

A Quick Overview of Forensic DNA Analysis

Prabhat Kumar*

National Forensic Sciences University, University in Gandhinagar, Gujarat, India

Commentary

The determination of a DNA profile for legal and investigative purposes is known as DNA profiling. As technology develops and enables for more information to be obtained with less beginning material, DNA analysis methods have evolved multiple times over the years. The mathematical evaluation of the rarity of the created profile within a population underpins modern DNA analysis. While DNA profiling is well known as a technique in forensic investigations, it can also be utilised for non-forensic applications [1]. Non-forensic applications of DNA profiling include paternity testing and human genealogy research. The restriction fragment length polymorphism analysis was the first real method of DNA profiling. In the United Kingdom, RFLP analysis was used for the first time in forensic casework in 1985. Variable Number Tandem Repeats (VNTRs) were employed in this sort of analysis to identify between individuals. VNTRs are found throughout the genome and are made up of the same DNA sequence repeated many times. Individuals may have a varying number of repeats at a given site in the genome. For example, individual A could have four repeats while person B could have five. The discrepancies were detected using a technique known as gel electrophoresis. Smaller fragments would travel further through the gel than larger ones, thereby separating them. These differences were used to identify between individuals, and RFLP analysis offers a high degree of individualising power when many VNTR sites are performed simultaneously. The RFLP analysis methodology was exceedingly time consuming, and amplification methods such as polymerase chain reaction could not be employed due to the length of the repeats used, which ranged between 9 and 100 base pairs. This limited RFLP to samples with a larger amount of DNA to begin with and did not function well with degraded samples. Before being phased out and replaced by newer procedures, RFLP analysis was the principal type of analysis conducted in the majority of forensic laboratories [2].

The FBI completely abandoned it in 2000, replacing it with STR analysis. DQ alpha testing, which was developed in 1991, was the first forensic DNA technology to use the polymerase chain reaction. This technique required significantly fewer cells than RFLP analysis, making it more suitable for crime scenes that lacked the enormous amounts of DNA evidence previously required. The DQ alpha 1 locus (or site) was also polymorphic, with various alleles that might be utilised to narrow the pool of individuals who could have produced that result and increase the likelihood of exclusion. In 1993, the DQ alpha locus was coupled with other loci in polymarker, a commercially available kit [3]. Polymarkers were forerunners of modern multiplexing kits, allowing several loci to be analysed with a single product. While polymarker was more sensitive than RFLP analysis, it did not have the same discriminatory strength as the previous RFLP testing. Scientists attempted to revert to a VNTR-based study paired with PCR technology known as amplified fragment length polymorphisms by 1995. (AmpFLP) the first attempt to combine VNTR analysis with PCR for forensic casework was AmpFLP. This approach employed shorter VNTRs, ranging from 8 to 16 base pairs, than RFLP analysis. AmpFLP's reduced base

pair sizes were developed to work well with the PCR amplification process. It was believed that this technique would combine the discriminating strength of RFLP analysis with the ability to analyse materials with less template DNA or that were otherwise degraded. However, only a few loci have been verified for forensic applications to operate with AmpFLP analysis because forensic labs swiftly moved on to other methodologies, limiting its discriminating ability for forensic materials. The technology was never widely adopted, although it is still employed in smaller countries since it is less expensive and easier to set up than newer approaches. By the late 1990s, laboratories had begun to transition to newer technologies, such as STR analysis. These utilised even shorter DNA fragments and could be amplified more reliably using PCR while retaining and increasing the discriminatory strength of the prior approaches [4]. The major type of forensic DNA analysis performed in current DNA laboratories is Short Tandem Repeat (STR) analysis. STR analysis expands on previous techniques such as RFLP and AmpFLP by reducing the size of the repeat units to 2 to 6 base pairs and merging numerous loci into a single PCR reaction. These multiplexing test kits may generate allele values for dozens of different loci across the genome at the same time, reducing the amount of time required to obtain a complete, individualising profile.

STR analysis has established itself as the gold standard for DNA profiling and is widely employed in forensic applications. STR analysis can also be limited to the Y chromosome alone. Because the Y chromosome is identical down the paternal line, Y-STR analysis can be employed in paternity or family searches (except in cases where a mutation occurred). Certain multiplexing kits combine autosomal and Y-STR loci into a single test, minimising the time required to gather a substantial amount of data. Mitochondrial DNA sequencing is a specialised technology that employs the distinct mitochondrial DNA found in all cells. This DNA is carried down the maternal line and is not unique to each person. However, due to the large number of mitochondria found in cells, mtDNA analysis can be employed for highly degraded samples or samples where STR analysis would not yield enough data to be informative. mtDNA can also be found in places where autosomal DNA is not present, such as hair shafts. Few laboratories process mitochondrial samples due to the higher risk of contamination when working with mtDNA. Those that do have particular methods in place to further segregate various samples from one another in order to avoid cross-contamination [5].

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*Address for Correspondence: Prabhat Kumar, National Forensic Sciences University, University in Gandhinagar, Gujarat, India, E-mail: Kumar.Pra@gmail.com

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