WORKSHOP

Left at the Scene of the Crime: Introduction to Forensic Science

EDVOTEK®

Designed for the Classroom

SINCE 1987

Introduction

There's a break-in at the lab! Your students become crime scene investigators as they inspect evidence and use forensic tools like DNA fingerprinting to catch a criminal. This exciting workshop will help you incorporate biotechnology and electrophoresis into your classroom.

Left at the Scene of the Crime

Late one night Dr. Elektra Phoresis worked on an important biotechnology experiment in the laboratory. She was very close to creating a groundbreaking vaccine that could save many lives. After working in the lab all day, Elektra decided to go home to eat dinner and get a good night's rest. The next morning the lab was in shambles. The scientist found that many important pages were ripped from her lab notebook. Furthermore, security footage showed that someone had stolen some critical reagents from the laboratory. A ransom note was left behind, demanding money in exchange for the lab notebook pages.

Upon investigating the crime scene, Officer Evie Dence identified a broken window in the laboratory as a potential entry point by the suspect. The forensic scientists believe the perpetrator may have been cut on the broken glass, as several blood-like samples were found around the crime scene. The potential biological samples were collected as evidence to be analyzed. Using handwriting analysis, the ransom note will also be examined as evidence.

Background Information

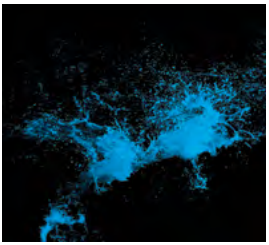
Excerpts from EDVO-Kits 191 & 130

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 tissues and hair are 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

Often trace amounts of blood cannot be detected by the naked eye, however it can easily be enhanced and made visible by spraying the area with certain chemical enhancers such as Leucocrystal violet. This reagent will react with blood to generate a purple/violet color. In this experiment, students will use Leucocrystal Violet to differentiate between trace blood samples and on different objects simulating materials recovered from a crime scene.



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). 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.

FORENSIC HANDWRITING ANALYSIS

Forensic handwriting analysis helps law enforcement officials, people in the court system, and historians identify the person responsible for a handwritten document. Forensic scientists analyze documents to check for signs of forgery, to see if any changes were made to a document after it was originally written, or simply to figure out who wrote or signed the document in the first place. They analyze historical writings, wills, autographs, or signed papers and handwritten notes left at a crime scene. When they make their reports, they call these papers "questioned documents."

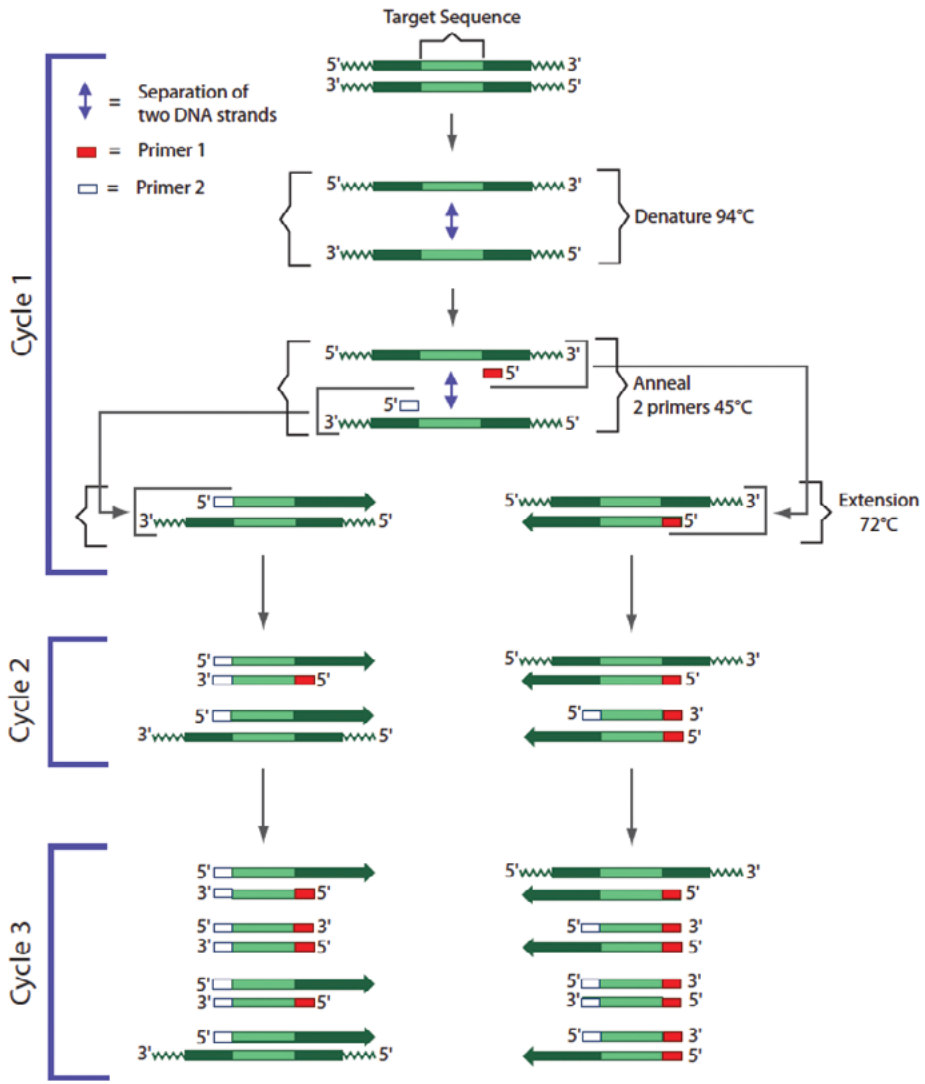


Figure 1: Polymerase Chain Reaction

Every questioned document is unique, so forensic scientists look at many different features of it during their investigation. Handwriting analysts examine the paper for clues about the motion, position, and pressure of the hand that wrote it, as well as the shape and spacing of the letters. This can tell them if the person wrote the note in a hurry or took their time, if they were old or young, and if they were sick or well among other things. To link a document to a specific person, the questioned document is compared to a confirmed sample of that person's signature or handwriting. This could be a previously written document or handwriting samples obtained during an investigation.

Rather than look for similarities, however, examiners actually look for differences. If the two pieces of writing do not have differences, it is possible they are from the same writer.

Questioned document examiners look at four different aspects of handwriting (see Figure 2 for examples):

1. How the writer forms letters, including slants or slopes, letter sizes, tremors, or embellishments (fancy capital letters, different ways to cross the letter "T" or dot the letter "i", or unusual shapes for letters that go below the line like "g" or "y").
2. The appearance and direction of the lines, including ink smoothness, ink darkness, and whether the line is straight or waivers up or down. These indicate the pressure and speed of the writing.
3. Spacing between the letters and words and the spacing of the margins.
4. The content, including grammar, punctuation, phrasing, and spelling.

Requested samples are often written in front of an examiner. If handwriting examiners witness a sample being written, they can determine whether a person is making natural motions or whether they are making motions designed to disguise their normal handwriting.

Non-requested samples are taken from a writer's home or office and include checks, lists, or notes. Even though the sample was done in private, an examiner can identify things that mean the writer was trying to disguise their handwriting, including shakiness in the writing, different thicknesses at the beginnings and ends of words, and unnatural spacing between letters.

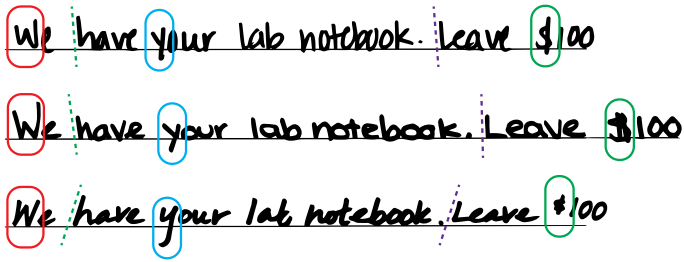
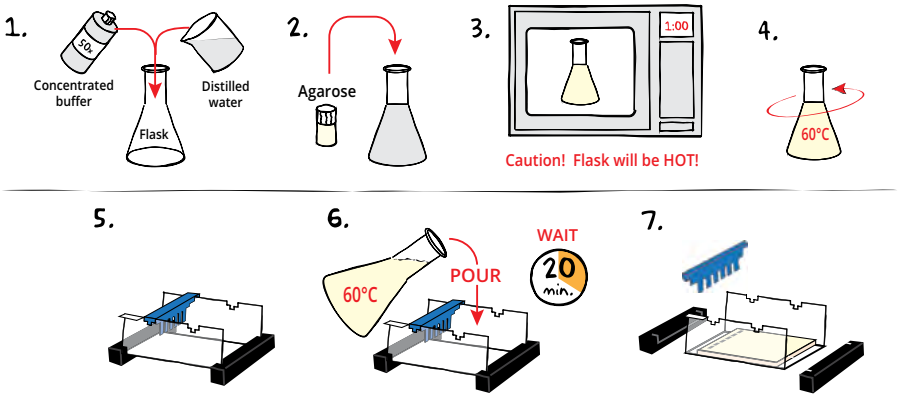


Figure 2: Four different aspects of handwriting.

Figure 3: Analysis of an altered check.

Experiment Procedures: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

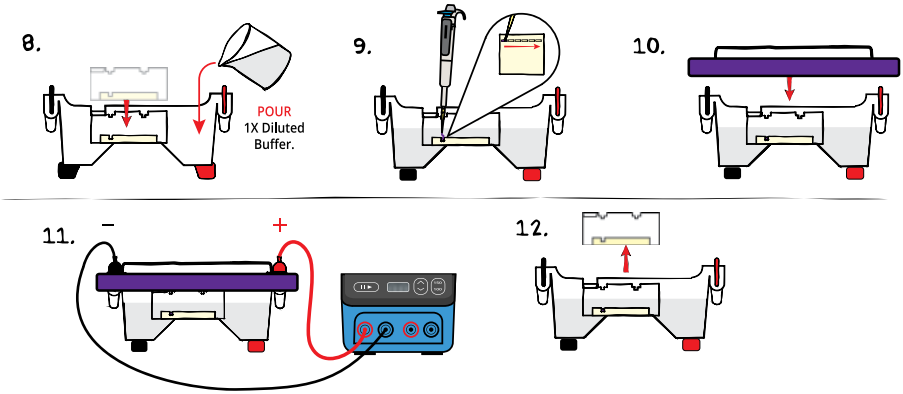
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **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).*

Experiment Procedures: Agarose Gel Electrophoresis



RUNNING THE GEL

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 Table 1, 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.

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

Lane	Label	Sample Name
1	A	DNA Standard Marker
2	B	Crime Scene PCR reaction
3	C	Suspect 1 PCR reaction
4	D	Suspect 2 PCR reaction
5	E	Suspect 3 PCR reaction
6	--	------

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

Experiment Procedures: Blood Splatter Analysis

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

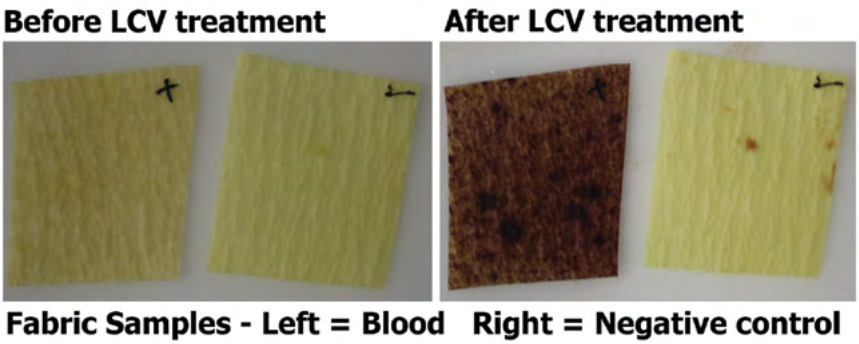
2. **PLACE** the item on a flat, clean surface.

3. Use the fine-mist sprayer to gently **SPRAY** the targeted area on the object with the Leuco-crystal 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, below:

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



Experiment Procedures: Handwriting Analysis

1. **OBTAIN** the writing samples from the 4 suspects. These can be found in Appendix A or as a separate printout from your instructor.

2. One at a time, **OBSERVE** the suspect writing samples, recording your observations in your laboratory notebook. In particular, pay attention to identifying characteristics within each handwriting sample, including:

    - 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 various letters to measure the slope. Do the individual letters slant to the left or the right? Are the sentences themselves level or do they slant up or down off of a straight line?

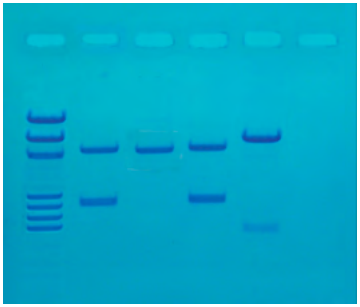
    - Special characteristics – Are there any specific things in the writing that make is stand out? Are the lowercase "i" or "j" letters dotted in a special way? Are there any letters drawn in an unusual or distinguishing style, unique punctuation, or consistent spelling mistakes?

3. Once you have made observations for each suspect, **OBTAIN** a copy of the ransom note. Using the criteria that you established for the suspect handwriting samples, **RECORD** any defining features from the ransom note.

4. **COMPARE** the handwriting characteristics of the writing samples to the ransom note and identify a potential suspect for the crime. You are now ready to perform "Paper Chromatography of Ink Samples".

Experimental Results and Analysis

AGAROSE GEL ELECTROPHORESIS



Lane	Tube	
1	A	DNA Standard marker
2	B	Crime scene PCR reaction
3	C	Suspect 1 PCR reaction
4	D	Suspect 2 PCR reaction
5	E	Suspect 3 PCR reaction

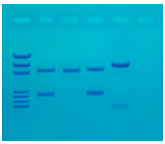
The DNA standards in Lane 1 make it possible to measure the DNA bands obtained from the PCR reactions. The results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA and Suspect 2 match. In criminal investigations, several known variable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

HANDWRITING ANALYSIS

The results from the handwriting analysis will identify multiple similarities and differences between the samples and ransom note. The handwriting from Suspect C should most closely match the ransom note. Potential distinguishing marks include punctuation and flourishes on letters, common misspellings, and the spacing or slant of letters.

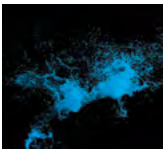
Related Products

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

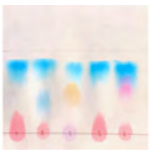


For 10 groups. Trace amounts of blood are often sufficient to identify the individual responsible for any number of crimes, including murder, burglary, or assault. Enhancement procedures can make a small stain of body fluid or tissue visible to the naked eye. In this experiment, students will act as 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

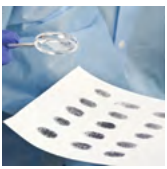
For 10 groups. In today's forensic science laboratory, toxicologists identify drugs and toxins in samples collected from crime scenes, victims, and potential suspects. If present, the toxicologist also determines whether the drug or toxin contributed to a person's behavioral changes or death. In this 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

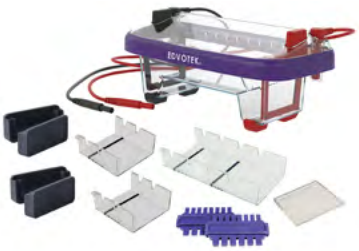
Whose Fingerprints Were Left Behind?



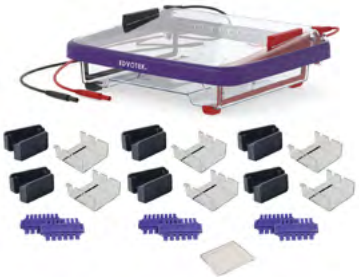
For 10 groups. After a crime has been committed, the evidence left behind can identify a potential culprit, although a single piece of evidence is not usually enough to convict someone. Even in this age of DNA, fingerprints and blood stains are still important at helping to identify a criminal. In this 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



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



M36 HexaGel™
For 1 to 6 Lab Groups
Cat# 515

DuoSource™ 150
75/150 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 MiniPipet™
Cat. # 587-2

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