Comparative Forensic Analysis of Multiple Biological SamplesUsing VNTR Profiling

Abstract

While blood samples consistently provide high-quality DNA, saliva and hair present unique challenges related to DNA yield, inhibitors, and degradation, necessitating specialised extraction and analysis strategies. This review paper assesses the effectiveness and applicability of Variable Number Tandem Repeat (VNTR) profiling across diverse biological samples, including blood, saliva, and hair, for forensic individual identification and criminal investigations. The report explains the fundamental principles of VNTR profiling, outlines the standardised protocols for DNA extraction from each sample type, and compares the quality and reproducibility of the generated DNA fingerprints in terms of DNA yield, amplification success, and band clarity.The development and widespread use of more sophisticated techniques like Short Tandem Repeat (STR) analysis, mitochondrial DNA (mt-DNA) analysis, and Y-chromosome STRs (Y-STRs) were made possible by the drawbacks of VNTR profiling, especially its need for large, undamaged DNA samples and time-consuming procedures. Even though they were eventually superseded, VNTRs set important precedents for DNA evidence in the court system, both scientifically and legally.

Keywords – VNTR profiling, Short Tandem Repeat, mitochondrial DNA analysis, biological samples, DNA fingerprints

1. Introduction

1.1. Overview of forensic DNA profiling

One of the most important methods in contemporary forensic science for identifying people and resolving criminal cases is DNA profiling, sometimes known as genetic fingerprinting.The basic principle of forensic DNA analysis is that every individual has a unique genetic makeup, except for identical twins. This uniqueness makes DNA a highly powerful form of evidence, capable of linking suspects to crime scenes, identifying victims, and establishing family relationships. DNA profiling in the mid-1980s revolutionized forensic capabilities (Briody, 2005). Before this breakthrough, identification relied on "classical" markers like ABO blood groups, serum proteins, and red blood cell enzymes. These older methods had low variability, limited stability, and poor resolution, often failing to provide enough detail for definitive individual identification. The introduction of DNA profiling, therefore, was not just an incremental step but a fundamental shift that greatly improved the accuracy and discriminatory power of forensic science(Alketbi,2023).

1.2. Historical significance of VNTRs

The pioneering work of Sir Alec Jeffreys in 1985 led to the development of the Variable Number Tandem Repeat (VNTR) method, which produced the first "DNA fingerprints". VNTRs are defined as repetitive, non-coding regions of DNA, typically composed of short sequences (20 to 50 bases) that are unique to everyone (Crawfordand Beaty,2013). These regions are inherited from biological parents, and their varying lengths create a distinct pattern of bars, forming a DNA profile or fingerprint. This methodology rapidly gained traction in forensic medicine and criminal investigations. A pivotal early application was the Colin Pitchfork case in the United Kingdom in 1986, which stands as a landmark example of VNTR profiling's power(Parven,2012).In this instance, DNA evidence cleared an innocent defendant who had first confessed in addition to conclusively identifying the real criminal. In addition to criminal justice, VNTR profiling was initially useful in humanitarian contexts, such as immigration situations, where it was applied to build family ties. A significant precedent for the acceptability of DNA evidence in court was set by the quick and broad legal and popular acceptance of VNTR profiling, which was highlighted by its use in both criminal and humanitarian situations (Odah, 2024).

2. Principles of variable number tandem repeat (VNTR) profiling

2.1. Molecular basis of VNTRs

Short nucleotide sequences arranged as tandem repetitions in particular regions of the human genome are known as variable number tandem repeats, or VNTRs. These repeating units are also called minisatellites, and they usually have a length of 10 to 100 base pairs. One distinguishing feature of VNTRs is that they are highly polymorphic genetic markers, with the quantity of these repeated units varying greatly between people (Nakamura*et al*., 1987). The primary causes of the variation in the number of repeats at VNTR loci include molecular events such as gene conversion, unequal crossing over during meiosis, and slipped-strand mispairing during DNA replication. These mechanisms ensure a high degree of polymorphism, which is the fundamental basis of their utility in individual identification. While this high mutation rate contributes to genetic diversity, it also implies a dynamic nature that could theoretically lead to slight variations between generations or even somatic cells. However, for forensic purposes, VNTRs are considered stable enough to provide reliable individual identification. The inherent mechanisms of VNTR variation guarantee a high degree of polymorphism, which is the bedrock of their utility in individual identification(Jin, 1994).

2.2. VNTRs as genetic markers for individual identification

The unique patterns formed by the varying lengths of VNTR regions create a distinctive "DNA profile" or "fingerprint" for everyone. These patterns act as inherited alleles, allowing them to be used effectively for personal or parental identification. When multiple independent VNTR markers are analyzed, the probability of two unrelated individuals sharing the identical allelic pattern becomes exceedingly low, thereby conferring a high discriminatory power to the technique. For instance, the chance of encountering identical patterns in an unrelated individual can be as low as 1 in 30 billion. VNTRs are particularly advantageous in forensic identification because they are in non-coding regions of the genome(Rasekh,2021). Because of this trait, they are less vulnerable to the forces of natural selection, which may otherwise cause varying frequencies in different populations. Forensic applications needing wide applicability benefit from VNTRs' ability to retain varied frequencies across populations. Although VNTR loci can display hundreds or perhaps hundreds of alleles, only 15 to 25 could usually be successfully identified due to practical constraints in laboratory procedures (Lunt*et al*., 1998).The necessity of examining several loci in order to attain adequate

discriminatory strength was directly impacted by this discrepancy between theoretical genetic diversity and actual resolution. With the following introduction of Short Tandem Repeat (STR) technology, this crucial development—the multi-locus analysis principle—was further refined (Uguen*et al*., 2024). The practical limitations of identifying every allele highlight how crucial it is for technology to keep improving in order to effectively utilise genetic markers in forensic science.

3. Extracting DNA from biological material

Every DNA profiling study begins with the extraction of high-quality, efficient DNA from the biological source. The integrity and purity of the extracted DNA are crucial for the effectiveness of downstream analyses, such as VNTR profiling. However, because of their varied origins and frequently damaged states, forensic materials pose special difficulties.

3.1. Standardized protocols for blood samples

Blood is frequently found at crime scenes due to its high cellular content and is believed to be an excellent source of DNA. While liquid blood may give substantial quantities of DNA, typically around 30,000 ng/mL, bloodstains can only provide 200 ng/cm²(Vitosevic*et al*., 2019). Standardised procedures are essential for the collection, preservation, and extraction operations in order to guarantee the best possible sample quality and integrity.

Methodology:

Collection: In order to prevent clotting, liquid blood samples are usually obtained intravenously and promptly combined with anticoagulants, such as ethylenediamine tetra-acetic acid (EDTA). After that, these samples are either stored for a short time (5–7 days) at 4°C or for a longer time (-20°C–-80°C). Before packaging, bloodstains taken from crime scenes must be completely air-dried to avoid deterioration and the growth of mould**.**

Lysis: To liberate the DNA, the first laboratory step entails lysing the cells and dissolving the nuclear membranes. Proteolytic enzymes, such as Proteinase K, are frequently added to specialised lysis buffers to help break down proteins that could obstruct DNA extraction or other processes.

Extraction/Purification: High amounts of pure DNA are known to be produced using conventional organic extraction techniques like silica-column extraction.Nevertheless, these techniques require dangerous chemicals and are time-consuming and labour-intensive.

Modern forensic laboratories increasingly prefer automated systems utilizing silica membrane or magnetic bead-based kits due to their efficiency, speed, and compatibility with diverse sample types.

Quality Control: Post-extraction, the quantity and purity of the isolated DNA are rigorously assessed. soafter isolation we perform Gel electrophoresis to assess DNA integrity and size distribution.

Blood consistently produces high-quality DNA, making it the "gold standard" for DNA profiling. Hemoglobin, a strong PCR inhibitor found in blood samples, can obstruct later amplification stages(Sidstedt*et al*.,2018). Thus, efficient inhibitor removalwhich is frequently accomplished by using lysis buffers that have been tuned or by using extra purification procedures such centrifugation to pellet hemoglobin precipitatesis essential to the successful extraction of DNA from blood. The continuing endeavor to strike a balance between enhancing DNA recovery and limiting inhibitory effects is demonstrated by the continued development of techniques to mitigate these inhibitors, which shows how forensic methodologies are continuously refined to enhance the value of even "ideal" material.

3.2. Standardized protocols for saliva samples

Saliva is a valuable non-invasive source of DNA in forensic investigations, primarily containing buccal epithelial cells and leukocytes.

Methodology:

Collection: Saliva can be collected via passive drool, cotton swabs, or oral rinses. For oral rinses, individuals typically swish a saline solution vigorously to dislodge cheek cells, which are then spit into a sterile container. If the saliva is present as a dried stain on an item, it should be air-dried completely before packaging.

Lysis & Purification: Like blood, DNA extraction from saliva often employs method silica-based columns. Centrifugation is a common step to pellet the buccal cells and separate them from smaller bacterial cells and other contaminants.

While saliva offers a convenient and non-invasive collection method, its forensic utility is complicated by several factors. Research shows that the quantity of human amplifiable DNA in saliva-derived DNA can be substantially lower than that in blood (e.g., 37.3% for saliva vs. 87.57% for blood)(Song*et al*., 2023). Although the overall purity of DNA extracted from

saliva can be comparable to blood, it is highly susceptible to bacterial contamination, which can interfere with human DNA amplification and downstream analysis. This necessitates advanced methods to selectively amplify human DNA and robust quality control to differentiate human from bacterial DNA. Beyond identifying a person, the existence of a salivary microbiome opens up new possibilities for forensic analysis, such as describing a person's eating patterns, smoking habits, or alcohol use(Yadav*et al*.,2024).

3.3. Standardized protocols for hair samples

Hair is a frequently encountered form of evidence at crime scenes. The primary source of nuclear DNA in hair is the root or follicle, which contains living cells. Hair shafts, particularly those that have been naturally shed (telogen phase), contain very little nuclear DNA(Linch*et al*.,1998). However, hair shafts are rich in mitochondrial DNA (mt-DNA), which is present in multiple copies per cell, making it a viable target for analysis when nuclear DNA is scarce.

Methodology:

Collection: Hairs should be collected meticulously using clean forceps, and each group of hairs should be packaged separately to prevent cross-contamination. It is crucial to avoid damaging any hair root samples, and if hairs are mixed with body fluids, they should be air-dried before packaging.

Lysis & Purification: Extracting DNA from hair, especially from shafts, is challenging. Complete cell lysis often requires pretreatment with strong proteolytic enzymes like Proteinase
K and reducing agents such as dithiothreitol (DTT) to break down the dense keratin structure. Extraction method silica-bead method. Commercial kits, such as Promega's DNA IQ™ System, are specifically designed to process these difficult samples and remove PCR inhibitors(Hu*et al*.,2015).

Hair, particularly shed hair shafts, presents significant challenges in forensic DNA analysis due to low nuclear DNA yield and susceptibility to degradation from environmental exposure and programmed cellular processes(Rana, 2025). Hair pigment melanin is an important barrier and a potent PCR inhibitor. Taq polymerase can be bound by melanin, which might disrupt its activity and result in problems including variant dropout or overall amplification failure (Vajpayee et al., 2025). Mt-DNA analysis, which is more prevalent and robust in hair

shafts than nuclear DNA but has a lesser discriminatory power, is frequently used in forensic hair analysis to get over these difficulties. These biological and chemical difficulties directly led to the switch from nuclear DNA to mt-DNA analysis for hair shafts, showing how the biological sample's characteristics determine which genetic marker is best to use, even if it provides less individualised information.

3.4. Best techniques and obstacles in DNA extraction

Common problems with DNA extraction in forensic science can have a big influence on the accuracy and success of later DNA profiling. These consist of:

Sample Quality and Integrity: When forensic samples are exposed to environmental elements like heat, UV light, and microbiological activity, they frequently deteriorate or are not well kept. Low levels of fragmented DNA are produced by degraded samples, which might not be appropriate for some uses.

Inhibitors: Inhibitors that can disrupt the extraction procedure or downstream Polymerase Chain Reaction (PCR) amplification are commonly found in biological samples (Hedman and Radstrom, 2012). Proteins, lipids, polysaccharides, urea from urine, polyamines from semen, heme from blood, and melanin from highly pigmented tissues including skin and hair are a few examples. Poor DNA yields, tainted results, incomplete DNA profiles, or even false negative reactions can result from these inhibitors.

Contamination: Samples can get contaminated by environmental factors, bacterial DNA, or cross-contamination from other samples or lab workers. Inaccurate results or mixed DNA profiles could result from contamination.

Several best practices are necessary to lessen these difficulties and guarantee accurate DNA extraction.:

Standardizing Protocols: Maintaining consistency is crucial. Reproducibility and reduced variability are ensured by creating and strictly following Standard Operating Procedures (SOPs) for sample collection, storage, and extraction (Roy*et al*., 2025). Maintaining adherence to these guidelines requires regular training for laboratory staff.

Using Fresh Reagents and Calibrated Equipment: The effectiveness of extraction is directly impacted by reagent quality. For best results, use high-quality, fresh reagents and calibrate your equipment frequently.To prevent contamination, reagents must be properly kept, and sterile procedures must be followed.

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Implementing Quality Control Measures: Monitoring the success of extraction is aided by implementing quality control procedures all along the way. This involves using gel electrophoresis to measure DNA concentration and purity as well as to evaluate DNA integrity (Lucena*et al*., 2016). END POINT PCR is also capable of detecting inhibition.

Optimizing Lysis and Purification:DNA can be successfully separated from inhibitors by using lysis buffers made especially for the kind of sample and adding extra purification procedures (such as proteinase K treatment, RNase treatment, and magnetic bead-based techniques) (Tan and Yiap, 2009). Inhibition can be overcome by diluting the DNA sample or by adding bovine serum albumin (BSA).

Preventing Contamination: Strict controls on contamination are essential. This entails utilising specialised tools and filter pipette tips, regularly changing gloves, physically separating work locations for various DNA analysis steps, and doing negative controls for every extraction batch.

The success of DNA profiling is not solely dependent on the extraction method but on a holistic approach encompassing proper sample collection, preservation, and stringent contamination control. Degradation and inhibitors represent major hurdles. The emphasis on "standardized forensic protocols" extends far beyond the laboratory, beginning at the crime scene. The quality of the initial sample collection and preservation directly dictates the feasibility and success of downstream DNA extraction and profiling. This highlights that forensic DNA analysis is a chain, and the weakest link, such as improper collection leading to degradation or contamination, can compromise the entire process, regardless of the sophistication of laboratory techniques(Alketbi,2024). This further emphasises how forensic science is multidisciplinary and necessitates smooth cooperation between the crime scene and the courtroom.

4. VNTR profiling methodology and DNA fingerprint generation

Following DNA extraction, the subsequent steps in VNTR profiling involve quantification, fragmentation, amplification, separation, and detection to generate a unique DNA fingerprint.

4.1. DNA Quantification

After DNA extraction, accurate quantification of the isolated DNA is a critical step to ensure optimal amounts for downstream amplification. While using too much DNA can result in profiles that are uninterpretable because of overloading effects, using too little DNA can result in a partial or nonexistent profile. Additionally popular is END POINT PCR, which has the benefit of concurrently measuring human DNA and determining whether PCR inhibitors are present (Sidstedt*et al*., 2020).

A crucial gatekeeper in the forensic process is DNA quantitation. It is a prediction technique that directly affects the success of further amplification and the interpretability of the final DNA profile; it is not just a number measurement. Real-time PCR's capacity to identify inhibitors at this early stage is especially useful because it enables forensic analysts to take corrective action before moving on to more resource-intensive amplification steps, such as diluting the sample or adding ameliorating agents like bovine serum albumin (BSA). Particularly for difficult or impaired samples, this proactive strategy maximises resource allocation and greatly increases the possibility of producing a useable DNA profile (Alketbi,2024).

4.2 Restriction fragment length polymorphism (RFLP) and restriction enzyme digestion

The Restriction Fragment Length Polymorphism (RFLP) look at is frequently used for VNTR profiling. With this technique, certain restriction endonucleaseswhich function as "molecular scissors"are applied to the isolated DNA. These enzymes recognise and break double-standard DNA at specific, targeted nucleotide sequences that border the VNTR regions. Because each person has a different amount of tandem repetitions in a VNTR region, the length of the generated DNA fragments vary as well, resulting in length polymorphisms (RFLPs), which are seen as a distinct pattern for each individual. A commonly used restriction enzyme in forensic DNA analysis is Hae-III, which cuts DNA at the sequence 5'-GGCC-3'(Budowle*et al*.,1990).

While restriction enzymes offer high specificity in cutting DNA at precise sites, RFLP technology has a significant limitation: it requires a large amount of intact, high molecular weight DNA (e.g., 500 ng over multi-locus arrays or at least 100 ng for RFLP). This requirement poses a considerable challenge for forensic samples, which are frequently degraded or available only in minute quantities at crime scenes(Budowle*et al*., 2006). The fact that RFLP depends on sizable, undamaged DNA fragments for efficient restriction enzyme activity and subsequent separation highlights a serious flaw in its frequently impaired

application to actual forensic evidence. A crucial advancement in forensic DNA profiling, this intrinsic constraint directly prompted the creation of substitute, more sensitive PCR-based techniques that could operate with far smaller and even degraded DNA quantities (Alketbi, 2023).

4.3. Polymerase Chain Reaction (PCR) Amplification of VNTRs (Amp-FLPs)

By making it possible to exponentially amplify target DNA segments and produce millions of copies from even small amounts of starting material, the Polymerase Chain Reaction (PCR) transformed DNA profiling (Alam*et al*., 2025). It made it possible to analyse trace or deteriorated materials that would have been impossible to profile using conventional RFLP methods, this capability was especially revolutionary for forensic science.

To get around the high DNA quantity requirement of standard RFLP, PCR-based VNTR analysisalso known as Amplified Fragment Length Polymorphisms, or Amp-FLPsinvolves amplifying shorter VNTR sections, such as 8 to 16 base pairs (Sheeja*et al*., 2020). Extension (DNA polymerase synthesises new DNA strands), denaturation (heating to separate DNA strands), and annealing (cooling to allow primers to bind to the target region) are the three mainsteps that are repeated in the PCR process.

The advent of PCR significantly increased the range of forensic samples that could be examined, transforming DNA profiling from a method used mostly on pristine samples to one that could be used on actual crime scenes. However, problems still exist even using PCR. Amplification may be hampered by environmental or biological sample inhibitors (such as urea, melanin, or heme), which could result in incomplete DNA profiles or total amplification failure (Wilson,1997). Furthermore, analyzing samples with very low copy number (LCN) DNA also presents difficulties, increasing the risk of stochastic effects like allele dropout or preferential amplification. This highlights a continuous cycle of technological advancement addressing old limitations but often revealing new ones, requiring further methodological refinements and careful statistical interpretations.

4.4. Gel Electrophoresis and Southern blotting

Following restriction enzyme digestion (and optional PCR amplification), the resulting DNA fragments are separated based on their size using agarose gel electrophoresis. DNA molecules go through the gel matrix's pores in the direction of the positive electrode because they are negatively charged. Smaller DNA fragments move more rapidly and travel further through

the gel than larger fragments, resulting in a continuous separation of DNA fragments according to their size(Smithand Cantor, 1987).

During electrophoresis, the agarose gel is soaked in a basic solution to denature (convert to single strands) the segregated DNA fragments therein. These single-stranded DNA fragments are then transferred from the gel to the surface of a nylon membrane through a process known as Southern blotting, named after its inventor, Edwin Southern(Southern, 2006). This blotting procedure is crucial as it immobilizes the DNA fragments on the membrane, preserving their spatial arrangement exactly as they were separated on the gel.

Although at first apparent, the visual aspect of gel electrophoresis for VNTRs naturally reduced the accuracy of allele size and caused measurement error. This "imprecise resolution" directly affected the statistical power and certainty of a match, necessitating the development of statistical criteria for determining whether two bands could be considered similar. This restriction was a major factor in the move towards automated, more accurate electrophoretic techniques, like capillary electrophoresis in STR analysis, which greatly improves objectivity and reproducibility in forensic DNA analysis by providing discrete numerical data rather than visual patterns.

4.5. Combining probe hybridisation with DNA fingerprint pattern recognition

Following Southern blotting to immobilise the DNA fragments on the nylon membrane, hybridisation with certain probes is the next step. According to Heath et al., (1993).These probes are synthetic oligonucleotides, which are DNA or RNA sequences that complement certain VNTR regions of interest. These probes, which are usually tagged with a fluorescent chemical or a radioactive label for detection, are added to a solution and incubated in the membrane. The probes attach to their complementary DNA sequences on the membrane to produce stable duplexes under particular salt and temperature conditions.

Only the radioactivity or fluorescence linked to the targeted DNA loci is left on the membrane after hybridisation, as unbound probes are removed by washing. Next, the probe hybridisation sites are identified. This process, called autoradiography, entails positioning the membrane adjacent to an X-ray film in a light-tight container for radioactive probes (Fischer and Werner, 2020). The black band pattern called as the "DNA fingerprint" or "autoradiograph" is generated by radioactive decay and is captured on X-ray film. The entire DNA profile is a collection of these autoradiographs from various loci.

Multi-locus probes, which hybridised to many VNTR loci at once and produced intricate and occasionally challenging-to-understand patterns, were frequently used in early techniques. Because of this intricacy, single-locus probeswhich target a distinct DNA sequence that surrounds a particular VNTRere developed and are preferred because they make it easier to identify individual alleles.

Despite its sensitivity, the use of radioactive probes for detection posed serious health and disposal hazards to lab workers. Furthermore, the visualization process required lengthy exposure times, often adding several days to the overall assay duration. These practical and safety hurdles in VNTR profiling pushed the field towards the adoption of non-radioactive detection methods (such as chemiluminescence and fluorescence) and the development of faster, automated systems(Surzycki, 2008). This evolution not only improved laboratory safety and efficiency but also facilitated the high-throughput analysis necessary for building large-scale forensic DNA databases, reflecting a broader trend in scientific methodology towards safer, faster, and more scalable techniques.

5. Comparative analysis of VNTR profiling across biological samples

The efficiency and utility of VNTR profiling in forensic investigations are significantly influenced by the type and condition of the biological sample from which DNA is extracted. Different features in terms of DNA production, quality, amplification success, and the clarity of the resulting DNA fingerprint patterns are revealed by comparing samples of blood, saliva, and hair.

5.1. VNTR profiling comparisons between biological samples

The quantity and quality of DNA obtained vary across different biological matrices, directly impacting the success of VNTR profiling.

Blood: Blood is always thought of as a great source of DNA as it usually produces huge amounts of high-quality genomic DNA. Liquid blood samples can provide approximately 30,000 ng/mL of DNA, while dried bloodstains yield about 200 ng/cm²(Vitosevic*et al*.,2019).The purity of the extracted.DNA from blood, as indicated by the A260/A280 ratio, typically falls within the ideal range of 1.8-2.0, signifying minimal protein or contaminant contamination(Janavi, 2017).

Saliva: Saliva can also be a source of high-quality genomic DNA, with some studies demonstrating yields comparable to blood, particularly when collected using specialized kits.

Liquid saliva can yield around 5,000 ng/mL of DNA. However, a significant consideration for saliva is that the proportion of *human amplifiable* DNA can be lower than in blood (e.g., a mean of 37.3% for saliva-derived DNA compared to 87.57% for blood-derived DNA) (Mahaboob, 2019).While the overall purity (A260/A280 ratio) of saliva DNA can be like blood, it may contain degraded RNA, which can affect quantification results if not treated with RNase, though it typically does not impede downstream applications.

Hair: The presence and integrity of the hair root have a major impact on the DNA yield from hair samples. While naturally shed hairs, which frequently lack a substantial root sheath, give far less nuclear DNA—typically 1–12 ng/root—plucked hairs with complete follicles can yield anything from 1–750 ng/root. Instead of nuclear DNA, the majority of the DNA present in hair shafts is mitochondrial DNA (mt-DNA) (Brandhagen et al., 2018). It is challenging to get high-quality nuclear DNA profiles because hair DNA is so prone to deterioration from environmental exposure and cellular activities.

Sample-specific extraction procedures and downstream analysis techniques are required due to the different DNA yields and quality among samples (Barcenilla*et al*., 2024). For example, although blood is strong, saliva needs to be handled carefully to prevent bacterial contamination and reduce the amount of human DNA, and hair frequently needs mt-DNA analysis because nuclear DNA is few. This suggests that in forensic practice, a "one-size-fits-all" strategy is inadequate. To overcome innate biological and environmental difficulties, laboratories must instead use a customised, flexible approach for every type of sample, optimising extraction and analysis (Bojko,2024). This demonstrates the intricacy of forensic casework, where the type and state of the evidence frequently determine the strategy to be used.

Table 1: Comparison of DNA yield and purity from Blood, Saliva, and Hair samples

Sample Type	Typical DNA Yield	A260/A280 Ratio (Purity)	Key Challenges / Considerations
Blood (Liquid)	~30,000 ng/mL	1.8 – 2.0	Heme inhibitors, degradation if not properly stored
Blood (Stain)	~200 ng/cm²	1.8 – 2.0	Heme inhibitors, degradation over time
Saliva (Liquid)	~5,000 ng/mL (amplifiable DNA: ~37.3% human)	1.8 – 2.0 (may contain bacterial DNA contamination, RNA affecting ratio)	Lower human DNA yield if untreated
Hair (with root, plucked)	~1 – 750 ng/root	1.8 – 2.0	Low nuclear DNA, degradation, melanin inhibitors
Hair (shed/shaft, nuclear DNA; high mt-DNA content)	~1 – 12 ng/root	1.8 – 2.0	Extremely low nuclear DNA, high degradation, melanin inhibitors

5.2. Comparison of amplification success and band clarity

The quality and amount of the extracted DNA, in addition to the presence of inhibitors, are strongly related to the success of DNA amplification and the level of clarity of the resulting DNA fingerprint patterns.

Blood: Blood samples lead to high amplification success rates and clear, well-defined band patterns(Anderson*et al.*,1999). This is mostly because, if strong inhibitors like heme are successfully eliminated during the extraction procedure, there is usually a high yield of undamaged DNA.

Saliva: Amplification success and genotyping call rates from saliva can be significantly lower compared to blood(Abraham*et al.*,2012). This reduction is often correlated with a lower percentage of human amplifiable DNA in saliva samples. Furthermore, the abundant bacterial DNA present in saliva can compete with human DNA during amplification, potentially interfering with the generation of clear human DNA profiles.

Hair: Amplifying nuclear DNA from hair, especially from hair shafts, is frequently unsuccessful due to the extremely low quantity of nuclear DNA and its high susceptibility to degradation(Brandhagen*et al.*,2018). Melanin, a pigment found in hair, acts as a potent PCR

inhibitor, which can lead to allele dropout (failure to detect one or both alleles) or complete amplification failure, severely compromising the quality and completeness of the DNA profile. Consequently, for hair shafts, mitochondrial DNA (mt-DNA) amplification is often pursued due to its higher copy number and greater resilience to degradation, resulting in more successful amplification, although with lower discriminatory power (Robertsand Calloway,2007).

The direct correlation between DNA yield/quality and amplification success/band clarity highlights that the initial state of the biological sample is paramount for obtaining a usable DNA profile. Degraded or inhibited samples inevitably lead to partial or unclear profiles, which in turn reduce their evidentiary value in forensic investigations(Noor*et al*.,2024). This strong correlation means that the "efficiency" of VNTR profiling is not just about the technique itself, but about the recoverability of high-quality, amplifiable DNA from the forensic sample. This highlights the significance of strong quality control and interpretation guidelines in forensic laboratories and has important ramifications for the interpretation of results in court, since incomplete or ambiguous profiles create ambiguity and necessitate careful statistical assessment.

5.3. Assessment of reliability and discriminatory power

There is very little chance that two unrelated people will have the same VNTR profile because VNTR profiling has a very high degree of discriminating power (Hilty *et al*., 2005). For example, it has been calculated that there is a 1 in 30 billion chance of finding identical patterns in an unrelated person. The number of distinct VNTR loci analysed closely correlates with this selective power; the more loci analysed, the smaller the chance of a random match (Weir, B.S., 1992). VNTRs' dependability as genetic markers is further enhanced by their somatic and germline durability.

However, a number of factors can influence how reliable VNTR profiling is. The confidence in proclaiming a match may be impacted by the measurement inaccuracy and lack of precise resolution of VNTR values in the electrophoretic method of separation (Hurd*et al*., 2002). Additionally, the method necessitates comparatively large quantities of intact DNA, and results may be compromised by contamination during sample processing or analysis. Finding the Random Match Probability (RMP) is an important stage when evaluating the importance of a DNA match. A statistical tool known as RMP calculates the possibility of a DNA profile in each group will be similar to the profile of a randomly chosen individual. (Koehler*et al*.,

1994). This computation makes use of statistical models, most frequently the product rule, and DNA allele frequencies derived from population databases. The size and genetic makeup of the reference population, as well as the possibility of laboratory errors, can affect RMP estimations (Nelson*et al*., 2008).

According to Murphy (2007), the shift from a straightforward "match" to a statistically sound "random match probability" for VNTRs demonstrates the growing scientific rigour and judicial scrutiny given to forensic DNA evidence. Although VNTRs have a great deal of discriminatory power, determining RMP is difficult and has generated statistical debates, especially when it comes to topics like family and demographic stratification. According to Li and Wang (2015), this intricacy suggests that a "match" is always a probabilistic assertion rather than a definitive statement of identification. This emphasises how important population genetics and biostatistics are to forensic science, going beyond simple pattern recognition to evaluate evidence quantitatively.

6. Applications for VNTR profiling in criminal investigations and individual identification

A revolutionary period in forensic research began with the advent of VNTR profiling, which had an impact on a variety of sectors requiring individual or lineage identification in addition to direct criminal identification.

Early Successes: The 1986 Colin Pitchfork case is a landmark illustration of the significant influence of VNTR profiling. Importantly, VNTR analysis cleared an innocent person who had confessed to the crimes in this historic case, in addition to identifying and convicting the real criminal (O'Dwyer, 2013). DNA evidence's legitimacy and public acceptance in court processes were greatly enhanced by its simultaneous potential to convict the guilty and clear the innocent.

Broader Forensic Utility: In addition to this seminal case, VNTR profiling was frequently used for:

Identify Suspects and Link Crimes: In order to create links, samples of bodily fluids or cells (such as blood, saliva, hair, or semen) taken from crime scenes were profiled and in comparison to criminal DNA databases or samples acquired from suspects.

Identify Human Remains: VNTR profiling proved invaluable in identifying bodies or body parts that were unidentifiable through conventional means, such as those that were badly decomposed or fragmented after disasters.

Paternity Testing and Immigration Cases: The inherited nature of VNTR patterns made them particularly useful for determining familial relationships, aiding in paternity disputes, and verifying family connections for immigration purposes.

Anthropological Genetics: Because of their polymorphism, VNTRs were useful in anthropological genetics, where they let researchers reconstruct evolutionary history, investigate the origins of humans and migration patterns, and even identify clan allegiance among native populations.

Microbial Forensics: The principle of VNTR analysis has extended beyond human identification. Multiple Loci VNTR Analysis (MLVA) emerged as a valuable technique in microbial forensics, used to distinguish strains of bacterial pathogens for outbreak surveillance and to trace sources of contamination.

The widespread adoption and success of VNTR profiling in diverse legal and scientific contexts, ranging from criminal justice to family law and anthropology, illustrate its foundational role in establishing DNA as a powerful, versatile, and legally admissible form of evidence(Alketbi,2023). This multifaceted application demonstrates that the technology's value transcended its initial forensic purpose, contributing to a broader understanding of human identity and relationships.

7. VNTR profiling's limitations and the development of DNA technology

Despite its groundbreaking impact and high discriminatory power, VNTR profiling, particularly in its Restriction Fragment Length Polymorphism (RFLP) based format, possessed inherent limitations that drove the development and adoption of more advanced DNA technologies.

7.1. Challenges with sample requirements and analysis complexity

The primary challenges associated with RFLP-based VNTR profiling included:

High DNA Quantity Requirement: A significant drawback was the necessity for a large amount of high molecular weight, undegraded DNA. RFLP methods typically require at least 100 ng of DNA, and multi-locus typing could demand 500 ng or more(Hashim*et al*.,2019). Such quantities are often difficult to obtain from typical crime scene evidence, which may be sparse.

Sensitivity to Degraded Samples: VNTR profiling did not perform well with degraded DNA. The large fragment sizes generated by restriction enzymes are easily fragmented by environmental exposure (e.g., heat, UV light, humidity, microbial activity), making it challenging to obtain complete or interpretable profiles(Tiedje*et al*., 1999).

Time-consuming and Labour-intensive: The RFLP process was infamously time-consuming and labour-intensive, frequently requiring weeks to provide results**.**This was due to multiple steps, including DNA digestion, lengthy gel electrophoresis, Southern blotting, and prolonged exposure times for radioactive probes(Wheeler*et al*., 2019).

Hazardous Reagents: Early methods frequently used radioactive probes (e.g., P32) for detection, which posed health and safety risks to laboratory personnel and created challenges for radioactive waste disposal(Hall*et al*., 2007).

Interpretation Complexity: Multi-locus VNTR patterns could be complex, especially when dealing with mixed samples from multiple contributors, making interpretation challenging. These inherent limitations of VNTRs (large DNA requirement, sensitivity to degradation, timeconsuming nature, reliance on hazardous materials) directly led to their gradual replacement by newer technologies. This illustrates a classic pattern of scientific progress where limitations in one technology drive the innovation and adoption of a superior one. The "retirement" of RFLP-VNTR as the primary forensic method, despite its foundational importance, serves as a powerful case study in technological evolution within forensic science(Walsh,2009). It demonstrates that even highly discriminatory methods can become obsolete if they fail to meet practical demands related to sample availability, processing time, and safety. This continuous drive for efficiency and robustness is fundamental to the advancement of forensic capabilities.

7.2. Transition to Short Tandem Repeats (STRs) and other markers

The limitations of VNTR profiling spurred the development and widespread adoption of more advanced DNA technologies, notably Short Tandem Repeats (STRs), along with specialized markers like mitochondrial DNA (mt-DNA) and Y-chromosome STRs (Y-STRs).

Short Tandem Repeats (STRs): In contrast to VNTRs (10-100 base pairs), STRs, commonly referred to as microsatellites, have substantially shorter repeat units (usually 2-6 base pairs). (Vergani, 2021). This smaller size conferred several significant advantages, leading to STR analysis becoming the current gold standard in forensic DNA analysis:

Smaller Sample Requirement: STR analysis can successfully amplify and profile DNA from very small amounts of starting material, often less than 1 ng, making it highly suitable for trace evidence commonly found at crime scenes(Clayton*et al*., 1998).

Compatibility with Degraded DNA: Due to their shorter fragment sizes, STRs are much better suited for analysis of degraded DNA samples, as they are more likely to remain intact even when the overall DNA is fragmented(Schneider*et al*., 2004).

Multiplexing Capability: The capacity of STRs to amplify several STR loci at once in a single reaction (multiplex PCR) is one of their main advantages.(Schoske*et al*., 2003). This significantly increases the overall discriminatory power, as the probabilities of individual loci are multiplied, and dramatically speeds up the analysis process. For instance, forensic laboratories in the United States typically analyze 13 or more core STR loci (CODIS loci)(Hares, 2012).

Automation and Speed: STR analysis is highly amenable to automation, utilizing robotic platforms for DNA extraction and capillary electrophoresis for fragment separation. This automation leads to much faster turnaround times, often hours or days compared to weeks for RFLP-VNTR(Karudapuram*et al*., 2007).

High Discriminatory Power: While an individual STR locus may be less discriminatory than a VNTR locus, the ability to multiplex many STR loci results in an extremely high overall discriminatory power, often yielding probabilities on the magnitude of 1 x 10^-15 or lower for a random match(Lins*et al*.,1998).

Mitochondrial DNA (MT-DNA) Analysis: For highly degraded samples or those lacking nuclear DNA (e.g., hair shafts without roots, old bones, charred remains), mt-DNA analysis provides a valuable alternative (Pajnic, 2020). Mt-DNA is inherited maternally, making it useful for tracing maternal lineage and identifying missing persons through maternal

relatives. In comparison with nuclear DNA, mt-DNA analysis has a lesser discriminatory power despite being robust.

Y-Chromosome STRs (Y-STRs): Polymorphic areas on the Y chromosome that are passed down directly from father to son are known as Y-Chromosome STRs, or Y-STRs. They are particularly useful in sexual assault cases to differentiate male DNA from overwhelming quantities of female DNA from the victim, and for tracing paternal lineages.The development and adoption of STRs, mt-DNA, and Y-STRs represent not merely replacements for VNTRs but a diversification of forensic tools(Dash, H.R0.et al, 2023). Each marker type possesses specific strengths and weaknesses, making a multi-faceted approach necessary for comprehensive forensic analysis. The evolution of DNA profiling techniques from VNTRs to a suite of specialized markers signifies a maturation of the field, moving from a single "gold standard" to a more nuanced, adaptive strategy(Seth-Smith, *et al*., 2025). This implies that modern forensic science employs a toolkit approach, selecting the most appropriate genetic marker(s) based on the quantity, quality, and specific context of the biological evidence, thereby maximizing the chances of obtaining probative information from even the most challenging samples.

Table 2: Advantages and Disadvantages of VNTR vs. STR Profiling

Feature/Aspect	VNTR Profiling (RFLP-based)	STR Profiling
Repeat Unit Length	10–100 base pairs (minisatellites)	2–6 base pairs (microsatellites)
Typical DNA Quantity Required	Large amounts (e.g., >100 ng, >500 ng for multi-locus)	Small amounts (e.g., <1 ng)
Suitability for Degraded Samples	Poor (requires high molecular weight DNA)	Good (smaller fragments amplify better)
Processing Time	Weeks (labor-intensive, lengthy probe exposure)	Hours/Days (faster turnaround)
Detection Method	Southern blotting, radioactive/chemiluminescent probes, autoradiography	PCR amplification, capillary electrophoresis, fluorescent detection
Automation Potential	Low	High (robotic platforms)
Multiplexing Capability	Limited (complex patterns with multi-locus probes)	High (multiplexing 13+ loci)
Discriminatory Power (per locus vs. overall)	High per locus, but overall power limited by practical number of loci	Extremely high overall (current gold standard)
Common Use in Forensics	Historically significant, now largely superseded	Current gold standard in modern forensics
Associated	Hazardous reagents	Potential for LCN issues,

Risks/Challenges	(phenol/chloroform), radioactive waste, complex interpretation	measurement error, and interpretation of mixtures

8. Conclusion

Variable Number Tandem Repeat (VNTR) profiling represents a pivotal milestone in the history of forensic science, laying the foundational principles for modern DNA-based individual identification. The method, pioneered by Sir Alec Jeffreys, revolutionized criminal investigations by offering unprecedented discriminatory power, capable of generating unique genetic "fingerprints" for individuals. Early applications, such as the landmark Colin Pitchfork case, unequivocally demonstrated VNTR profiling's capacity to both identify perpetrators and exonerate the innocent, thereby establishing critical legal and societal precedents for the acceptance of DNA evidence.The comparative analysis of VNTR profiling across different biological samplesblood, saliva, and hairreveals varying efficiencies and inherent challenges. Blood samples consistently yield the highest quantities of high-quality DNA, making them the most reliable source for profiling, provided that potent inhibitors like heme are effectively managed during extraction. Saliva, while offering a non-invasive collection method, presents complexities due to lower human amplifiable DNA content and susceptibility to bacterial contamination, demanding specialized purification techniques. Hair samples, particularly hair shafts, pose the greatest challenge due to their low nuclear DNA yield, propensity for degradation, and the presence of melanin, a significant PCR inhibitor. Consequently, profiling hair often necessitates a shift to mitochondrial DNA analysis, which, while more robust, offers less individualizing information.

The limitations inherent in RFLP-based VNTR profiling, including the requirement for large quantities of undegraded DNA, its time-consuming and labor-intensive nature, and the use of hazardous radioactive materials, drove the continuous evolution of DNA technologies. This led to the development and widespread adoption of Short Tandem Repeat (STR) profiling, which became the new gold standard due to its superior sensitivity, compatibility with degraded samples, multiplexing capabilities, and amenability to automation. Additionally, specialised markers like Y-chromosome STRs (Y-STRs) and mitochondrial DNA (mt-DNA) have become essential adaptive methods for examining difficult or particular kinds of forensic evidence.

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