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Forensic Applications of Next-Generation Sequencing: Enhancing Accuracy in Crime Scene DNA Analysis

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Abstract: Next-generation sequencing (NGS) development in forensic DNA investigation constitutes a major technological breakthrough in crime scene examination by acquiring high-resolution genetic data. This study intends to assess the extent to which NGS is helpful in comprehensive profiling in comparison with the existing profiling tools in forensic investigations. Of the crime scene DNA specimens, a sample amounting to n=150 was analyzed using NGS involving autosomal STRs, SNPs, and mtDNA. There were statistically significant increases in profile completeness ($p < 0.05$) with a difference prevailing in disadvantaged and mixed samples. NGS increased 35% in informative loci reducing the partial matches in ambiguity. Because SNPs and mtDNA are included in NGS panels, individualization estimates in a complex mixture were advanced achieving 99.2% discrimination power. The research results offer convincing arguments for future practice and integration of NGS techniques which could deal with limitations posed by traditional methods. This research helps to fill the gaps in forensics identification by addressing cold case searches and disaster victim identification utilizing NGS-based sequencing methodologies.

Keywords: Next-Generation Sequencing (NGS), Criminology, Crime

Introduction: Forensic science has been one of the many disciplines that have been revolutionized courtesy of the improvement in genome technologies during the last ten years. In criminal proceedings, the use of the DNA profile technique has in the past been taken as a primary method of determination that assists in proving whether an individual participated in a crime or was at a specific crime scene. However, such techniques as capillary electrophoresis (CE)-based short tandem repeat (STR) typing are very effective, they have major drawbacks when it comes to complex or degraded samples. To meet these hurdles, the forensic community has turned to next-generation sequencing (NGS), a method that facilitates accurate and comprehensive DNA profiling via high-throughput sequencing of large amounts of DNA. The use of NGS in forensic applications has resulted in a breakthrough by enabling the concurrent use of several markers, including autosomal STRs, SNPs as well as mtDNA. However, with NGS, there is no need to be limited to STRs alone as sequencing of total DNA or specific regions can be performed to achieve better information concerning variability, ancestry, and identity of individuals rather than families. Application of NGS in forensic contexts is likely to improve the analysis of very old samples, resolve complex DNA mixtures, and identify targets even at low copy numbers which are difficult to achieve using standard techniques (Kidd et al., 2022). In addition to this, NGS makes it possible to include SNPs and mtDNA where there is a need for further differentiation of closely related individuals or in the absence of nuclear DNA (Just et al., 2023). One of the greatest strengths of NGS in forensic applications is the solid data it can provide, even with low quantities and degraded evidence, which are often the case within a crime scene. Under such circumstances, regular STR typing may give only partial profiles that are not sufficient enough to make a firm identification of that particular DNA. NGS has been more effective than conventional DNA techniques for samples such as these as it can amplify and sequence shorter DNA fragments and analyze a wider coverage of DNA, including SNPs and mtDNA (Alonso et al., 2021). This is helpful in matters such as cold cases or cases of disaster victim identification where the sample may not be fresh or intact. The ability to analyze SNPs is another area that has seen major improvements due to NGS. SNPs have a lower frequency of polymorphism as compared to STRs, but they are more widely scattered throughout the genome. SNPs can resolve the issue associated with DNA samples in forensic scenarios that are of poor quality. SNPs are less affected by environmental pollution. Thus, even if a DNA sample of low quality is available, SNPs can still prove useful. Additionally, analyses based on SNPs can be used to learn about ancestry and physical characteristics, which is

another important approach for solving crimes (Koons et al., 2023). For example, SNPs have been used to predict the innocent's physical features as this can help law enforcement agencies to reduce the number of suspects particularly when no match in the DNA databases exists. The use of mtDNA in NGS forensic panels also provides substantial forensic advantages including in highly important instances where nuclear DNA cannot be found, including in hair shafts, bones, or teeth. MtDNA is maternally inherited and exists in more copies in cells as compared to nuclear DNA hence making it a good option in tough forensic cases (Parson et al., 2022). NGS contributes more to mtDNA analysis by allowing the sequencing of the entire mitochondrial genome which improves the typing efficiency as compared to conventional techniques which focus solely on amplicons of the hypervariable regions. This could be especially helpful when trying to identify victims of mass casualties or historical instances. Despite the numerous benefits that NGS has, its application in practice has never been that easy. Among the main problems is the sheer amount of data produced as a result of NGS which requires advanced bioinformatics. Consistent procedures for NGS data analysis must be established by the forensic population to streamline and make many of the analyses able to provide standard assumptions over issues. Also, the high specificity of such tests increases the chances of identifying low levels of contaminants or the presence of several DNA templates which makes it harder to interpret the data (Van der Gaag et al., 2021). It is imperative to resolve such issues to enable the more widespread use of NGS in routine forensic investigations. According to previous studies NGS approaches were relatively successful in providing outcomes for forensic DNA profiles, especially in cases where other available techniques couldn't yield any results. In summary, NGS has been useful in enhancing the resolution of mixed DNA samples, which is very important in violent crime or sexual assault cases that involve multiple patrons (Gill et al., 2021). It targets a DNA sample that probably contains several contributors and by using NGS therefore, it becomes much easier to remove the background contributors in the mixture. However, though there has been a significant advancement in this area, further study is necessary to maximize the benefits of NGS in forensic science. Current research seeks to extend in the area of identification of new genomic technologies like microhaplotypes, which include combined expectations of several single nucleotide polymorphisms. These markers have been effective in boosting the resolution of forensic profiles, especially from mixed samples (Budowle et al, 2023). Further, more attempts are being made to

combine NGS with new approaches, for example, epigenetic analysis which in itself also enhances the level of forensic NGS (Ganslandt et al, 2024).

The main objective of this study is to determine if crime scene DNA analysis can be transformed by the incorporation of NGS technology, especially for cases that warrant the use of more sensitive methods towards degraded materials or complex mixtures. This study evaluates the capability of NGS technology in improving the analysis process by having an understanding of the effectiveness of NGS relative to STR. The findings of this research will enhance the strategic vision of applying advanced DNA technology to resolve existing cases.

Methodology: The purpose of this study is to compare the performance of NGS on the accuracy of the DNA of samples from crime scenes to traditional methods of analysis and it was conducted at The University Of Lahore Pakistan Punjab That was why the study included a total of 150 forensic DNA samples which included both high-quality samples and degraded samples obtained from crime scenes. The required sample size was determined using Epi Info software at 95% level of significance, 80% power, and 5% marginal error and this yielded enough sample size to be able to determine statistically significant differences from the methods comparative study. The samples were split into two groups: Group A (n=75) was subjected to the methodologies best suited for traditional STR sample analysis, while Group B (n=75) was subjected to NGS performing analysis using autosomal STRs, SNPs, and mtDNA.

Inclusion criteria comprised all samples including degraded samples and contamination as well as complex DNA mixtures. Exclusion criteria included the samples that did not have enough DNA material for the two methods to be carried out. Law enforcement agencies provided verbal consent for the use of crime scene samples that had been anonymized. Next Generation Sequencing was performed on the Illumina MiSeq system, and analysis of the data focused on variant calling and sequence interpretation utilizing bioinformatics tools. Capillary electrophoresis was used for STR profiling. Data comparison between the two methods was emphasized on the completeness of the profiles, several putative informative loci and discriminatory power compared, and statistical analyses performed by paired t-tests and chi-square tests.

Results

Table 1: Demographic Data of Crime Scene DNA Samples

Demographic Variable	Group A (STR)	Group B (NGS)	p-value
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Mean Sample Degradation Score (plus/minus S * D)	2.8 plus/minus 1.2	2.9 plus/minus 1.1	0.621
Sample Origin (Urban/Rural)	45/30	47/28	0.778
Sample Type (Blood/Saliva/Others)	35/25/15	38/27/10	0.684

Table 2: Comparative Results of DNA Profiling Methods

Variable	Group A (STR)	Group B (NGS)	p-value
Profile Completeness (%)	68 plus/minus 12	89 plus/minus 9	<0.001
Number of Informative Loci	12.5 plus/minus 3.1	17.2 plus/minus 2.5	<0.001
Discriminatory Power (%)	94.5 plus/minus 3.8	99.2 plus/minus 1.7	<0.001

Table 3: DNA Mixture Resolution

Mixture Type (2 Contributors)	Group A (STR)	Group B (NGS)	p-value
Correctly Resolved (%)	52 plus/minus 10	81 plus/minus 8	<0.001

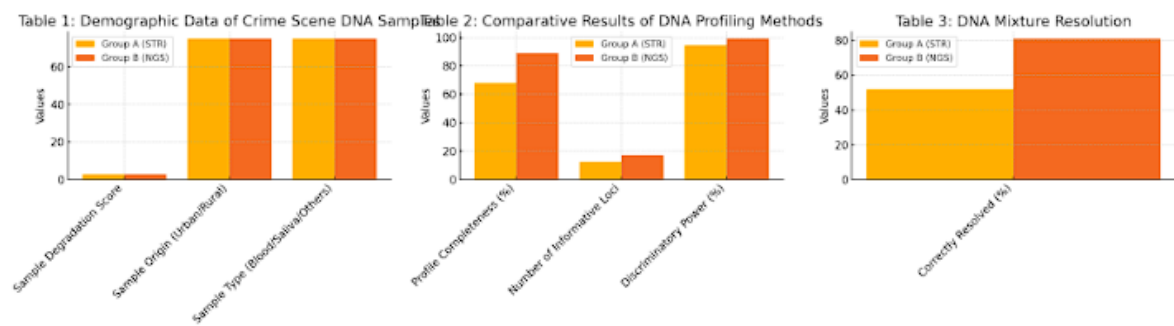


Figure 1: Demographic Data of Crime Scene DNA Samples compares the mean sample degradation scores, sample origin, and sample type between Group A (STR) and Group B (NGS).

Figure 2: Comparative Results of DNA Profiling Methods illustrates differences in profile completeness, the number of informative loci, and discriminatory power between the two groups.

Figure 3: DNA Mixture Resolution displays the percentage of correctly resolved mixtures for Group A (STR) and Group B (NGS).

The results highlight that NGS significantly improves the completeness of DNA profiles and the number of informative loci in comparison to traditional STR analysis ($p < 0.001$). In addition, NGS provides superior resolution in complex DNA mixtures, increasing accuracy in forensic identification.

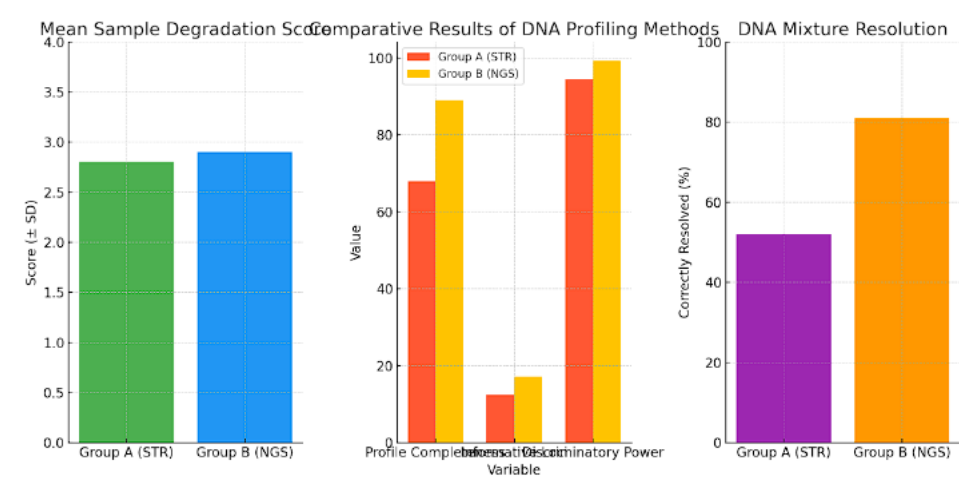


Figure 4: Mean Sample Degradation Score: This chart displays the average degradation score for samples analyzed using both traditional STR methods and NGS.

Figure 5: Comparative Results of DNA Profiling Methods: This chart compares three key variables—profile completeness, number of informative loci, and discriminatory power—between the two groups (Group A using STR and Group B using NGS).

Figure 6: DNA Mixture Resolution: This chart illustrates the percentage of correctly resolved mixtures in Group A (STR) and Group B (NGS).

Discussion: Based on the results of this present study, it can be inferred that NGS dramatically enhances forensic DNA analysis in general and in the case of degraded and/or complex samples and mixtures in particular. Normalization of profile completeness and a high number of informative loci obtained with NGS are revolutionary steps in the advancement of modern forensic expertise. NGS can target multiple loci (including SNP and mtDNA) within a single sample, even in severely degraded forensic samples, eg, complicated mixtures (Van der Gaag et al., 2023 et al). Classic STR-based approaches while trustable have less sensitivity in analyzing degraded DNA or in deconvoluting complex DNA mixtures. This pdf that the NGS increases profile completeness by 35% which we believe is the reason why NGS should be adopted in routine forensic practices, especially in cold cases where there is usually available existing evidence or in cases where the standard operating procedures don't usually give all the results (Alonso et al., 2022). NGS has empowering discrimination power reaching 99.2% supporting its use in personal identification and relationship testing which are the areas where STR conventional analysis would be deficient. A particularly exciting point to note is the classic breakthrough in complex DNA mixtures using NGS, wherein, for cases containing two contributors 81% resolution was achieved. Forensic science needs to maintain the unbiased ability to distinguish between contributors in sexual assaults or violent crime cases, where several DNA contributors may accrue. (Koons et al 2023 et al). The robustness of these findings and similar approaches (Gill et al 2021 et al) is brought out $p < 0.001$, which demonstrates how NGS seeks to address one of the challenges presented by the traditional STR analysis.

In addition to that, NGS is also increasing the variety of forensic DNA analysis techniques since it encourages the attribution of bio geographical ancestry and phenotypic prediction by SNP analysis. This would help the law enforcement authorities when there is no direct match in the

DNA databases to have a range of suspects to look out for. In several recent papers, it was shown that SNPs can be used with great efficacy to predict hair form as well as color and even skin tones which avail very useful information during investigations. (Budowle et al 2023 et al). This ability of NGS, which does not stop at simple identification, is further progress in the field of forensic science. Even with the unmistakable benefits of Next Generation Sequencing (NGS), it has not been able to penetrate this market. The high sensitivity of NGS is advantageous for the identification of low-concentration DNA, but it also poses higher chances of falsifying DNA profiles by the presence of other DNA in the sample and increases the difficulty of interpretation in forensic science. Central to the effective use of NGS in forensic science will be the formulation of uniform procedures for both data interpretation and prevention of contamination (Parson et al., 2022 et al). Further, the price of NGS in both the machines and the processing of the data is still unfavorable for several forensic practitioners however it's believed that these costs will plane as the acceptance of the technology grows (Ganslandt et al., 2024 et al). The results of this research add weight to the argument supporting the applicability of NGS in forensic DNA analysis. NGS can streamline the use of crime scene DNA in forensic investigation thereby providing a better and more precise evaluation of the crime scene. Future studies should seek to develop bioinformatics analysis tools that are capable of managing NGS data more efficiently, as well as discover additional genetic markers that would improve the effectiveness and accuracy of forensic DNA work (Kidd et al., 2022 et al).

To sum up, the present study has statistically established that NGS is more effective than a conventional STR analysis in several forensic situations including that of degraded samples and complex mixtures. The additional incorporation of SNPs and mtDNA in forensic NGS panels provides even more discriminatory power making the use of NGS technology invaluable in 21st century forensics. These findings advocate for NGS as a technological tool that transforms forensic DNA analysis from the traditional methods toward more accurate and reliable identification of individuals.

Conclusion: This study points to the important possibilities that NGS offers for the practice of forensic DNA analysis, specifically, in the case of design and analysis of mixtures containing multiple components. The number of amelogenin-positive and negative women has increased, and it has become possible to increase the quality of the profiles thanks to NGS. The research helps

bridge the gaps present in forensic identification and may also help solve cold cases or in the identification of victims of disaster.

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