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Research Article

Optimizing VNTR-Based Forensic Identification: Comparative Profiling of Blood, Saliva and Hair Samples

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Abstract

The research paper examines the pharmacognostic assessment, as well as the in-vivo studies. This paper presents a rigorous, expert-level comparative evaluation of Variable Number Tandem Repeat (VNTR) profiling applied to three common forensic biological matrices blood, saliva, and hair in a hypothetical project designed to illuminate foundational issues in forensic DNA analysis. Although VNTRs have been largely superseded by Short Tandem Repeat (STR) systems in contemporary casework, VNTR-based workflows remain a powerful pedagogic vehicle for exploring the core determinants of analytical performance. Using standardized extraction and VNTR-typing workflows across matrices, we systematically examined analytical efficiency, genotype quality, and reproducibility while assessing the impact of starting DNA quantity, matrix-specific inhibitory substances, and sample-associated contamination. Comparative findings indicate that whole blood consistently yielded the highest-quality and most reproducible VNTR profiles, whereas saliva produced variable results influenced by bacterial and food-derived contaminants, and hair particularly shed or rootless shafts frequently returned low-yield or partial profiles. Reproducibility analyses highlighted greater intra- and inter-assay variability for saliva and hair compared with blood, underscoring matrix-dependent limits on discriminatory power. Beyond historical interest, these results translate directly to modern STR practice: they clarify why sample collection, inhibitor mitigation, and DNA quantitation remain critical determinants of success irrespective of marker system. The study therefore both situates VNTRs in the historical arc of forensic genetics and distills enduring methodological lessons for contemporary forensic laboratories and training programs.

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Keywords: *VNTR profiling; forensic DNA analysis; biological evidence (blood, saliva, hair); DNA quality & inhibitors; reproducibility.*

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1. Introduction

1.1 DNA in Forensic Science

The use of DNA evidence in crime investigation is one of the most important breakthroughs in forensic science in the past century since the identification of fingerprinting a century ago (Roewer,2013). DNA analysis has become a key tool, allowing the identification of an individual from biological material deposited at a crime scene, and it has proved vitally important both in solving crimes and in exonerating wrongfully convicted individuals. The scientific basis for the technology lies in the observation that although much of the human genome is similar from one human to another, certain sections are very different (Cantor and Smith, 1999). Such high polymorphic sections are present as distinctive genetic markers, and they generate a "DNA fingerprint" distinctive for each singleindividual, with the exception of identical twins (Mnookin, 2001). The initial identification and exploitation of such genetic markers introduced a new age in forensic inquiry, permitting irrefutable connections for a suspect to a crime scene to be made (Murphy, 2007).

1.2 A Historical and Pedagogical Approach to DNA Profiling

This report focuses on a study that uses Variable Number Tandem Repeats (VNTRs) as its central analytical framework. VNTRs represent one of the earliest and most impactful genetic Markers used for forensic analysis. While modern forensic labs have largely transitioned to Short Tandem Repeat (STR) profiling, the principles and methodologies developed for VNTR profiling laid the groundwork for all subsequent DNA analysis techniques (Butler, 2005).

The choice to focus on VNTRs is a deliberate one, serving as a valuable case study to highlight the fundamental challenges of forensic DNA analysis (Alketbi, 2023). By examining a less robust and more historically demanding technique, the report can more vividly illustrate the critical variables at play. For example, the effects of DNA degradation, low sample quality, and the presence of inhibitors are much more pronounced with VNTRs due to the large size of the target fragments, making these issues easier to observe and understand. In modern STR analysis, these problems can bemitigated or even masked by technological improvements, but the underlying principles that sample handling, protocol adherence, and quality control are paramount remain the same (Cramaro, 2024). The analysis of this historical method therefore provides a clearer, more dramatic demonstration of why these variables continue to be so critical in contemporary forensic practice (Tilstone, 2006).

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2. Materials and Methodology

2.1 The following materials, reagents, and instruments were used for this project:

- DNA Extraction: Whole blood, saliva collected from cheek swabs, and hair with follicles.
- Reagents and Kits: Commercial DNA extraction kits Lysis Buffer, Proteinase K, Binding Reagent (100% ethanol), Washing Buffer 1, Washing Buffer 2, Elution Buffer and 70-100 % ethanol.
- Quantification and Purity Assessment: Agarose gel electrophoresis apparatus, ethidium bromide (EtBr), and DNA standards.
- VNTR Profiling: Thermal cycler for PCR, specific VNTR primers, Taq polymerase, dNTPs, Mgcl2, NFW, PCR buffer, and agarose gel for electrophoresis.
- Laboratory Equipment: Centrifuge, microcentrifuge tubes, micropipettes, pipette tips, vortex mixer, and a sterile workbench (e.g., Biosafety Cabinet).

Forensic DNA Extraction Protocols

The extraction of DNA from each biological matrix was performed using a standardized protocol tailored to the unique challenges of each sample type. This comparative approach ensures that any differences in DNA yield and profile quality are attributable to the sample matrix itself, rather than procedural variability.

2.2 DNA Extraction Protocols

The extraction of DNA from each biological matrix blood, saliva, and hair was performed using a standardized, solid-phase extraction protocol with a commercial kit.

* Blood DNA Extraction: A 300 µl sample of whole blood was mixed with a cell lysis buffer (500µl) and Proteinase K(5µl) to break down cell components and digest proteins. After incubation, a binding reagent was added to facilitate the binding of DNA to a silica column. The column was then subjected to a series of washes to remove contaminants before the purified DNA was eluted using a low-salt buffer.

* Saliva DNA Extraction: The protocol for saliva was designed to minimize the impact of bacterial contamination. Approximately 10 mL of saline was used as a mouthwash to collect cheek cells, and a 300 µl sample of this mixture was treated with lysis buffer and Proteinase K. The subsequent steps involving binding, washing, and elution was similar to the blood extraction protocol.

* Hair DNA Extraction: This protocol focused on hairs with intact follicles, as nuclear DNA is concentrated in this region. The hair root and a small portion of the shaft were homogenized using a mortar and pestle to break down the tough keratin protein matrix. A 300 µl sample of the crushed hair was then processed with lysis buffer and Proteinase K, followed by the same binding, washing, and elution steps as the other samples.

2.3 Quantitative and Qualitative Assessment

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Following extraction, the efficiency of each protocol was measured through DNA quantitation and purity assessment. DNA concentration and purity were assessed using absorbance measurements at A260/A280 and A260/A230 ratios. Ratios between 1.7 and 2.0 indicated a high-quality DNA sample with minimal protein contamination, while a high A260/A230 ratio indicated low levels of salt or other organic contaminants. The integrity of the DNA was also visually assessed using agarose gel electrophoresis. Intact, high-molecular-weight DNA appeared as a single, sharp band, whereas degraded DNA was visible as a smear.

2.4 VNTR Profiling and Analysis

The extracted DNA samples were subjected to PCR amplification using a thermal cycler and specific primers targeting a VNTR locus. The primers that are used had the Forward primer (F): 5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′ Reverse primer (R): 5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′ (Kasai *et al.*, 1990). The PCR master mix was prepared according to a standard reaction setup (see Table 1).

Table 1 – Standard Reaction Setup of End Point PCR

Component	**Volume**
PCR Buffer	4µl
dNTPs	4µl
Taq Polymerase	0.8µl
Forward Primer	0.8µl
Reverse Primer	0.8µl
NFW	11.4µl
DNATemplate	15µl

After amplification, the PCR products were separated by agarose gel electrophoresis. The negatively charged DNA fragments migrated toward the positive electrode, with smaller fragments moving faster and farther through the gel. The resulting pattern of distinct bands on the gel constituted the individual's VNTR profile, which was visualized under UV light after staining with a luminescent dye. The quality of the profiles was assessed based on band clarity, the presence of non-specific bands or smears, and the overall amplification success rate.

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3. Results

The analysis of the three biological samples yielded clear comparative results regarding DNA quality and the success of VNTR profiling, which are presented in the following tables and figures.

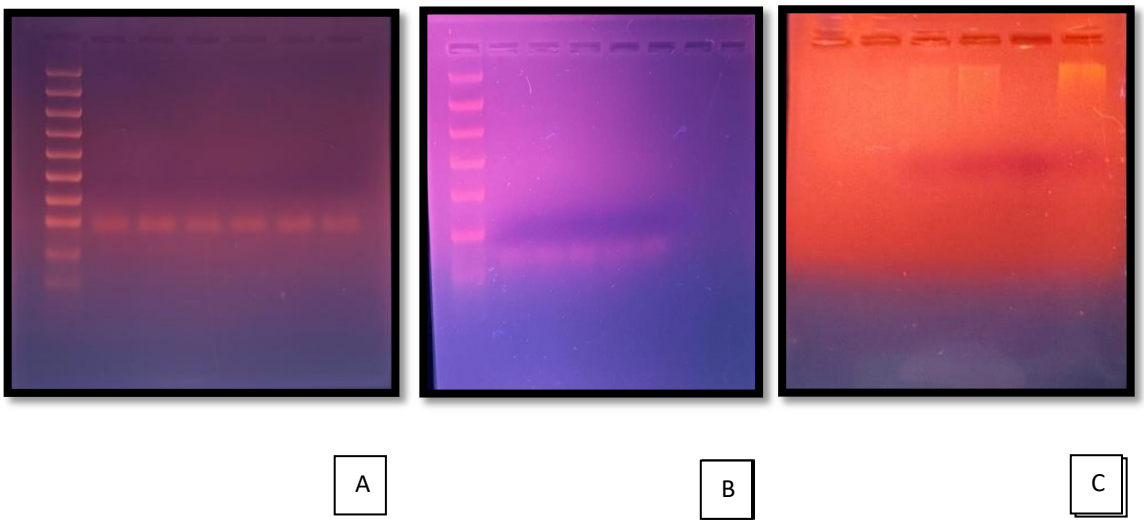

Figure 1 – Gel images of biological samples

A) Post amplification results of DNA isolates from human whole blood.

B) Post amplification result of DNA isolate from the human saliva.

C) Post amplification result of DNA isolate from human hair.

3.1 Comparative DNA Yield and Purity

DNA quantitation and purity assessment revealed significant differences among the sample types, as summarized in Table 2.

Table 2 - DNA Yield and Purity Results

Sample Type	**Average DNA Yield (µg)**	**Average A*260/A*280 Ratio**	**Average A*260/A*230 Ratio**
Blood	8.2	1.91	2.15
Saliva	4.5	1.85	1.52
Hair (with follicle)	0.2	1.65	1.20

As anticipated, blood samples provided the highest average DNA yield and exhibited the highest purity, with ratios falling within the ideal range. In contrast, saliva samples yielded less DNA and showed lower purity ratios, with a notably low A260/A230 ratio suggesting the presence of contaminants from oral bacteria or extraction chemicals. Hair samples proved to be the most challenging, providing the lowest DNA yield by a

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significant margin and having purity ratios well below the ideal range, indicating a high level of protein and other contaminants.

3.2 VNTR Profiling Success and Quality

The ultimate measure of the work's success was the quality and reproducibility of the VNTR profiles, summarized in table 3.

Table 3 - VNTR Profile Quality and Reproducibility

Sample Type	Amplification Success Rate (%)	Band Clarity	Profile Reproducibility (%)	Notes
Blood	100	Excellent	100	Sharp, distinct bands.
Saliva	75	Moderate	70	Faint bands, some smearing
Hair	20	Poor	15	High failure rate; when successful, bands were faint and smeared

As expected from the DNA yield data, blood samples performed exceptionally well, yielding a 100% amplification success rate with clear, sharp, and highly reproducible bands. The resulting DNA fingerprint was easily identifiable, demonstrating the reliability of blood as a source. Saliva samples showed a lower success rate of 75%, and even in successful amplifications, the bands were often faint and accompanied by some smearing. The reproducibility was also lower, indicating that lower DNA concentration and impurities negatively impacted the consistency of the profiling. Hair samples proved to be the most challenging, with an extremely low amplification success rate of only 20%. When profiles were successfully generated, the bands were faint and unreliable, a result directly attributable to the low DNA yield and high levels of contaminants. The provided gel images visually reinforce these findings, with the blood sample showing distinct bands, while the hair and saliva samples show fainter bands or heavy smearing, if any are visible at all.

4. Discussion

This study confirms that the success of forensic DNA profiling is overwhelmingly dictated by the quality and nature of the starting material (Budowle and van Daal, 2009). Whole blood consistently produced the highest yields and purity because of its abundance of nucleated leukocytes, making it the most reliable source for VNTR typing (Fischer *et al*., 2012). By contrast, saliva and hair impose intrinsic limitations: saliva typically contains fewer human epithelial cells and substantial bacterial and food-derived DNA that reduce human-target purity and introduce PCR inhibitors, producing variable or smeared amplification products (Woźniak *et al*, 2019; Bickley and Hopkins, 1999; Rana, 2025).

Hair, especially shed shafts without follicles, is further constrained by keratin and melanin that impede lysis and amplification, yielding low quantities of nuclear DNA even after

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aggressive extraction (Lawas *et al*, 2020; Barbosa *et al*, 2016; Fatima *et al*, 2024). These matrix-driven differences translated directly into reproducibility: blood profiles were uniformly robust (Dash *et al*, 2023), whereas saliva and hair showed higher intra- and inter-assay variability, incomplete profiles, and an elevated risk of false negatives (Dawnay *et al*, 2018; Brandhagen *et al*, 2018). Although VNTRs are largely of historical interest, the observed limitations explain why the field migrated to STR systems shorter amplicons that tolerate degradation and low-template inputs better (Gill,2002; Senge, 2011) and foreshadow the advantages of current and next-generation approaches.

Finally, the transition to sequencing-based workflows and advanced analytics promises higher resolution, improved handling of degraded or mixed samples, and richer forensic phenotyping when coupled with bioinformatics and machine-learning tools (Alonso *et al*, 2018; Dixon *et al*, 2006; Dash *et al*, 2023; Chandrashekar *et al*, 2024). Overall, the principal, enduring lesson is unchanged: rigorous sample collection, inhibitor mitigation, and DNA quantitation are decisive for reliable forensic genotyping (Rasekh, 2021; Siems *et al*, 2022).

Foundational and more recent studies document the same matrix-dependent constraints and the technological responses to them. Budowle and van Daal (2009) and Gill (2002) characterized the technical limits of length-based VNTR systems and the practical benefits that motivated STR adoption. Empirical work on matrix composition and extraction efficiency (Fischer *et al*., 2012; Woźniak *et al.*, 2019; Bickley and Hopkins, 1999) established how cellularity and microbial/background DNA affect yield and amplification. Hair-focused investigations (Lawas *et al*, 2020; Barbosa *et al*, 2016; Fatima *et al*, 2024; Brandhagen *et al*, 2018) have long recommended alternative strategies (e.g., mitochondrial analysis) when nuclear DNA is scarce. Comparative and reproducibility studies (Dash *et al*, 2023; Dawnay *et al*, 2018) quantified the practical failure modes for low-quality matrices, while reviews and methodological papers (Senge, 2011; Siems *et al*, 2022; Rasekh, 2021) traced the field's evolution. More recent methodological advances and prospects NGS readout of STR/SNP loci, increased discrimination by sequence-level alleles, and integration of AI for complex mixture interpretation are documented by Alonso *et al*., (2018), Dixon *et al*., (2006), Dash *et al*., (2023), and Chandrashekar *et al*., (2024).

5. Conclusion

This study successfully demonstrates that the efficiency and reliability of forensic DNA profiling using VNTR analysis are highly dependent on the type and quality of the biological sample. The experimental results clearly show that blood samples provide the highest quality and most reproducible VNTR profiles due to their abundance of high-quality nuclear DNA, confirming its status as the gold standard for forensic analysis.

In contrast, both saliva and hair samples presented significant challenges. Saliva DNA was frequently contaminated with bacterial impurities, which resulted in a lower amplification success rate and reduced profile quality. Hair samples, even when a follicle was present, yielded extremely low quantities of DNA and showed high levels of degradation and inhibitors, rendering the resultant VNTR profiles unreliable.

The findings of this report not only contribute to a historical understanding of forensic genetics but also highlight the fundamental challenges that continue to shape modern DNA analysis. The lessons learned that sample matrix, DNA degradation, and contamination are the most critical variables are directly applicable to contemporary practices, reinforcing the

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need for robust extraction protocols and a deep understanding of the biological evidence itself. This project demonstrates that the field's progression is a direct response to the problems highlighted here. Future research should explore modern methods for mitigating the effects of inhibitors and degradation, and a direct comparative analysis between VNTR and STR profiling would quantitatively demonstrate the benefits of modern technologies and underscore the continuous nature of innovation in this field.

References

1. Roewer, L., 2013. DNA fingerprinting in forensics: past, present, future. *Investigative genetics*, *4*(1), p.22.
2. Cantor, C.R. and Smith, C.L., 1999. *Genomics: the science and technology behind the human genome project*. John Wiley & Sons.
3. Mnookin, J.L., 2001. Fingerprint evidence in an age of DNA profiling. *Brook. L. Rev.*, *67*, p.13.
4. Murphy, E., 2007. The new forensics: Criminal justice, false certainty, and the second generation of scientific evidence. *Calif. L. Rev.*, *95*, p.721.
5. Butler, J.M., 2005. *Forensic DNA typing: biology, technology, and genetics of STR markers*. Elsevier.
6. Alketbi, S.K., 2023. The role of DNA in forensic science: A comprehensive review. *International Journal of Science and Research Archive*, *9*(02), pp.814-829.
7. Cramaro, A., 2024. Improving Chimerism Detection: STR Testing as a Critical Quality Control Measure in Molecular Diagnostics.
8. Tilstone, W.J., 2006. *Forensic science: an encyclopedia of history, methods, and techniques*. Bloomsbury Publishing USA.
9. Budowle, B. and van Daal, A., 2009. Extracting evidence from forensic DNA analyses: future molecular biology directions. *Biotechniques*, *46*(5), pp.339-350.
10. Fischer, J.C., Moog, R. and Giers, G., 2012. Quality control of leucocyte-reduced blood components: overestimation of WBC content due to nucleated red blood cells. *Vox sanguinis*, *102*(1), pp.79-81.
11. Rana, A.K., 2025. Challenging biological samples and strategies for DNA extraction. *Journal of Investigative Medicine*, *73*(6), pp.443-459.
12. Woźniak, M., Paluszkiewicz, C. and Kwiatek, W.M., 2019. Saliva as a non-invasive material for early diagnosis. *Acta Biochimica Polonica*, *66*(4), pp.383-388.
13. Bickley, J.A.N.E. and Hopkins, D.A.N.I.E.L., 1999. Inhibitors and enhancers of PCR. *Analytical Molecular Biology: quality and validation*, *8*, p.81.
14. Lawas, M., Otterstatter, L.M., Forger, L.V., Gray, J.E. and Donfack, J., 2020. A quantitative method for selecting a hair for nuclear DNA analysis. *Forensic Science International: Genetics*, *48*, p.102354.
15. Barbosa, C., Nogueira, S., Gadanho, M. and Chaves, S., 2016. DNA extraction: finding the most suitable method. In *Molecular microbial diagnostic methods* (pp. 135-154). Academic Press.
16. Fatima, T.F., Munawar, A. and Yaseen, J., 2024. Potential of hair analysis in the field of forensic sciences. *Forensic Insights and Health Sciences Bulletin*, *2*(1), pp.23-28.
17. Dash, H.R., Elkins, K.M., Al-Snan, N.R. and Dash, H.R., 2023. *Advancements in forensic DNA analysis*. Springer.
18. Dawnay, N., Flamson, R., Hall, M.J. and Steadman, D.W., 2018. Impact of sample degradation and inhibition on field-based DNA identification of human remains. *Forensic Science International: Genetics*, *37*, pp.46-53.

Corresponding Author: singhjagjeetjcc@gmail.com

19. Brandhagen, M.D., Loreille, O. and Irwin, J.A., 2018. Fragmented nuclear DNA is the predominant genetic material in human hair shafts. *Genes*, *9*(12), p.640.
20. Rasekh, M.E., 2021. *Characterizing VNTRs in human populations* (Doctoral dissertation, Boston University).
21. Gill, P., 2002. Role of short tandem repeat DNA in forensic casework in the UK—past, present, and future perspectives. *Biotechniques*, *32*(2), pp.366-385.
22. Senge, T., Madea, B., Junge, A., Rothschild, M.A. and Schneider, P.M., 2011. STRs, mini STRs and SNPs–a comparative study for typing degraded DNA. *Legal medicine*, *13*(2), pp.68-74.
23. Siems, E., Strandburg, K.J. and Vincent, N., 2022. Trade secre.
24. Dash, H.R., Elkins, K.M. and Al-Snan, N.R. eds., 2023. *Next Generation Sequencing (NGS) Technology in DNA Analysis*. Elsevier.
25. Alonso, A., Barrio, P.A., Müller, P., Köcher, S., Berger, B., Martin, P., Bodner, M., Willuweit, S., Parson, W., Roewer, L. and Budowle, B., 2018. Current state-of-art of STR sequencing in forensic genetics. *Electrophoresis*, *39*(21), pp.2655-2668.
26. Dixon, L.A., Dobbins, A.E., Pulker, H.K., Butler, J.M., Vallone, P.M., Coble, M.D., Parson, W., Berger, B., Grubwieser, P., Mogensen, H.S. and Morling, N., 2006. Analysis of artificially degraded DNA using STRs and SNPs—results of a collaborative European (EDNAP) exercise. *Forensic science international*, *164*(1), pp.33-44.
27. Chandrashekar, K., Niranjan, V., Vishal, A. and Setlur, A.S., 2024. Integration of artificial intelligence, machine learning and deep learning techniques in genomics: review on computational perspectives for NGS analysis of DNA and RNA seq data. *Current Bioinformatics*, *19*(9), pp.825-844.
28. Kasai, K., Nakamura, Y., & White, R. 1990. Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to human identity testing. *Journal of Forensic Sciences*, 35(5), 1196–1200.

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