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Title	Enhancing Trace DNA Profile Recovery in Forensic Casework Using the Amplicon RX Post-PCR Clean-up Kit
Type	Article
URL	https://clok.uclan.ac.uk/54822/
DOI	https://doi.org/10.2139/ssrn.5116732
Date	2025
Citation	Aljanahi, Naeema S., Alketbi, Salem K, Almheiri, Maryam M., Alshehhi, Suaad A., Sanqoor, Afra N. and Alghanim, Hussein J. (2025) Enhancing Trace DNA Profile Recovery in Forensic Casework Using the Amplicon RX Post-PCR Clean-up Kit. Scientific Reports, 15.
Creators	Aljanahi, Naeema S., Alketbi, Salem K, Almheiri, Maryam M., Alshehhi, Suaad A., Sanqoor, Afra N. and Alghanim, Hussein J.

It is advisable to refer to the publisher's version if you intend to cite from the work. https://doi.org/10.2139/ssrn.5116732

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Enhancing trace DNA profile recovery in forensic casework using the amplicon RX post-PCR clean-up kit

Naeema S. Aljanahi, Salem K. Alketbi✉, Maryam M. Almheiri, Suaad A. Alshehhi, Afra N. Sanqoor & Hussein J. Alghanim

This study evaluated the effectiveness of the amplicon RX post-PCR clean-up kit in enhancing trace DNA profile recovery from forensic casework samples amplified using the GlobalFiler PCR amplification kit. The impact of post-PCR clean-up on allele recovery and signal intensity was assessed in both trace casework samples and control samples across a range of DNA concentrations. The results showed that the amplicon RX method significantly improved allele recovery compared to the 29-cycle protocol ($p = 8.30 \times 10^{-12}$) and achieved slightly better results than the 30-cycle protocol ($p = 0.019$). Additionally, the Amplicon RX method demonstrated a significant increase in signal intensity ($p = 2.70 \times 10^{-4}$), reflecting improved sensitivity in detecting trace DNA profiles compared to the 30-cycle protocol. In the evaluation of control samples, the amplicon RX method consistently outperformed both the 29- and 30-cycle protocols, especially at lower DNA concentrations (D3: 0.001 ng/µL). While the performance of all methods declined at the lowest concentration (D4: 0.0001 ng/µL), the Amplicon RX method still demonstrated superior allele recovery ($p = 0.014$ compared to 29 cycles; $p = 0.011$ compared to 30 cycles). Therefore, the Amplicon RX method should be widely adopted in forensic laboratories to enhance the analysis of extremely low-template and compromised samples. These findings highlight the potential of the amplicon RX post-PCR clean-up kit to improve trace DNA analysis in forensic casework. Further research is recommended to validate these results and explore its broader application in forensic DNA analysis, particularly in complex DNA mixtures and extremely low-template samples.

Keywords Forensic science, Trace DNA, Touch DNA, DNA recovery, GlobalFiler PCR amplification kit, Amplicon RX post-PCR clean-up kit

Forensic DNA analysis has transitioned from an academic exploration to a globally recognized practice, playing a crucial role in criminal investigations by providing critical genetic evidence[1–5]. Touch DNA refers to the minute quantities of DNA deposited on surfaces or objects through direct contact, often when an individual touches or handles an item[1]. Unlike body fluids such as blood, saliva, or semen, touch DNA is not visible to the naked eye and may originate from sources like skin cells or sweat[6,7]. Often referred to as “trace DNA” due to its small quantity, the precise origin of touch DNA remains a subject of ongoing research. It is hypothesized to consist of material from anucleate corneocytes (dead skin cells), nucleated cells (from the hands or transferred from other parts of the body), or cell-free DNA found in sweat[7].

Touch or trace DNA is of particular significance in forensic investigations, as it can be recovered from a broad array of touched items or surfaces, enabling the establishment of links between suspects and criminal activities. Common sources of trace DNA include tools, weapons, clothing, and various other objects handled by individuals[8–12]. However, the recovery of touch DNA poses challenges compared to other forms of biological evidence due to several influencing factors, such as the nature of the surface, collection methods, and extraction techniques[13–15], as well as the effects of time and environmental conditions[16–18]. Despite these challenges, trace DNA samples, often characterized by limited quantity, degradation, and contamination, continue to represent a significant obstacle in forensic casework[19–22].

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The advent of PCR-based amplification techniques, such as the GlobalFiler PCR Amplification Kit, has markedly enhanced the sensitivity and reliability of DNA analysis[23,24]. Nevertheless, trace DNA samples often contain impurities, including residual primers, dNTPs, enzymes, and other PCR reagents, which can adversely affect downstream applications and compromise the reliability of generated DNA profiles[25]. In response to these challenges, post-PCR clean-up methods were developed to purify amplified DNA, thereby removing inhibitory substances and enhancing the recovery of trace DNA[25–27]. One such promising method is the Amplicon Rx Post-PCR Clean-up kit (Independent Forensics), which has demonstrated effectiveness in eliminating contaminants while preserving DNA integrity[28]. However, the application of this method in purifying GlobalFiler PCR products within forensic casework samples remains underexplored.

In forensic DNA laboratories, profiles are generated from both evidence and reference samples using advanced molecular biological techniques, such as multiplex PCR[29]. Commercial kits typically recommend a 25 µL PCR reaction volume, although only a fraction of this volume is used for capillary electrophoresis (CE) analysis. Consequently, a substantial portion of the PCR product is often discarded, potentially limiting the quality of DNA profiles for samples with limited DNA quantities. Moreover, the process of electrokinetic injection, which introduces amplified DNA fragments into the capillary, frequently encounters inhibitors present in the PCR reaction. These inhibitors can reduce the CE signal, and simply increasing the quantity of PCR product added to the formamide/size standard solution does not resolve this issue[25]. As such, concentration and purification of the amplicons are critical to enhancing the CE signal, and this is where the Amplicon Rx Post-PCR Clean-up kit offers considerable potential[28].

Trace DNA samples typically contain very small amounts of DNA, which often result in insufficiently robust profiles for identification. Some of these limitations can be addressed through improvements in the collection of touch DNA using validated techniques[30–37]. However, to generate DNA profiles that meet laboratory acceptance thresholds, it is essential to recover sufficient data beyond this threshold. The Amplicon Rx Post-PCR Clean-up kit (Independent Forensics) facilitates this by allowing for the recovery of the remaining 90–95% of amplicons in the PCR reaction tube[28]. This approach ensures that all DNA fragments generated during multiplex PCR are effectively utilized, improving the efficiency of electrokinetic injection into the capillary and significantly enhancing the intensity of dye-labeled amplicon signals.

This study aimed to evaluate the effectiveness of the Amplicon Rx Post-PCR Clean-up kit in enhancing the sensitivity of multiplex short tandem repeat (STR) DNA profiling without increasing the number of PCR cycles in trace DNA casework samples amplified using the GlobalFiler PCR Amplification Kit. Specifically, the study compared the performance of the Amplicon Rx Post-PCR Clean-up kit on GlobalFiler PCR products amplified for 29 cycles with the conventional 30-cycle protocol. Additionally, control experiments were conducted using single-source DNA samples that underwent serial dilutions to assess the kit's performance across a range of DNA concentrations. The objective was to determine which protocol, in conjunction with the Amplicon Rx Post-PCR Clean-up kit, more effectively improves trace DNA profile recovery. By evaluating both trace casework samples and control dilutions, this research provides valuable insights into the potential benefits of the Amplicon Rx Post-PCR Clean-up kit for forensic DNA workflows. Enhancing the sensitivity of multiplex STR DNA profiling without altering the number of PCR cycles could contribute to more accurate and reliable DNA profiles from trace casework samples, thereby improving the overall effectiveness of forensic DNA analysis.

Materials and methods

DNA recovery, extraction, and quantification

A total of 55 samples were collected from a variety of touched or used items, including tools (e.g., screwdrivers and weapons such as knives, axes, machetes, and bats), stolen items (e.g., mobile phones, wallets, and handbags), wearable items (such as clothing, shoes, sandals, jewelry, and glasses), packaging materials (e.g., plastic bags or containers used to transport drugs), vehicles (e.g., cars and motorcycles), and other touched items. Sample collection was performed using a cotton swab moistened with approximately 100–150 µL of molecular-grade water, which was sprayed onto the swab using a plastic spray bottle[1,30].

The collected samples were processed using the PrepFiler Express DNA extraction kit (Thermo Fisher Scientific) with the Automate Express liquid handling system (Thermo Fisher Scientific), following the manufacturer's protocol. Complete swab heads were utilized, and the final elution volume was set to 50 µL. DNA concentrations were measured using the Investigator Quantiplex Pro DNA Quantification Kit, according to the manufacturer's recommendations, on the QuantStudio 5 Real-Time PCR (qPCR) system (Thermo Fisher Scientific).

Control samples

Four single-source casework trace DNA samples, previously quantified using the same method as mentioned above, were selected for a control experiment comparing the Amplicon Rx Post-PCR Clean-up kit with both 29-cycle and 30-cycle PCR protocols. The initial DNA concentrations (ng/µL) of these samples were: Sample 1 (S1) – 11.2197 ng/µL, Sample 2 (S2) – 5.7061 ng/µL, Sample 3 (S3) – 7.6795 ng/µL, and Sample 4 (S4) – 1.7046 ng/µL. Each sample was diluted to a standardized concentration of 1 ng in 100 µL using TE buffer, applying the $C1V1 = C2V2$ dilution equation.

Subsequently, each of the four control samples underwent a series of four sequential dilutions to create a range of DNA concentrations for testing. For the first dilution (D1), each sample was diluted to 0.1 ng/µL: S1 = 8.9 µL of stock DNA in 91.1 µL TE, S2 = 17.5 µL of stock DNA in 82.5 µL TE, S3 = 13.0 µL of stock DNA in 87.0 µL TE, and S4 = 58.7 µL of stock DNA in 41.3 µL TE. For the second dilution (D2), 10 µL of D1 was diluted in 90 µL of TE to achieve a concentration of 0.01 ng/µL. This process was repeated for the third (D3) and fourth (D4) dilutions, yielding concentrations of 0.001 ng/µL and 0.0001 ng/µL, respectively. Each series of dilutions

for the four samples was duplicated three times, resulting in a total of 48 samples per protocol, which were then used for the control experiment to evaluate the Amplicon Rx Post-PCR Clean-up kit.

DNA amplification
Following quantification, the casework trace DNA samples, which had concentrations below 0.0028 ng/μL (ranging from 0.1 to 2.8 pg), were subjected to amplification using the GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific, Cat. No. 4476135) on a Veriti Thermal Cycler (Thermo Fisher Scientific, Cat. No. 4375305). Amplifications were performed for both 29 cycles and 30 cycles, following the manufacturer's recommended conditions. Each PCR reaction used 15 μL of extracted DNA (representing the maximum volume of DNA) combined with 10 μL of PCR reaction mixture, consisting of 7.5 μL of Master Mix and 2.5 μL of Primer Set, resulting in a total reaction volume of 25 μL. Control samples underwent the same amplification protocol as the casework trace samples.

Post-PCR clean-up and capillary electrophoresis
For capillary electrophoresis (CE), 1 μL of PCR-amplified product from both the 29-cycle ($n = 55$) and 30-cycle ($n = 55$) samples was mixed with 9.5 μL of Hi-Di formamide and 0.5 μL of GeneScan 600 LIZ Size Standard (Thermo Fisher Scientific) per sample. For post-PCR purification using the Amplicon Rx protocol (Fig. 1, $n = 55$), 120 μL of Amplicon Rx binding buffer was added to 24 μL of the PCR product from the 29-cycle amplification after removing 1 μL for initial CE analysis. The mixture was loaded onto an Amplicon Rx spin column and centrifuged at 12,000 × g for 3 min. The flow-through was discarded, and the column was transferred to a new collection tube. Elution was achieved by adding a mixture of 20 μL of Hi-Di formamide and 0.5 μL of GeneScan 600 LIZ Size Standard. The column was incubated at room temperature for 5 min, followed by centrifugation at 12,000 × g for 2 min to recover the amplicons. Finally, 11 μL of the eluted product was subjected to CE analysis.

The samples were analyzed using an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific) with a 36-cm capillary array and POP-4 polymer (Thermo Fisher Scientific) under standard injection conditions (1.2 kV, 24 s). STR data were sized and typed using GeneMapper ID-X Software Version 1.6 (Thermo Fisher Scientific), adhering to in-house validation guidelines with a minimum detection threshold of 75 RFUs. Control samples followed the same post-PCR clean-up and CE protocols as the casework trace samples.

Data analysis
Statistical analysis was performed using Python, where paired t-tests were conducted to compare the experimental conditions. Paired t-tests were chosen to evaluate the differences between the three protocols—29 cycles, 30 cycles, and Amplicon Rx—as the same samples were tested across different conditions, allowing for direct comparison. The t-statistic values were calculated based on the difference in means between conditions and the variance of the differences within samples. The p-values were derived from the t-distribution. Summary statistics were calculated for each method, including mean, standard deviation, minimum, and maximum values, which were essential for understanding the differences in allele recovery and peak height intensity (RFU) among the methods.

Additionally, comparisons across a dilution series (D1-D4) were performed to evaluate the performance of each method at varying DNA concentrations. All data analysis and visualization were performed using Python libraries, including 'scipy' for statistical tests and 'matplotlib' for graphical representation. Line plots were used to visualize the average number of alleles recovered across dilutions, while bar charts and box plots were employed to depict the differences in RFU values between the methods, providing insights into method performance at varying DNA concentrations and signal intensities.

Quality control was maintained by including negative controls in the collection and extraction methods, which were confirmed to be free of DNA upon quantification and amplification. Positive controls and allelic ladders were used to verify the accuracy of size determinations and STR typing, with the results aligning with the manufacturer's standards. The inclusion of these controls ensured the reliability, integrity, and accuracy of the experiment.

Results
Analysis of trace casework samples
A comparison of allele recovery between the three methods—29 PCR cycles, 30 PCR cycles, and the Amplicon Rx post-purification—on trace casework samples (ranging from 0.1 to 2.8 pg of DNA) demonstrated that the method significantly influenced the number of alleles recovered. The mean number of alleles recovered was 12.6 for the 29-cycle protocol, 22.4 for the 30-cycle protocol, and 26.3 for the Amplicon Rx post-purification method, which yielded the highest recovery. Statistical analysis confirmed a significant improvement in allele recovery when increasing from 29 to 30 PCR cycles ($p = 6.69 \times 10^{-13}$) and when using the Amplicon Rx post-purification method over the 29-cycle protocol ($p = 8.30 \times 10^{-12}$). The difference between the 30-cycle protocol and the Amplicon Rx post-purification method was smaller but remained statistically significant ($p = 0.019$), as shown in Fig. 2.

The peak height intensity (RFU) of 40 single-source trace samples was evaluated to compare the results obtained from the 30-cycle method and the Amplicon Rx post-purification (RX) treatment. A significant difference in average RFU signal was observed between the two methods ($p = 2.70 \times 10^{-4}$). The average RFU recorded for the 30-cycle method was 253.6, while the RX method resulted in an average RFU of 453.7.

The RX method produced higher average RFU but also introduced greater variability in peak heights across samples compared to the more consistent but lower signal observed with the 30-cycle method (Figs. 3 and 4).

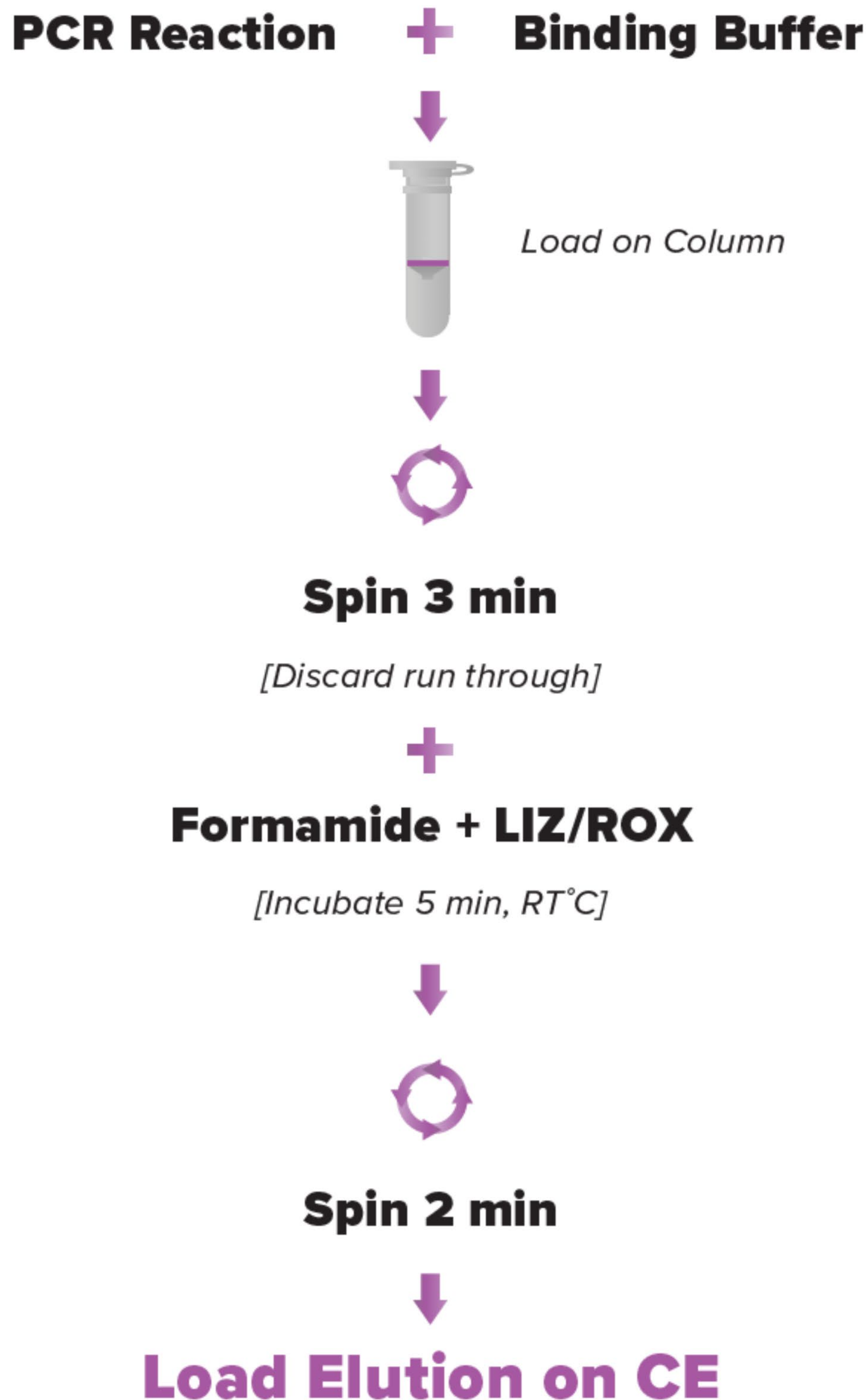

Fig. 1. An overview of the Amplicon Rx protocol. This post-PCR processing step is essential for purifying amplified DNA and removing inhibitory substances that can interfere with downstream applications. The protocol begins by adding the Amplicon Rx binding buffer to the PCR reaction, facilitating the binding of DNA fragments to the spin column matrix. The reaction mixture is then loaded onto the Amplicon Rx spin column and centrifuged to remove any residual contaminants from the liquid phase. Following centrifugation, the column is transferred to a fresh collection tube, where a mixture of Hi-Di formamide and GeneScan 600 LIZ Size Standard (Thermo Fisher Scientific) is added to elute the purified amplicons. After incubation, a second round of centrifugation is performed to recover the DNA fragments. The purified elution is then prepared for capillary electrophoresis (CE) analysis, enabling precise sizing and detection of short tandem repeat (STR) loci, which are crucial for forensic DNA profiling.

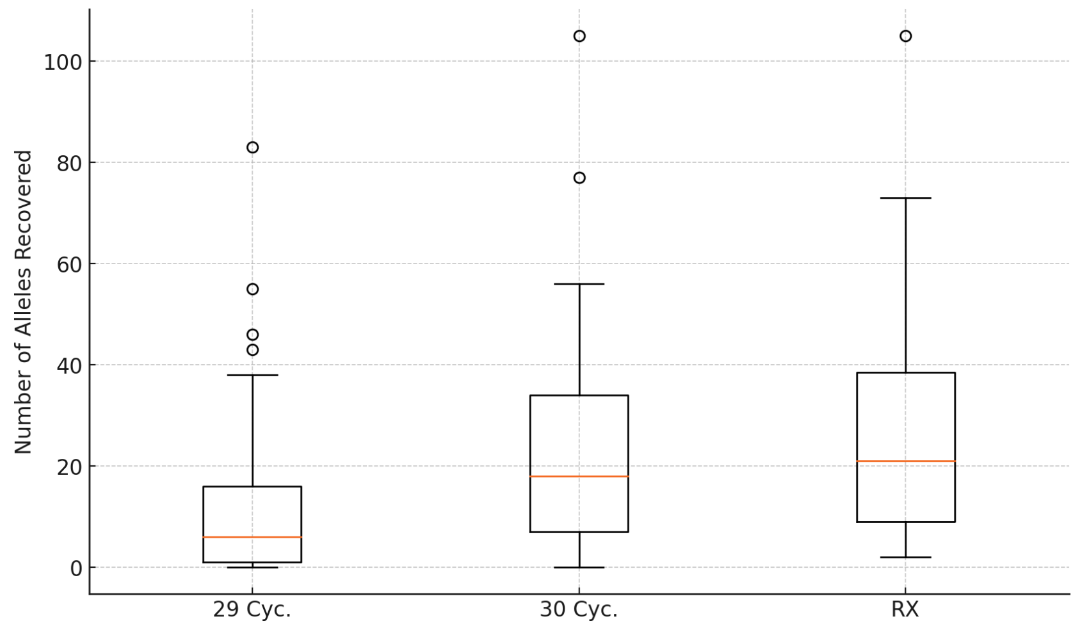

Fig. 2. The distribution of allele recovery from amplified and post-purified trace DNA samples ($n = 165$) using the GlobalFiler PCR Amplification Kit. The samples were amplified under three conditions: 29 PCR cycles (29 Cyc.), 30 PCR cycles (30 Cyc.) following the manufacturer's protocol, and post-PCR purification using the Amplicon Rx protocol (RX). The box plot illustrates the variability in allele recovery across the three methods. While the 30-cycle protocol showed improved recovery over 29 cycles, the RX post-purification method yielded the highest allele recovery with less variability, indicating more consistent performance. The mean number of alleles recovered was 12.6 for 29 cycles, 22.4 for 30 cycles, and 26.3 for the RX method. Statistical comparisons yielded the following results: 29 cycles vs. 30 cycles (t-statistic = − 9.36, $p = 6.69 \times 10^{-13}$), 29 cycles vs. RX (t-statistic = − 8.67, $p = 8.30 \times 10^{-12}$), and 30 cycles vs. RX (t-statistic = − 2.41, $p = 0.019$).

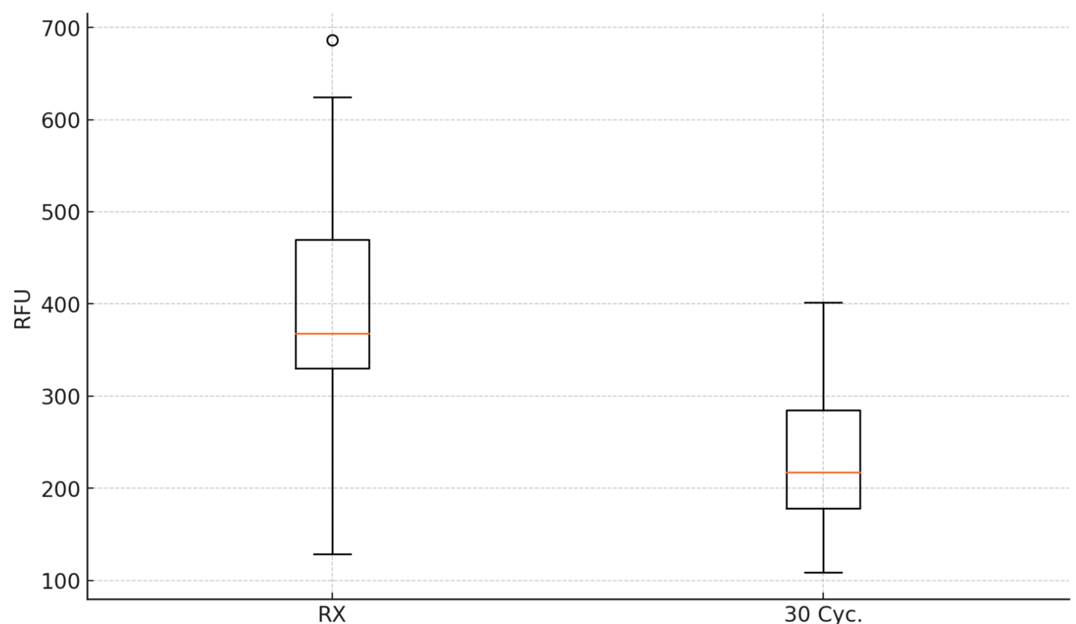

Fig. 3. The distribution of RFU values detected from trace DNA samples ($n = 40$) using the 30-cycle method and the Amplicon Rx post-purification protocol (RX). The box plot highlights the variability in signal intensity between the two methods, with the RX method producing a higher average RFU and greater variability in peak heights compared to the 30-cycle method. The mean RFU for the 30-cycle method was 253.6, while for the RX method, it was 453.7. A statistically significant difference in RFU was observed between the two methods (t-statistic = − 4.383, $p = 2.70 \times 10^{-4}$).

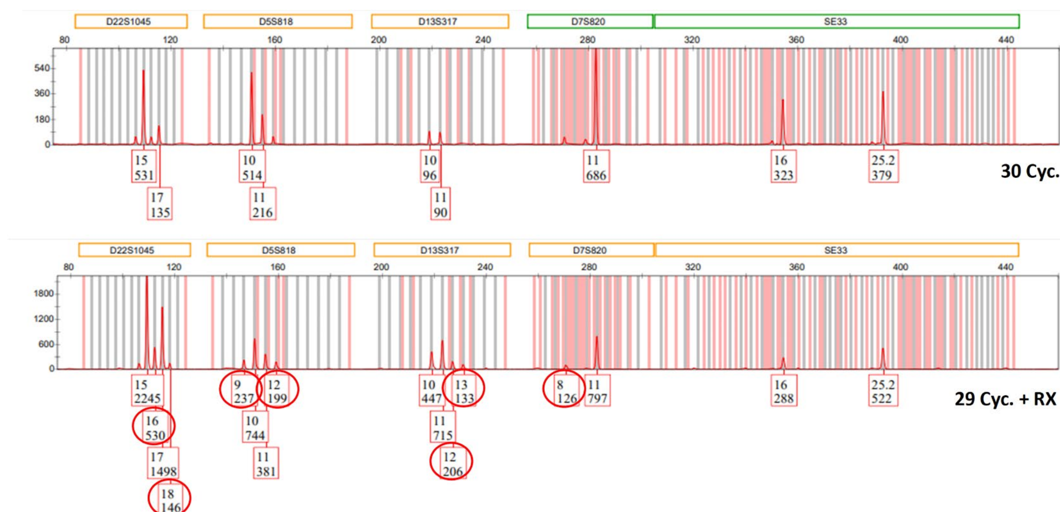

Fig. 4. Comparison of electropherograms at five loci (D22S1045, D5S818, D13S317, D7S820, and SE33) obtained from an amplified and post-purified trace DNA sample (DNA concentration 0.0017 ng/μL) using the GlobalFiler PCR Amplification Kit. The samples were amplified with 29 PCR cycles (29Cyc.) and 30 PCR cycles (30Cyc.) according to the manufacturer's protocol, with additional post-purification performed using the Amplicon Rx protocol (RX). The electropherograms illustrate the marked improvements in allele recovery (circled in red) and peak height intensity (RFU) achieved using the Amplicon Rx Post-PCR Clean-up Kit.

Evaluation of control samples

Allele recovery from four single-source diluted trace control DNA samples (0.1–0.0001 ng/μL) was evaluated to compare the results obtained using the 29-cycle method, 30-cycle method, and the Amplicon Rx post-PCR clean-up treatment. The analysis revealed a significant difference in allele recovery between the Amplicon Rx method and both PCR protocols.

The Amplicon Rx method recovered an average of 29 alleles, compared to 22 for the 29-cycle protocol and 24 for the 30-cycle protocol, with no meaningful difference observed between the 29- and 30-cycle methods. No statistically significant difference was observed between the 29-cycle and 30-cycle protocols ($p = 0.167$). Statistically significant differences were observed between the Amplicon Rx method and the 29-cycle protocol ($p = 0.014$) and between the Amplicon Rx method and the 30-cycle protocol ($p = 0.011$). While both the 29- and 30-cycle methods showed similar recovery levels, the Amplicon Rx method exhibited a wider range of recovery, particularly at lower DNA concentrations (Fig. 5).

The control samples underwent a series of four sequential dilutions (D1–D4) to generate a range of DNA concentrations for testing, beginning with 0.1 ng/μL for D1, followed by D2 (0.01 ng/μL), D3 (0.001 ng/μL), and D4 (0.0001 ng/μL). The performance of the three methods—29 Cycles, 30 Cycles, and Amplicon Rx—varied significantly across these dilutions. At the highest concentration (D1), all three methods performed similarly, with an average of 40 alleles recovered for each method. In D2, the 29 Cycles method recovered an average of 39.25 alleles, while the 30 Cycles method recovered 38 alleles, and the Amplicon Rx method maintained consistent performance, recovering 40 alleles. However, as the DNA concentration decreased in D3, the performance of the 29 Cycles and 30 Cycles methods dropped sharply, recovering an average of 6 and 10.75 alleles, respectively. In contrast, the Amplicon Rx method recovered an average of 33 alleles at D3. Statistical testing confirmed significant differences between the methods at this dilution ($p < 0.05$). At the lowest concentration (D4), the Amplicon Rx method showed a decline, recovering an average of 5.75 alleles, compared to 1 allele for the 29 Cycles method and 2 alleles for the 30 Cycles method. These results are illustrated in Fig. 6.

Discussion

The recovery and analysis of trace DNA profiles from forensic casework samples present significant challenges due to the limited quantities and potential degradation of DNA[4,7]. This study evaluated the effectiveness of the Amplicon RX Post-PCR Clean-up Kit in enhancing trace DNA profile recovery using the GlobalFiler PCR Amplification Kit. Specifically, the impact of the Amplicon RX method on DNA profile recovery, allele detection, and peak height intensity was assessed.

Consistent with previous studies, the Amplicon RX Post-PCR Clean-up Kit significantly improved allele recovery from trace DNA samples[25]. By effectively removing PCR inhibitors and impurities, the Amplicon RX method increased sensitivity and enhanced the detection of low-template DNA, resulting in the recovery of more alleles. Notably, it performed better than the 29-cycle protocol and, in some cases, produced results comparable to the standard 30-cycle protocol. This highlights the value of integrating post-PCR clean-up in forensic workflows, particularly in cases where time and cost savings are essential.

While higher PCR cycle numbers (such as the 30-cycle protocol) can boost sensitivity, they can also increase the risk of allele drop-in, particularly in low-quantity samples. Allele drop-in can occur due to contamination,

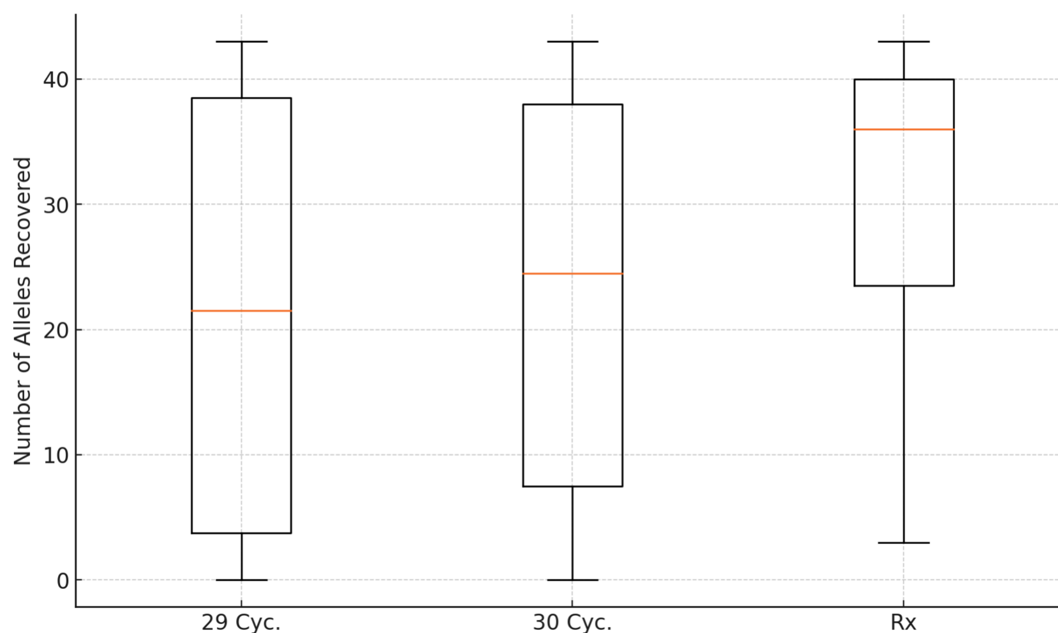

Fig. 5. The distribution of allele recovery from amplified and post-purified single-source diluted trace control DNA samples ($n = 144$) using the GlobalFiler PCR Amplification Kit. The samples were amplified under three conditions: 29 PCR cycles (29 Cyc.), 30 PCR cycles (30 Cyc.) following the manufacturer's protocol, and post-PCR purification using the Amplicon Rx protocol (RX). The box plot illustrates the variability in allele recovery across the three methods. The average number of alleles recovered was 22 for the 29-cycle protocol and 24 for the 30-cycle protocol, showing no significant difference between the two methods. In contrast, the Amplicon Rx method recovered an average of 29 alleles. Statistical comparisons yielded the following results: 29 cycles vs. 30 cycles (t-statistic = − 1.45, $p = 0.167$), 29 cycles vs. RX (t-statistic = − 2.77, $p = 0.014$), and 30 cycles vs. RX (t-statistic = − 2.89, $p = 0.011$).

template switching, or the amplification of spurious DNA fragments, complicating profile interpretation[38]. Incorporating a post-PCR clean-up step, such as the Amplicon RX method, can mitigate this risk by removing unwanted primers, dNTPs, and other PCR artefacts. This not only reduces contamination but also enhances the accuracy of the resulting DNA profiles. As with any forensic method, careful validation and quality control measures are essential to ensure reliability. Regular use of positive and negative controls, along with monitoring for allele drop-in, helps maintain the integrity of the DNA profiles.

One key observation from this study is the significant increase in peak height intensity (RFU) observed with the Amplicon RX method compared to the 30-cycle method. Enhanced signal amplification was particularly evident in low-template samples, where the Amplicon RX method consistently produced stronger signals. This aligns with earlier research, such as Sinelnikov and Reich (2016), which reported improved peak height intensities following the use of post-PCR clean-up techniques[25]. The increased RFU values observed suggest that the Amplicon RX method is especially effective in improving signal quality, making it a valuable tool in forensic casework involving trace or degraded DNA samples.

In the control sample analysis, the Amplicon RX method demonstrated consistent allele recovery across dilutions D1 to D3 (0.1 ng/μL to 0.001 ng/μL), recovering significantly more alleles compared to the 29- and 30-cycle methods. Statistical comparisons revealed the Amplicon RX method's superiority at lower DNA concentrations, particularly in D3, where the performance gap was most pronounced (Fig. 6). These findings emphasize the method's reliability in cases involving challenging low-template samples.

However, the Amplicon RX method showed limitations at the lowest DNA concentrations tested. While it demonstrated superior allele recovery up to D3 (0.001 ng/μL), performance declined at D4 (0.0001 ng/μL). At this concentration, fewer alleles were recovered, indicating that the clean-up process may be less efficient with extremely low-template DNA. While the 29- and 30-cycle methods also underperformed at D4, the difference in allele recovery was less pronounced. This suggests that while the Amplicon RX method is highly effective at concentrations of 0.001 ng/μL (D3), its utility at 0.0001 ng/μL (D4) is limited. Further research is needed to assess its performance in this range.

Despite this limitation, the Amplicon RX method remains a valuable tool for improving DNA profile recovery in challenging trace DNA samples. Its ability to enhance both allele recovery and signal quality, coupled with its potential to reduce the risk of allele drop-in, makes it an important addition to forensic laboratories. Future studies could explore combining post-PCR clean-up techniques with methods like reduced-cycle PCR and DNA repair enzymes to further optimize DNA recovery from degraded or low-template samples. Reduced-cycle PCR minimizes artefact formation, while DNA repair enzymes can restore damaged DNA before amplification, increasing the likelihood of recovering complete and reliable profiles. This combination could help balance

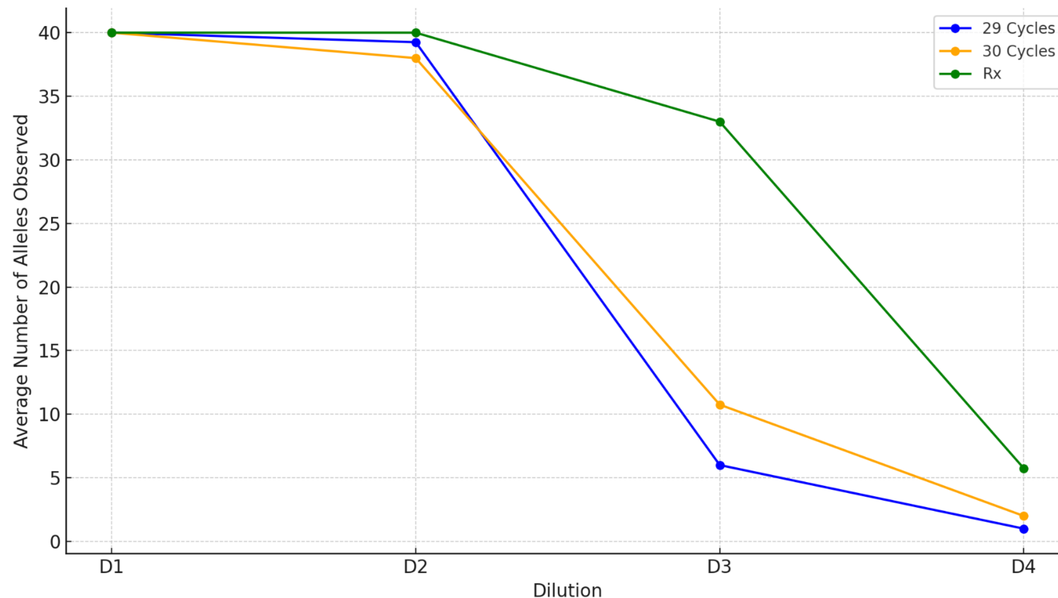

Fig. 6. The distribution of allele recovery from amplified and post-purified single-source diluted trace control DNA samples ($n = 144$) using the GlobalFiler PCR Amplification Kit. The samples were amplified under three conditions: 29 PCR cycles (29 Cyc.), 30 PCR cycles (30 Cyc.) following the manufacturer's protocol, and post-PCR purification using the Amplicon Rx protocol (RX). The line plot illustrates the variability in allele recovery across the four dilutions (D1–D4) for the three methods. The average number of alleles recovered was 40 for all methods at D1, 39.25 for 29 cycles, 38.0 for 30 cycles, and 40.0 for RX at D2. In D3, the averages were 6.0 for 29 cycles, 10.75 for 30 cycles, and 33.0 for RX, demonstrating the superior performance of the RX method at lower DNA concentrations. At D4, the averages dropped to 1.0 for 29 cycles, 2.0 for 30 cycles, and 5.75 for RX. Statistical comparisons at D3 yielded the following results: 29 cycles vs. 30 cycles (t-statistic = − 3.61, $p = 0.036$), 29 cycles vs. RX (t-statistic = − 1.51, $p = 0.001$), and 30 cycles vs. RX (t-statistic = − 6.85, $p = 0.006$).

sensitivity with artefact reduction, especially in low-DNA samples, providing more accurate and robust results in forensic analyses.

In the Analysis of Trace Casework Samples, the Amplicon RX method successfully enhanced allele recovery from trace DNA at low quantities. This is significant because forensic casework samples often contain degraded or limited amounts of DNA, making recovery and analysis challenging. The method's ability to improve the detection of alleles in low-template samples demonstrates its potential to provide accurate and reliable profiles in complex forensic cases. By recovering more alleles even from low-concentration samples, the Amplicon RX method proves its robustness and utility in real-world forensic scenarios, where trace DNA is often critical.

Future research could also examine the Amplicon RX method's performance in more complex DNA mixtures. Control samples with mixtures of up to four or five contributors could simulate complex forensic cases. Such studies would allow a comprehensive evaluation of the method's ability to recover true alleles while minimizing artefacts, such as false positives (allele drop-in). The focus should be on determining whether the Amplicon RX method can resolve complex signals, such as allele stacking and overlapping peaks, without generating excessive artefacts. Additionally, assessing its performance with low-template DNA in mixture scenarios could provide further insight into its reliability in a wider range of forensic applications.

In sum, while the Amplicon RX method has proven effective in individual low-template samples, future research should expand its application to more complex forensic scenarios, such as DNA mixtures, to fully assess its robustness and accuracy in diverse casework conditions.

Conclusion

The evaluation of the Amplicon RX Post-PCR Clean-up Kit on GlobalFiler PCR products confirms its effectiveness in improving trace DNA profile recovery from forensic casework samples, especially those with very low DNA quantities. The kit enhances sensitivity, increases allele recovery, and boosts peak height intensity, leading to more accurate and reliable DNA profiles. This study demonstrates that by optimizing the recovery of trace DNA, the Amplicon RX method assists forensic practitioners in extracting valuable genetic information from challenging samples. Given its ability to significantly improve the recovery of touch or trace DNA profiles, the Amplicon RX method should be widely adopted in forensic laboratories to enhance the analysis of low-template and compromised samples. Moving forward, further investigations should explore the application of the Amplicon RX method in diverse forensic scenarios and sample types, including complex DNA mixtures, to fully validate its effectiveness and broaden its utility across different casework conditions.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 28 November 2024; Accepted: 24 January 2025
Published online: 27 January 2025

References

1. Alketbi, S. K. Analysis of Touch DNA. Doctoral thesis, University of Central Lancashire. Available at: https://clok.uclan.ac.uk/46154/. (2023).
2. Butler, J. M. *Advanced Topics in Forensic DNA Typing: Methodology* (Academic, 2012).
3. Alketbi, S. K. The role of DNA in forensic science: A comprehensive review. *Int. J. Sci. Res. Arch.* **09**(02), 814–829 (2023).
4. Alketbi, S. K. Emerging technologies in forensic DNA analysis. *Perspect. Legal Forensic Sci.* **1**(1), 10007. https://doi.org/10.70322/plfs.2024.10007 (2024).
5. Goodwin, W., Linacre, A. & Hadi, S. *An Introduction to Forensic Genetics* 2nd edn (Wiley-Blackwell, 2011).
6. Alketbi, S. K. The affecting factors of touch DNA. *J. Forensic Res.* **9**, 424 (2018).
7. Burrill, J., Daniel, B. & Frascione, N. A review of trace Touch DNA deposits: Variability factors and an exploration of cellular composition. *Forensic Sci. Int. Genet.* **39**, 8–18 (2019).
8. Daly, D. J., Murphy, C. & McDermott, S. D. The transfer of touch DNA from hands to glass, fabric, and wood. *Forensic Sci. Int. Genet.* **6**(1), 41–46 (2012).
9. Alketbi, S. K. Collection of Touch DNA from rotten banana skin. *Int. J. Forensic Sci.* **5**(4), 000204 (2020).
10. Verdon, T. J., Mitchell, R. J. & van Oorschot, R. A. H. Evaluation of tapelifting as a collection method for touch DNA. *Forensic Sci. Int. Genet.* **8**(1), 179–186 (2014).
11. Alketbi, S. K. & Alsoofi, S. Dual recovery of DNA and fingerprints using Minitapes. *J. Forensic Sci. Criminal Invest.* **16**(1), 555929 (2022).
12. Sessa, F. et al. Touch DNA: impact of handling time on touch deposit and evaluation of different recovery techniques: An experimental study. *Sci. Rep.* **9**, 9542 (2019).
13. Alketbi, S. K. & Goodwin, W. The effect of surface type, collection, and extraction methods on Touch DNA. *Forensic Sci. Int. Genet. Suppl. Ser.* **7**(1), 704–706 (2019).
14. Alketbi, S. K. & Goodwin, W. The effect of sandy surfaces on Touch DNA. *J. Forensic Legal Invest. Sci.* **5**, 034 (2019).
15. Tozzo, P., Mazzobel, E., Marcante, B., Delicati, A. & Caenazzo, L. Touch DNA sampling methods: Efficacy evaluation and systematic review. *Int. J. Mol. Sci.* **23**(24), 15541 (2022).
16. Alketbi, S. K. & Goodwin, W. The effect of time and environmental conditions on Touch DNA. *Forensic Sci. Int. Genet. Suppl. Ser.* **7**(1), 701–703 (2019).
17. Shahzad, M. et al. Evaluation of storage conditions and the effect on DNA from forensic evidence objects retrieved from lake water. *Genes* **15**(3), 279 (2024).
18. Alketbi, S. K. & Goodwin, W. The impact of deposition area and time on Touch DNA collected from fabric. *Forensic Sci. Int. Genet. Suppl. Ser.* **8**(1), 45–47 (2022).
19. Gill, P. et al. Genotyping and interpretation of STR-DNA: low-template, mixtures and database matches—Twenty years of research and development. *Forensic Sci. Int. Genet.* **18**, 100–117 (2015).
20. Hanssen, E. N., Lyle, R., Egeland, T. & Gill, P. Degradation in forensic trace DNA samples explored by massively parallel sequencing. *Forensic Sci. Int. Genet.* **27**, 160–166 (2017).
21. Alketbi, S. K. Maintaining the chain of custody: anti-contamination measures for trace DNA evidence. *Int. J. Sci. Res. Arch*. **08**(02), 457–461 (2023).
22. Budowle, B., Eisenberg, A. J. & van Daal, A. Validity of low copy number typing and applications to forensic science. *Croat Med. J.* **50**(3), 207–217 (2009).
23. Wang, D. Y. et al. Developmental validation of the GlobalFiler® express PCR amplification kit: A 6-dye multiplex assay for the direct amplification of reference samples. *Forensic Sci. Int. Genet.* **19**, 148–155 (2015).
24. Ludeman, M. J. et al. Developmental validation of GlobalFilerTM PCR amplification kit: A 6-dye multiplex assay designed for amplification of casework samples. *Int. J. Legal Med.* **132**(6), 1555–1573 (2018).
25. Sinelnikov, A. & Reich, K. Amplicon Rx™, Post-PCR clean-up and concentration specifically for forensic DNA multiplex STR PCR reactions. *Eur. J. Forensic Sci.* **3**(1), 15–21 (2016).
26. Irwin, D. L. & Mitchelson, K. R. Comparison of PCR product clean-up methods for high-throughput capillary electrophoresis. *DNA Sequen. Optim. Prep. Cleanup* **2**, 201 (2006).
27. Forster, L., Thomson, J. & Kutranov, S. Direct comparison of post-28-cycle PCR purification and modified capillary electrophoresis methods with the 34-cycle low copy number(LCN) method for analysis of trace forensic DNA samples. *Forensic Sci. Int. Genet.* **2**(4), 318–328 (2008).
28. Amplicon Rx™ Post-PCR Clean-up Kit User Manual. Available at: https://www.ifi-test.com/amplicon-rx/ (Accessed: 15/06/2024).
29. Van Oorschot, R. A. et al. DNA transfer in forensic science: A review. *Forensic Sci. Int. Genet.* **38**, 140–166 (2019).
30. Alketbi, S. K. & Goodwin, W. Validating touch DNA collection techniques using cotton swabs. *J. Forensic Res.* **10**, 445 (2019).
31. Alketbi, S. K. An innovative solution to collect Touch DNA for direct amplification. *J. Forensic Sci. Criminal Invest.* **16**(1), 555928 (2022).
32. Stefanović, A., Šorgić, D., Cvetković, N., Antović, A. & Ilić, G. Precision Touch DNA sampling on plastic bag knots for improved profiling of packer and holder contributions. *Forensic Sci. Int. Genet.* **71**, 103033 (2024).
33. Alketbi, S. K. The impact of collection method on Touch DNA collected from fabric. *J. Forensic Sci. Criminal Invest.* **15**(5), 555922 (2022).
34. Mapes, A. A., Kloosterman, A. D., van Marion, V. & de Poot, C. J. Knowledge on DNA success rates to optimize the DNA analysis process: From crime scene to laboratory. *J. Forensic Sci.* **61**(4), 1055–1061 (2016).
35. Alketbi, S. K. & Goodwin, W. Collection methods for Touch DNA direct amplification. *J. Forensic Legal Invest. Sci.* **9**, 072 (2023).
36. Abdullah, A., Szkuta, B. & Meakin, G. E. Effect of swabbing technique and duration on forensic DNA recovery. *Sci. Just.* **63**(3), 343–348 (2023).
37. Alketbi, S. K. *Collection Techniques of Touch DNA Deposited on Human Skin Following a Strangulation Scenario* (International Journal of Legal Medicine, 2023).
38. McNevin, D., Edson, J., Robertson, J. & Austin, J. J. *Reduced Reaction Volumes and Increased Taq DNA Polymerase Concentration Improve STR Profiling Outcomes from a Real-World Low Template DNA Source: Telogen Hairs*11, 326–338 (Forensic Science, 2015).

Acknowledgements

Sincere gratitude is extended to the colleagues in the Biology and DNA Section of the General Department of Forensic Science and Criminology at Dubai Police for their unwavering dedication and invaluable support

throughout the development of this review. Their expertise, insights, and continuous encouragement were essential in shaping the ideas and analyses presented in this manuscript. Appreciation is also extended to the broader forensic science community for cultivating a collaborative environment that fosters innovation and excellence in research.

Author contributions

S.K.A. conducted data analysis and wrote the main manuscript text. N.S.A., M.M.A., S.A.A., and A.N.S. performed DNA analysis. H.J.A. provided supervision. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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