DNA Evidence: Basics of Analyzing

Overview of Steps in Analyzing DNA Evidence

Several basic steps are performed during DNA testing regardless of the type of test being done. The general procedure includes: 1) the isolation of the DNA from an evidence sample containing DNA of unknown origin, and generally at a later time, the isolation of DNA from a sample (e.g., blood) from a known individual; 2) the processing of the DNA so that test results may be obtained; 3) the determination of the DNA test results (or types), from specific regions of the DNA; and 4) the comparison and interpretation of the test results from the unknown and known samples to determine whether the known individual is not the source of the DNA or is included as a possible source of the DNA.

Any probative biological sample that has been stored dry or frozen, regardless of age, may be considered for DNA analysis.

Each additional test at a previously untested locus (location or site) in the DNA provides another opportunity for the result of "exclusion" if the known individual being used for comparison is not the source of the DNA from an evidence sample of unknown origin. If, however, the known individual is the source of the DNA on the evidence sample, additional testing will continue only to include that individual as a possible source of the DNA. When a sufficient number of tests have been performed in which an individual cannot be excluded as the source of the DNA by any of the tests, a point is reached at which the tests have excluded virtually the world's population and the unique identification of that individual as the source of the DNA has been achieved.

Steps in DNA Sample Processing

Following is a review of the steps involved in processing forensic DNA samples with STR markers. STRs are a smaller version of the VNTR sequences first described by Dr. Jeffreys. Samples obtained from crime scenes or paternity investigations are subjected to defined processes involving biology, technology, and genetics.

Biology

Following collection of biological material from a crime scene or paternity investigation, the DNA is first extracted from its biological source material and then measured to evaluate the quantity of DNA recovered. After isolating the DNA from its cells, specific regions are copied with a technique known as the polymerase chain reaction, or PCR. PCR produces millions of copies for each DNA segment of interest and thus permits very minute amounts of DNA to be examined. Multiple STR regions can be examined simultaneously to increase the informativeness of the DNA test.

Technology

The resulting PCR products are then separated and detected in order to characterize the STR region being examined. The separation methods used today include slab gel and capillary electrophoresis (CE).
Fluorescence detection methods have greatly aided the sensitivity and ease of measuring PCR-amplified STR alleles. After detecting the STR alleles, the number of repeats in a DNA sequence is determined, a process known as sample genotyping.

The specific methods used for DNA typing are validated by individual laboratories to ensure that reliable results are obtained and before new technologies are implemented. DNA databases, such as the one described earlier in this chapter to match Montaret Davis to his crime scene, are valuable tools and will continue to play an important role in law enforcement efforts.

**Genetics**

The resulting DNA profile for a sample, which is a combination of individual STR genotypes, is compared to other samples. In the case of a forensic investigation, these other samples would include known reference samples such as the victim or suspects that are compared to the crime scene evidence. With paternity investigations, a child's genotype would be compared to his or her mother's and the alleged father(s) under investigation. If there is not a match between the questioned sample and the known sample, then the samples may be considered to have originated from different sources. The term used for failure to match between two DNA profiles is 'exclusion.'

If a match or 'inclusion' results, then a comparison of the DNA profile is made to a population database, which is a collection of DNA profiles obtained from unrelated individuals of a particular ethnic group. For example, due to genetic variation between the groups, African-Americans and Caucasians have different population databases for comparison purposes.

Finally a case report or paternity test result is generated. This report typically includes the random match probability for the match in question. This random match probability is the chance that a randomly selected individual from a population will have an identical STR profile or combination of genotypes at the DNA markers tested.

**Types DNA Evidence Analysis**

- Polymerase Chain Reaction (PCR)
- Short Tandem Repeats (STR)
- Y-Chromosome
- Mitochondrial DNA

**DNA Typing — Polymerase Chain Reaction (PCR)**

The evolution of DNA testing advanced significantly when Dr. Kary Mullis discovered that DNA could be copied in the laboratory much as it is in the natural world.
The copying process, known as polymerase chain reaction (PCR), uses an enzyme (polymerase) to replicate DNA regions in a test tube. By repeating the copying process, a small number of DNA molecules can be reliably increased up to billions within several hours.

RFLP analysis requires a biological sample about the size of a quarter, but PCR can be used to reproduce millions of copies of the DNA contained in a few skin cells. Since PCR analysis requires only a minute quantity of DNA, it can enable the laboratory to analyze highly degraded evidence for DNA. On the other hand, because the sensitive PCR technique replicates any and all of the DNA contained in an evidence sample, greater attention to contamination issues is necessary when identifying, collecting, and preserving DNA evidence. These factors may be particularly important in the evaluation of unsolved cases in which evidence might have been improperly collected or stored.

**DNA Typing — Short Tandem Repeat (STR) Analysis**

Short tandem repeat (STR) technology is a forensic analysis that evaluates specific regions (loci) that are found on nuclear DNA. The variable (polymorphic) nature of the STR regions that are analyzed for forensic testing intensifies the discrimination between one DNA profile and another. For example, the likelihood that any two individuals (except identical twins) will have the same 13-loci DNA profile can be as high as 1 in 1 billion or greater.

The Federal Bureau of Investigation (FBI) has chosen 13 specific STR loci to serve as the standard for CODIS. The purpose of establishing a core set of STR loci is to ensure that all forensic laboratories can establish uniform DNA databases and, more importantly, share valuable forensic information. If the forensic or convicted offender CODIS index is to be used in the investigative stages of unsolved cases, DNA profiles must be generated by using STR technology and the specific 13 core STR loci selected by the FBI.

**DNA Typing — Y-Chromosome Analysis**

Several genetic markers have been identified on the Y chromosome that can be used in forensic applications. Y-chromosome markers target only the male fraction of a biological sample. Therefore, this technique can be very valuable if the laboratory detects complex mixtures (multiple male contributors) within a biological evidence sample. Because the Y chromosome is transmitted directly from a father to all of his sons, it can also be used to trace family relationships among males. Advancements in Y-chromosome testing may eventually eliminate the need for laboratories to extract and separate semen and vaginal cells (for example, from a vaginal swab of a rape kit) prior to analysis.

**DNA Typing — Mitochondrial Analysis**

Mitochondrial DNA (mtDNA) analysis allows forensic laboratories to develop DNA profiles from evidence that may not be suitable for RFLP or STR analysis. While RFLP and PCR techniques analyze DNA extracted from the nucleus of a cell, mtDNA technology analyzes DNA found in a different part of the cell, the mitochondrion (see exhibit 1). Old remains and evidence lacking nucleated cells — such as hair shafts, bones, and teeth — that are unamenable to STR and RFLP testing may yield results if mtDNA analysis is
performed. For this reason, mtDNA testing can be very valuable to the investigation of an unsolved case. For example, a cold case log may show that biological evidence in the form of blood, semen, and hair was collected in a particular case, but that all were improperly stored for a long period of time.

Although PCR analysis sometimes enables the crime laboratory to generate a DNA profile from very degraded evidence, it is possible that the blood and semen would be so highly degraded that nuclear DNA analysis would not yield a DNA profile. However, the hair shaft could be subjected to mtDNA analysis and thus be the key to solving the case. Finally, it is important to note that all maternal relatives (for example, a person's mother or maternal grandmother) have identical mtDNA. This enables unidentified remains to be analyzed and compared to the mtDNA profile of any maternal relative for the purpose of aiding missing persons or unidentified remains investigations. Although mtDNA analysis can be very valuable to the investigation of criminal cases, laboratory personnel should always be involved in the process.