Left at the Scene of the Crime:

An Introduction to Forensic Science

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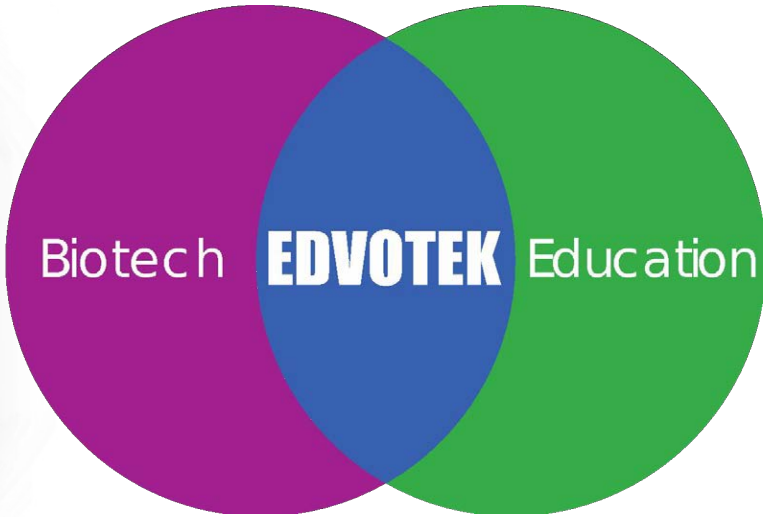

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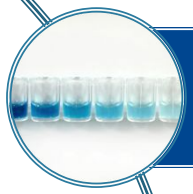
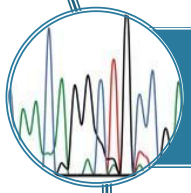
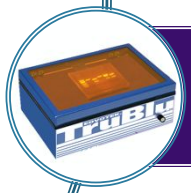


Experiments



Reagents



Equipment

Resources

What is Forensic Science?

- **Forensic science (forensics)** is the application of scientific knowledge to answer questions of interest within the legal system.
- Evidence from accidents and crime scenes is collected and analyzed by forensic scientists.
- Forensic scientists act as expert witnesses.

What kind of evidence can be analyzed?

Could someone have been drugged?
Cat. #195
Cat. #193

Can we identify this handwriting?
Cat. #196

Whose blood is this?
Cat. #130
Cat. #S-51
Cat. #225
Cat. #109
Cat. #371

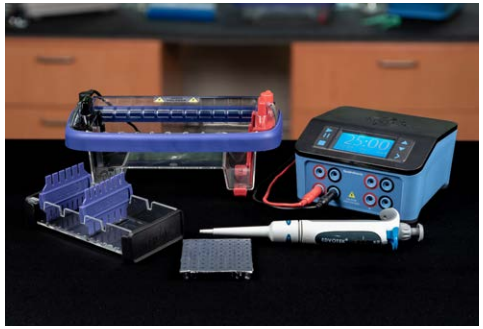
Are there fingerprints? Do they match a criminal's?
Cat. #S-91

What kind of blood is that?
Cat. #192
Cat. #140

Is that blood?
Cat. #191
Cat. #194

Forensic Scientists Use Techniques from Many Scientific Disciplines

Blood Typing



Gel electrophoresis

Polymerase Chain Reaction (PCR)

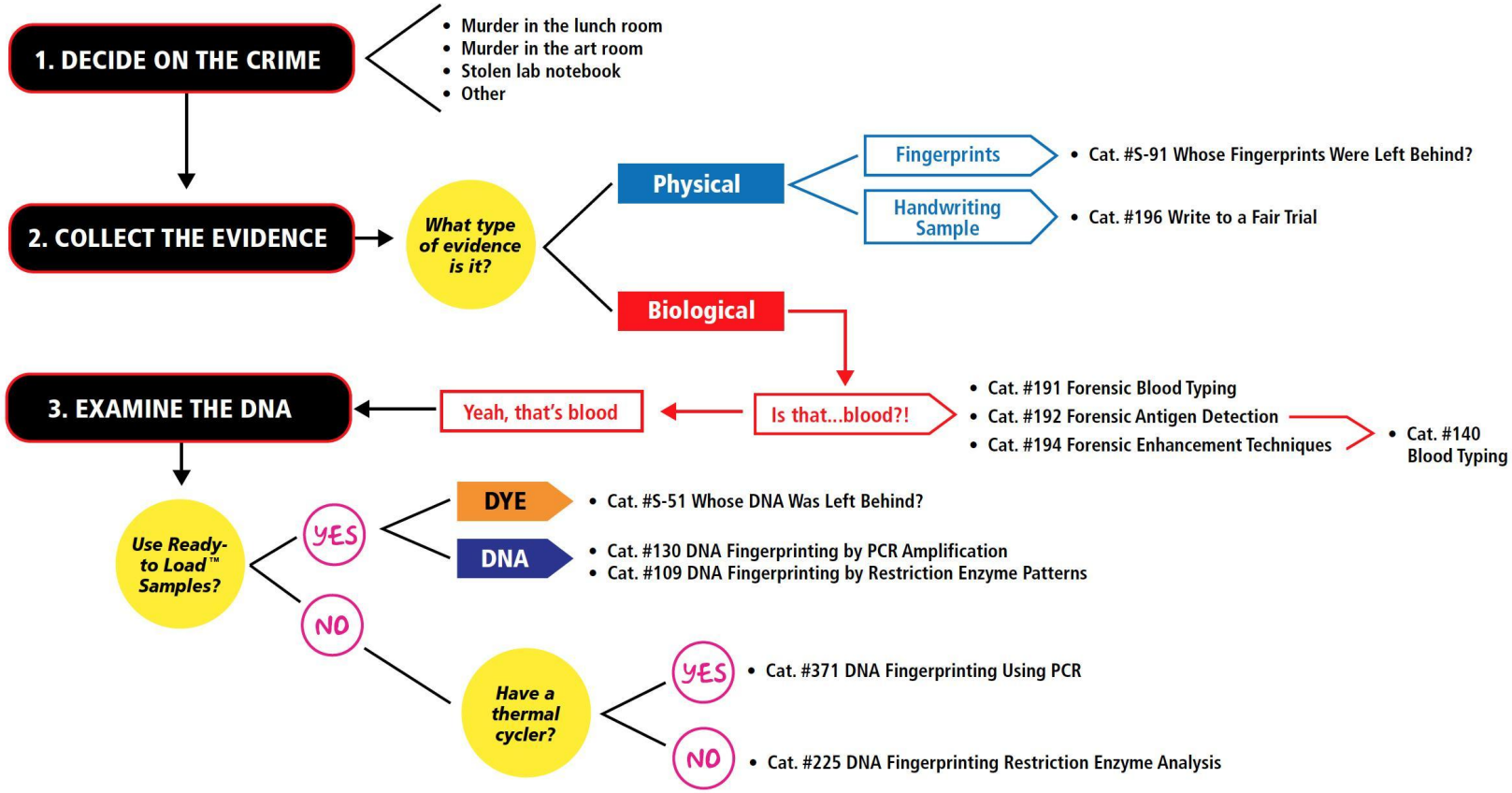
Clockwise from top: #140, #541, #504

- **Anthropology** – Analyze remains in advanced stages of decomposition
- **Genetics/Molecular Biology** – Uses biological material to determine serological and DNA profiles
- **Toxicology** – Identifies poisons, drugs and/or alcohol in tissues and body fluids.
- **Chemistry** – Analysis of trace physical evidence
- **Pathology** – Determine cause of death post-mortem

A Murder in the Art Room!

- Mr. Olson, the beloved principal of Edvotek High School, was found murdered in the art classroom early one morning.
- Police were called and the crime scene was sealed. Evidence was collected for analysis back in the forensics lab.
- Potential evidence: Blood spatter and blood transfer, handwritten note in the principal's office.

Setting up a Forensics Unit



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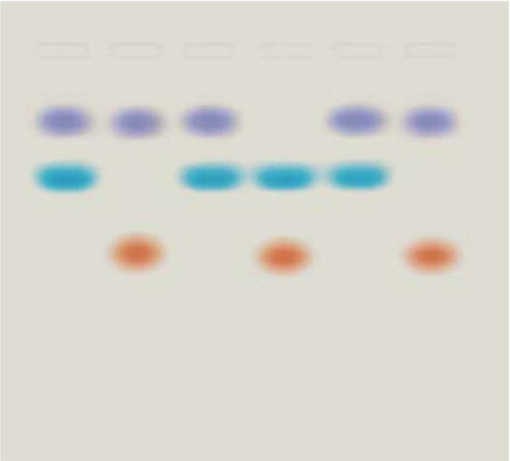
Crime Scene Investigation Using Forensic Techniques

DNA Fingerprinting
(EDVO-KIT #S-51)



Write to a fair trial: Forensic Handwriting Analysis
(EDVO-KIT #196)

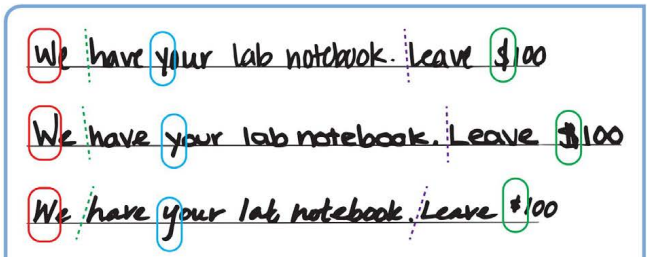
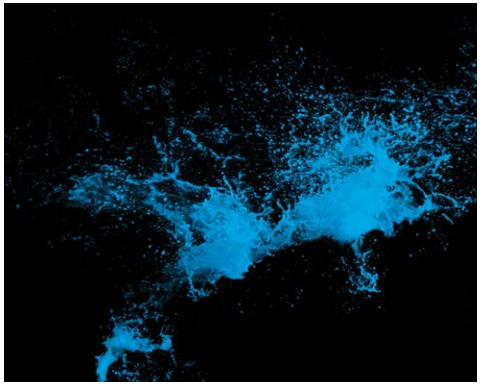


Figure 1: Four different aspects of handwriting.

Forensic Blood Enhancement Techniques
(EDVO-KIT #194)



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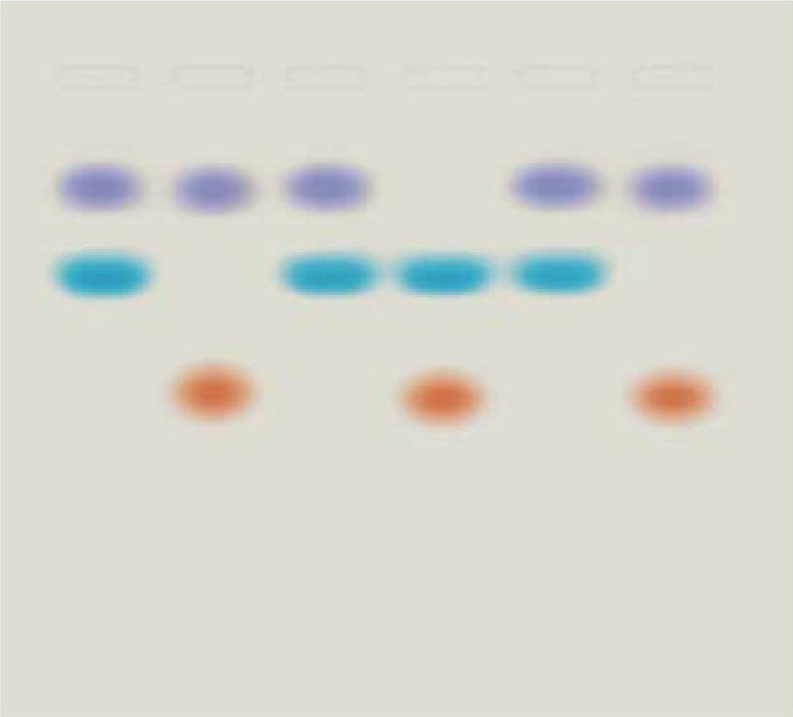
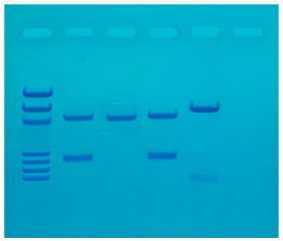
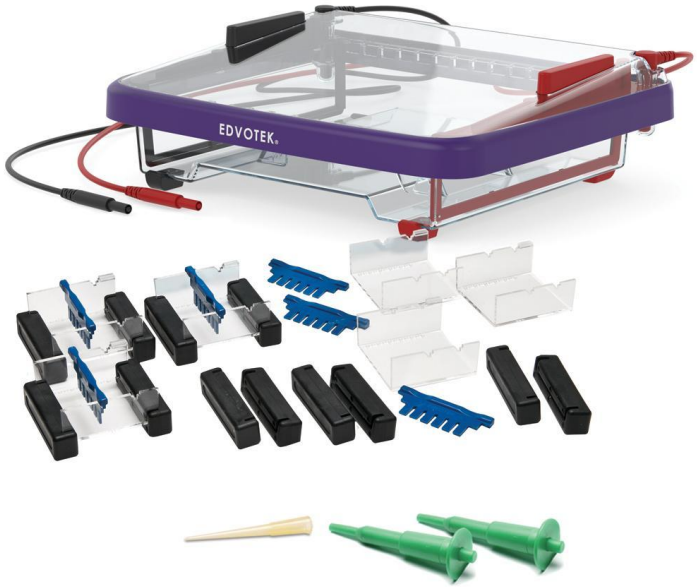
Whose DNA was Left Behind?

- Simulation of DNA Fingerprinting featuring Ready-to-Load™ dye samples
- "DNA" profiles for the crime scene and the suspects were created.
  - DNA was extracted from the sample, amplified by PCR and digested with restriction endonucleases.



What Do I Need to Perform Electrophoresis Experiments?





LabStation™ #5062

- Horizontal electrophoresis apparatus
- D.C. power source
- Micropipet or transfer pipet
- Agarose
- Electrophoresis Buffer
- Samples – dye, DNA, RNA
  - PCR products
- A way to visualize samples

Casting the Agarose Gel

1. Prepare the tray for gel casting by sealing the ends with rubber end caps.

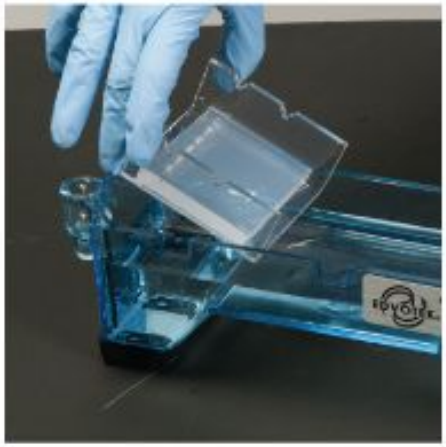
2. Place a comb in the appropriate notches.

3. Prepare the agarose gel solution. Cool to 60°C and then pour the gel.

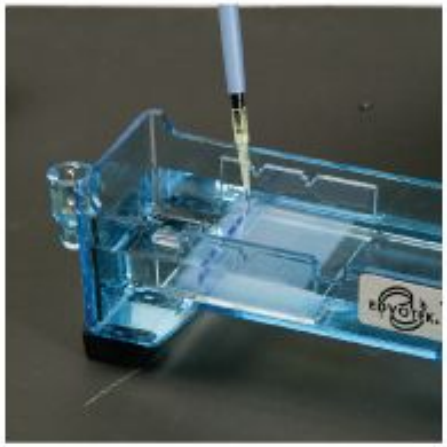
4. After approx. 20 min. the gel will solidify. Remove the comb from the gel tray.

Performing Electrophoresis

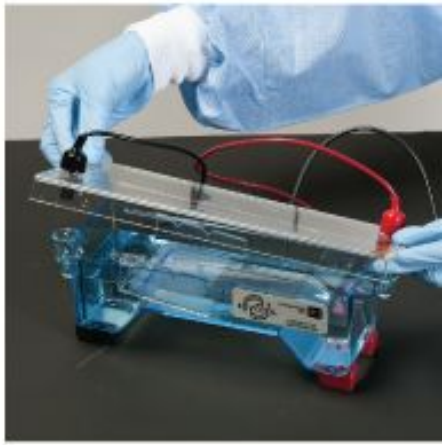
5. Slowly remove the rubber end caps. Be very careful not to damage or tear the gel!



6. Place the gel (on its tray) into the electrophoresis chamber. The gel should be completely submerged under electrophoresis buffer.

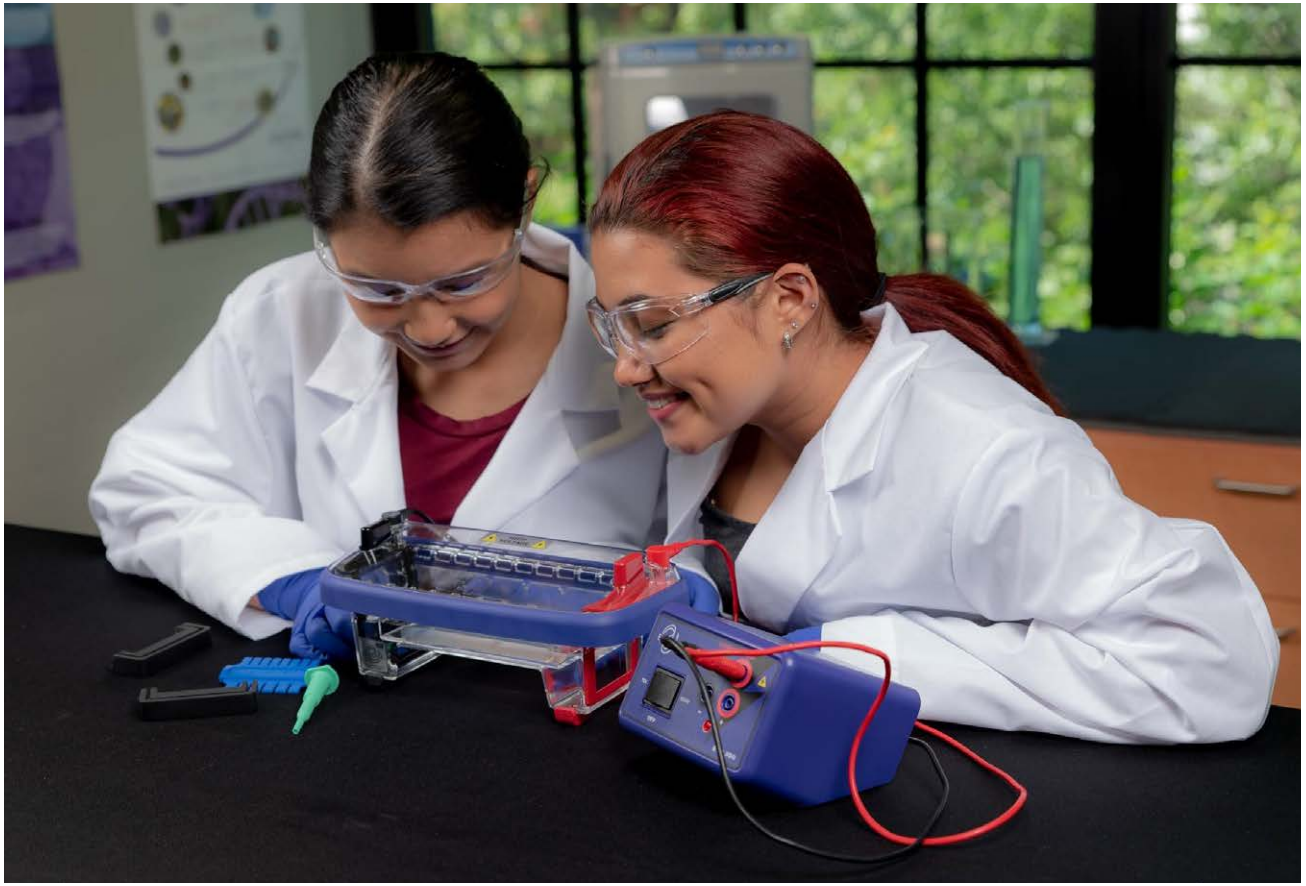


7. Load samples into wells in consecutive order, starting with the first well on the left.



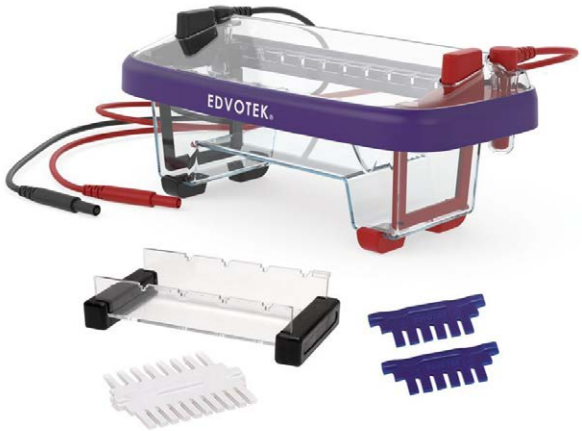
8. After samples are loaded, attach the safety cover, connect the leads to the D.C power source and set the power source at the required voltage.

Let’s run our gels!

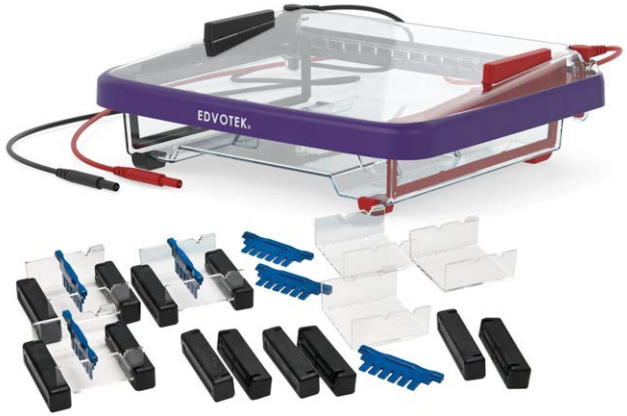


Load 35 microliters of sample per well

Electrophoresis Chambers for Classrooms of all Sizes



Cat. # 502
Model M12
Two gels



Cat. # 515
Model M36
Six gels

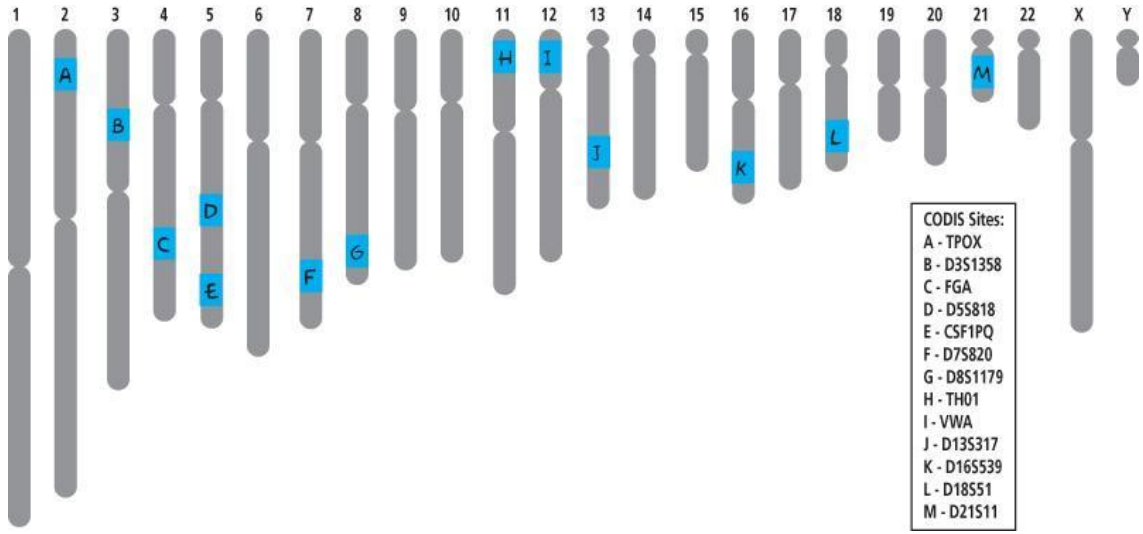
Power Supplies Provide Current for Electrophoresis

Cat. #509
DuoSource™ 150
(75/150 V)

Cat. #5010
QuatraSource
(10-300V)

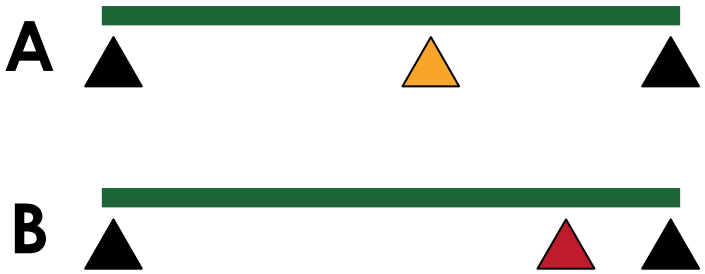
DNA Sequences are Unique

CODIS – Combined DNA Index System



- The probability of a random match between two samples is $1X10^{18}$
  - Single Nucleotide Polymorphisms (SNPs)
  - Restriction Fragment Length Polymorphisms (RFLPs)
- **DNA profiling** is a forensic technique used to identify individuals by analyzing differences within DNA.

RFLPs Are Used as Landmarks for DNA Fingerprinting

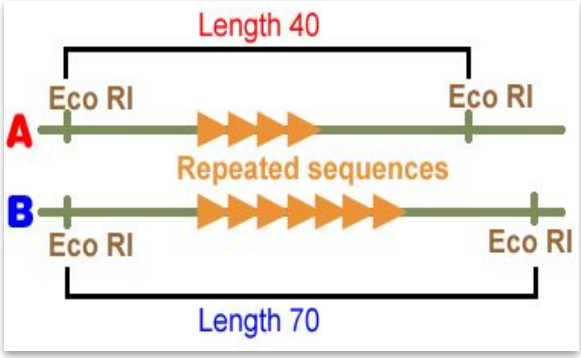


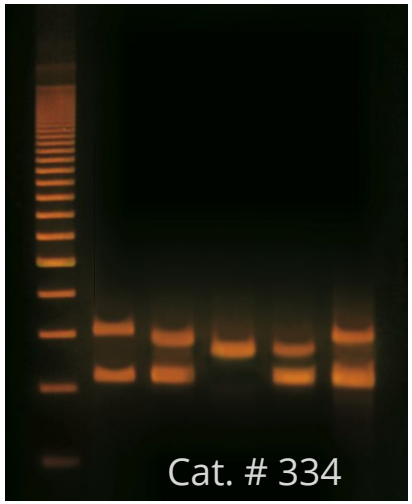
Cat. # 109

- **Restriction Fragment Length Polymorphisms (or RFLPs)** are heritable differences in the nucleotide sequence.
- Some RFLPS add a restriction enzyme cut site to the DNA.

RFLPs Are Used as Landmarks for DNA Fingerprinting

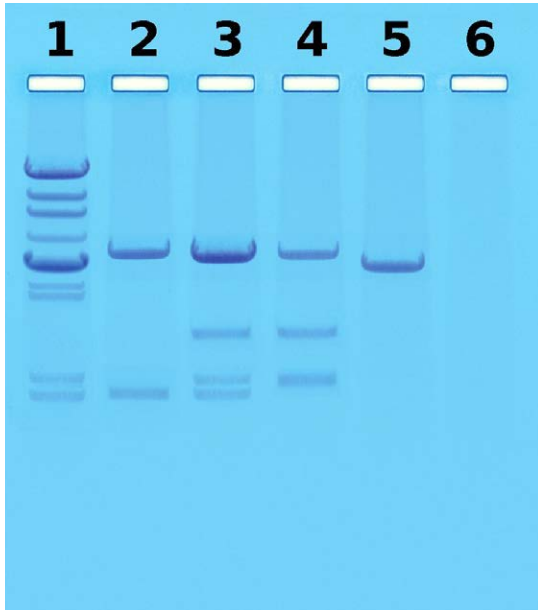
- **Variable Number of Tandem Repeats (or VNTRs)** comprise short, repetitive DNA sequences present in multiple copies between two restriction sites
  - 15-35 base pair sequences
  - VNTRs appear between five and 100 times.





DNA Fingerprinting is Used to Determine Paternity

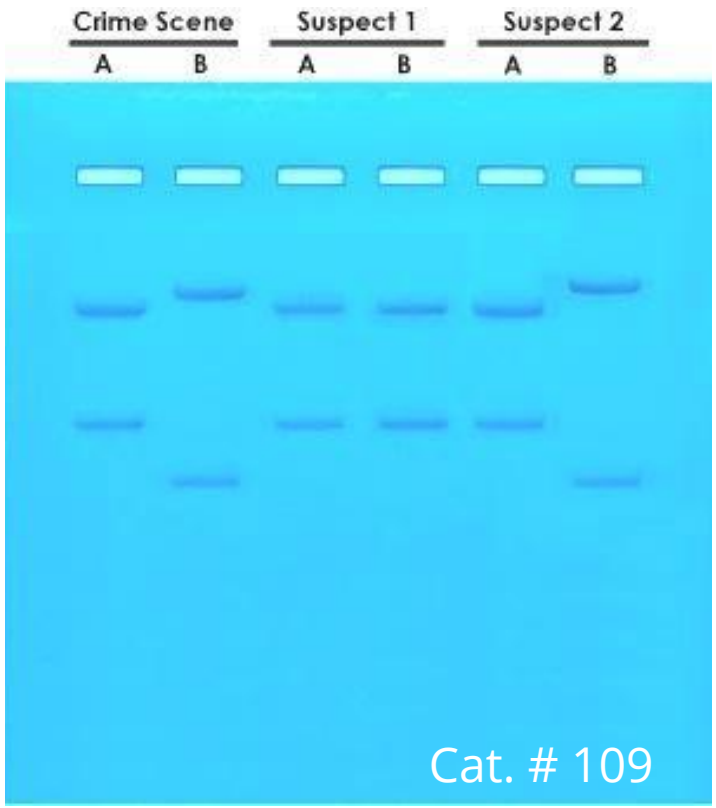
- Parentage can be determined from a child's DNA profile.
- DNA fragments in the child's sample that are absent from the mother's DNA must be contributed by the biological father.



1 – Standard DNA Fragments
2 – Mother's DNA Digest
3 – Child's DNA Digest
4 – Father 1 DNA Digest
5 – Father 2 DNA Digest
(Cat. #114)

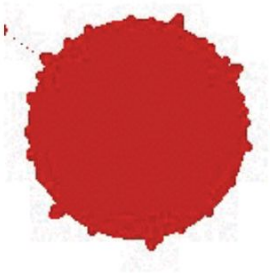
DNA Fingerprinting is Used to Solve Crimes

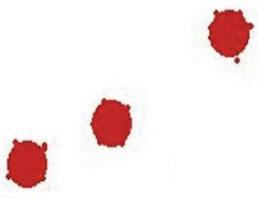
- Forensic scientists identified a biological sample at the scene of a crime.
- DNA was extracted from the sample, amplified by PCR and digested with restriction endonucleases to create a "DNA Fingerprint".
- A match suggests that the suspect was at the crime scene.

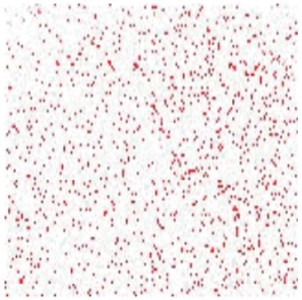


Forensic Blood Spatter Analysis is a presumptive test

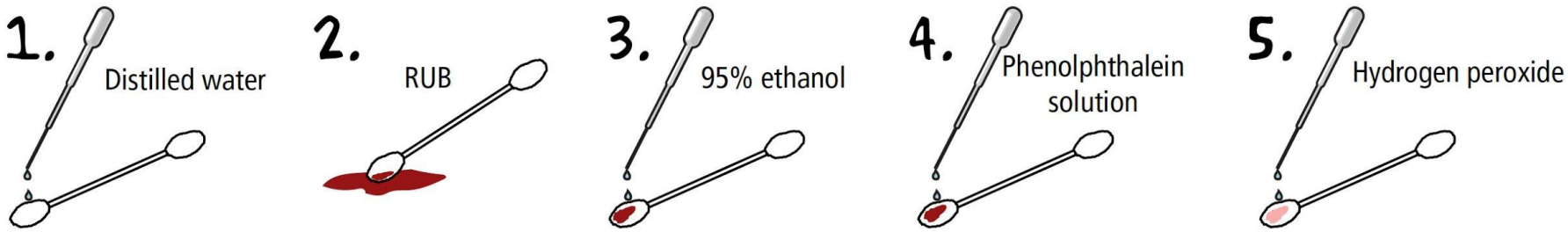
- Blood spatter evidence can be found in crime scenes, often around the victim or where the violence occurred
- Drops of blood will take on a different appearance based on how or what was used and the velocity of impacts.
- Often, trace amounts of blood cannot be detected by eye but can be enhanced with simple chemical tests:
  - **Leucocrystal violet**
  - **Phenolphthalein**







Kastle-Meyer Test: Phenolphthalein



1. **EXAMINE** the object for the red-brown staining and general characteristics. **PLACE** the item on a flat, clean paper towel.
2. **MOISTEN** a cotton swab by dipping in distilled water.
3. **RUB** the cotton swab across the fabric sample until red.
4. **ADD** two drops of 95% Ethanol to the swab using a bulb pipet.
5. **ADD** two drops of phenolphthalein solution to the swab using a new pipet.
6. **ADD** two drops of hydrogen peroxide to the swab with another new pipet. If blood is present the swab will immediately turn pink.
7. **COLLECT** the next crime scene sample and repeat steps 1-6.

Leucocrystal Violet (LCV)

1. **WORK** with one item at a time to avoid cross contamination or sample mix-up. **EXAMINE** the object for the red-brown staining and general characteristics.

2. **PLACE** the item on a flat, clean paper towel.

3. **USE** the fine-mist sprayer to gently **SPRAY** the targeted area on the object with the Leucocrystal Violet (LCV) solution from a distance of about 2-3 inches.

4. **ALLOW** the samples to sit for 30 seconds before analyzing. LCV generates a purple/violet color and indicates the presence of blood.

5. **RECORD** your sample ID and observations in the table on page 8 of the workshop manual.

Sample ID	Leucocrystal Violet +/-
Crime scene #1	
Crime scene #2	
Crime scene #3	
Crime scene #4	

Before LCV treatment **After LCV treatment**

Fabric Samples - Left = Blood **Right = Negative control**

Forensic Handwriting analysis

- One of the pieces of evidence collected after the discovery of Mr. Olson was a note directing him to the art room. Due to the potential connection to the crime the note was gathered as evidence for later analysis.

- Handwriting investigators examine pieces of evidence for clues about the motion, position, an pressure of the hand that wrote it, as well as the shape and spacing of the letters.

- To link a document to a specific person, the questioned document is compared to a confirmed sample of that person's signature or handwriting.

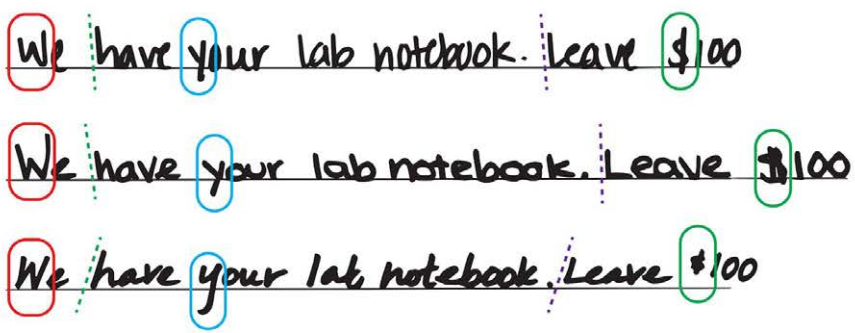


Figure 1: Four different aspects of handwriting.

Forensic Handwriting analysis

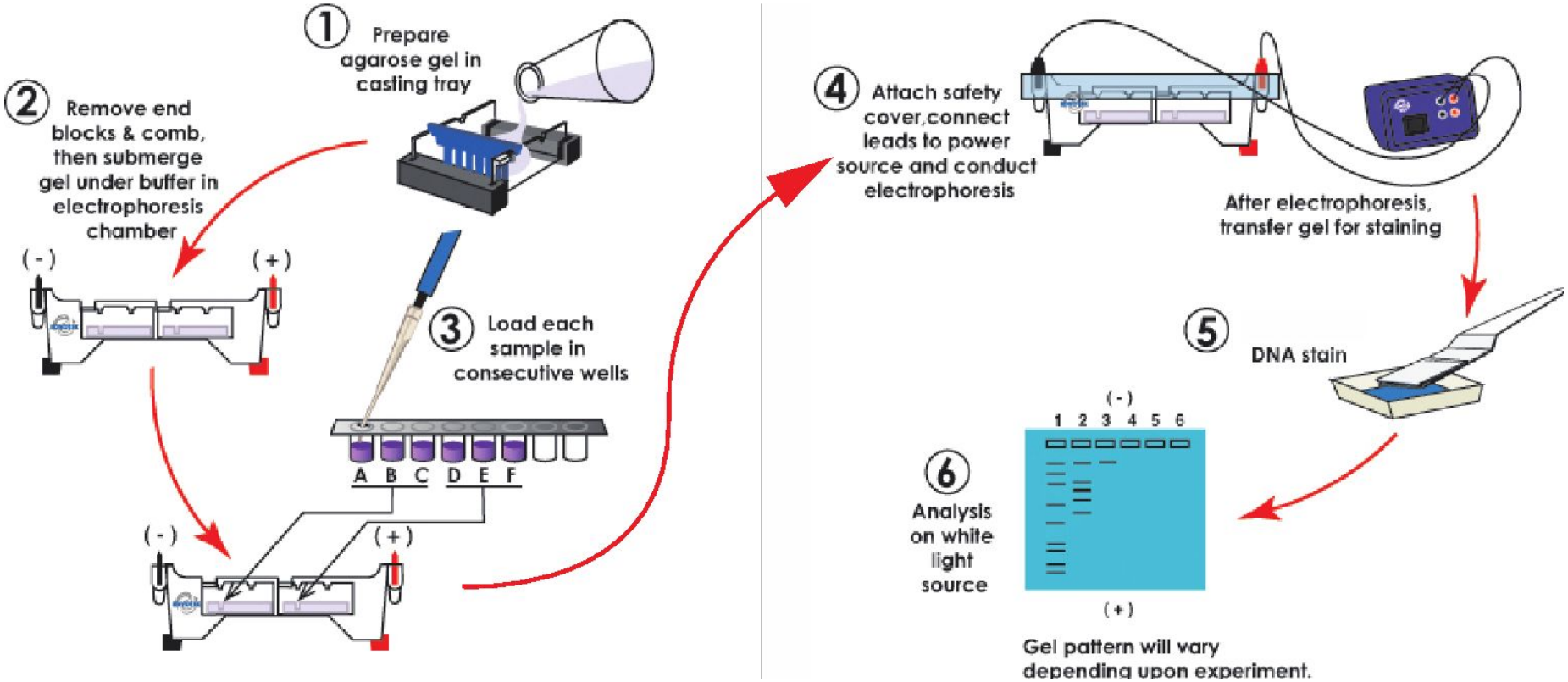
1. **OBTAIN** the writing samples from the 3 suspects and the note left for Mr. Olson.

2. **OBSERVE** the suspect writing samples, recording your observations. **Size** – Measure the height and width of specific letters in the handwriting. For example, use a ruler to measure the height of an uppercase "L", or a lowercase "y" in each writing sample.
   - **Spacing** – Use a ruler to measure the distance between letters or words.
   - **Slope** – Draw a line through the various letters to measure the slope. Do the individual letters slant to the left or the right? Do the sentences themselves slant up or down off a straight line?
   - **Special characteristics** – Are there any specific things in the writing that make it stand out? Are the lowercase "i" or "j" letters dotted in a special way? Are there any letters drawn in an unusual or distinguishing style, unique punctuation, or consistent spelling mistakes?

3. **OBTAIN** a copy of the note. Using the criteria you established for the suspect handwriting samples, **RECORD** any defining features from the note.

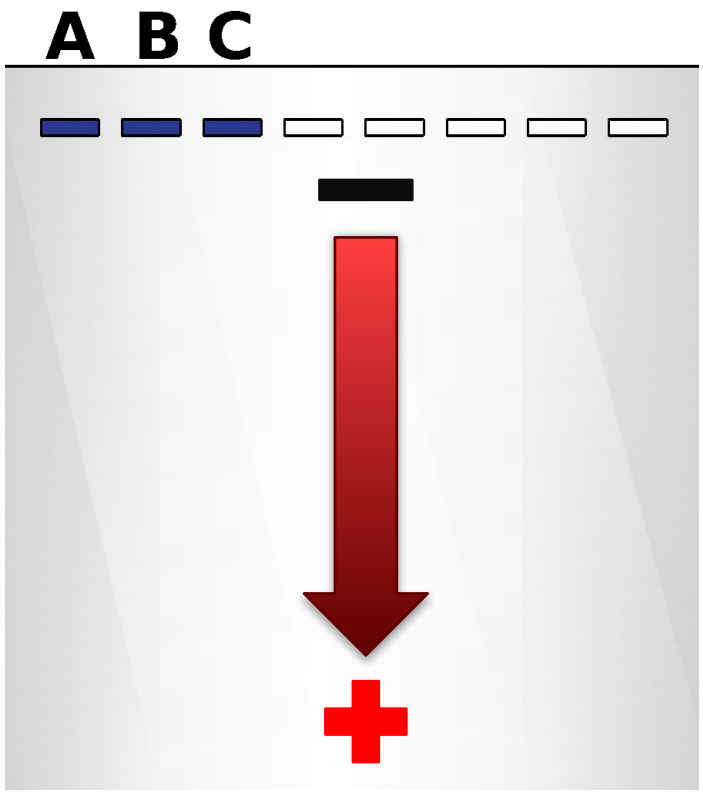
4. **IDENTIFY** a suspect for further analysis.

Summary of Electrophoresis



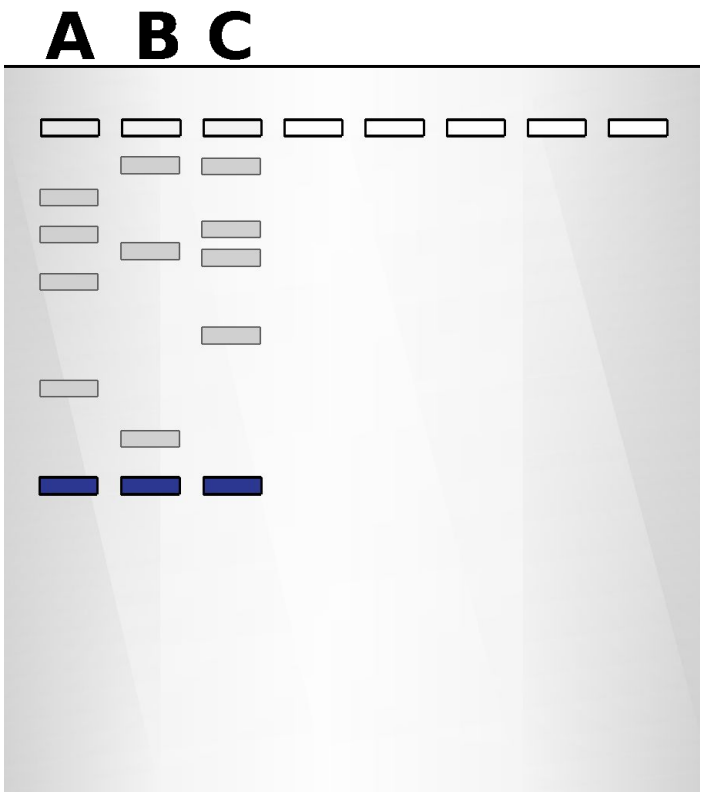
Electrophoresis Separates DNA Fragments By Size

- The sugar-phosphate backbone of DNA has a strong negative charge.
- When an electrical current is passed through the gel, the current drives the DNA fragments through the gel towards the positive electrode.



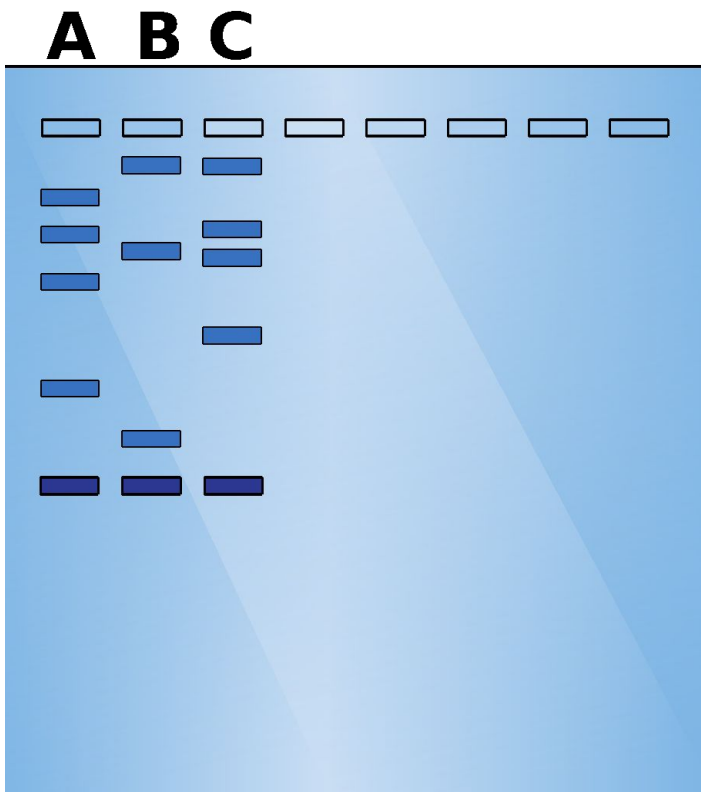
Electrophoresis Separates DNA Fragments By Size

- 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.



Electrophoresis Separates DNA Fragments By Size

- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
- UV-reactive dyes simulate DNA fragments, eliminating post-staining time.



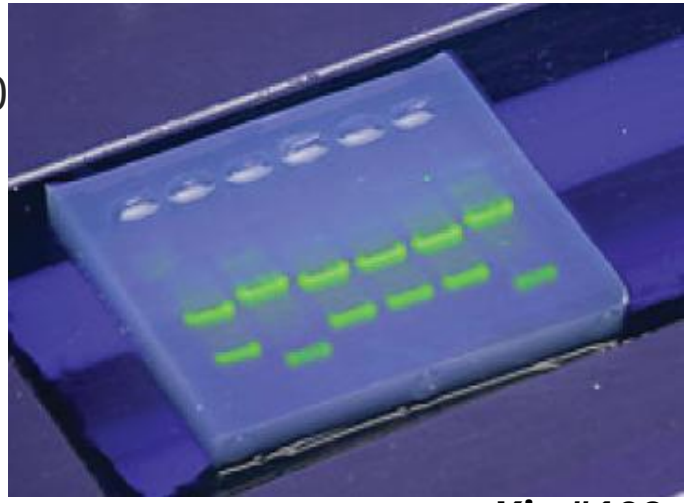
SYBR® Safe DNA Stain

In-gel Staining

- Melt agarose and cool to 65°C.
- Add concentrated Sybr® Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
- Run DNA samples through gel – no post staining or destaining necessary!

Post-electrophoresis Staining

- Dilute concentrated stain to 1:20,000 (5 µL per 100 mL distilled water).
- After electrophoresis, place gel in tray. Cover gel with diluted Sybr® Safe stain.
- Stain gel for 10 – 15 minutes.



Kit #109
Transilluminator #558
SybrSafe® Stain #608

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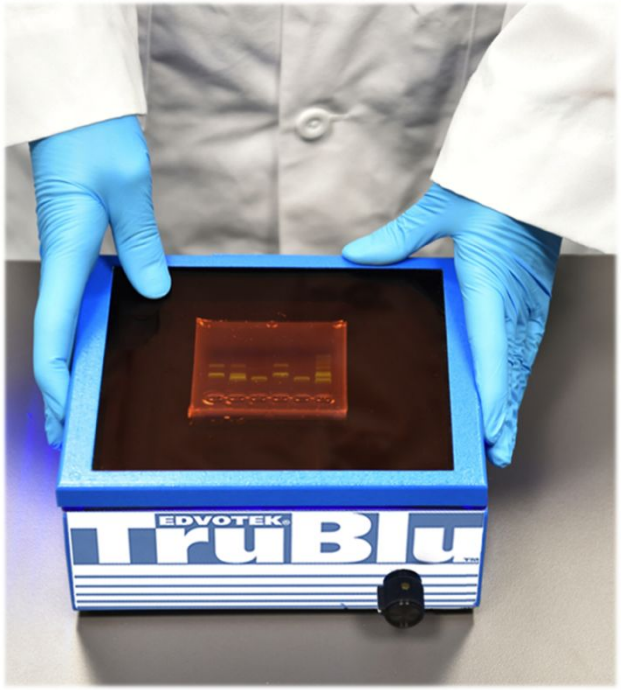
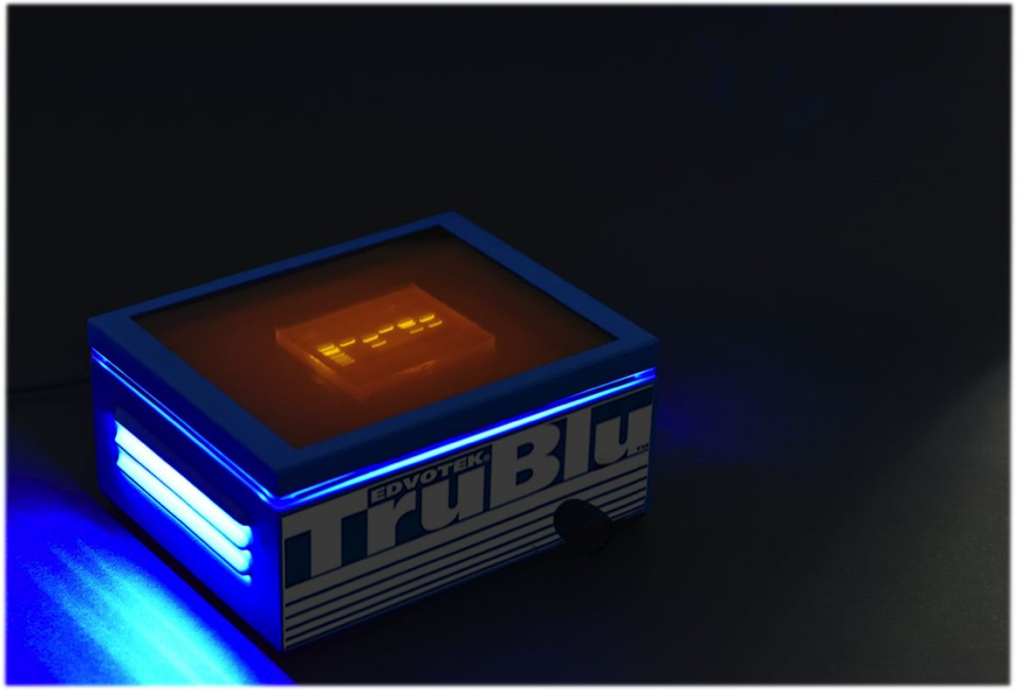
TruBlu™ Bluelight Transilluminator

Optimized for **SYBR® Safe** stained gels • Large viewing area • No harmful UV





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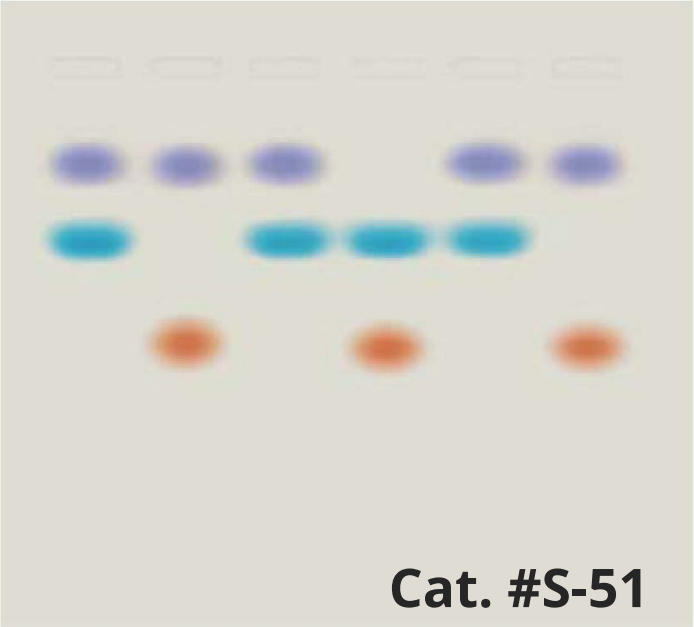
Which Suspect Was at the Crime Scene?

DNA	CS	CS	S1	S1	S2	S2
Enzyme	1	2	1	2	1	2

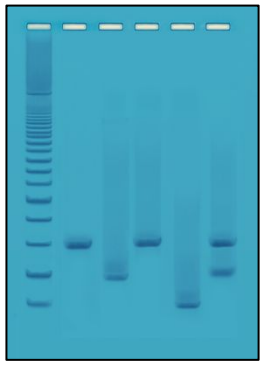


- A match provides strong evidence that the suspect was present at the crime scene.
  - Alone, this evidence does not **prove** the suspect committed the crime.
- If a suspect's DNA profile does not match that of the crime scene, that person may be eliminated from the inquiry.

Crime Scene Investigation

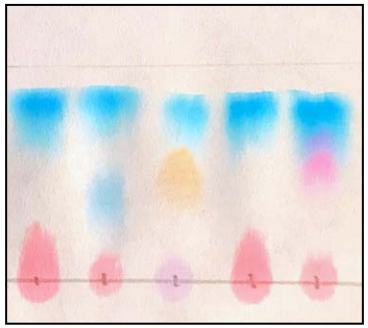
- **Forensic science uses scientific methodology to answer legal questions.**
- **Forensic Blood Spatter Analysis is a presumptive test for blood that can help rule out suspects and identify details related to the crime.**
- **DNA Fingerprinting identifies heritable differences in an individual's DNA.**
- **Handwriting analysis can identify the author of a handwritten document and ensure authenticity.**
- **Together, the results of these tests can be used as evidence in the court of law to help solve crimes.**

Bring the Exciting World of Forensics to your Classroom!

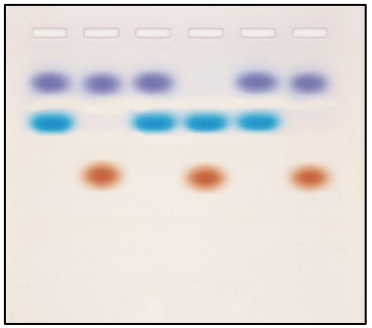


DNA Fingerprinting using PCR (#371)

Forensic Blood Typing (#191)



Forensic Handwriting Analysis (#196)



Whose DNA Was Left Behind? (#S-51)

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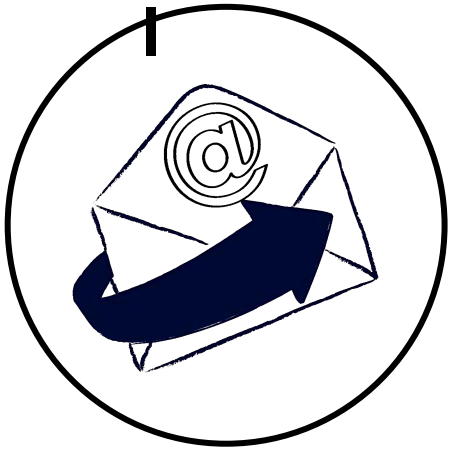

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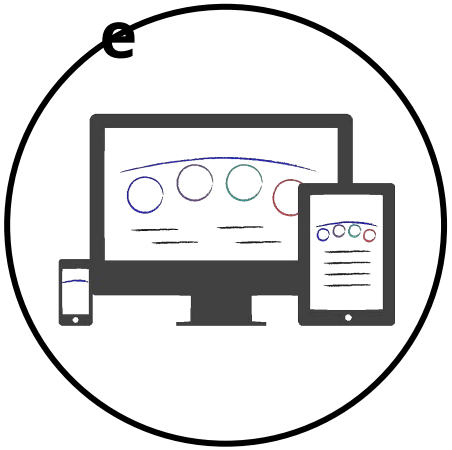

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