# Crime Scene and DNA Basics for Forensic Analysts

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Welcome to the Crime Scene and DNA Basics for Forensic Analysts course


This course provides information in the two lessons *Evidence at the Crime Scene* and *History and Types of Forensic DNA Testing*.

The first lesson addresses the importance of documenting, protecting, and preserving the scene and what types of evidence can be found there and methods used for its collection and preservation.

The second lesson addresses the historical use and disadvantages of restriction fragment length polymorphisms (RFLP), the method and sequence of steps in which DNA profiles are developed, and the concept of short tandem repeats (STR) testing and its advantages over earlier methods.

Disclaimer

The opinions and points of view expressed in this training program represent a consensus of the authors and do not necessarily reflect the official position of the U.S. Department of Justice.

This project was developed by the National Forensic Science Technology Center under Award No. 2004-DN-BX-0079 awarded by the National Institute of Justice, Office of Justice Programs.

Evidence at the Crime Scene: Introduction


Responding to a crime scene is a critical step in the scientific investigation of a case. Unless the crime scene response is handled correctly, the investigation may be severely compromised. Investigators and crime scene specialists are responsible for identifying, securing, collecting, and preserving the evidence that is submitted to the crime laboratory. The investigator's knowledge in crime scene documentation and the variety of methods for the collection and processing of all types of evidence is crucial. Additionally, many times the investigator must make timely decisions whether to obtain written consent or a search warrant, so that the evidence will be admissible and not subject to a motion to suppress.

Objectives

Upon successful completion of this unit of instruction, the student shall be able to:

- Understand the importance of protecting and preserving the scene to ensure the integrity of the physical evidence within.
- Understand the different types of crime scenes and the types of evidence encountered in these scenes.
- Understand the importance of scene documentation through photography, video recording, and sketching.
- Understand the different methods used for collection and preservation of evidence.

Types of Evidence

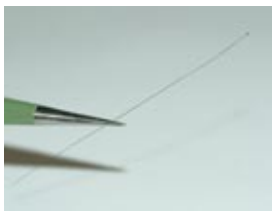
Evidence can be divided into two categories:

- Testimonial - statements or the spoken word from the victim(s) or witness(es).
- Physical - also referred to as real evidence, consists of tangible articles such as hairs, fibers, latent fingerprints, and biological material.

The concept known as the "Locard's Exchange Principle" states that every time someone enters an environment, something is added to and removed from it. The principle is sometimes stated as "every contact leaves a trace," and applies to contact between individuals as well as between individuals and a physical environment. Law enforcement investigators are therefore taught to always assume that physical evidence is left behind at every scene. This will be generally true, and the amount and nature of the evidence created will be largely dependent on the circumstances of the crime.

Examples include:

- Biological material - blood, semen or saliva
- Fibers
- Paint chips
- Glass
- Soil and vegetation
- Accelerants
- Fingerprints
- Hair
- Impression evidence – shoe prints, tire tracks or tool marks
- Fracture patterns – glass fragments or adhesive tape pieces
- Narcotics



Oftentimes, evidence tells a story and helps an investigator re-create the crime scene and establish the sequence of events. Physical evidence can corroborate statements from the victim(s), witness(es) and/or suspect(s). If analyzed and interpreted properly, physical evidence is more reliable than testimonial evidence; testimonial evidence is more subjective in nature. An individual's perception of events and memory of what happened can be incomplete or inaccurate. Physical evidence is objective and when documented, collected, and preserved properly may be the only way to reliably place or link someone with a crime scene. Physical evidence is therefore often referred to as the "silent witness."

There are three types of crime scenes:

- Oudoor Crime Scene
- Indoor Crime Scene
- Conveyance

Outdoor

An outdoor crime scene is the most vulnerable to loss, contamination, and deleterious change of physical evidence in a relatively short period of time. Individuals with access to the scene can potentially alter, destroy or contaminate evidence. The risk is greatest when investigators fail to secure the crime scene properly.

Destruction or deterioration of evidence due to environmental conditions such as heat, cold, rain, snow and wind are problems associated with outdoor scenes. Evidence that cannot be protected under these conditions should be collected expeditiously without compromising its integrity. Investigators who encounter a combination of an indoor and outdoor scene should give priority to processing the outdoor component.

Nighttime outdoor crime scenes are especially problematic. Regardless of the quality of the light source used to illuminate the scenes, the lack of sunlight can lead to investigators inadvertantly missing or destroying evidence. Whenever possible, outdoor crime scenes should be held and secured until daylight for processing.

Indoor

Compared to an outdoor scene, evidence at an indoor scene is generally less susceptible to loss, contamination and deleterious change. Indoor crime scenes are usually easier to secure and protect, and securing a scene can be as simple as closing a door.

The methods used by forensic laboratories have evolved so that very small amounts of biological material can produce a usable DNA profile. This, however, means that the potential for detecting DNA traces deposited by contamination at crime scenes becomes a factor. Contamination of any crime scene can easily occur if proper precautions, such as limiting the number of people inside the scene, are not taken. For example, first responders, emergency medical personnel, patrol supervisors, crime scene investigators, and medical examiners are all potential sources of contamination and/or loss of evidence.

Conveyance

Conveyance is defined as "something that serves as a means of transportation." Types of crimes committed in conveyances include, but are not limited to:

- Vehicle Burglary
- Grand Theft
- Car Jacking
- Narcotics Violation
- Sexual Battery
- Homicide

It is important that the crime scene investigator recognize that physical evidence recovered from these scenes may extend well beyond the conveyance itself. The flight path of the perpetrator may reveal evidence important to the investigation. For example, impression evidence, such as shoe or footprints in soil, may be found leading away from the scene, and property removed from the conveyance may be deposited or dropped as the perpetrator flees the scene. Cigarette butts are sometimes found in and around the conveyance. The nature of the crime may give the investigator an idea of the type of evidence present.

To protect the scene against inclement weather and other factors that may contribute to evidence loss and/or destruction, a conveyance such as a vehicle may be transported to the laboratory after proper documentation

has been completed.

Location & Collection of Evidence

Items of physical evidence are not always visible to the naked eye and may be easily overlooked. A deliberate, methodical, disciplined approach to collection and preservation of evidence is essential. One exception may be if evidence integrity is at risk, and under those circumstances it is important that rapid decisions be made to prevent its degradation and/or loss.

It is imperative that the investigator obtain as much information as possible regarding the circumstances of the crime prior to entering the scene. Statements from witnesses, victims, or first responders can provide a broader understanding of the investigation. The investigator can develop an approach to the scene based on this information and the nature of the crime. For example, at the scene of a burglary, attention may focus on the point of entry. Fragments of wood, metal, or broken glass may be discovered, along with fingerprints, blood, and fibers from clothing deposited when the perpetrator forced entry.

In the case of a violent crime such as a sexual assault, attention may be directed to the clothing and the person of the victim(s) and the suspect(s). An investigator might find body fluids, stains, torn clothing, fingerprints, fibers, hair, and other trace materials in the areas where the attack took place. Potential evidence such as saliva, bite marks, semen, hair, skin tissue under the finger nails, and other trace materials may be found on the victim(s). Transferred evidence such as cosmetics, vaginal fluid, hair from the victim, and blood may also be found on the suspect.

Once potential evidence is located and documented, the next step is to collect and package the items in a manner that prevents contamination, loss, and deleterious change.

Biological evidence requires care to guard against the possibility of cross contamination either by the investigator or by other biological specimens at the scene. Equipment is available to crime scene investigators which aide in the prevention of cross contamination.

Types of equipment include:

- Tyvek white paper body suit
- Paper mask that covers nose and mouth
- Eye protection
- Latex or Nitrile gloves
- Sleeve protectors
- Shoe covers
- Hair net

The investigator should prioritize the order in which evidence is collected. Biological evidence, trace materials, and evidence of a fragile nature should be collected first. Collection methods used to gather and package this evidence vary. The use of an alternate light source (ALS) or oblique lighting may be necessary. A sample detected with the ALS should be properly packaged with a notation alerting the analyst that it is a luminescent sample.

Preservation of Evidence

From crime scene to forensic laboratory to courtroom, all evidence must be inventoried and secured to preserve its integrity. Evidence admissibility in court is predicated upon an unbroken chain of custody. It is important to demonstrate that the evidence introduced at trial is the same evidence collected at the crime scene, and that access was controlled and documented.

An understanding of the rules governing chain-of-custody is vital for an investigator. For example, in a sexual assault incident, the victim is typically transported to another location to have a sexual assault examination performed. Many jurisdictions have established teams to perform these examinations, and they go by several names, such as: Sexual Assault Victim Examination (S.A.V.E.), Sexual Assault Nurse Examiners (S.A.N.E), Sexual Assualt Response Team (S.A.R.T) . The examination involves the collection of the victim's clothing, hair samples, swabs for body fluids, and documentation of bruising and bitemarks. The materials collected are packaged by the team members.

Proper evidence packaging includes:

- Appropriate packaging and labeling of all items
- Each item properly sealed and marked
- Correct and consistent information recorded on label and procedural documentation

The evidence is turned over to the investigator for submission to a department's property and evidence section. A receipt documenting the transfer is obtained. Generally, submissions to the forensic laboratory are done on a request for analysis form, listing the evidence items, and a documented chain of custody. Each individual assuming custody of the evidence from collection through analysis signs the chain of custody document. Many departments have automated this process using an information management system, whereby all transfers are securely done using barcodes. The chain of custody report will identify each individual contributing to the analysis of the evidentiary materials.

Once the analysis is complete, the evidence is either returned to the submitting agency or stored by the laboratory. The chain of custody will document this disposition. All law enforcement reports, photographs, lab analysis reports, and chain of custody documents are kept in the case file, which can be made available to the prosecution and is subject to discovery by defense counsel.

Think of the chain of custody as a chain, if one link should be broken, the chain is broken, and the evidence collected may be ruled as inadmissible.

Helpful Hints to Safeguard the Chain of Custody:

- Limit the number of individuals handling evidence.
- Confirm that all names, identification numbers, and dates are listed on the chain of custody documents.
- Ensure that all evidence packaging is properly sealed and marked prior to submission.
- Obtain signed or otherwise secure receipts upon transfer of evidence.

## Collection Techniques

The importance of avoiding cross contamination cannot be overemphasized. The investigator performing the collection must ensure tools are clean or sterilized and that gloves are changed between handling each sample.

Collection methods differ depending on the type of evidence and the substrate upon which it is found. It is preferable to collect evidence in its original state. If the evidence is fragile or can easily be lost, the entire object should be collected and packaged, if size and circumstances permit.

Some laboratories recommend the submission of substrate controls. Substrate controls are clean samples of the collection materials or unstained portions of the material the biological evidence is deposited on. The laboratories can use these to troubleshoot contamination, Polymerase Chain Reaction (PCR) inhibition, or interference with fluorescence.

The investigator should consult the local forensic laboratory and refer to the department standard operating procedures regarding collection and preservation of biological evidence.

Procedures for Evidence Collection

Blood & Other Body Fluids

| ***Type of Collection*** | ***Procedure*** |
|---|---|
| Cuttings | Removal of a section of the item containing the stain using a sterile or clean cutting device. |
| Wet Absorption | A sterile swab, gauze pad, or threads are slightly moistened with sterile distilled water. An effort should be made to concentrate the stain in a localized portion of the swab or pad. For example, when using a swab, the stain should be concentrated on the tip. The collection medium is concentrated into the stain and allowed to air dry. Some laboratories recommend following the first moistened swabbing with a second dry swabbing to ensure thorough sample collection. Both swabs are retained and submitted for analysis. |
| Scraping Method | Using a clean razor blade or scalpel, the sample is scraped into a clean piece of paper that can be folded and packaged in a paper envelope or other appropriate packaging. |
| Lifting with Tape | For dried blood stains on a non-absorbent surface, fingerprint lifting tape may be placed over the stain and lifted off. The stain is transferred to the adhesive side of the tape, which may then be secured on a clear piece of acetate for submission to the laboratory. |

Hair & Fiber Collection

| ***Type of Collection*** | ***Procedure*** |
|---|---|
| Visual Collection | On some surfaces, hairs and fibers can be seen with the naked eye. Using clean forceps and trace paper, the sample can be removed from the surface and placed into a clean piece of paper that can be folded and packaged in a paper envelope or other appropriate packaging. |

| | |
|---|---|
| Tape Lifting | Water or methanol soluble tapes are available for the collection of trace hair and fiber evidence. The tape is applied to the location of the suspected sample, removed, and packaged. |
| Vacuuming Method | The area where the suspected samples are located are vacuumed up and caught in a filtered trap attached to the vacuum. These samples are packaged in clean trace paper for submission to the laboratory. Vacuuming is the least desirable collection method because there is a risk of cross contamination if the equipment is not properly cleaned between each use. |

Reference Sample Collection

Reference samples should be collected from individuals who might be linked to the crime scene where DNA evidence is found. Reference samples can be used for elimination or comparative analysis. For example, buccal swab samples taken from the suspect and/or victim, a known source, should be compared to biological evidence found at the crime scene to eliminate or place them at the scene.

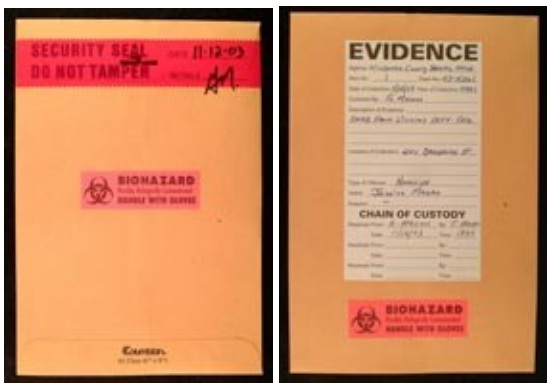
Procedures for Reference Sample Collection

| ***Type of Collection*** | Procedure |
|---|---|
| Buccal Swab | Sterile swabs or other buccal collection devices are rubbed against the inside cheek of the individual's mouth to collect epithelial cells for analysis. |
| Liquid Blood Samples | Generally collected in purple topped vacuum tubes that contain the preservative ethylenediamine tetraacetic acid (EDTA). |

Packaging & Storage

Biological evidence should be dried before packaging to minimize sample degradation. Packaging in paper is preferred; however, some laboratories allow packaging in plastic if the sample is *thoroughly* dried.

Liquid samples, such as water from a toilet bowl or pipes, should be properly documented and packaged in sterile glass or plastic containers and refrigerated as soon as possible.

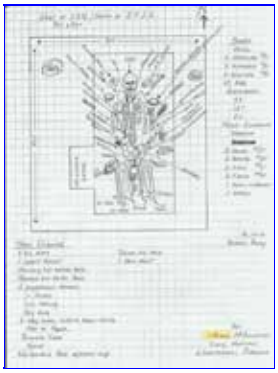


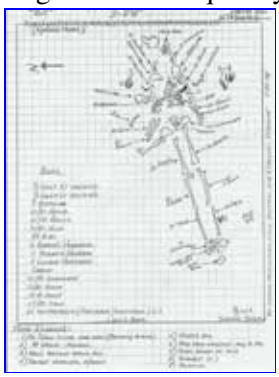

Documentation - Chain of Custody

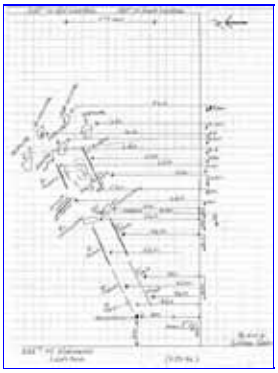
Documentation of the scene begins with the first responder. Police officers are taught the importance of taking notes from the time of arrival. The crime scene investigator documents the scene in the form of still and video photography. Sketches are completed at the scene to illustrate relationships between articles of evidence not easily depicted by photography. The following methods of crime scene documentation are used to provide an accurate representation of the scene.

Methods of Crime Scene Documentation

| Type of Documentation | Guidelines |
|---|---|
| Note Taking | It is important that the responding officers note the condition of the scene as it existed upon their arrival. Note taking should be continuously updated during the course of the investigation.<br><br>Investigator's notes might include such factors as:<br><br>• Victim and witness statements<br>• Who was present at the scene<br>• Lighting conditions<br>• Open doors and windows<br>• Odors<br>• Signs of activity such as food preparation<br>• Date and time indicators such as newspapers or mail<br>• General descriptions of the scene and surrounding area |
| Photography and Videography | The primary means of crime scene documentation is still photography. Police officers should have an understanding of the importance of keeping the scene preserved, and not moving anything until it is photographed. The photographer must be able to testify that the photograph is a true and accurate representation of the scene at the time the photograph was taken. Crime scene photographs should reveal a detailed, chronological story of the scene. |
| Sketching | Photographs may not always depict spatial relationships between objects; sketches are used to supplement photographs. Sketches can more easily depict the overall layout of the scene and the relationships between objects. Investigators usually complete hand-drawn, rough sketches while at the crime scene. These sketches contain all the necessary information for the investigator to subsequently complete a finalized version. |







For courtroom presentation, hand drawn sketches may be converted using computerized programs such as computer aided design (CAD), which provide a dynamic, professional appearance.

Types of sketches may include:

- Entire Scene - the complete scene with measurements
- Bird's-eye View – an overhead view of the scene
- Elevation Sketch
- Cross Projection Sketch
- Three Dimensional Sketch

## History and Types of Forensic DNA Testing: Introduction

DNA testing is a relatively recent technological advance in the field of forensic biology. The technology has a remarkable power to discern genetic differences, pretty well to the point of individualization. Coupled with the development of increasingly sensitive methods, DNA testing is now an essential part of the crime laboratory's armamentarium in the investigation of crimes against the person.

### Objectives

Upon successful completion of this unit of instruction, the student shall be able to do the following:

- Understand the history of forensic science and the use of restriction fragment length polymorphisms (RFLP)
- Understand the method in which DNA profiles are developed, including the use of oligonucleotides as probes
- Understand the disadvantages of RFLP analysis
- Understand the concept of short tandem repeats (STR) testing, including multiplexing and the ability to label nucleotides with fluorescent tags
- Describe the history of forensic DNA testing
- Describe the advantages of STR typing over earlier methods
- Describe the sequence of steps involved in DNA typing

photo courtesy of the National Institute of Standards and Technology

### DNA Typing by RFLP Analysis

DNA typing was introduced into forensic science in the mid-1980s, arising from discoveries made in biomedical research. Ray White, an American geneticist at the University of Utah, identified regions of DNA that did not code for proteins but were highly variable between individuals.

Early research included the use of restriction enzymes to cut strands of DNA at specific locations and produce DNA fragments of defined lengths. White separated the fragments based on size, calling the variations restriction fragment length polymorphisms (RFLP). In 1980, White and colleagues described the first polymorphic RFLP marker and proposed methods for mapping the human genome based on RFLP technology.01

The first forensic science applications of the technique arose from the work of Alec Jeffreys who found that RFLP technology could be used to develop patterns of restricted DNA that were more or less specific to an individual. At the time, his work focused on paternity testing. In 1985, the British police from West Midlands approached Jeffreys to assist them in a rape-homicide case. Jeffrey's work resulted in the release of a wrongfully convicted man and the apprehension and conviction of the true perpetrator. Soon thereafter, RFLP DNA evidence contributed to the convictions of Tommy Lee Andrews in Florida and Timothy Wilson Spencer in Virginia.02

The pieces of DNA cut by the restriction enzymes contain genes and non-coding DNA. The non-coding DNA includes regions consisting of direct repeats of the same sequence of bases, referred to as tandem repeats. The number of repeats of the sequence is genetically determined and, provided that the sequence is long enough and is repeated a sufficient number of times, will affect the length of the restriction fragment. These regions are called variable number of tandem repeats (VNTR) loci.

Variable Number of Tandem Repeats (VNTRs)

Since each VNTR region consists of repeats of a specific sequence of bases, complementary oligonucleotides can be synthesized. These oligonucleotides, when labeled with a marker such as $P^{32}$ or a chemiluminescent compound, are known as probes.

There are five basic steps to developing DNA profiles using VNTRs:

1. Extracting DNA
2. Cutting DNA into fragments using restriction enzymes
3. Separating the fragments based on size using gel electrophoresis
4. Transferring the fragments to a nylon membrane (southern blotting), causing immobilization
5. Locating and identifying the fragments by applying a solution containing the probe of interest, which then hybridizes to the immobilized DNA. Visualization of the fragments requires a lengthy exposure of the probe to a detection system and can add several days to the assay time.


**Example of 16-base pair tandem repeats in the D1S80 locus.**
**The sequence is usually repeated between 14 and 40 times.**

Probes

Probes will bind specifically to complementary VNTR fragments. Unbound and non-specifically bound probe is removed using a washing process. The RFLP profile is then visualized by exposing the membrane to film or through the use of equipment, such as the Kodak imaging station.

Visit the Kodak website to read more about the KODAK Image Station 4000R Digital Imaging System.

The technique originally developed by Jeffreys used multi-locus probes which could hybridize to several VNTR sites.

Alternatively, profiles can be developed by using a single probe process. The membrane containing the immobilized fragments is treated with one probe and visualized. This single probe is then removed and another probe is applied to the membrane. This was the most common method used in the United States, and the process generally consisted of four to six probes. Single locus probe procedures are easier to interpret and allow for simplified court presentation.

Disadvantages

While highly discriminatory, RFLP analysis of VNTRs has several drawbacks, including:

- The process is extremely laborious and time-consuming
- Radioactive probes pose health and disposal risks (although chemiluminescent technology eliminated this risk)
- A relatively large amount of sample is required to perform the tests
- The method requires high molecular weight, un-degraded DNA
- The use of yield gels is an essential, but time consuming, step in the analysis not only to estimate the amount of DNA recovered but also to determine the suitability of the sample for analysis

*Read more about yield gels in the DNA Extraction & Quantitation PDF file.*

RFLP allele assignment was performed by incorporating a reference sizing ladder in the electrophoresis and comparing the fragments to the size markers. Various approaches were developed to deal with the intrinsic variation in the method, including the use of windows or bins to express the range within which the true fragment size lay. Apart from the undesirability of having to explain why there was not a definitive size assigned to a fragment, the inherent continuous distribution of allelic states posed challenges for the national DNA database software (CODIS).

Polymerase Chain Reaction (PCR)

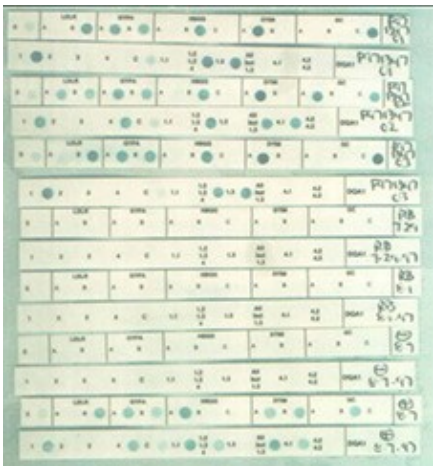
In 1983, Kary Mullis developed the technique known as the polymerase chain reaction (PCR),03 which ultimately revolutionized molecular biology, including forensic DNA analysis. Through PCR, forensic DNA analysis essentially became more rapid and sensitive. The problems of analysis time, use of radioactive materials, large sample size, presence of un-degraded high molecular weight DNA, and the need to deal with sizing variations were all dealt with by the various PCR techniques.

*Read more about Quantitative PCR in the DNA Extraction & Quantitation PDF file.*

One of the first forensic PCR tests was based on identification of human leukocyte antigen (HLA). The HLA markers are proteins of known sequence; the genes coding for each antigen can be identified. A PCR-based assay for one of the HLA loci, DQ-Alpha, was developed in 1991 and was used in crime laboratories. The kit, developed by the Cetus Corporation and marketed by Roche, was simple to use and required minimal equipment.

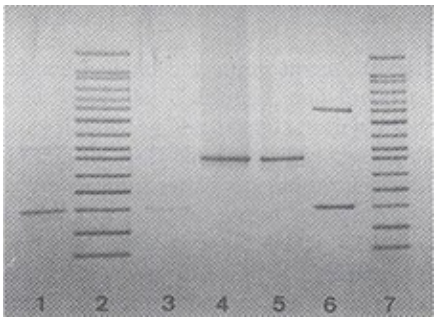
DQ-Alpha

DQ-Alpha has four main alleles, numbered 1 through 4. The DQ-Alpha 1 and 4 alleles have sub-types (1.1, 1.2, 1.3 and 4.1, 4.2, 4.3). However, the strips could not recognize the specific 1.2 allele nor could they distinguish between the 4.2 and 4.3 alleles.



The concept was extended to incorporate a number of other loci to be typed concomitantly with DQ-Alpha, and marketed as Polymarker in 1993. A kit containing reagent for both DQ-Alpha and Polymarker became quite popular. The system was sensitive and easy to use but even the combination kit did not afford the discrimination power of RFLP.

AmpFLPs




**AmpFLP (D1S80) visualized using silver staining**

The next phase in the development of DNA for use in forensic science returned to the concept of VNTRs. Some VNTR regions are relatively short and can be amplified by PCR.

Sometimes called AmpFLPs (amplified fragment poymorphisms), examples that were evaluated for use in crime laboratories included:

- D1S80
- YNZ 22 (D17S5)
- 3'ApoB

AmpFLPs did not have the discrimination power of RFLP or the simplicity of DQ-Alpha/Polymarker and were not widely used.

Short Tandem Repeats (STR)

Later in the 1990s, short tandem repeat (STR) testing appeared in forensic DNA analysis. In keeping with the name, STRs are VNTR-like regions that have very short sequences, ranging approximately 2 to 6 base pairs (bp). Although individual STR loci are not as discriminatory as RFLP markers, the short size and number of available STRs allowed scientists to amplify and analyze three or more loci simultaneously (multiplexing).

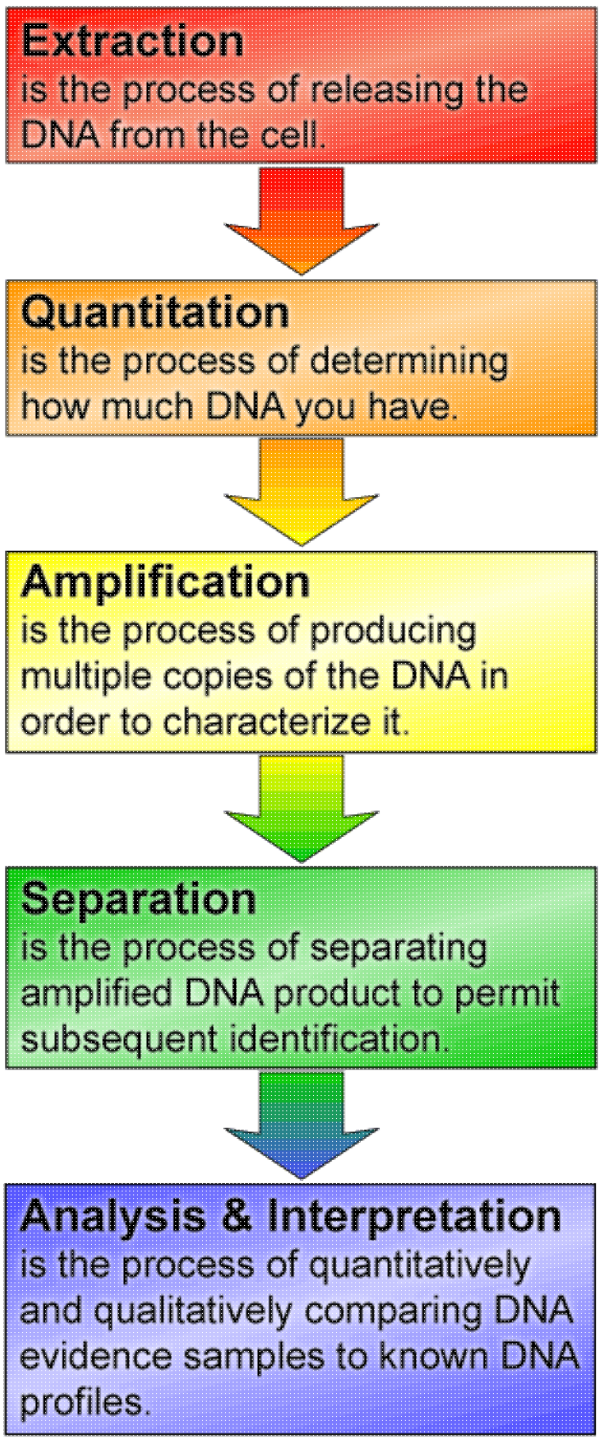
*Read more about STRs in the DNA Amplification PDF file.*

STR analysis is the current method of choice for DNA testing in crime laboratories and yields results that are nearly equivalent to individualization. The keys to the success of STR typing are multiplexing and the ability to label nucleotides with fluorescent tags. It should be noted that early work on STRs did not involve multiplexing, but rather visualized the separated fragments by the use of silver staining or a special type of

green dye.

DNA Analysis Flowchart

DNA analysis follows a sequence of steps as represented in the accompanying flowchart. Each step in the analysis process will be addressed in subsequent subjects/modules within the *President's DNA Initiative DNA Analyst Training*.



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Bill Tilstone

Bill Tilstone has a B.Sc. and Ph.D. from the University of Glasgow, Scotland. He was on the faculty of the Forensic Science program at the University of Strathclyde for 12 years before moving to Adelaide, Australia, to be Director of the State Forensic Science facility. He was appointed Executive Director of the newly created National Forensic Science Technology Center in 1996, and now serves NFSTC as Director of Instructional Technology and Education.

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Online Links

KODAK Image Station 400R Digital Imaging System

http://www.kodak.com/US/en/health/s2/products/imgStation4000R/index.jhtml