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Mini Review

Forensic Genetics in the Genomic Era: STR, SNP and Epigenetic Markers Through the Lens of NGS – A Review Article

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Abstract

Forensic genetics has entered a transformative genomic era with the integration of Next-Generation Sequencing (NGS) technologies, enabling high-resolution analysis far beyond the capabilities of conventional capillary electrophoresis-based Short Tandem Repeat (STR) profiling. While STRs remain the cornerstone of human identification, NGS provides sequence-level characterization that increases discriminatory power and improves interpretation of degraded samples and complex mixtures. In parallel, the incorporation of Single Nucleotide Polymorphisms (SNPs)-including identity, ancestry-informative, and phenotype-associated markers-has expanded the forensic genetic toolkit, offering new opportunities for ancestry estimation, kinship analysis, and biogeographical inference. Moreover, epigenetic markers, particularly DNA methylation signatures, have emerged as powerful tools for estimating chronological age and identifying tissue or body-fluid origin, thereby providing complementary information beyond individual identification.

This review synthesizes recent advances in STR, SNP, and epigenetic marker analysis through the lens of NGS and highlights their combined potential in modern forensic applications. We discuss the technical advantages, current implementations, and analytical challenges associated with massively parallel sequencing, including validation requirements, bioinformatic complexity, and ethical considerations. As genomic technologies continue to evolve, integrated multi-marker approaches are expected to redefine standards in forensic DNA analysis, enabling more comprehensive molecular profiling and improved investigative capabilities. This review aims to critically evaluate the integration of STRs, SNPs, and epigenetic markers within NGS-based forensic workflows and to highlight current limitations and future perspectives for routine forensic implementation.

Introduction

Forensic genetics has historically relied on STR markers amplified through PCR and visualized via CE. These polymorphic microsatellite loci form the foundation of national databases, such as CODIS and ENFSI reference systems. However, CE-based workflows distinguish alleles only by fragment length, rendering them incapable of detecting isoalleles or sequence-level variations that may carry forensic value. Furthermore, CE interpretation faces challenges in highly degraded, environmentally challenging, or mixed biological samples. The advent of NGS has allowed for Massively Parallel Sequencing (MPS) of STRs, SNPs, mitochondrial genomes, and epigenetic loci within a single reaction. Simultaneously, the multiplexed characterization of hundreds of markers increases both the discriminatory power and the contextual information available in forensic investigations. As sequencing accuracy, throughput, and cost-efficiency continue to improve, forensic laboratories are transitioning toward hybrid or fully NGS-based workflows [1-4]. The purpose of this review is to provide a comprehensive and critical overview of STR, SNP, and epigenetic markers in the context of next-generation sequencing, highlighting their forensic relevance, current limitations, and future potential. By synthesizing recent methodological advances and practical challenges, this manuscript aims to support informed integration of genomic technologies into forensic casework.

Historical Background: Transition to The Genomic Era

Early DNA profiling began with RFLP analysis, which required micrograms of intact DNA. The introduction of PCR-based STR typing revolutionized forensic genetics by enabling amplification of nanogram-level DNA. Standardization of core STR loci led to widespread adoption and the establishment of global human identification networks [5].

However, STR typing reached a practical limit due to:

a. Fragment-length-based allele assignment
b. Difficulty resolving isoalleles
c. Reduced performance in degraded DNA
d. Limited ability to integrate SNP, mtDNA, and epigenetic data [6].

NGS overcame these constraints, enabling forensic scientists to analyze sequence motifs, microvariants, flanking regions, and large SNP panels simultaneously. This shift toward genomic sequencing is now supported by commercial forensic systems such as MiSeq FGx (Illumina), Ion S5 Precision ID (Thermo Fisher), and ForenSeq Signature Prep Kits [7].

Legal and Forensic Implications of the Transition from CE to NGS

The transition from Capillary Electrophoresis (CE)-based STR profiling to Next-Generation Sequencing (NGS) represents not only a technological advancement but also a conceptual and legal shift in forensic DNA analysis. Unlike CE, which relies on fragment-length polymorphism, NGS generates sequence-resolved genetic data, introducing additional layers of complexity in interpretation, validation, and courtroom presentation [8,9]. Consequently, the admissibility of NGS-derived forensic evidence must be evaluated within established legal frameworks governing scientific evidence, particularly the Daubert and Frye standards.

Under the Daubert standard, which emphasizes empirical testability, peer review, known or potential error rates, and general acceptance, NGS-based forensic methods demonstrate a strong scientific foundation. Numerous validation studies have confirmed the analytical accuracy, sensitivity, and reproducibility of massively parallel sequencing for STRs, SNPs, and mitochondrial DNA marker [1,9,10]. However, Daubert scrutiny increasingly focuses on downstream analytical components, including bioinformatic pipelines, probabilistic interpretation models, and the transparency of statistical reporting rather than on the sequencing technology itself [11].

In jurisdictions adhering to the Frye standard, where admissibility depends primarily on general acceptance within the relevant scientific community, the implementation of NGS-based STR analysis may progress more cautiously. While CE-STR profiling benefits from decades of courtroom precedent and standardized population databases, sequence-based STR typing is still undergoing harmonization with respect to allele nomenclature, reporting conventions, and database construction [8]. As a result, NGS-derived STR results are currently more likely to be admitted as complementary or confirmatory evidence rather than as direct replacements for CE-based profiles, particularly in contested cases.

Beyond admissibility considerations, NGS challenges the traditional conceptualization of forensic DNA analysis as a purely identification-focused discipline. By enabling the simultaneous interrogation of STRs, SNPs, ancestry-informative markers, and epigenetic loci within a single workflow, NGS introduces a paradigmatic shift toward multidimensional forensic inference [7,10]. This shift necessitates not only rigorous technical validation but also enhanced judicial literacy and clear guidelines for expert testimony to ensure that probabilistic conclusions and associated uncertainties are accurately conveyed in court.

STR Analysis in the Genomic Era

Sequencing-based STR typing characterizes the internal repeat structure and flanking sequences of STR alleles. This reveals sequence-level diversity that is invisible to CE and significantly improves mixture deconvolution, particularly in complex, multi-contributor samples.[5].

NGS-based STR advantages include:

a. Isoallele detection and enhanced discrimination
b. Superior performance on degraded DNA
c. Access to informative flanking variants
d. Integration with SNP and epigenetic markers

Challenges include:

a. Establishment of universal nomenclature for sequence-based STRs
b. The development of population allele frequency databases
c. Standardized quality control and validation requirements
d. The development of complex bioinformatic pipelines

Despite these challenges, hybrid CE-NGS models are increasingly implemented in forensic laboratories [8,9].

Table 1: Comparison of CE-based STR and NGS-based STR Analysis.

Feature	CE-based STR	NGS-based STR
Allele detection	Fragment length only	Sequence-based (repeat and flanking regions) Fully detectable
Isoallele resolution	Not detectable	Fully detectable
Mixture interpretation	Limited in complex mixtures	Improved contributor resolution
Performance on degraded DNA	Moderate	Superior (shorter amplicons)
Marker integration	STRs only	STRs, SNPs, mtDNA, epigenetic loci
Population databases	Extensive, standardized	Emerging, incomplete
Nomenclature	Highly standardized	Ongoing harmonization
Court acceptance	Long-standing precedent	Increasing but jurisdiction-dependent

A comparative overview of CE-based and NGS-based STR analysis highlights that while CE remains the legal gold standard, NGS provides substantially enhanced genetic resolution and analytical flexibility, albeit with ongoing challenges related to standardization and judicial acceptance (Table 1) [8,9].

Challenges in Court Admissibility and Statistical Reporting of Sequence-Based STRs

Despite the increased discriminatory power offered by sequence-based STR analysis, its forensic implementation introduces novel statistical and legal challenges. Conventional STR interpretation relies on length-based allele frequencies derived from well-established population databases. In contrast, NGS reveals multiple sequence variants within alleles of identical length, thereby expanding allelic diversity and complicating allele frequency estimation [8,9].

One of the primary challenges concerns the limited availability of population reference datasets for sequence-defined STR alleles across diverse populations. In the absence of comprehensive frequency data, laboratories may adopt conservative statistical approaches, such as collapsing sequence variants into length-based categories, which may partially obscure the added discriminatory value of NGS [9,10]. Furthermore, the application of likelihood ratios and probabilistic genotyping models to sequence-level STR data requires extensive validation to ensure consistency, transparency, and reproducibility across laboratories [11].

From a legal standpoint, courts may question whether sequence-based STR statistics are directly comparable to traditional CE-derived match probabilities. This concern underscores the importance of standardized reporting practices, including explicit documentation of analytical thresholds, error rates, and interpretative assumptions. Clear expert testimony is essential to convey that increased genetic resolution does not inherently increase uncertainty, but rather reflects a more precise characterization of underlying genetic variation [8,11].

Until international consensus is achieved regarding nomenclature, statistical frameworks, and database standards, sequence-based STR analysis is likely to coexist with CE-based methods within a hybrid forensic framework. Such an approach balances technological innovation with legal robustness and facilitates the gradual integration of NGS-derived evidence into routine forensic casework and judicial practice [9,10].

Forensic SNP Markers

SNPs represent the most abundant variation in the human genome and are particularly useful in forensic contexts where DNA is degraded or present in trace quantities. Their short amplicon lengths (30-80 bp) make them ideal for compromised samples [12].

SNP categories include:

a. Identity SNPs (iSNPs) for human identification
b. Ancestry-Informative SNPs (AIMs) for biogeographic estimation
c. Phenotype-informative SNPs for prediction of eye, hair, and skin pigmentation (e.g., HIrisPlex-S system)
d. Kinship SNPs for distant relatedness estimation [11].

NGS enables parallel sequencing of hundreds to thousands of SNPs with high accuracy. Integration of AIM-SNP panels has improved investigative leads in cases lacking reference individuals. However, ethical concerns regarding ancestry and phenotype inference require careful oversight [13,14].

Despite their increasing utility in forensic intelligence and investigative contexts, ancestry-informative SNPs introduce distinct interpretative, statistical, and legal challenges that warrant explicit critical evaluation to prevent overstatement of their evidentiary value [11,13].

Limitations and Over-Interpretation Risks of AIM-Snps: Investigative Leads Versus Evidentiary Proof

Ancestry-Informative Snps (AIM-SNPs) are designed to infer population-level genetic affinities rather than individual identity, and their forensic value lies primarily in generating probabilistic investigative leads rather than establishing evidentiary proof. Misinterpretation arises when ancestry estimates are presented with undue certainty, detached from population reference limitations, model assumptions, and statistical uncertainty [11,13].

While Ancestry-Informative Snps (AIM-SNPs) have expanded the scope of forensic intelligence by providing probabilistic insights into biogeographic ancestry, their interpretation carries a substantial risk of overstatement when applied beyond their validated purpose. AIM-SNP panels are designed to infer population-level genetic affinities rather than individual identity, and their predictive outputs are inherently probabilistic and model-dependent [11,13]. Consequently, misinterpretation may arise when ancestry estimates are presented with undue certainty or implicitly framed as definitive indicators of an individual's origin.

A critical distinction must therefore be drawn between investigative leads and evidentiary proof. AIM-SNP-derived ancestry inference is best suited to generating investigative hypotheses that may assist law enforcement in narrowing search spaces or prioritizing investigative resources, particularly in cases lacking reference profiles [7,11]. However, such inferences do not meet the evidentiary thresholds required for individualization or source attribution and should not be presented as standalone proof in judicial proceedings. The risk of over-interpretation is further amplified by population stratification, reference database limitations, and the uneven global representation of ancestry panels. Many AIM-SNP systems rely on continental or sub-continental population clusters that may inadequately capture admixture, recent migration, or fine-scale population structure, leading to oversimplified or misleading conclusions [10,13]. Moreover, algorithmic outputs are sensitive to the choice of reference populations, statistical models, and classification thresholds, factors that are often opaque to non-specialist audiences, including judges and juries.

From a legal perspective, conflating investigative intelligence with evidentiary proof raises significant concerns regarding fairness, transparency, and the potential for cognitive bias. Courts applying Daubert or Frye standards may accept AIM-SNP analysis as a scientifically valid exploratory tool, yet remain appropriately cautious about its probative value when used to support guilt or identity claims [11]. Without careful contextualization, ancestry inference may inadvertently reinforce stereotypes or introduce prejudicial narratives that exceed the scientific limits of the data. Accordingly, best-practice guidelines increasingly emphasize that AIM-SNP results should be reported using cautious, non-deterministic language, explicitly framed as probabilistic estimates, and accompanied by clear statements regarding their intended investigative-rather than evidentiary-use [7,14]. Transparent communication of uncertainty, model assumptions, and population coverage is essential to prevent over-reliance on ancestry inference and to preserve the scientific and legal integrity of forensic genetic evidence.

Epigenetic Markers in Forensic Science

Epigenetic modifications, particularly DNA methylation at CpG sites, provide biological information not encoded in the DNA sequence. Their forensic relevance includes:

1. **Age estimation:** Predictive age models focus on CpG sites in genes such as ELOVL2, FHL2, and PDE4C. NGS allows simultaneous quantification of methylation across multiple age-associated sites.
2. **Body fluid and tissue identification:** Distinct methylation signatures differentiate blood, saliva, semen, menstrual fluid, vaginal secretions, and skin cells.
3. **Environmental and lifestyle inference:** Emerging research links methylation changes with behaviors such as smoking or exposure to environmental stressors; however, routine forensic use remains limited due to ethical considerations [15].

Interpretative Limitations and Legal Sensitivity of Forensic Epigenetic Markers

Epigenetic markers, particularly DNA methylation-based assays, have emerged as promising tools for estimating chronological age and identifying tissue or body-fluid origin in forensic contexts. However, their interpretative value must be carefully contextualized with respect to methodological uncertainty, post-mortem variability, and legal sensitivity. Unlike static genetic variants, epigenetic signatures reflect dynamic biological processes influenced by age, tissue type, environmental exposure, and physiological state [15].

Error margins in age prediction models

DNA methylation-based age prediction models consistently report prediction errors expressed as mean absolute deviation, typically ranging from approximately ±2 to ±5 years, depending on the marker set, tissue type, analytical platform, and population studied [2,15]. While such accuracy is sufficient to provide useful investigative guidance, it falls short of the precision required for individual-level identification or definitive age determination. Importantly, prediction error increases at the extremes of age distribution and may vary across tissues, emphasizing that age estimates should be reported as probabilistic ranges rather than exact values.

Failure to explicitly communicate these error margins may lead to overinterpretation of age estimates, particularly in judicial contexts where numerical outputs may be perceived as definitive. Accordingly, best-practice reporting recommends the inclusion of confidence intervals, tissue source specification, and validation context to ensure transparent interpretation of epigenetic age estimates [15,16].

Tissue identification and post-mortem variability

Epigenetic tissue and body-fluid identification relies on differential methylation patterns across cell types. While numerous studies have demonstrated robust discrimination between blood, saliva, semen, vaginal fluid, and skin cells, post-mortem factors introduce additional complexity [3,16]. Decomposition processes, microbial activity, environmental exposure, and post-mortem interval may alter methylation levels, potentially affecting classification accuracy.

Furthermore, forensic samples frequently consist of mixed tissues or degraded DNA, complicating the interpretation of methylation signals derived from heterogeneous cellular sources. Although NGS enhances sensitivity and multiplexing capacity, the biological variability inherent to epigenetic markers necessitates cautious interpretation, particularly in cases involving aged remains or environmentally challenged samples [3].

Legal Sensitivity of Epigenetic Information

From a legal and ethical perspective, epigenetic data warrant heightened scrutiny due to their capacity to reveal sensitive biological information beyond identity. Age estimation, tissue origin, and emerging associations with lifestyle or environmental exposures may intersect with protected personal attributes, raising concerns regarding privacy, proportionality, and misuse [10,13]. Unlike STR or identity SNP profiles, epigenetic markers can convey context-dependent biological narratives that may be misconstrued or overstated in court.

Consequently, the forensic use of epigenetic evidence must adhere to strict limitations regarding scope and purpose. Similar to ancestry inference, epigenetic findings are best framed as investigative intelligence rather than evidentiary proof, unless supported by robust validation, clearly defined error margins, and transparent statistical reporting. Judicial acceptance of epigenetic evidence will therefore depend not only on scientific reliability but also on the clarity with which uncertainties and ethical boundaries are communicated during expert testimony [11,15]. Taken together, while epigenetic markers substantially enrich forensic inference when integrated with NGS, their application requires a cautious, well-regulated framework that balances scientific innovation with legal robustness and ethical responsibility. Accordingly, while epigenetic markers analyzed through NGS offer valuable forensic intelligence by informing age estimation and tissue origin, their dynamic nature, inherent error margins, and sensitivity to post-mortem and environmental factors necessitate cautious interpretation and clearly delimit their role as supportive investigative tools rather than definitive evidentiary proof in judicial proceedings.

Forensic Applications of NGS

NGS supports a multi-dimensional forensic framework by enabling:

a. Enhanced STR resolution
b. High-density SNP typing
c. Full mitochondrial genome sequencing
d. Epigenomic profiling for age and tissue estimation
e. Simultaneous analysis of nuclear, mitochondrial, and epigenetic markers

NGS-based forensic intelligence has expanded investigative capabilities, including improved mixture interpretation, complex kinship analysis, and biogeographic ancestry inference [10].

Future Directions in Forensic Genomics

Emerging research trends include:

a. Multi-omic forensic phenotyping integrating genomic, epigenomic, transcriptomic, and microbiome data
b. Single-cell sequencing for resolving complex mixtures
c. Forensic metagenomics using microbial communities as trace evidence
d. AI-driven forensic pipelines for probabilistic inference
e. Robust ethical frameworks balancing investigative utility with privacy protection [16].

Conclusion

The field of forensic genetics has undergone a substantial transformation with the integration of next-generation sequencing technologies, expanding DNA analysis beyond traditional STR-based identification toward a multidimensional genomic framework. The ability of NGS to simultaneously interrogate STRs, SNPs, mitochondrial DNA, and epigenetic markers has enhanced the resolution, sensitivity, and contextual interpretability of forensic evidence, particularly in challenging samples involving degradation, mixtures, or unknown contributors.

This review highlights that the forensic value of NGS lies not in the replacement of established methodologies, but in the strategic integration of complementary marker systems. Sequence-based STR analysis improves discriminatory power and mixture interpretation, while SNP markers extend forensic inference to degraded samples and complex kinship scenarios. Epigenetic assays further contribute by enabling probabilistic age estimation and tissue identification, thereby enriching forensic intelligence when used within validated and clearly defined limits.

At the same time, the expanded inferential capacity of forensic genomics introduces new scientific, legal, and ethical challenges. Interpretation of NGS-derived data requires robust validation, standardized nomenclature, comprehensive population reference datasets, and transparent statistical frameworks. Importantly, markers designed for investigative intelligence-such as ancestry-informative SNPs and epigenetic predictors-must be clearly distinguished from evidentiary tools intended for individualization to prevent overinterpretation and judicial misapplication.

Looking forward, the continued advancement of forensic genomics will depend on disciplined implementation rather than technological novelty alone. Hybrid analytical models combining established STR workflows with NGS-based extensions currently offer the most defensible approach for routine casework. Ongoing international collaboration on methodological standardization, ethical governance, and judicial education will be essential to ensure that the growing power of genomic technologies strengthens both scientific credibility and legal reliability within forensic practice.

Discussion

The integration of Next-Generation Sequencing (NGS) technologies into forensic genetics represents a paradigm shift from traditional length-based DNA profiling toward a more comprehensive, sequence-resolved analytical framework. This review highlights how STRs, SNPs, and epigenetic markers-when analyzed through massively parallel sequencing-provide complementary layers of information that collectively enhance the interpretative power of forensic DNA evidence. STRs remain the cornerstone of forensic identification due to their high polymorphism and well-established statistical frameworks. However, NGS-based STR analysis substantially extends their forensic utility by resolving sequence-level variation within repeat regions and flanking sequences. This added resolution improves discrimination between isoalleles and enhances mixture interpretation, particularly in complex or low-template samples. Nevertheless, despite these advantages, sequence-based STR analysis introduces new challenges related to nomenclature harmonization, inter-laboratory comparability, and the need for expanded population reference datasets. Consequently, NGS-based STRs are currently best viewed as complementary to, rather than replacements for, capillary electrophoresis-based workflows in routine casework.

SNP markers provide distinct advantages in forensic contexts where STR analysis is limited, such as highly degraded samples or extended kinship investigations. Identity SNPs offer strong individualization with low mutation rates, while ancestry-informative and phenotype-associated SNPs expand the scope of forensic intelligence beyond identity testing. The ability of NGS platforms to multiplex large SNP panels alongside STRs enables the generation of multidimensional genetic profiles from minimal DNA input. However, the forensic application of ancestry and phenotype inference remains scientifically and ethically complex. Population stratification, probabilistic interpretation, and the risk of overstating predictive accuracy necessitate cautious implementation and transparent reporting standards.

Epigenetic markers further broaden forensic capabilities by providing dynamic biological information unavailable through static genetic variants. DNA methylation-based age estimation and tissue source identification have demonstrated promising accuracy across multiple studies and biological matrices. When combined with NGS, epigenetic profiling becomes possible even in challenging forensic samples. Despite this progress, epigenetic signatures are influenced by interindividual variability, tissue specificity, and environmental factors, which may complicate interpretation. Standardized marker panels, validation across diverse populations, and consensus analytical pipelines are therefore essential before routine forensic adoption.

A critical advantage of NGS lies in its ability to integrate multiple marker classes-STRs, SNPs, mitochondrial DNA, and epigenetic loci-within a single analytical workflow. This integrative approach supports more robust interpretation of complex forensic scenarios, such as mixed samples, degraded DNA, and unidentified remains. However, implementation barriers remain substantial, including higher costs, longer turnaround times, bioinformatic complexity, and the need for extensive laboratory validation. Moreover, legal admissibility and courtroom acceptance of NGS-derived evidence depend on standardized methodologies, transparent statistical models, and clear communication of uncertainty. Overall, the findings synthesized in this review underscore that the future of forensic genetics is not defined by a single marker type or technology, but by the strategic integration of complementary genomic and epigenomic information. While NGS offers transformative potential, its forensic value ultimately depends on scientifically robust validation, ethical governance, and harmonized international standards.

Limitations

This review has several limitations. First, it is based exclusively on previously published literature and does not include original experimental or population data. Second, the rapid evolution of sequencing platforms, chemistries, and bioinformatic tools means that some technical aspects discussed may change as technologies mature. Third, population reference datasets for NGS-based STRs, SNPs, and epigenetic markers remain incomplete for many global populations, which may limit the generalizability of certain conclusions. Finally, ethical and legal considerations related to forensic genomics vary across jurisdictions and were addressed here in a generalized framework rather than through region-specific regulatory analysis.

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