Limit Detection of Short Tandem Repeats (STR) Analysis on Touch DNA Samples

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Abstract: *Forensic short tandem repeats (STR) profiling on touch DNA samples has emerged as a primary method for human identification. The stability and uniqueness of STR combination from the targeted locus in each individual make it a precision marker for human identification. Touch DNA samples can be found in traces of biological material shed from a person. This work aimed to identify the lowest concentration limit required for generating an interpretable DNA profile and the sensitivity of the STR loci applied. Touch DNA samples were collected from donors who were asked to hold a rope for 5 min. A double swab technique was used to lift the touch samples from the rope. These samples are subjected to DNA extraction and quantification. Two STR amplification cycles, 29 and 34 cycles, were used. DNA concentration greatly influences the success of amplifying the target allele at each STR locus to be interpreted into a complete DNA profile, shown by its allele peak. Touch DNA concentration > 0.25 ng can produce a complete DNA profile. LCN method successfully amplified touch DNA with a concentration 0.0625–0.25 ng/μL. Limit detection of touch DNA analysis is 0.25 ng/μL. Low-copy DNA can still be analyzed within 0.0625–0.25 ng/μL.*

Keywords: *short tandem repeats; touch DNA; DNA concentration*

■ INTRODUCTION

The evolution of DNA analysis in forensic investigations began with the groundbreaking discovery of DNA fingerprints in 1985, utilizing the RFLP technique for DNA profiling. The introduction of STR polymorphisms in the early 1990s represented a significant advancement, enabling efficient PCR-based analysis of low DNA concentrations (1–20 ng), and crucial for forensic cases. This shift revolutionized forensic science by enhancing sensitivity and discriminatory power, making short tandem repeat (STR) analysis via PCR the gold standard for individual identification from trace DNA samples. The inclusion of internationally recognized combined DNA index system (CODIS) core loci, initially 13 and expanded to 20 in 2017, further strengthens DNA profiling's accuracy and utility in criminal and humanitarian contexts, including disaster victim identification efforts [1-8].

Locard's exchange principle, articulated by Dr. Edmond Locard, states that every interaction between an individual and their environment results in trace material transfer. This means that contact between a person and their surroundings always results in the transfer of small amounts of physical evidence from one to the other. This foundational concept in forensic science emphasizes the reciprocal exchange of evidence, such as fibers, hairs, bodily fluids, and microscopic particles, during such interactions [1]. Forensic experts leverage this principle to reconstruct events, establish connections between individuals and crime scenes, and contribute to the resolution of criminal cases by meticulously examining trace evidence. The Locard's exchange principle underscores the interconnectedness between individuals and their surroundings, serving as a fundamental basis for scientifically grounded crime scene analysis and investigative practices.

DNA analysis on touch traces, pioneered by van Oorschot and Jones in 1997, marked a significant breakthrough in forensic science [9]. The application of touch DNA analysis has opened new avenues for forensic investigations, allowing for the identification of potential contributors to genetic material left behind at crime scenes. Touch DNA, or trace DNA, constitutes genetic material acquired through physical contact, presenting challenges related to its ambiguous DNA quantity and cellular origin. Crime scenes commonly yield touch DNA samples marked by inherently limited quantities, frequently falling below the threshold of 0.1 ng. This intrinsic limitedness in touch DNA specimens highlights a forensic challenge, necessitating meticulous and highly sensitive analytical techniques for reliable detection and profiling in criminal investigations [10-11].

The genetic profiles extracted from limited quantities of DNA present a challenge, often resulting in imperfect or partial representations and, in some instances, an inability to generate any identifiable DNA profile. The limitations of these diminutive samples, primarily due to their small size, contribute to incomplete genetic information. Factors such as degradation, contamination, or the limitedness of gene material can further complicate the generation of comprehensive profiles. As a consequence, forensic analysts face the intricate task of navigating these challenges, employing advanced methodologies and stringent quality control measures to enhance the accuracy and reliability of genetic profiles obtained from inherently constrained DNA samples.

Low copy number (LCN) PCR is a method that increases amplification cycles from standard PCR cycles to maximize the amplification of low-quantity DNA. Generally, this method is used to amplify DNA samples less than 0.2 ng. The PCR cycle used in the LCN method is 34 cycles, compared to the standard of 29 cycles [12-14]. However, research conducted by Kloosterman and Kersbergen [15] revealed that the LCN method causes an increased risk of contamination, allele removal, locus removal, increased stuttering, and the risk of excessive stochastic effects.

Given these challenges, establishing a standardized framework within laboratories is vital. This framework outlines appropriate methodologies for examining and interpreting results, ensuring consistency and reliability across forensic analyses. In the context of this study, a specific focus has been placed on defining the detection limit for touch DNA concentrations. This limit signifies the threshold at which a profile, considered as interpretable or full, can still be reliably generated. Additionally, our investigation involves a comprehensive evaluation of the sensitivity of STR loci, specifically targeting the 20 CODIS STR loci. This evaluation investigates their responsiveness to LCN DNA, acknowledging the significance of understanding the performance characteristics of these loci when dealing with minute DNA quantities. Through meticulous analyses and a commitment to methodological rigor, this study aims to contribute valuable insights to the development of robust and reliable methodologies for the forensic examination of touch DNA samples.

■ EXPERIMENTAL SECTION

Materials

Materials used in this study include touch DNA samples from nine donors. It was because the experiment comprised nine individuals based on the formula of

sample size, consisting of four females and five males, whose shedder levels were undisclosed. This study used the Eq. (1) of linear correlation between quantitative variables.

$$n = 4 + \left(\frac{\left(\frac{Z\alpha}{2} + Z\beta \right)}{0.5 \ln\left(\frac{1+r}{1-r} \right)} \right)^2 \quad (1)$$

Sample size calculation for trials that involve the estimate of linear correlation between two quantitative variables is dependent solely on the linear correlation coefficient. Each person as sample has a predetermined shedder status categorized as inclusion criteria of this sample research. The shedder status of those samples was divided into three levels based on DNA concentration [16-17]: high (> 0.25 ng/μL), moderate (0.0625–0.25 ng/μL), and low (< 0.0625 ng/μL). We also did not consider gender or secondary data from this research sample. Simulating scenarios pertinent to criminal activities involving the binding of victims with ropes, we employed non-porous plastic ropes for our experimentation. DNA analysis was conducted with PrepFiler™ BTA Forensic DNA Extraction kit, Quantifiler™ Trio DNA Quantification kit, GlobalFiler™ Amplification kit, μL HI-Di™ formamide, and 0.3 GeneScan™ 600 LIZ® Size Standard v2.0.

Instrumentation

Instruments used in this study include Applied Biosystem 7500 Real-Time PCR, Applied Biosystem ProFlex PCR System, Applied Biosystem 3500 Genetic Analyzer with GeneMapper Id.X v4 software, and some laboratory equipment such as laminar air flow (LAF), thermomixer, pipette, and tips.

Procedure

Ethical clearance

This research has obtained ethical clearance from the Faculty of Dental Medicine Health Research Ethical Clearance at Universitas Airlangga. The ethical clearance certificate is numbered 191/HRECC.FODM/II/2023, affirming that the study has undergone rigorous ethical review and meets the required standards for the protection of human subjects and the ethical conduct of research. This clearance underscores the commitment to maintaining ethical standards throughout the research process, ensuring the welfare and rights of participants involved in the study.

Touch DNA sampling

To maintain a pristine experimental environment, the plastic rope underwent thorough sterilization using DNAZap™ PCR DNA degradation solution in accordance with the prescribed protocol. Following sterilization, the rope was exposed to UV light in a LAF for 30 min to eliminate any residual DNA adhering to its surface [18]. To prevent cross-contamination, all nine donors were instructed to wash their hands with soap and then dry them with sterile tissues. Afterward, donors were permitted to participate in various activities for an hour, as their daily activities in different rooms but at the same room temperature, with explicit instructions to avoid touching others or wearing gloves. After 1 h, each donor was asked to gather in a room for touch DNA sampling. Each donor held a rope and performed rope-tying movements for a duration of 5 min. This sample collection approach aimed to maximize the collection of touch DNA samples on the rope and match them with criminal incidents related to rope tying.

Touch DNA recovery

The recovery of touch DNA from objects was executed employing the double swab technique. This method involves wetting a Nylon Swab (specifically 4N6 FlOQSwabs® Crime Scene) with nuclease free water. Subsequently, pressure is applied to the rope, which the donor has held, and the wet swab is employed to collect the initial sample. Following this, a dry nylon swab is used to wipe the same area previously sampled with the wet swab. This meticulous process ensures a comprehensive collection of touch DNA, enhancing the likelihood of capturing a diverse range of genetic material from the object under scrutiny [19-20].

DNA analysis

DNA extraction from all samples was conducted using the PrepFiler™ BTA Forensic DNA extraction kit, following the prescribed swab protocol. The concentrations of the extracted DNA were quantified in

real-time using quantitative PCR, specifically employing the Quantifiler™ Trio DNA Quantification kit as per the product manual [21]. After DNA quantification, 13 and 20 CODIS core STR loci were analyzed using the GlobalFiler™ Amplification kit, with a reaction volume set at 25 µL. The amplification process involved two cycles: the initial 29 PCR cycles, following the standard kit method, and a subsequent 34 cycles, representing the maximum cycle count for the LCN DNA method [22].

The resulting amplification product fragments underwent separation and detection on an ABI 3500 Genetic Analyzer (Life Technologies). For this, 1 µL of the PCR product was combined with a mixture comprising 8.7 µL HI-Di™ formamide and 0.3 GeneScan™ 600 LIZ® Size Standard v2.0 [23]. It is important to note that the DNA profiles used for comparison and positive controls were obtained from buccal swabs collected from each donor, serving as the reference samples. This meticulous and standardized approach in DNA analysis and profiling ensures the reliability and accuracy of the results. At the same time, the inclusion of positive controls enhances the robustness of the analytical process.

Statistical analysis

The data acquired took the form of DNA concentration and the count of loci, encompassing both 13 and 20 CODIS loci where both alleles were successfully amplified. Statistical analyses were conducted utilizing SPSS 16 software. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Subsequently, the non-parametric Pearson test was employed to explore the relationship between DNA concentrations and the successfully amplified alleles at both the 13 and 20 CODIS loci.

The Kruskal-Wallis test was performed to determine the effect of DNA concentration on allele expression at the locus. This test allows for an examination of whether there are significant differences in allele expression among various DNA concentration levels. Additionally, the Wilcoxon test was applied to investigate potential disparities in the count of loci between the 13 CODIS loci and the expanded set of 20 CODIS loci. Furthermore, this analysis was extended to encompass standard PCR, executed at 29 cycles, and the LCN PCR method, performed at 34 cycles. The Wilcoxon test aimed to determine if there were notable distinctions in the number of successfully amplified loci between these two PCR methodologies across the specified cycle counts.

■ RESULTS AND DISCUSSION

The results of this study focus on the relationship between DNA concentration and the number of successfully amplified alleles at each examined locus. Additionally, it explores the impact of additional PCR cycles on LCN DNA. Consequently, these findings contribute to establishing the limit of detection for touch DNA analysis. Studies have shown that the minimum STR loci for human identification is 13 CODIS core loci, which are CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11 along with the Amelogenin locus [24]. Higher discriminate power of STR loci is needed to assist in missing person investigation that need paternity DNA analysis [25]. Therefore, in this current study, we analyze the potential use of 13 core CODIS loci for touch DNA samples if the analysis of all 20 core CODIS loci cannot be achieved perfectly and show a full DNA profile.

Fig. 1 and 2 illustrate that the average number of amplified alleles at the 13 and 20 STR loci varies based on DNA concentration. The Pearson correlation test revealed a significant correlation between the DNA concentration variable and the number of successfully amplified alleles at both the 13 and 20 CODIS loci (Sig. (2-tailed) < 0.05). The results of the Kruskal-Wallis Test, with an Asymp. Sig. value of 0.000 < 0.05, lead to the rejection of the null hypothesis (H_0) and acceptance of the alternative hypothesis (H_1). This implies a significant difference in the number of alleles among different DNA concentration ranges (> 0.25, 0.0625–0.25, and < 0.0625 ng/µL) for both the CODIS 13 and 20 loci. Notably, a higher DNA concentration (> 0.25 ng/µL) is associated with a greater number of amplified loci. Based on the findings of this study, the selection of the number of STR loci does not show significant differences for touch DNA. While DNA profiles can be concluded from 13 STR loci, this research indicates that the low concentration of touch DNA has similar effects on both

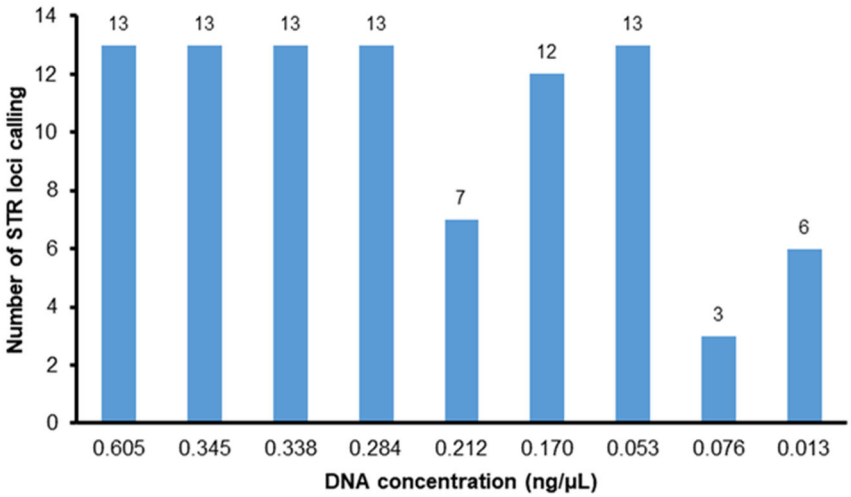


Fig 1. The number of amplified alleles at 13 STR CODIS loci (n = 108)

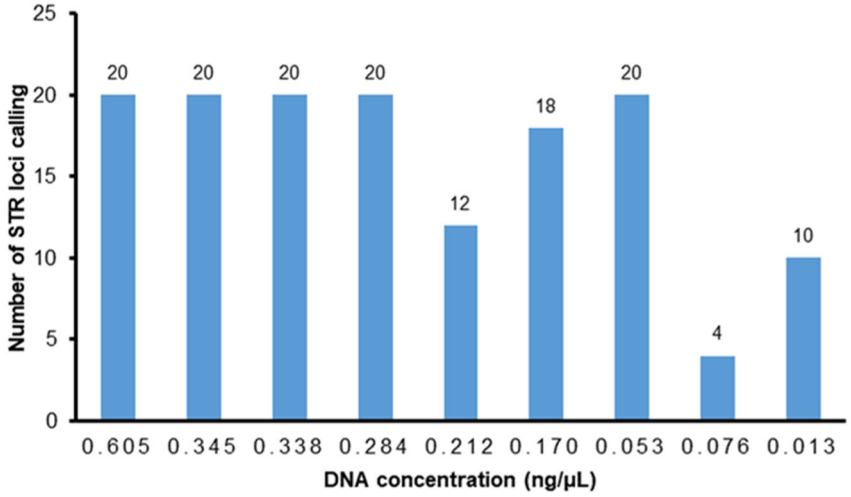


Fig 2. The number of amplified alleles at 20 STR CODIS loci (n = 108)

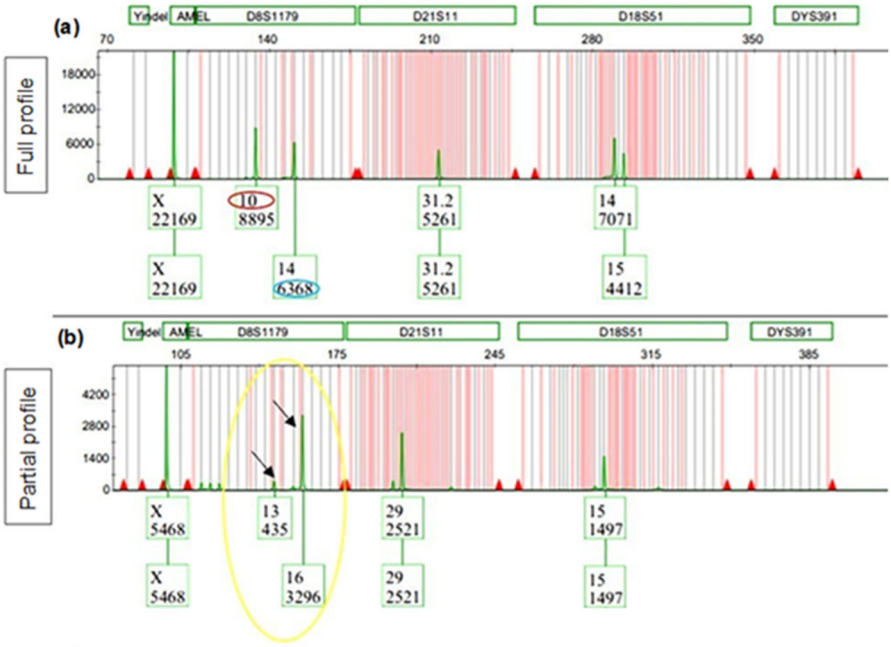
13 and 20 STR loci. Therefore, following previous literature stating that analyzing more STR loci enhances discriminative power [25]. Given its inherently low concentration, it is recommended that all 20 CODIS STR loci be used as human identification markers to increase the reliability of touch DNA results.

Table 1 highlights that each locus exhibits a distinct tendency for successful amplification based on the size of the target allele fragment. Longer fragment sizes are prone to drop out more than shorter fragment sizes, contributing to the variability observed in the amplification success across different loci. A well-interpreted DNA profile is characterized by the presence of one pair of alleles, both homozygote and heterozygote, at each examined locus. The evolution of the standard target locus from 1997 to 2017 transitioned from 13 CODIS core loci to the inclusion of 20 CODIS core loci [3]. The amplification of each STR fragment involves specific primers and is subsequently detected in a capillary electrophoresis system, presenting as peak fluorescent labeled alleles (Fig. 3).

The success of the amplification process depends on the quality and quantity of the DNA template. Notably, longer target alleles are more susceptible to degradation, posing a challenge for the primers to recognize and amplify these alleles effectively. Consequently, alleles at the locus may fail to amplify, leading to the generation of an imperfect DNA profile [26]. This phenomenon is

Table 1. The tendency of locus drop out at each STR loci

No.	Fragment size (bp)	STR locus	Repeats type	Sequence	Locus drop out (%)
1	138.52–183.23	D5S818	Tetranucleotide	AGAT	6.48
2	76.53–113.68	D2S441	Tetranucleotide	[TCTA][TCAA]	7.41
3	88.25–120.94	D22S1045	Trinucleotide	ATT	8.33
4	179.06–218.29	TH01	Tetranucleotide	TCAT	8.33
5	96.35-141.43	D3S1358	Tetranucleotide	[TCTG][TCTA]	9.26
6	118.68–171.49	D19S433	Tetranucleotide	AAGG	9.26
7	160.00–207.31	D1S1656	Tetranucleotide	[TAGA][TAGG]	9.26
8	182.88–239.64	D21S11	Tetranucleotide (complex)	[TCTA][TCTG]	9.26
9	198.94–243.22	D13S317	Tetranucleotide	TATC	9.26
10	223.55–378.44	FGA	Tetranucleotide (complex)	CTTT	9.26
11	114.23–171.40	D8S1179	Tetranucleotide	[TCTA][TCTG]	10.19
12	216.50–268.41	D12S391	Tetranucleotide (complex)	[AGAT][TAGAC]	10.19
13	262.53–298.45	D7S820	Tetranucleotide	GATA	10.19
14	156.56–209.39	vWA	Tetranucleotide	[TCTG][TCTA]	11.11
15	261.17–342.24	D18S51	Tetranucleotide	AGAA	11.11
16	283.09–318.44	CSF1PO	Tetranucleotide	TAGA	11.11
17	85.36–129.59	D10S1248	Tetranucleotide	GGAA	12.04
18	227.28–267.78	D16S539	Tetranucleotide	GATA	12.04
19	281-54–349.84	D2S1338	Tetranucleotide	AAGG	12.04
20	338.11–378.44	TPOX	Tetranucleotide	GAAT	12.04



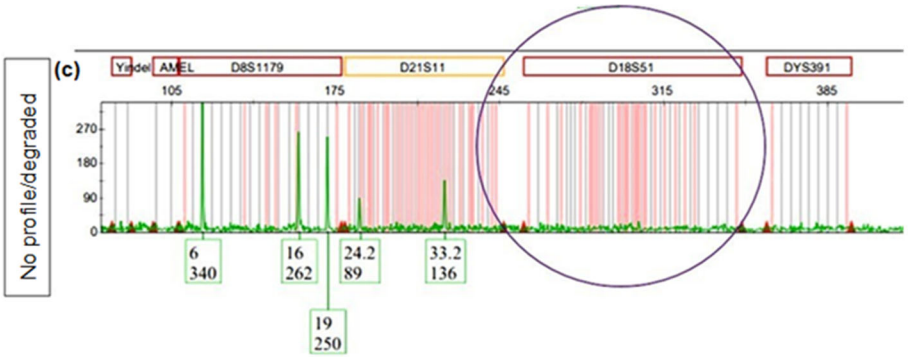


Fig 3. The electropherogram depicts STR profiles amplification and subsequent capillary electrophoresis from touch DNA samples. (a) red circle, at the number 10, as the allele or repeat number of the STR base, and blue circle, at the number 6368, signifies the length of the base in base pairs for the respective allele. (b) yellow circles and black arrows draw attention to irregularities in peak heights and denote imbalance peaks. (c) a purple circle is employed to indicate the presence of a drop-out allele, underscoring instances where a particular allele fails to amplify

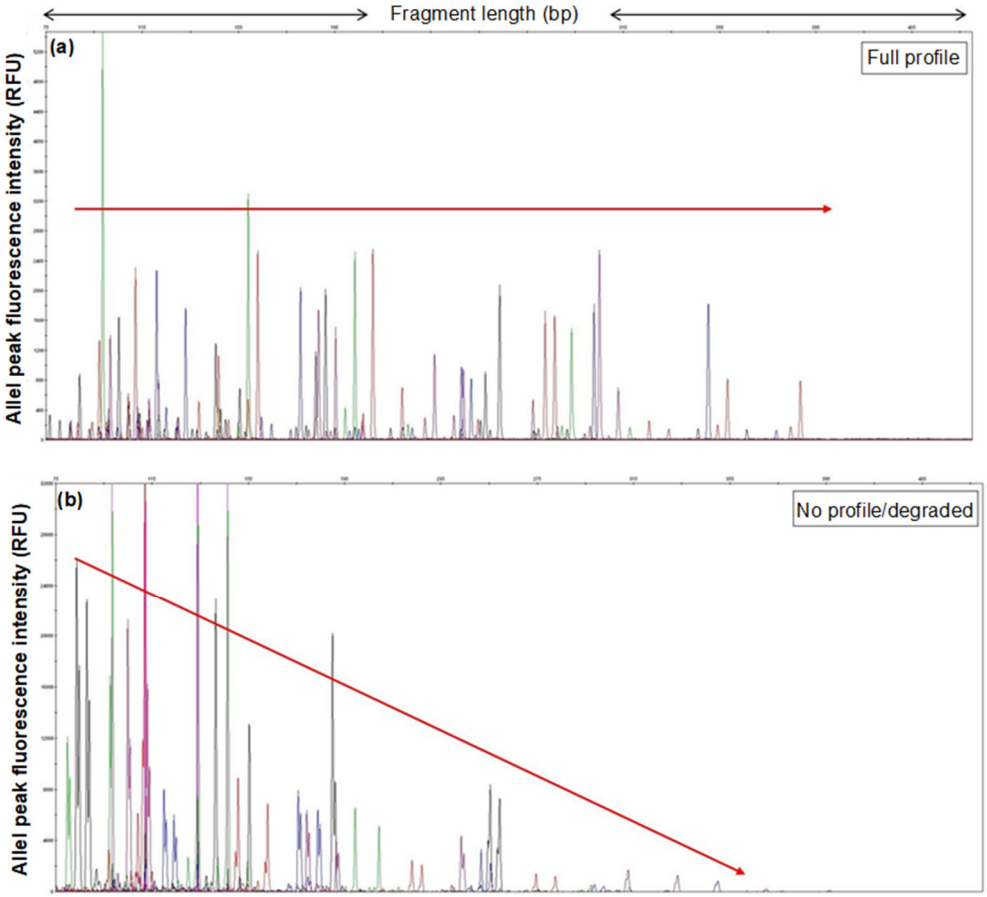


Fig 4. Amplification of touch DNA with (a) 0.0625–0.25 and (b) < 0.0625 ng/µL. The degradation slope shown in touch DNA concentration under 0.0625 ng/µL

visually depicted in Fig. 4, illustrating the impact of allele degradation on the amplification success and the resultant profile quality. Understanding these intricacies is crucial for accurate and reliable DNA profiling in forensic contexts.

From Fig. 4, we can see that the amplification of

touch DNA is illustrated in two scenarios: samples with DNA concentrations ranging of (a) 0.0625–0.25 ng/μL and (b) < 0.0625 ng/μL. The graphical representation highlights a degradation slope observed in touch DNA concentrations < 0.0625 ng/μL. In the upper segment, where DNA concentrations of 0.0625–0.25 ng/μL, the amplification process appears more robust, suggesting a relatively higher concentration of genetic material. On the contrary, the degradation slope becomes evident in the lower segment, where DNA concentrations are <0.0625 ng/μL. This signifies a decline in the amplification efficiency, likely due to the challenges associated with working with lower DNA concentrations. The observed degradation slope underscores the complexities inherent in amplifying touch DNA samples with deficient concentrations.

Touch DNA refers to trace genetic material obtained from skin epidermal cells through physical contact. The DNA in these samples can originate from anucleate or fragmentary keratinocytes from the hands, nucleated epithelial cells from the hands or other body parts, and extracellular DNA [27]. The nature of this touch DNA in its characteristics is highly dependent on environmental conditions, which generally leads to it being found in low quantities. Such challenges are integral to forensic analyses of trace DNA, requiring specialized techniques and methodologies to mitigate the impact of degradation and ensure accurate genetic profiling. The graphical representation serves as a visual aid in understanding the relationship between DNA concentration and the efficiency of the amplification process in touch DNA samples.

The findings from this research describe the relationship between DNA concentration and the success of amplifying target alleles at specific loci, thereby influencing the interpretability of resultant DNA profiles. It is imperative to contextualize these observations within the broader scientific discourse, where numerous studies [26] have consistently identified a crucial range of DNA concentrations—typically between 0.2 and 2 ng—yielding intact DNA profiles. Importantly, this current study reinforces and aligns with the established thresholds, emphasizing that DNA concentrations falling below 0.1 ng pose formidable challenges in terms of interpretability, primarily due to heterozygote imbalance, as visually exemplified in Fig. 3.

The congruence between the empirical results of this study and the existing scientific consensus is of particular significance. Specifically, the study underscores that the optimal concentration of touch DNA for generating interpretable profiles, both at the standard 13 CODIS loci and the expanded 20 CODIS loci, lies above the threshold of 0.25 ng. In contrast, the intermediary DNA concentration range of 0.065–0.25 ng manifests a spectrum of DNA profiles characterized by heterozygote peak imbalances, hindering the complete reading of allele profiles at these loci.

The observed heterozygote peak imbalances in the intermediate DNA concentration range can be attributed to stochastic PCR amplification dynamics. This phenomenon suggests that preferential amplification may occur at the beginning of the amplification cycle or alleles with smaller sizes may be favored during the amplification process [26]. This study's nuanced insights into the interplay of DNA concentration, amplification success, and the resulting interpretability of DNA profiles offer valuable contributions to the forensic scientific community. By emphasizing the critical importance of maintaining optimal DNA concentrations, especially in the context of touch DNA analysis, this research contributes to the refinement of forensic practices and methodologies, ultimately enhancing the accuracy and reliability of DNA profiling in the realm of criminal investigations.

The outcomes of the Wilcoxon test examining the difference in the number of amplified alleles between two distinct PCR methods reveal a statistically significant influence, as indicated by the Asymp. Sig. (2-tailed) value of 0.000, which is below the conventional significance threshold of 0.05. This inference is corroborated by the visual representation in Fig. 5, illustrating the impact of utilizing the LCN method as opposed to the standard method.

The comprehensive analysis of Fig. 5 provides additional insights into the interplay between DNA concentration and the effectiveness of the amplification

process through additional PCR cycles. In instances where the DNA concentration falls within the range of 0.0625–0.25 ng/μL, the execution of supplementary PCR cycles successfully amplifies all target loci. However, a distinctive pattern emerges with DNA concentrations < 0.0625 ng/μL. Despite the implementation of additional PCR cycles, not all target loci could be successfully amplified.

This observed trend underscores the complex relationship between DNA concentration and amplification efficacy, emphasizing the challenges posed by lower DNA concentrations. The statistical significance of the Wilcoxon test further underscores the impact of employing the LCN method, emphasizing its influence on the amplification outcomes in comparison to the standard method. These findings contribute valuable insights into the nuanced dynamics of PCR methodologies in the context of varied DNA concentrations, focused on the challenges associated with low DNA concentrations and the potential benefits of employing specialized amplification methods like LCN to overcome these challenges. By employing the standard method with 29 cycles and the LCN method with 34 cycles, the study aims to emphasize potential differences in the amplification efficiency and the number of loci successfully amplified under these conditions. This comparative analysis provides valuable insights into the performance characteristics of each method across different shedding levels, contributing to a comprehensive understanding of the factors influencing loci amplification in touch DNA samples with varying DNA concentrations.

STRs represent DNA regions characterized by 2–6 base pair-long repeated units, commonly referred to as alleles. The variability in the number of repeats among individuals results in unique allelic profiles, with most alleles differing in size by the length of a single repeat unit. Tetranucleotide repeats, featuring a 4-base repeat structure, are the most frequently targeted STR loci. Their relatively small size (<500 bases) renders them amenable to the amplification of low-quality and low-quantity DNA, and even smaller amplicon sizes have been strategically designed for profiling highly degraded DNA samples [16,28].

As indicated in Table 1, the analysis of touch DNA samples with diminished DNA quantity reveals instances of locus dropout, particularly pronounced in large fragment sizes (> 250 bp). These loci, being core loci, play a crucial role in the specific discrimination of individuals and should not be overlooked. To address the challenge of locus dropout in touch DNA samples,

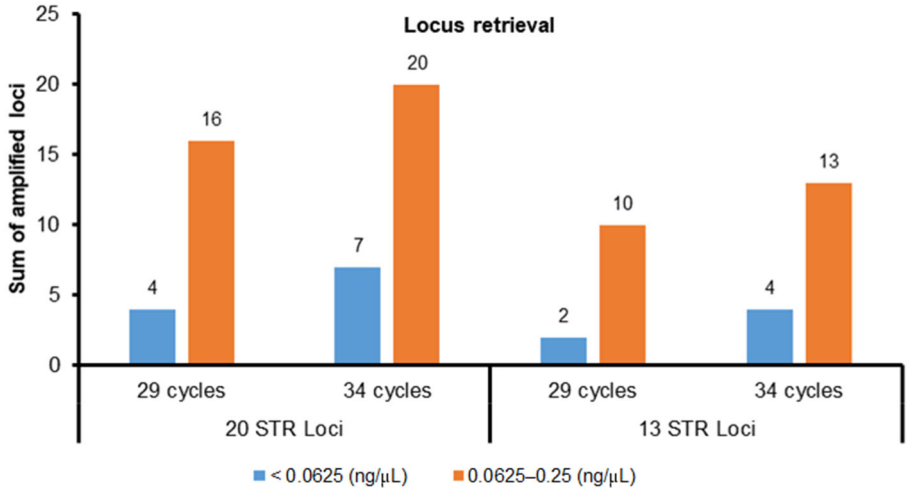


Fig 5. The examination of differences in the number of loci can be conducted through two distinct amplification methods: the standard method employing 29 cycles and the LCN method utilizing 34 cycles. This analysis is based on DNA concentrations obtained from individual shedders categorized as intermediate (0.0625–0.25 ng/μL) and low (< 0.0625 ng/μL)

a strategy involving smaller target sizes to reduce allele size at each locus is imperative. The implementation of the LCN method, involving an increase in amplification cycles from 29 to 34, has proven effective in enhancing the integrity of DNA profiles. Notably, 20% of touch DNA samples in this study transitioned from generating partial profiles at both 13 CODIS loci and 20 CODIS loci to complete profiles.

However, it is essential to acknowledge the inherent risks associated with the LCN method. High stutter peaks pose potential errors in allele reading and the interpretation of mixtures in touch DNA samples, introducing challenges linked to peak imbalance [26]. Consequently, while the LCN method demonstrates utility in optimizing DNA profile completeness, the associated risks require careful consideration.

Validation studies on commercial STR kits for DNA profiling have shown that the range of template DNA concentrations required to produce reliable DNA profiles is 0.2 to 2–3 ng, with an optimal DNA concentration of 1 ng [17]. Likewise, the finding of this research shows that touch DNA concentration within the range of 0.0625 to 0.25 ng, with LCN method, can still produce a fully interpretable DNA profile. However, the quality of the profile is not as interpretable as the DNA profile from the DNA template with 0.20–0.25 ng in concentration. This is because the LCN method is highly susceptible to stochastic effects, which can result in allelic drop-in, leading to potential misinterpretation. Drawing from the study's insights, we recommend establishing a limited concentration of touch DNA samples within the range of 0.20–0.25 ng to yield consistent, well-interpreted, full DNA profiles. This recommendation takes into account both the challenges posed by locus dropout in low-quantity DNA samples and the risks associated with implementing the LCN method, striving for a balanced approach in forensic DNA analysis.

One major limitation of this study is the inherently low quantity and potential degradation of touch DNA samples. These samples often fall below the threshold of 0.1 ng, making complete and accurate genetic profiles difficult. The low concentration poses challenges for reliable amplification and profiling, leading to partial or no identifiable DNA profiles in some cases. The use of LCN PCR, while increasing the amplification success of low DNA quantities, introduces risks such as contamination, allele drop-out, and stutter peaks. These stochastic effects can lead to erroneous allele calls and complicate the interpretation of mixed DNA samples. Longer alleles are more prone to drop out due to degradation, affecting the DNA profile's completeness. This variability in amplification success across different loci can hinder the ability to generate full profiles, particularly for touch DNA samples with lower DNA concentrations. Based on these limitations, further research is needed to develop and refine methodologies that enhance the sensitivity and specificity of DNA analysis from low-quantity samples. This could include optimizing PCR protocols by adding more enzymes or different sets of primers and improving the robustness of existing methods against stochastic effects with algorithm analysis.

■ CONCLUSION

The study identified the detection limitations of touch DNA, focusing on the ability of DNA concentration ranges (> 0.25, 0.0625–0.25, and < 0.0625 ng) to be amplified on STR CODIS loci as targeted DNA fragments. The comprehensive relationship and correlation analyses between DNA concentration and the number of CODIS loci (20 and 13) yielded statistically significant associations. Based on the study, the concentration of touch DNA samples between 0.20 and 0.25 ng consistently produces well-interpreted results. These findings serve as a robust guideline for forensic practitioners, emphasizing the crucial need to understand the limitations imposed by DNA sample quantity before embarking on the amplification process. Despite the promising potential of the LCN method to elevate the integrity of DNA profiles, the study acknowledges and addresses the inherent risks associated with its implementation. Notably, the LCN method may introduce challenges, including the potential for dropouts in touch DNA samples and the presence of various risks, such as high stutter peaks, which can influence the accuracy of allele

reading and the interpretation of mixtures. The study, therefore, advocates the adoption of 20 STR loci in the profiling of touch DNA samples. This strategic choice is positioned to provide an enhanced discriminatory capability and mitigate the risks associated with low DNA concentrations. Such a recommendation aligns with the ongoing pursuit of optimal forensic practices and methodologies, contributing to the broader discourse on advancing forensic science and ensuring the fidelity of DNA profiling outcomes in touch DNA analysis.

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■ CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

■ AUTHOR CONTRIBUTIONS

Vira Saamia is the primary researcher. Ahmad Yudianto and Muktiningsih Nurjayadi supervised the research and manuscript writing. Novitasari contributed to the manuscript revision. Abdul Hadi Furqoni was responsible for data analysis. All authors read and approved the final manuscript.

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