immensely important clinical procedure. It is one of the first procedures performed during blood transfusions and surgery, and is also important in forensic science. Blood typing is an example of an agglutination assay, the precipitation of antigens on red blood cells and antibodies in the blood. When both components are present at a similar concentration they are in a state known as equivalence. In an equivalent state, neither the antibody nor the antigen is in excess, and the antigen-antibody complex forms large networks that precipitate out of solution. Importantly, the precipitate is easy to detect by eye, making agglutination assays both easy and cost-effective to perform. The most common blood typing system relates to the presence of the A and B antigens on the red blood cells. This system, known as the ABO blood types, produces four possible blood types: A, B, AB, and O (Figure 4). Individuals with only A antigens will have type A blood, while someone with B antigens has type B blood. The A and B antigens are co-dominant, so a person can have both antigens on their red blood cells, leading to the AB blood type. If an individual has neither A nor B antigens they have type O blood. A person with type A blood will recognize red blood cells with the A antigen as "self". However, if that person gets a blood transfusion with type B blood, the new red blood cells will be recognized by the immune system as foreign and will cause an immune response. Antibodies targeting the B antigens (anti-B antibodies) will bind to the B antigen on the transfused cells and agglutinate. In many cases, this severe immune response can be deadly. Therefore, it is important for hospitals and clinics to maintain records of patient blood types.

| Blood Type | Antigen on Red Blood Cells | Antibody in Blood | Percentage of Population |
|---|---|---|---|
| A | A | anti-B | 42% |
| B | B | anti-A | 10% |
| AB | A & B | none | 4% |
| O | O | anti-A & anti-B | 44% |

**Figure 1:** Types of Blood in the Population

The same reaction that can lead to severe immune responses in a patient is used for clinical and forensic blood typing experiments. For example, type B blood can be easily recognized by the agglutination between the anti-B antibodies and the B antigen. When something that is suspected to be blood is found at a crime scene, detectives will work quickly to secure the evidence and send it to a forensic lab for testing. In the lab, forensic scientists will perform presumptive and confirmatory tests for blood, potentially recommending additional testing such as DNA profiling.

**DNA FINGERPRINTING**

In humans, DNA is packaged into 23 pairs of chromosomes that are inherited from an individual's biological parents. Although most of this genetic material is identical in every person, small differences, or "polymorphisms", in the DNA sequence occur throughout the genome. For example, the simplest difference is a Single Nucleotide Polymorphism (or SNP). Changes in the number and location of restriction enzyme sites result in Restriction Fragment Length Polymorphisms (or RFLPs). Short repetitive stretches of DNA at specific locations in the genome can vary in number to produce STRs (Short Tandem Repeats) and VNTRs (Variable Number of Tandem Repeats). Although most polymorphisms occur in non-coding regions of DNA, those that disrupt a gene can result in disease. Medical diagnostic tests can identify specific polymorphisms associated with disease.

Analyzing several different polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another. Because polymorphisms are inherited, DNA fingerprints can also be used to determine paternity/maternity (and other familial relationships). The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are utilized to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. After DNA is extracted from these samples, 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.

Early fingerprinting analysis involved restriction digestion of the isolated DNA. Following electrophoresis of the digested sample, the DNA is transferred to a nylon membrane during a process known as Southern blotting. Sequence-specific DNA probes are used to visualize the membrane-bound DNA. If the DNA is not digested by the restriction enzyme, the probes will only hybridize to a single DNA segment. If a restriction site occurs within this sequence, the probe will hybridize with multiple bands of DNA. VNTRs are identified when a probe labels DNA at a dissimilar molecular weight.

Although RFLP analysis is very precise, it is time-consuming and requires large amounts of DNA. To address these problems, forensic scientists use the polymerase chain reaction (PCR) to produce DNA fingerprints. PCR allows researchers to quickly create many copies of a specific region of DNA *in vitro* (summarized in Figure 1-1). This technique requires 500-fold less DNA than traditional RFLP analysis and it can be performed in an afternoon.

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

**ESCAPE ROOM SCENARIO**

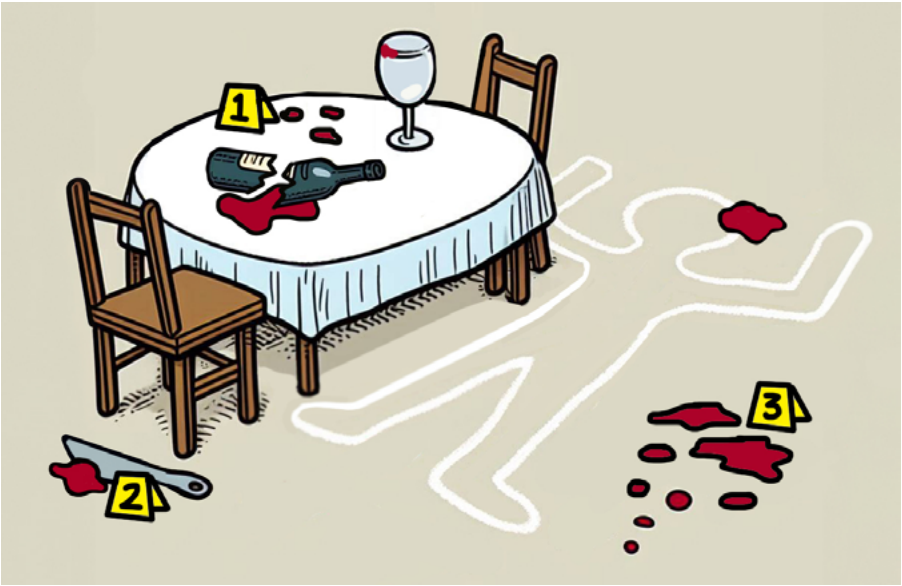
Laura Smith has been arrested for being the main suspect in the murder of her husband, Jeremy Smith. She has been questioned multiple times and continues to plead innocent to the crime. Laura's lawyer is trying to prove her innocence and has convinced the Charleston Police Department to further investigate the crime scene. You are the best forensic blood analyst that money can buy in Charleston. Your job is to determine if the evidence collected at the crime scene contains blood, what blood type the blood evidence is and to use DNA fingerprinting to determine who committed the crime. Will your forensics skill help prove Laura's innocence?

**The following information may help you with your forensic assessment of the crime.**

On Saturday night, police dispatch received a distressed phone call from a woman in her mid-30's, claiming to have found her husband dead in their kitchen upon returning home. Dispatch sent officers to the scene shortly after. Clay, the local police sheriff, was called down to the murder scene at the small suburban condo in Charleston, South Carolina. When he arrived at the condo, Clay found a deceased man in the kitchen. There was a major head laceration on the deceased man. Upon initial inspection of the scene the following primary evidence was collected: glass shards (from a broken bottle of red wine) with what appears to be blood splatter on the kitchen table (Sample 1), a knife near the table with apparent blood on it (Sample 2), potential blood spatter on the hardwood floor near the kitchen table (Sample 3), and a wine glass with women's lipstick on it. Clay and his crime scene investigators collected the evidence from the scene and the coroner was able to identify the body as Jeremy Smith.

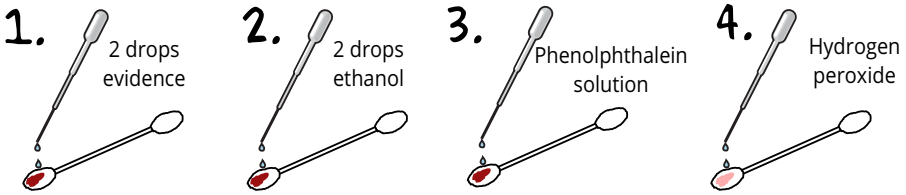
Jeremy Smith was a 32 year old man, who was married to 33 year old Laura Smith. Laura was the distressed woman from the police dispatch phone call. When questioned about her whereabouts during the crime, she claimed to have gone to an urgent care to take care of a deep wound she obtained. Laura insists that she accidentally cut herself while cooking dinner with Jeremy. Clay and his partner Amy, questioned the couples' neighbors. Most of the neighbors complained about their constant arguments. Their fights often disrupted the peaceful neighborhood. However, neighbors also noted that Jeremy was also constantly fighting with the HOA president, Sarah Ann. She is known for being a stickler on the HOA rules and ever since the Smiths moved in, there have only been fights between Jeremy and Sarah. The meeting notes from the HOA meetings prove that the two have a history of being verbally aggressive towards each other.

Clay and Amy have determined that Laura and Sarah Ann are the main suspects of this crime. The head laceration was determined to be what caused Jeremy's death. The glass shards in Jeremy's head wound matched the broken glass from the wine bottle found at the crime scene. According to eyewitness reports, Laura and Sarah Ann were seen at the scene of the crime 1 hour prior to the crime. Alcohol was detected in both Sarah Ann and Laura's blood samples. It was confirmed by the local urgent care that Laura was treated for a knife wound.



## Module I: Kastle-Meyer Test

The first steps of your forensic analysis will use the Kastle-Meyer test as a presumptive test for blood. You will test the three crime scene samples to see if they are positive or negative for the presence of blood.




1. Use the transfer pipet to **ADD** two drops, or 40 µL of blood evidence to the swab.

2. Use a transfer pipet to **ADD** two drops, or 40 µL, of 95% ethanol to the swab. **NOTE** any color change. **PLACE** the pipet and remaining ethanol to the side for testing additional samples.

3. Use a new pipet to **ADD** two drops, or 40 µL, of the phenolphthalein solution to the swab. **NOTE** any color change. No color change is expected even if blood is present. **PLACE** the pipet and remaining phenolphthalein to the side for testing additional samples.

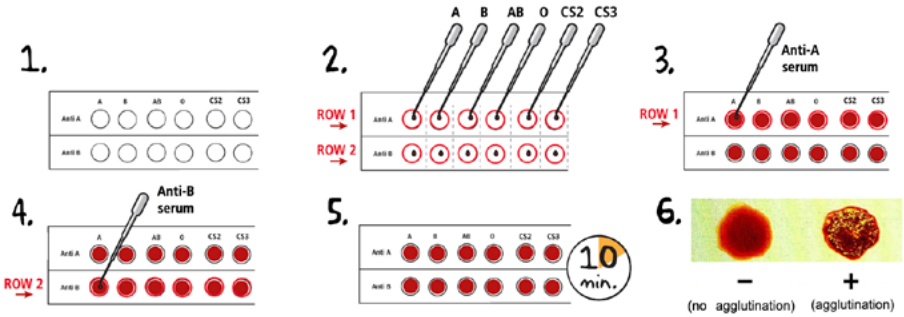
4. Use a new pipet to **ADD** two drops, or 40 µL, of hydrogen peroxide to the swab. **NOTE** any color change. An immediate pink color is expected if blood is present. **RECORD** your results in the chart below.

5. **REPEAT** steps 1-4 for each blood evidence sample.

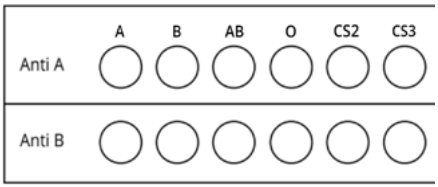
| Crime Scene Sample # | Positive or Negative for Blood |
|---|---|
| **CS1** | |
| **CS2** | |
| **CS3** | |

## Module II: Blood Type Test

Now that some samples from the crime scene have been identified as likely blood using the Kastle-Meyer test, it is necessary to confirm the identity of the samples. In this Module, you will perform ABO testing on the blood samples from the crime scene. Note that Jeremy is blood type O, Sarah Ann is blood type A, and Laura is blood type A.




1. **PLACE** a microtiter plate piece as shown below.




2. **ADD** 50 µL (or two drops from a transfer pipet) of each control blood type sample into each of the two corresponding wells. For example, control A blood type goes into the two wells under the letter "A". Repeat the same procedure for the control blood types B, AB, O, and the two positive blood samples from the crime scene. Each well requires 50 µL.

3. Use a new pipette tip to **ADD** 50 µL of Anti-A serum into each of the wells in row #1. The same tip or transfer pipet can be used for all samples in row #1.

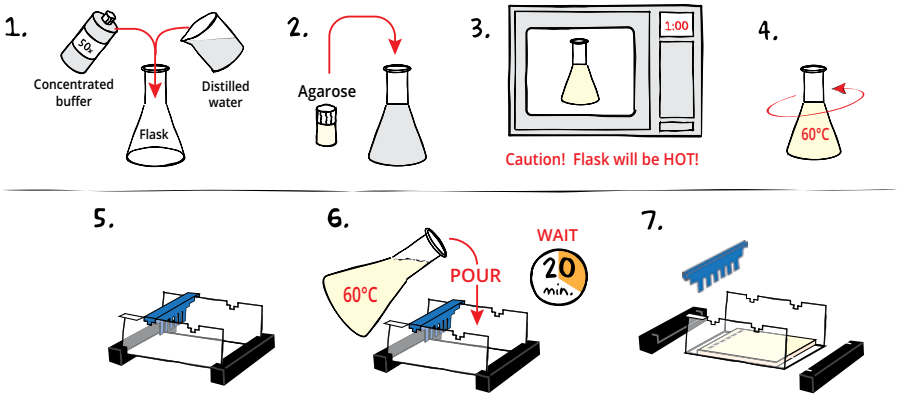
4. Use a new pipette tip to **ADD** 50 µL of Anti-B serum into each of the wells in row #2. The same tip or transfer pipet can be used for all samples in row #2.

5. Let the samples **INCUBATE** undisturbed on the lab bench for 5-10 minutes.

6. **COMPARE** the crime scene evidence with the control blood samples.

## Module III: DNA Fingerprinting

After determining the blood collected from the knife is Type A you must use DNA fingerprinting to find out which suspect the blood came from. You send both Sarah Anne and Laura's blood samples as well as the blood collected from the knife to an external DNA lab. Here they will extract DNA from each sample and amplify it by PCR. In this module, you will use gel electrophoresis to analyze the PCR reactions obtained from the suspects and compare them to the blood found at the scene.



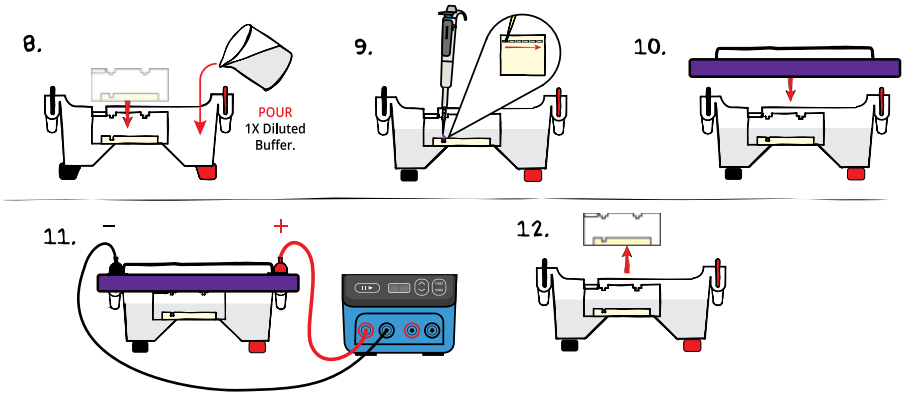

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution.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

Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

| Size of Gel Casting Tray | Concentrated Buffer (50x) | + Distilled Water | + Amt of Agarose | = TOTAL Volume |
|---|---|---|---|---|
| 7 x 7 cm | 0.6 mL | 29.4 mL | 0.24 g | 30 mL |
| 10 x 7 cm* | 0.9 mL | 44.1 mL | 0.36 g | 45 mL |
| 14 x 7 cm | 1.2 mL | 58.8 mL | 0.48 g | 60 mL |

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

## Module III: DNA Fingerprinting, continued




8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by the Table, at right.
10. **PLACE** safety cover on the unit. **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). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **PROCEED** to gel staining.

| LANE | TUBE | SAMPLE NAME |
|---|---|---|
| 1 | A | DNA standard marker |
| 2 | B | Crime scene blood collected from knife |
| 3 | C | Laura PCR Reaction |
| 4 | D | Sarah Ann PCR Reaction |

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

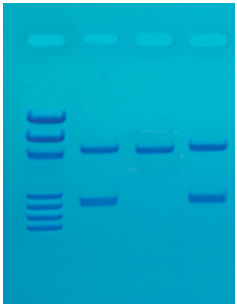
| EDVOTEK Model # | Total Volume Required | Dilution: 50x Conc. Buffer | + Distilled Water |
|---|---|---|---|
| EDGE™ | 150 mL | 3 mL | 147 mL |
| M12 | 400 mL | 8 mL | 392 mL |
| M36 | 1000 mL | 20 mL | 980 mL |

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

| | Electrophoresis Model: EDGE™ | M12 & M36 |
|---|---|---|
| Volts | Min/Max (minutes) | Min/Max (minutes) |
| 150 | 10/20 | 20/35 |
| 125 | N/A | 30/45 |
| 100 | 15/25 | 40/60 |

## Experimental Results and Analysis

### AGAROSE GEL ELECTROPHORESIS

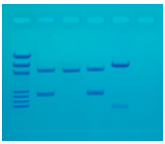


The DNA standards in Lane 1 make it possible to measure the DNA bands obtained from the PCR reactions. The results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA and the sample from Sarah Ann match. In criminal investigations, several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

| LANE | TUBE | SAMPLE NAME | MOLECULAR WEIGHTS |
|---|---|---|---|
| 1 | A | DNA standard marker | 6751, 3652, 2827, 1568, 1118, 825, 630 |
| 2 | B | Crime scene blood collected from knife | 3000, 1282 |
| 3 | C | Laura PCR Reaction | 3000 |
| 4 | D | Sarah Ann PCR Reaction | 3000, 1282 |

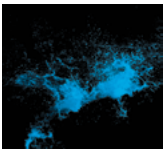
# Related Products

## DNA Fingerprinting by PCR Amplification



*For 8 gels.* Forensic DNA fingerprinting has become a universally accepted crime-fighting tool. Recent advances use the polymerase chain reaction (PCR) to amplify human DNA obtained from crime scenes. This experiment, based on a crime scene scenario, has an inquiry-based component.
***Cat. #130***

## Forensics Enhancement Techniques

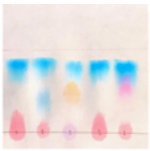


*For 10 groups.* Trace amounts of blood are often sufficient to identify the individual responsible for any number of crimes, including murder, burglary, or assault. Enhancement procedures can make a small stain of body fluid or tissue visible to the naked eye. In this experiment, students will act as detectives following the aftermath of a drug bust involving gang warfare over territory. Reagents that are routinely used as a first screen will be utilized to detect simulated blood and DNA. In addition, biological materials will be recovered from splatters, blood trajectory, and small droplets of simulated human materials.
***Cat. #194***

## Forensic Toxicology

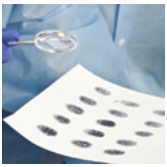
*For 10 groups.* In today's forensic science laboratory, toxicologists identify drugs and toxins in samples collected from crime scenes, victims, and potential suspects. If present, the toxicologist also determines whether the drug or toxin contributed to a person's behavioral changes or death. In this forensic science experiment, students will use the Enzyme Linked Immunosorbent Assay (ELISA) to analyze simulated crime scene samples for the presence of drugs.
***Cat. #195***


## Write to a Fair Trial: Handwriting Analysis



*For 10 groups.* Your lab notebook has been stolen, replaced with a ransom note demanding lunch money in exchange for its safe return! In this hands-on experiment, students will use principles of forensic handwriting analysis and paper chromatography to examine writing samples from 4 potential suspects. Only after careful analysis will they be able to solve the classroom crime.
***Cat. #196***

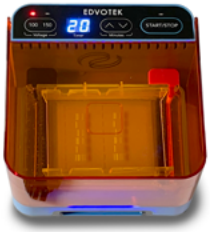
## Whose Fingerprints Were Left Behind?



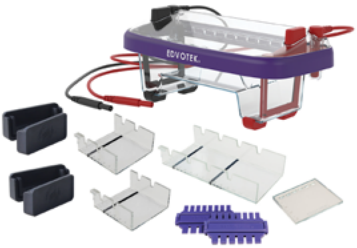
*For 10 groups.* After a crime has been committed, the evidence left behind can identify a potential culprit, although a single piece of evidence is not usually enough to convict someone. Even in this age of DNA, fingerprints and blood stains are still important at helping to identify a criminal. In this experiment your students will learn to detect and analyze fingerprints and then use these techniques to solve a classroom crime.
***Cat. #S-91***

**Details for all these products and MORE can be found on our website!**

## Related Products



**EDGE™ Integrated Electrophoresis System**
Runs one 10 x 7 cm gel
***Cat# 500***



**M12 Complete™ Electrophoresis Package**
For 1 or 2 Lab Groups
***Cat# 502-504***

**DuoSource™**
100/150 V, for 1 or 2 Units
***Cat# 509***

**QuadraSource™**
10-300 V, for 1 or 4 Units
***Cat# 5010***

**White Light LED Transilluminator**
***Cat# 552***

**EDVOTEK® Variable Micropipette**
5-50 µL Micropipette
***Cat. # 590***

**Fixed Volume MiniPipette™**
35 µL MiniPipette™
***Cat. # 587-2***

**TruBlu™ Jr Blue Light Transilluminator**
***Cat# 555***

**Long Wave UV Light**
***Cat# 969***

**Details for all these products and MORE can be found on our website!**