
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-Kit 190

Crime scene analysts play a crucial role in collecting and preserving evidence, as contamination can compromise investigations. They identify materials such as blood, trace substances, and even skin cells under a victim's nails to aid forensic analysis. Forensic scientists use various techniques to examine evidence, including **presumptive tests**, which indicate the presence of a substance, and **confirmatory tests**, which verify its identity. Microscopic and molecular analyses then compare evidence with potential suspects.

Advancements in genetics have transformed forensic science, allowing DNA analysis from even tiny biological samples. Techniques like PCR and DNA fingerprinting help solve crimes and bring criminals to justice.

FORENSIC SAMPLE ANALYSIS FOR BLOOD

Blood is often found at crime scenes, in spatters, drops, and drips. Analyzing these spatters is a field of study in itself! Most blood spatter evidence is found around the victim or where the violence occurred. Blood itself can be found on clothes, skin, or in the get-away vehicle. However, a red stain on the floor at a crime scene can't be immediately assumed to be human blood, nor can it be assumed to belong to the perpetrator. 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.

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.

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

Forensic scientists exercise caution before performing long, expensive DNA fingerprinting test on a sample that is not human blood. So, the first step when dealing with potential blood evidence is to confirm its human origin. This process begins with a simple presumptive test to distinguishes between blood and non-blood. Then, blood group typing is performed to confirm the sample is human and to help narrow down the suspects based on their blood group.

The Kastle-Meyer test, introduced in 1903, is the most used presumptive test for detecting blood. It uses phenolphthalin and hydrogen peroxide to identify the presence of blood in samples. Phenolphthalin is a reduced form of the acid-base indicator phenolphthalein (Box 1). Phenolphthalein changes from clear to pink in basic solutions. When phenolphthalein in a basic solution gains two electrons, it shifts from pink to clear. This reduced molecule is used for the Kastle-Meyer test.

To perform the test, the potential blood sample is collected on a cotton-tipped swab and then treated with a few drops of 95% ethanol to lyse, or break open, the cellular membranes. Next, phenolphthalin solution is applied, followed promptly by hydrogen peroxide. This sequence triggers a reaction where the iron in hemoglobin reacts with hydrogen peroxide, generating water

and free oxygen molecules. The hemoglobin loses electrons during this process, which are then donated back to hemoglobin by the phenolphthalein molecule. This causes the indicator to change from clear to pink, indicating blood.

The Kastle-Meyer test is valued for its speed, specificity for hemoglobin, and reliability, even with dilute samples. However, it may yield false positive results due to chemicals like iron and copper oxides that can react with the hydrogen peroxide. While it remains an efficient presumptive test, its susceptibility to false positives necessitates caution.

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 next step in blood testing is to confirm the identity of the sample using a test that definitively detects blood. One such confirmatory test is ABO blood group testing, which categorizes blood into types A, B, AB, and O based on the presence or absence of A and B antigens on the surface of red blood cells. Testing for blood groups relies on the precipitation of an antigen-antibody complex, called agglutination, using specific antibodies that bind to the surface proteins. Blood samples are mixed with antibodies to either the A or B antigens, and the samples are allowed to incubate. If either the antibody or the blood cells are in excess, there is no visible reaction. However, when both components are present at a similar concentration, the interactions between the antigens and the antibodies form large complexes that precipitate out of solution in a state known as equivalence. The mixture in the wells will look granular instead of smooth, which is easy to detect by eye. Only blood will produce this agglutination, which is why it is classified as a confirmatory blood test.

Although confirmatory tests like blood typing are more time-consuming and costly than presumptive tests, they offer superior accuracy. While it cannot pinpoint a specific perpetrator, blood typing can help narrow down suspects by identifying groups of individuals with matching blood types or by eliminating those with incompatible blood types, aiding in criminal investigations. The next step would be DNA fingerprinting, to identify the origin of the blood sample more conclusively.

DNA FINGERPRINTING

Once a sample has been confirmed to be human blood or tissue, the DNA is extracted and analyzed using 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. Analyzing several different polymorphisms within a person's genome generates a unique DNA "fingerprint" that can allow us to distinguish one individual from another, or even to determine 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. Following collection, the DNA is extracted from the cells, amplified using the Polymerase Chain Reaction (or PCR, box 2), and fragmented into smaller pieces using specific restriction enzymes. These fragments are analyzed using agarose gel electrophoresis, a technique which uses a porous

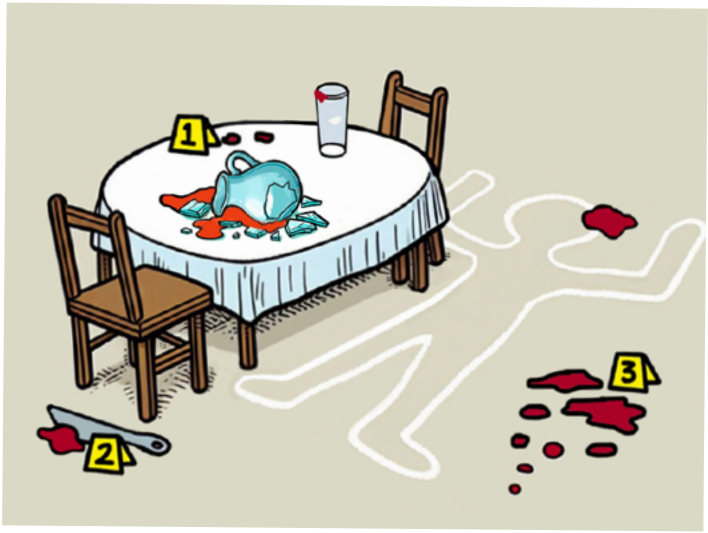
gel matrix and an electrical current to separate DNA fragments by size. After electrophoresis is completed, the gel is stained, and the banding patterns examined. Each band is like a puzzle piece that, when analyzed, reveals similarities and discrepancies between the crime scene and suspect samples. A match provides strong evidence that the suspect was present at the crime scene. This methodical process serves as a cornerstone in criminal investigations, offering a scientific means to establish connections and resolve cases with accuracy and precision.

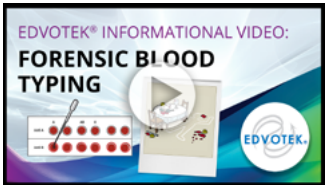
Data from crime scene evidence can suggest that a suspect was at a crime scene, but that data alone cannot convict a person of a crime. Many lines of evidence, including witness statements and alibis, must come together to build a case against a suspect. These results are used as evidence in the court of law.

ESCAPE ROOM SCENARIO

Monica Homewood has been arrested as the main suspect in the murder of her husband, Bryce Homewood. She has been questioned multiple times and continues to plead innocent to the crime. Monica'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 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 Monica's innocence?

The following information and evidence are required for your forensic assessment of the crime.





OFFICIAL POLICE REPORT **RESPONDING OFFICER:** Caitlin Marlow

DETAILS OF EVENT:

On Saturday night, police dispatch received a distressed phone call from a woman in her mid-30's, claiming to have returned home to find her husband dead in their kitchen. Dispatch sent Caitlin Marlow, the local police sheriff, to the crime scene in a small suburban condo in Charleston, South Carolina. Upon arriving at the condo, Sheriff Marlow found a deceased man with a traumatic head injury, including a deep laceration, in the kitchen.

Marlow and her crime scene investigators collected the evidence from the scene to be analyzed by the crime scene team. Upon initial inspection of the scene, the following primary evidence was collected: glass shards (from a broken iced tea pitcher) 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 tall glass with women's lipstick on it.

Later, the coroner was able to identify the body as Bryce Homewood. The cause of death was determined as a traumatic head injury. Glass shards in the head wound matched the broken glass from the iced tea pitcher found at the crime scene. A mysterious note with unintelligible text was found in the victim's pocket.

Marlow and her team of detectives questioned the neighbors in the condo complex. They determined Monica Homewood, Deena Granville, and Alex Johnson to be the three main suspects of this crime. According to eyewitness reports, both Monica and Deena were seen at the scene of the crime one (1) hour before the call to the police dispatch. Neighbors also noted that a local real estate agent, Alex Johnson, was seen talking with Mr. Homewood at the condo the night before.

THE VICTIM:

- **Bryce Homewood**, aged 32, was a graphic designer for an advertising agency. He was married to Monica Homewood. He was known for his sharp wit and humor, though sometimes he veered into the realm of abuse and/or harassment when angry.

SUSPECTS:

- **Monica Homewood**, 33, works as a pediatric nurse at a local children's hospital. She was the distressed woman from the police dispatch phone call. She is also married to the victim. Most of the neighbors complained about their constant arguments. Their fights often disrupted the peaceful condo building.

  When questioned about her whereabouts during the crime, Monica claimed to have gone to an urgent care to take care of a deep wound she claims she obtained accidentally while cooking dinner with Bryce. It was confirmed by the local urgent care that Monica was treated for a knife wound.

- **Deena Granville**, 34, is the neighbor and HOA president. She works as a marketing manager for a fashion retail company. She is known for being a stickler on the HOA rules and ever since the Homewoods moved in, there have only been fights between Bryce and Deena.

  Neighbors also noted that Bryce was also constantly fighting with Deena over excessive property management fees. The meeting notes from the HOA meetings prove that the two have a history of being verbally aggressive towards each other.

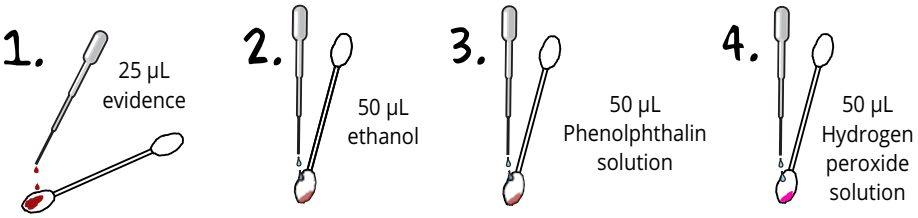
- **Alex Johnson**, 38 - Real Estate Agent. Alex Johnson has ongoing disputes with Bryce over a contested condo sale. At the time of the crime, he claimed to be attending a local real estate conference which investigators have yet to confirm. Neighbors noted Alex had a strained relationship with the victim due to their frequent clashes over business deals, but he always maintained a professional demeanor in their interactions.

SAMPLES PENDING FORENSIC ANALYSIS:

Potential blood evidence found at the crime scene. Tests to be performed include Kastle-Meyer testing, Blood Group Typing and DNA Fingerprinting.

Module I: Kastle-Maeyer 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 a transfer pipet to **ADD** 1 drop (or 25 µL) of blood evidence to the swab. NOTE: Using more than one drop (25 µL) of evidence may affect the results.

2. Use a transfer pipet to **ADD** 2 drops (or 50 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** 2 drops (or 50 µL) of the phenolphthalin solution to the swab. **NOTE** any color change. No color change is expected even if blood is present. **PLACE** the pipet and remaining phenolphthalin to the side for testing additional samples.

4. Use a new pipet to **ADD** 2 drops (or 50 µL) of hydrogen peroxide solution "H2O2" to the swab. **NOTE** any color change. A pink color is expected after several seconds if blood is present. **RECORD** your results in the chart below.

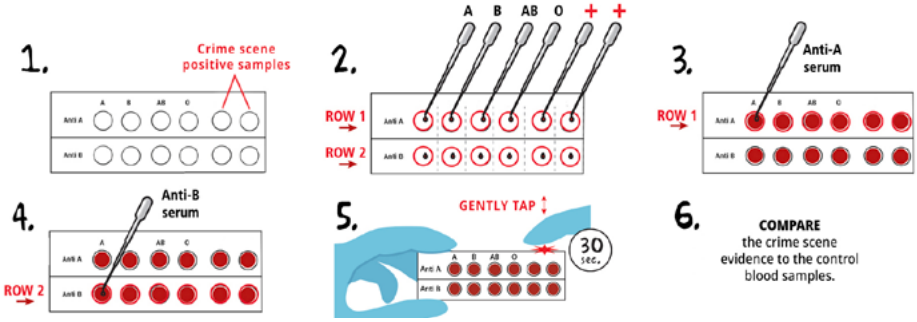
5. **REPEAT** steps 1-4 for each blood evidence sample.

IMPORTANT
For steps 2-4: When adding the detection reagents, hold the evidence swab at a steep angle, with the crime scene sample at the bottom.

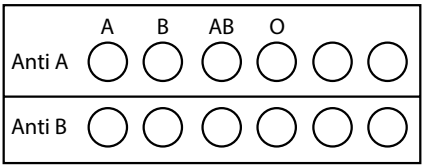
Crime Scene Sample #	Positive or Negative for Blood
CS1	
CS2	
CS3	

Module II: Blood Type Test

Now that some samples from the crime scene have been identified as blood using the Kastle-Meyer test, it is necessary to confirm the blood type of the samples. In this module, you will perform ABO testing on the blood samples from the crime scene. This will allow us to determine which crime scene blood samples are from the victim, and which samples were from the suspects. NOTE: Bryce is blood type O, Deena is blood type B, Alex is blood type A, and Monica is blood type B.



1. **PLACE** a microtiter plate piece as shown below. Using a permanent marker, label the plate. The column labels will include the four control blood types (A, B, AB, O) and any Module I crime scene samples that test positive for blood. The row labels will be Anti-A and Anti-B.



2. **ADD** 50 µL or two drops of each control blood type sample into each of the two corresponding wells. For example, control blood type A 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 or 2 drops 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.

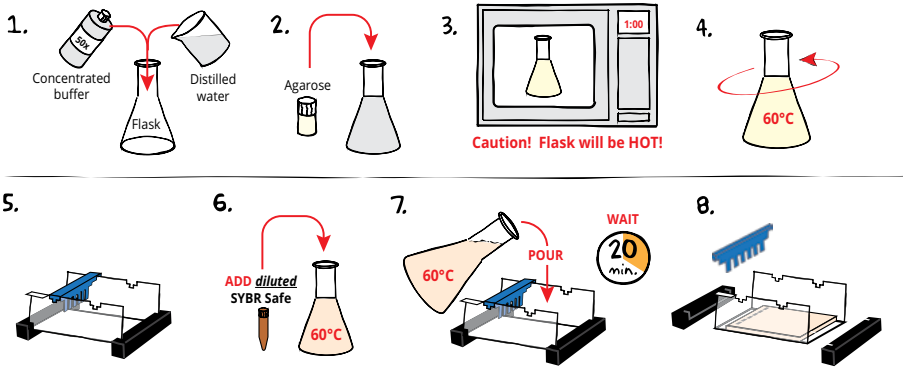
4. Use a new pipette tip to **ADD** 50 µL or 2 drops of Anti-B serum into each of the wells in row #2. The same tip or transfer pipet can be used for all samples in row #2.

5. Firmly **HOLD** the plate piece on the lab bench as shown. Very gently **TAP** the side of the plate with your finger near the positive crime scene samples to **MIX** for approximately 20-30 seconds. Do this VERY CAREFULLY to avoid spilling any of the samples from the wells. **PROCEED** to the next step.

6. To visualize the results, **PLACE** the microtiter plate on a white light transilluminator, or use a bright flashlight held above the plate. **COMPARE** the crime scene evidence with the control blood samples. **RECORD** your results.

Module III: DNA Fingerprinting

After using blood typing to determine which samples are from the suspect, you must use DNA fingerprinting to find out who the blood came from. You send the suspects' 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 samples obtained from the suspects and compare them to the blood found at the scene.



CASTING THE AGAROSE GEL

REMINDER: This kit requires 0.8% agarose gels cast with at least 4 wells.

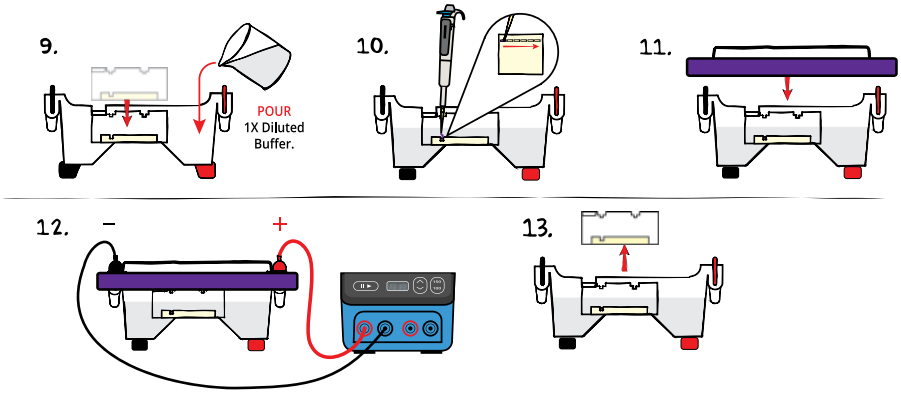
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD *diluted* SYBR® Safe** to the molten agarose and swirl to mix (see Table A).
7. **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.
8. **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™ with SYBR® Stain**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL	30 µL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL	45 µL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

Module III: DNA Fingerprinting, continued



RUNNING THE GEL

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

9. **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.
10. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well as indicated by the Gel Loading table.
11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** leads to the power source and **PERFORM** electrophoresis. (See Table C for time and voltage guidelines.)
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

VISUALIZING the SYBR® GEL

SLIDE gel off the casting tray onto the viewing surface of the transilluminator. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.

GEL LOADING TABLE

LANE	SAMPLE	SAMPLE NAME
1	A	DNA standard marker
2	B	Crime scene sample
3	C	Deena PCR Reaction
4	D	Monica PCR Reaction

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

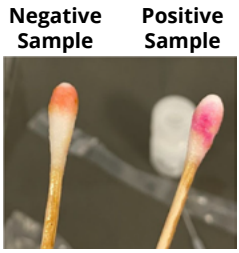
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

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

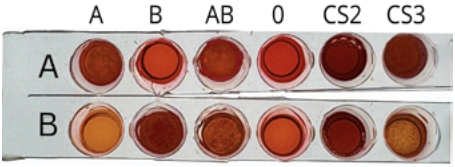
Experimental Results and Analysis

MODULE I ANALYSIS



CS1 was negative and did not produce any color change after adding H_2O_2, while CS2 and CS3 turned bright pink and were positive. Therefore, CS2 and CS3 should continue on for confirmatory testing.

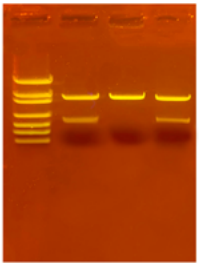
MODULE II ANALYSIS



The crime scene sample CS2 is type O and crime scene sample CS3 is type B blood. Both Deena and Monica are type B.

While blood typing can narrow down suspects, DNA testing would have to be performed to conclusively say if the blood belongs to either of the two suspects. Therefore, PCR samples from crime scene sample CS3 should be DNA tested alongside PCR samples from Monica and Deena.

MODULE III ANALYSIS



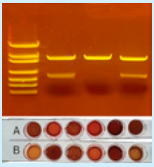
The DNA standards in Lane 1 make it possible to measure the DNA bands obtained from the PCR reactions. The results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA matches the sample from Monica. In criminal investigations, several known 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 sample	3000, 1282
3	C	Deena PCR Reaction	3000
4	D	Monica PCR Reaction	3000, 1282

Related Products

Cat. #190

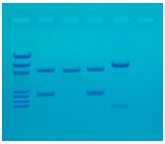
Forensic Escape Room: Design Your Own Biotech Adventure



For 10 groups. Explore the world of forensic science with this fun and exciting crime scene escape room! In this investigation, students decipher clues, solve puzzles, and unravel the evidence to free the innocent. Hands-on techniques include forensic blood detection, blood typing, and DNA fingerprinting. Comprehensive instructions on how to set up an escape room are included.

Cat. #130

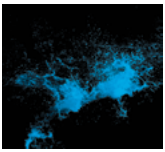
DNA Fingerprinting by PCR Amplification



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. #194

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.

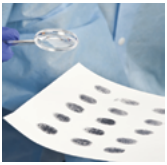
Cat. #195

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. #S-91

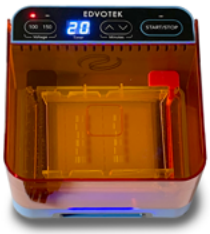
Whose Fingerprints Were Left Behind?



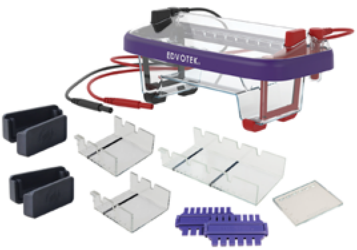
For 10 groups. After a crime has been committed, the evidence left behind can identify a potential culprit, although a single piece of evidence is not usually enough to convict someone. Even in this age of DNA, fingerprints and blood stains are still important at helping to identify a criminal. In this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

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



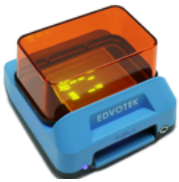
M12 Complete™ Electrophoresis Package
For 1 or 2 Lab Groups
Cat# 502-504

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



TruBlu™ Jr Blue Light Transilluminator
Cat# 555

Long Wave UV Light
Cat# 969

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

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