



Multi-Omics Approaches in Forensic Biology: Integrating Genomics, Epigenomics, Proteomics and Microbiomics for Human Identification and Crime Scene Reconstruction

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Abstract

Forensic biology has traditionally been based on the use of DNA profiling as the primary method for human identification and biological evidence analysis. While conventional Short Tandem Repeat (STR) profiling is highly reliable for individual identification, it alone cannot address several important forensic questions, including the biological origin of a sample, the age of the contributor, tissue or body-fluid identification, environmental association and aspects of crime-scene reconstruction. Recently developed omics-based technologies in molecular biology that allow the acquisition of multiple layers of information are capable of analyzing forensic biological evidence.

Multi-omics approaches integrate complementary molecular information from genomics, epigenomics, proteomics, and microbiomics, providing a more complete biological profile of an individual or evidence sample. Genomics is used for human identification with methods such as STRs, single nucleotide polymorphisms, mitochondrial DNA and massively parallel sequencing. Epigenomics, in particular DNA methylation analysis, has potential applications in forensic age estimation, tissue identification and characterization of biological variation. Proteomics can be used to detect protein biomarkers that can help to identify body fluids and tissues. Microbiomics can be used to study the microbial communities associated with individuals, biological materials and environments and has potential applications in individual association, crime scene reconstruction and postmortem interval estimation.

Combining these molecular layers may add value to the analysis of biological evidence, moving beyond traditional DNA profiling. However, a number of challenges still exist such as sample degradation, contamination, population and environmental variability, limited reference databases, complex bioinformatic analysis, methodological standardization, validation and ethical concerns due to the increasing amount of biological information that can be obtained from forensic samples. This review discusses the principles, applications, advantages and limitations of genomics, epigenomics, proteomics and microbiomics in forensic biology and focuses on their integration for human identification and crime-scene reconstruction. The review also highlights current research gaps and future prospects including massively parallel sequencing, single-cell analysis, artificial intelligence and integrated computational approaches. Thus, multi-omics is emerging as a promising complementary framework that may extend forensic biology from conventional genetic identification to comprehensive molecular characterization of biological evidence.

Keywords: Forensic Biology; Multi-Omics; Forensic Genomics; Epigenomics; DNA Methylation; Forensic Proteomics; Microbiomics; DNA Profiling; Body Fluid Identification; Human Identification; Crime Scene Reconstruction; Postmortem Interval Estimation; Massively Parallel Sequencing; Bioinformatics; Molecular Forensics

Abbreviations

DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; PCR: Polymerase Chain Reaction; STR: Short Tandem Repeat; SNP: Single Nucleotide Polymorphism; mtDNA: Mitochondrial DNA; Y-STR: Y-Chromosome Short Tandem Repeat; Y-SNP: Y-Chromosome Single Nucleotide Polymorphism; NGS: Next-Generation Sequencing; MPS: Massively Parallel Sequencing; RFLP: Restriction Fragment Length Polymorphism; VNTR: Variable Number Tandem Repeat; InDels: Insertion/Deletion Polymorphisms; CpG: Cytosine-phosphate-Guanine dinucleotide; MS: Mass

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Spectrometry; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; PMI: Postmortem Interval; AI: Artificial Intelligence; ML: Machine Learning; UV: Ultraviolet; ELS: Ethical, Legal and Social

Introduction

Forensic science is an interdisciplinary science that utilizes scientific principles and analytical techniques to the investigation of crime and the interpretation of evidence for legal purposes [14]. Within forensic science, forensic biology plays a significant role in the examination of biological evidence such as blood, saliva, semen, hair, skin cells, tissues, bones, teeth, and other cellular materials recovered from crime scenes [1, 2]. Such evidence can provide valuable information about the identity of individuals, biological relationships, body-fluid origin, tissue type, and circumstances surrounding a criminal event [1, 3, 4]. Among the various biological approaches, DNA analysis has become the cornerstone of modern forensic identification because of the high degree of discrimination provided by polymorphic genetic markers [1, 5]. However, the increasing complexity of criminal investigations has created a need for analytical approaches that can provide information beyond conventional DNA profiling [3, 4, 6].

DNA profiling was a key achievement in forensic biology [7, 8]. Early forensic identification methods were mostly based on blood-group systems, protein polymorphisms, enzyme analysis, and serological investigations [9-11]. Although these methodologies contributed greatly to forensic investigations, their discriminatory power was comparably limited [10, 11]. The introduction of Polymerase Chain Reaction (PCR) and Short Tandem Repeat (STR) analysis changed forensic genetics by enabling the generation of highly discriminating DNA profiles from very tiny volumes of biological material [5, 12]. STR profiling is currently commonly utilized for individual identification, criminal investigations, relationship testing, missing-person inquiries, and catastrophe victim identification [1, 2, 5]. Nevertheless, a standard DNA profile primarily gives information describing the genetic source of biological material and does not necessarily convey the whole biological or contextual aspects of the evidence [1, 3, 6].

For example, if a bloodstain was recovered from a crime scene, traditional STR profiling can be used to determine whether the DNA profile matches a particular individual [1, 5]. However, DNA profiling alone might not be able to answer several other important questions, such as if the stain is indeed blood, what tissue or biological fluid was involved, the approximate age of the contributor, or what environmental and microbial characteristics might be associated with the evidence [3, 4]. Similarly, additional molecular information could add to the interpretation of evidence in complex crime scenes with more than one person, degraded biological material or mixtures of biological traces [3, 4, 6, 13]. Such limitations have led researchers to search for alternative and complementary molecular approaches [3, 4, 6, 13-17].

Advancements in molecular biology, biotechnology, sequencing technologies, mass spectrometry, and computational biology have paved the way for the integration of omics-based methods in forensic science [13-17]. The term "omics" encompasses the thorough examination of a specific category of biological molecules or information [15, 17]. Genomics is concerned with DNA and genetic diversity, while epigenomics explores molecular changes

that influence gene function [3, 15]. Transcriptomics delves into RNA and gene expression patterns, proteomics focuses on proteins and peptides, and metabolomics investigates small molecules and metabolic byproducts [15, 17]. Microbiomics examines the composition and functional traits of microbial communities linked to humans and their surroundings [13, 15, 17].

In forensic biology, the use of omics technologies marks a transition from traditional methods that focus on individual biomarkers to the analysis of extensive molecular patterns [6, 15, 17]. Genomics is the most well-established technique for human identification within these methods [1, 2, 6]. Contemporary forensic genomics encompasses STR profiling, the analysis of Single Nucleotide Polymorphisms (SNPs), mitochondrial DNA studies, Y-chromosome analysis, and Massively Parallel Sequencing (MPS), also known as Next-Generation Sequencing (NGS) [2, 18-23]. MPS has broadened the scope of forensic genetic analysis by enabling the concurrent examination of numerous genetic markers and offering sequence data that can uncover additional variations beyond what is possible with traditional fragment-length analysis [6, 20, 23].

Epigenomics is a significant field within forensic research [3, 24]. Epigenetic changes, especially DNA methylation, can differ among tissues and individuals and may also systematically alter with age [3, 24-26]. As a result, DNA methylation markers have been explored for forensic purposes, including estimating biological age, identifying tissues, distinguishing body fluids, and differentiating genetically similar individuals [24-26]. Unlike traditional genetic variations, epigenetic patterns can evolve over an individual's lifetime, offering potentially valuable insights that a standard DNA profile cannot provide [3, 24]. Consequently, forensic epigenetics has become a crucial area of study for deriving additional information from biological evidence [3, 24-26].

Proteomics offers an additional molecular viewpoint by analyzing the proteins and peptides found in biological samples [4, 27]. Various tissues and bodily fluids possess unique proteins or protein combinations, which makes proteomic analysis a potentially valuable tool for determining the biological source of forensic stains [4, 27-29]. Mass spectrometry has emerged as a crucial analytical method in forensic proteomics, being explored for purposes such as identifying body fluids, characterizing tissues, determining species, identifying protein biomarkers, conducting proteomic genotyping, and estimating postmortem intervals [4, 27-31]. Consequently, proteomic analysis can provide insights into the characteristics of biological evidence and also enable the exploration of genetic variation through genetically variant peptides [4, 27].

Microbiomics is a developing discipline that focuses on studying microbial communities linked to individuals, biological substances, objects, and surroundings [32-35]. The human body hosts a variety of microbial communities that differ across anatomical locations, individuals, regions, and environmental settings [32-34]. Consequently, forensic research has shown interest in the microbiomes of the skin, mouth, gastrointestinal tract, and genitals [32-35]. Microbial profiling holds promise for applications such as linking individuals, identifying body fluids, characterizing environments, interpreting trace evidence, and estimating postmortem intervals [32-35]. The progression of microbial communities after death has also sparked significant interest, as these changes might offer insights into decomposition and the elapsed time since death [33-35]. Nonetheless, the use of microbiomes in forensic science faces considerable

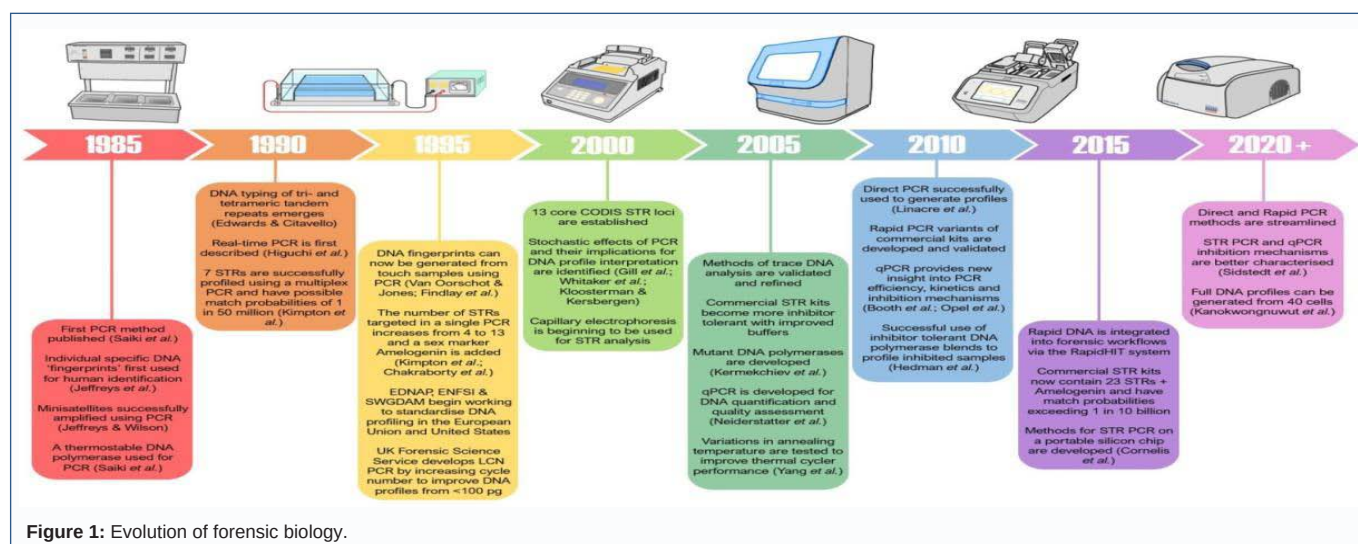


Figure 1: Evolution of forensic biology.

obstacles due to environmental variability, contamination, individual differences, and the scarcity of comprehensive reference databases [32-35].

The growing access to these molecular technologies has resulted in the idea of multi-omics, in which data from several biological levels are studied together [13-17]. Multi-omics is not based on a single molecular marker or analytical platform but aims at integrating complementary information obtained from genomics, epigenomics, proteomics, microbiomics and possibly other omics [13-17]. In forensic biology this approach opens the way to the conversion of the biological trace into a multidimensional information source [6, 13-17].

Genomics may identify the donors of biological materials, epigenomics can provide information on age or tissue characteristics [3, 24-26], proteomics can help to identify the nature of the biological fluid or tissue [4, 27-29] and microbiomics can give information on microbial or environmental associations [32-35]. So, the combination of these different molecular concentrations might give a more complete biological profile than regular DNA analysis [13-17].

Such an integrated approach is especially relevant to crime-scene reconstruction [6, 36]. Crime-scene reconstruction is the scientific interpretation of different types of evidence to determine the likely sequence and circumstances of events [36]. Biological evidence can give information about the people involved, the type of biological material deposited, possible interactions between people and objects, and changes that occur after death [1, 3, 4, 32-35]. Multi-omics could potentially combine genetic, epigenetic, protein-based and microbial information to contribute to this process [6, 13-17]. However, molecular results cannot be interpreted without considering other forensic evidence such as fingerprints, bloodstain patterns, trace evidence, digital evidence, scene documentation, and investigative information [36].

Multi-omics forensic analysis is promising but is still in its infancy [6, 13-17]. There are a number of technical and interpretive hurdles that must be cleared before it can be routinely and widely implemented [13-17, 37-42]. These include the limited number of forensic samples, degradation of DNA/RNA, instability of proteins, microbial contamination, environmental factors, population variation, tissue specificity, complex data analysis, lack of standardized

protocols, limited reference databases and extensive validation [37-50]. In addition, the combination of several molecular datasets can produce very complex biological information, leading to difficulties in the statistical interpretation and the courtroom presentation [13, 14, 16, 37-40]. But ethical and privacy considerations also become more important, as advanced molecular analysis can reveal information beyond simple individual identification [43, 45, 51].

The advent of massively parallel sequencing, high-resolution mass spectrometry, advanced bioinformatics, machine-learning algorithms and single-cell technologies will further expand the capabilities of forensic molecular analysis [6, 52-54]. These technologies could help forensic scientists to move toward a more complete characterization of biological traces [6, 13-17, 52]. However, the aim of multi-omics should not be viewed as a replacement for established DNA profiling [2, 6]. Instead, it should be viewed as a complementary framework that may provide additional layers of biological information and enhance the contextual interpretation of forensic evidence [6, 13-17].

The merging of the fields of genomics, epigenomics, proteomics and microbiomics is therefore a promising avenue for the future of forensic biology [6, 13-17]. An understanding of these approaches in depth may enable investigators to go beyond the traditional question of "Whose biological material is this?" to broader questions, such as "What biological material is present?", "What can be learned about its contributor?", and "What environmental or microbial information is associated with the evidence?", and "How these findings can be useful in the reconstruction of the crime scene?" [3, 4, 6, 13-17, 32-35]. This review discusses the principles, applications, advantages, limitations, current challenges and future prospects of multi-omics approaches in forensic biology with special focus on their potential contributions to human identification and crime scene reconstruction.

Evolution of Forensic Biology

Forensic biology has beautifully evolved from the study of visible biological traits and blood groups to highly advanced molecular and multi-omics technologies [1, 6-11]. Morphological analysis, blood grouping, microscopy and biochemical tests were used in the first forensic biological investigations [9-11]. The methods yielded useful information but were limited in their capacity to differentiate between individuals [9-11]. The development of molecular genetics, DNA profiling, Polymerase Chain Reaction (PCR), Short Tandem

Repeat (STR) analysis, Massively Parallel Sequencing (MPS) and other high-throughput techniques slowly led to an increase in the sensitivity, specificity and information obtained from biological evidence [5, 6, 7, 8, 12, 30 55-57]. In recent years, the field has expanded from traditional DNA profiling to genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics, laying the groundwork for a multi-omics approach to forensic investigation [3, 4, 6, 13-17, 52, 58]. The major stages in the evolution of forensic biology are summarized in Figure 1.

Early era: Serological and morphological approaches (1900-1960s)

The first systematic approaches to forensic biology were mostly based on blood-group analysis and serology. Karl Landsteiner described the ABO blood-group system in 1900, and this system has since become an important tool in forensic investigations and medico-legal examinations [9]. Blood grouping gave the investigators a knowledge of whether the biological material belonged to a particular blood group and so preliminary information for excluding or associating individuals [9, 10].

In the first half of the twentieth century, forensic laboratories used microscopic examination and biochemical tests to identify biological materials. Tests were developed for blood, semen and other body fluids to ascertain the biological nature of stains recovered from crime scenes [10]. However, these approaches generally had relatively low discrimination power because many unrelated individuals share the same blood group or biochemical characteristics [10].

Early forensic biology was therefore mostly useful for exclusion rather than individual identification: However, these methods laid down the basic premise that biological evidence that could be retrieved from a crime scene could provide scientifically interpretable information about persons and biological substances [9, 10].

Protein and enzyme-based methods (1960s-1980s)

The next major phase in the development of forensic biology was the study of genetic polymorphisms of proteins and enzymes [11]. The development of electrophoretic techniques enabled forensic scientists to analyze polymorphic proteins and enzymes found in blood and other biological materials [11].

Forensic identification of markers such as hemoglobin variants, serum proteins, transferrin, haptoglobin, Phosphoglucomutase (PGM), and other red-cell and serum enzyme systems was investigated [11]. These markers increased the discriminating power of biological evidence compared with ABO blood grouping alone [11].

However, protein and enzyme-based methods suffered from important limitations. In general, these required reasonably well-preserved biological material and sufficient amounts of sample [11]. They were also much less discriminating than that subsequently achieved by DNA analysis [1, 11]. Furthermore, environmental degradation can affect protein-based evidence [11]. These limitations led to a requirement for a more stable and highly polymorphic biological marker [1].

Emergence of DNA fingerprinting (1980s)

The big breakthrough was the discovery of DNA polymorphisms that could be used to tell individuals apart [7]. In 1984, Alec Jeffreys and co-workers discovered highly variable repetitive regions in human DNA and proposed the concept of DNA fingerprinting [7]. These regions were highly variable between individuals and were

called minisatellites or Variable Number Tandem Repeats (VNTRs) [7].

Soon after, the forensic potential of DNA fingerprinting was demonstrated. Gill, Jeffreys and Werrett (1985) reported the use of DNA fingerprinting for forensic biological evidence, where DNA from blood and semen stains could be used for individual identification [8]. This represented a fundamental change in forensic biology because DNA could provide substantially greater discriminatory power than traditional blood-group and protein markers [1, 8].

The importance of this development was not limited to identifying suspects. DNA analysis could also be used for victim identification, kinship analysis, paternity testing, missing-person investigations, and linking biological evidence from different crime scenes [1, 5].

PCR revolution and the development of STR profiling

Another major advance in forensic DNA analysis was the introduction of the Polymerase Chain Reaction (PCR) in the 1980s [55]. PCR allowed specific regions of DNA to be amplified from very small quantities of biological material [12, 55]. This was especially critical for forensic samples, since evidence from crime scenes is often limited, degraded, or contaminated with environmental compounds [12, 55].

With the introduction of PCR based forensic analysis it became possible to get DNA profiles from samples that would have been difficult or impossible to analyze with the earlier RFLP/VNTR methods [5, 12, 55]. The technology therefore greatly enhanced the sensitivity and practical utility of forensic DNA testing [5, 55].

Short Tandem Repeats (STRs) became the main markers used in forensic DNA profiling in the 1990s [5, 56]. STRs are short DNA sequences that are repeated a variable number of times at specific locations in the genome [5, 56]. The combined STR profile provides a high level of discrimination as individuals differ in the number of repeats at multiple loci [5, 56].

The development of multiplex PCR has allowed the amplification of multiple STR loci simultaneously, which increases the efficiency of forensic analysis [5, 56]. Automation of capillary electrophoresis further improved STR profiling in speed, reproducibility and standardization [5].

This combination of PCR + multiplex STR analysis + automated detection was the basis of modern forensic DNA identification [5, 56].

Expansion to mitochondrial DNA, Y-STRs and SNPs

As forensic DNA technology progressed, investigators began to use genetic markers other than the traditional autosomal STRs [19-21, 30, 57]. Mitochondrial DNA (mtDNA) was especially helpful when dealing with highly degraded samples or samples with low amounts of nuclear DNA since mitochondria are present in many copies per cell [57-59].

mtDNA analysis is especially useful in the study of hair shafts, bones, teeth and severely degraded remains [57, 59]. This is because mtDNA is maternally inherited, and less discriminative than a full autosomal STR profile [59]. MtDNA is generally used as a complementary, not standalone, identification method [59].

Another specialized approach was provided by Y-chromosome STRs (Y-STRs). The Y chromosome is passed down from father to son, and analysis of Y-STRs is especially helpful in the investigation of male DNA in samples with a high female DNA content e.g. some

sexual-assault evidence [30].

In the meantime, single nucleotide polymorphisms (SNPs) became important, as they are frequent in the whole human genome and can be analyzed with modern sequencing methods [19, 20]. SNPs are useful for inferring ancestry, testing identity, analyzing kinship and for forensic DNA phenotyping [19, 20].

Genomic and high-throughput era (2000s-2010s)

The advent of high-throughput molecular technologies ushered in a new era of forensic genomics, moving away from traditional forensic genetics [6, 52]. Advances in automated DNA analysis, multiplex PCR, better DNA databases and more sophisticated statistical interpretation mean that forensic laboratories could do more [5, 6].

Of particular importance was the development of Massively Parallel Sequencing (MPS), also known as Next-Generation Sequencing (NGS) [6, 52]. Differently from the conventional STR typing, mainly based on the length of the fragments, the MPS allows the determination of the actual nucleotide sequence of genetic markers and the simultaneous analysis of a high number of genetic targets [6, 52].

MPS has extended forensic genetic analysis to include:

- SNPs for STR sequence variation [6, 19]
- Mitochondrial DNA insertions/deletions polymorphisms (InDels) [6, 21]
- Y-chromosome markers [6, 20]
- ancestry-informative markers [6, 20]
- phenotype-associated genetic variants simultaneous use of several genetic marker systems [6, 20]

Forensic genomics has thus evolved from simple match/no-match evaluations of DNA profiles to extracting more information from the same biological trace [6, 20].

Emergence of forensic epigenomics

The discovery that gene activity could be regulated by chemical modifications that did not alter the underlying DNA sequence opened up a new dimension to forensic biology [3, 24]. DNA methylation is one of the most investigated epigenetic mechanisms [3, 24].

DNA methylation patterns can vary between tissues and may change in an ordered manner with biological aging [3, 24-26]. These traits have motivated research on the forensic application of epigenetics, particularly regarding age estimation and body-fluid/tissue identification [3, 24-26].

Thus, forensic epigenomics provides information that cannot be directly provided by conventional STR profiling [3, 24]. Although STR analysis is primarily used to answer the question “Who contributed the DNA?”, epigenetic analysis can help to answer questions such as “What is the approximate biological age of the contributor?” or “What type of tissue or biological material is present?” [3, 24-26].

This is a major conceptual shift from simply genetic identification to the molecular characterization of contributor and biological trace [3, 24-26].

Emergence of forensic proteomics

The advent of advanced mass spectrometry and proteomic

technologies has added another molecular layer to forensic biology [4, 53]. Proteomics is the study of proteins and peptides in biological samples on a large scale [4, 53].

Characteristic protein patterns are found in different biological fluids and tissues [4, 27, 53]. Therefore, proteomic approaches have been used to identify body fluids, characterize tissues, identify species, discover protein biomarkers and individualization [4, 53].

The particular interest in proteomics is because proteins are functional products of the cellular processes [4, 53]. So, protein profiles can offer information complementary to that obtained from DNA-based analysis [4, 53]. For example, DNA analysis can help identify the individual to which a stain belongs but protein analysis may help determine the biological nature of the stain or tissue source [4, 53].

The development of high-resolution mass spectrometry has further increased the potential of forensic proteomics, enabling researchers to simultaneously detect and characterize large numbers of peptides [4, 53].

Emergence of forensic microbiomics

Another important development in forensic biology is that the presence of microorganisms associated with humans and environments may provide potentially useful forensic information [59, 32-35, 60]. In our bodies we have the microbiome, complex communities of microbes that together are called the microbiome [32-35].

Microbial communities are associated with anatomic site and may vary between individuals [32-34]. Characteristic microbial communities also exist on environmental surfaces [32, 33]. These observations have propelled the research on forensic microbiology and microbiomics [59, 32-35].

Microbiome analysis has been studied for: individual association; identification of body fluids; characterization of environmental microbial communities; linking individuals to objects or locations; decomposition studies; estimation of the postmortem interval; and crime-scene reconstruction [32-35, 60].

Especially interesting is microbial succession during decomposition [33-35]. The microbial communities can change in predictable ways after death [33-35]. Such changes may potentially give information about the postmortem interval [33-35]. However, environmental conditions, temperature, geographical variation, contamination and individual differences can affect microbial profiles, therefore extensive validation is required before many microbiome-based applications become routine [32-35, 59].

Transition toward multi-omics forensic biology

The latest stage in the evolution of forensic biology is the integration of multiple molecular disciplines [6, 13-17]. In modern forensic research it is increasingly recognized that a biological trace carries information at several molecular scales and not only at the level of DNA [6, 13-17].

Genomics offers information on genetic identity and genetic variation [2, 6, 18-23].

Epigenomics can provide information on age, tissue and biological state [3, 24-26].

Proteomics is the study of proteins, body fluids and tissues [4, 53].

Microbiomics gives information on microbial communities and relationships to the environment [32-35, 60].

Transcriptomics can give information about patterns of gene expression and tissue characteristics [15, 17].

The integration of these different molecular layers is called multi-omics [13-17]. Recent advances suggest that forensic genetics is entering a “omics era”, where genomics, transcriptomics, epigenomics and microbiome profiling are increasingly being used to extract more information from biological traces [1, 6].

This represents a major shift in the objectives of forensic biology. Forensic DNA analysis was historically used primarily for individual identification [1, 2]. Current molecular forensic strategies are increasingly directed toward obtaining a wider biological profile from the same evidence [6].

Significance of this evolution for human identification and crime-scene reconstruction

Forensic biology has evolved to not only provide more accurate identification, but also to yield more contextual information that may be obtained from biological evidence [1, 3, 4, 6, 13-17].

Investigators have used conventional serological methods to classify biological material into broad categories [9-11]. DNA profiling enabled the identification of individuals with very high discrimination [1, 5, 7, 8, 56]. Modern omics approaches can add several additional dimensions to this information [6, 13-17].

For instance, a biological trace might be amenable to various approaches:

DNA → Who? [1, 2, 18-23]
↓
Epigenetic markers → Approx. age / tissue characteristics? [3, 24-26]
↓
Proteins → What body fluid/tissue? [4, 27-29, 31, 53]
↓
Microbiome → What microbe/environment relationship? [32-35, 60]
↓
Integrated Interpretation → What does the evidence tell us about what happened? [6, 13-17, 36]

This is especially important in crime scene reconstruction, where investigators need to see the evidence in its overall context and not just where it came from [36]. Ultimately multi-omics can help to identify relationships between biological traces, individuals, objects and locations [6, 13-17, 32-35]. But these applications require rigorous validation, appropriate reference populations, standardized analytical protocols and careful statistical interpretation before they can be considered as routine forensic evidence [13-16, 37-42, 43-45].

Concept of Multi-Omics in Forensic Biology

Introduction to the multi-omics concept

In the holistic study of a particular class of biological molecules or biological information, the term “omics” is used. After the development of genomics other omics fields such as transcriptomics,

proteomics, metabolomics, epigenomics and microbiomics have emerged [13-17]. Each omics discipline provides information on a different molecular layer of a biological system. However, the analysis of a single molecular layer may provide an incomplete picture of a complex biological process. This limitation resulted in the creation of multi-omics, an approach where datasets from two or more omics disciplines are analyzed together to gain a more comprehensive understanding of biological systems [9-11, 13-17].

Simply put, multi-omics is the study of multiple molecular layers within the same biological evidence and the integration of the information gathered from these layers. For instance, an individual's genetic variation can be informed by genomics, DNA methylation patterns can be informed by epigenomics, the proteins present in a biological sample can be informed by proteomics, and the microorganisms associated with the sample can be characterized by microbiomics. By combining these datasets, they may be able to provide information that cannot be obtained from any single omics approach alone [13-17].

The basic idea may be summed up as:

Biological evidence → multiple layers of molecules → data integration → complete biological profile

This is especially important in forensic biology, where a biological trace from a crime scene may contain information at various molecular scales [15]. Traditional DNA profiling is mostly used to address the question of identity, whereas multi-omics can provide additional information about the biological nature and properties of the evidence [1, 6, 13-17].

Why is multi-omics important in forensic biology?

Traditional forensic DNA profiling has revolutionized the identification of humans and is still the major molecular identification approach. However, a DNA profile typically provides information about the genetic profile of the contributor rather than a detailed description of the biological evidence [1, 5, 6].

For example, suppose we have a biological stain at a crime scene. Standard STR analysis can be used to determine whether the DNA profile is consistent with a particular individual [1, 5].

Other forensic questions, however, might be:

- What kind of biological material is present?
- What bodily fluid or tissue was responsible for the stain?
- What can we infer about the submitter?
- What is the probable biological age of the contributor?
- Are there environmental or microbial connections?
- Can the biological evidence help establish connections between people, objects and locations?
- Do various molecular features contribute jointly to the reconstruction of an event?

Multi-omics offers a conceptual framework to address some of these questions thru the integration of complementary molecular information [6, 13-17].

In recent years, the advent of genomics, transcriptomics, epigenomics and microbiome profiling in forensic genetics has been investigated to obtain further information from biological traces [6,

13-17].

Therefore, the importance of multi-omics in forensic biology is that it can transform a biological trace from a single source of identification information into a multi-dimensional source of biological information [6, 13-17].

Major molecular layers relevant to forensic multi-omics

Multi-omics is not one lab technique. Rather, it is a framework that can accommodate several molecular disciplines [13-17].

Genomics: Genomics is the DNA and genetic variation of an individual. Genomics is the most developed molecular layer in forensic science [2, 6, 18-23].

Main genomic approaches comprise:

- Short tandem repeat profiling
- Single Nucleotide Polymorphism (SNP) analyzes
- Mitochondrial DNA Testing
- Y chromosome analysis
- Insertion/deletion analysis
- High-Throughput Sequencing (HTS)
- Whole genome sequencing performed in selected research settings

The principal contribution of genomics to forensic biology is human identification and genetic association [2, 6, 18-23].

So:

Genomics - "Who does the biological evidence relate to?"

Epigenomics: Epigenomics is the study of molecular changes that regulate genes without modifying the underlying DNA sequence. DNA methylation is one of the most studied epigenetic mechanisms in forensic research [3, 24-26].

Patterns of DNA methylation differ between tissues and alter with age. Thus, forensic epigenomics has been explored for:

- Estimation of biological age
- Detection of body fluids
- Identification of tissue
- Bio-sample differentiation
- Biological Characterization Investigation

Therefore:

Epigenomics → "What biological information can we extract from the molecular state of the DNA

Forensic epigenetics is of particular interest as it might give information not available from conventional STR profiling alone [3, 24-26].

Transcriptomics: Transcriptomics is the study of RNA molecules and the patterns of gene expression RNA profiles have the potential to assist in determining the tissue or body fluid origin of forensic biological material, as different tissues express different sets of genes [15, 17].

For example, transcriptomic approaches have been explored

to identify: Blood, saliva, semen, Vaginal discharge, Menstruation, Other tissues.

Transcriptomics therefore gives information on which genes are actively expressed and which biological tissue may have contributed to a forensic sample [15, 17].

So,

Transcriptomics -- "What tissue or biological state is represented?"

Proteomics: Proteomics is the large-scale study of proteins and peptides found in biological samples.

Proteins are functional products of cellular processes. They can provide complementary information to DNA. Different body fluids and tissues have their characteristic protein compositions [4, 27-29, 31].

Proteomics could be useful for:

- Identification of body fluid
- Tissue recognition
- Protein biomarker discovery
- Species determination
- Personalization
- Investigation of post-mortem changes

Mass spectrometry is a key technology in forensic proteomics studies [4, 53].

So:

Proteomics → "What proteins are present and what properties do biological fluids have?"

Proteomic information can supplement genomic information, as DNA and proteins are different levels of biological organization [4, 53].

Microbiomics: Microbiomics studies the microbial communities of humans, biological materials and environments [32-35].

The human body contains complex microbial communities that vary by anatomical site and may also vary by individual and environment [32-35]. Therefore, microbial signatures have been examined in forensic studies of:

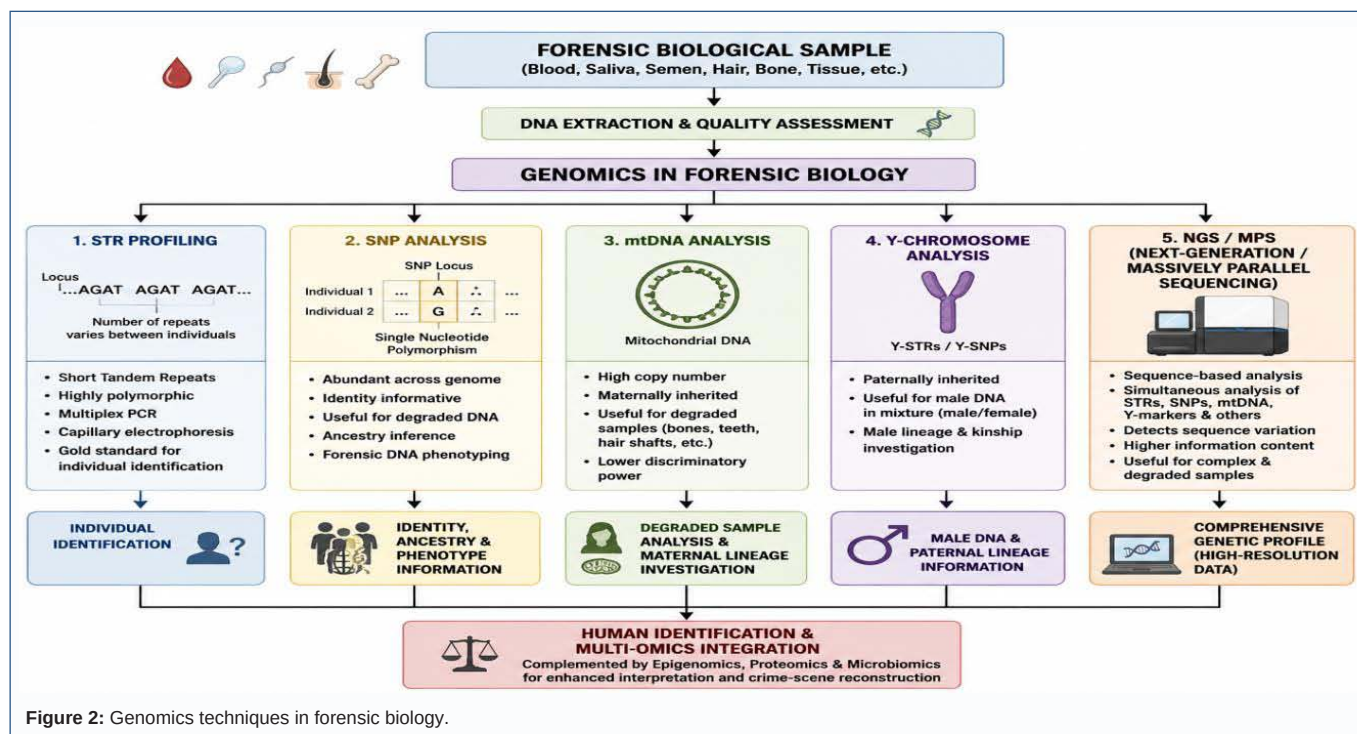
- Single Association
- Body fluid identification
- Environmental association
- Interpretation of trace evidence
- Studies of decomposition
- Estimation of postmortem interval
- Crime scene reconstruction

So:

Microbiomics → "What microbiota and environmental information are associated with the evidence?" [32-35, 60].

Genomics in Forensic Biology

Genomics is the study of DNA, genetic variation and genome



organization. It is the basis of modern forensic biology, particularly for the purpose of human identification [2, 6, 18-23]. In conventional forensic genomics, the main methods used are Short Tandem Repeats (STRs), Single Nucleotide Polymorphisms (SNPs), mitochondrial DNA (mtDNA), Y-chromosomal markers and Next-Generation/Massively Parallel Sequencing (NGS/MPS). These approaches yield complementary information depending on the type and condition of the biological evidence [2, 6, 18-23]. The major genomics techniques used in forensic biology are illustrated in Figure 2.

STR profiling

Short Tandem Repeats (STRs) are short sequences of DNA that are repeated a number of times at specific locations in the genome. STRs can be used to identify humans because the number of repeats can differ from person to person. Multiple STR loci are simultaneously analyzed using multiplex PCR to create a DNA profile [5, 12].

STR profiling is commonly used to identify suspects and victims, determine paternity and other family relationships, track missing persons, and compare evidence from crime scenes. Its main advantages include high discriminatory power, standardized analysis and extensive forensic databases. However, interpretation problems might be caused by degraded DNA, low-template samples, and complex DNA mixtures [9, 10, 5, 12].

STR profiling → (Mostly) used to identify an individual.

SNP analysis

Single Nucleotide Polymorphisms (SNPs) are variations at single nucleotide positions in the genome. SNPs are abundant throughout the genome and have many uses in forensics [19, 20].

SNPs may be used for:

- Human identification
- Inference of ancestry

- DNA forensic phenotyping
- Analysis of degraded DNA Kinship analysis

SNPs may be especially useful in highly degraded DNA than STRs, since many SNP assays focus on short DNA fragments. Some SNP panels can also give information on biogeographical ancestry and externally visible characteristics. However, predictions are probabilistic and depend on appropriate reference populations and validated marker panels [7, 11, 19, 20].

SNP analysis → Identity + ancestry + phenotype associated information.

Mitochondrial DNA (mtDNA)

Mitochondrial DNA is found in mitochondria and exists in many copies per cell. Its high copy number makes it useful for analysis of highly degraded biological materials including bones, teeth and hair shafts [57, 59].

mtDNA is passed down primarily thru the maternal line and is therefore useful for maternal line analysis and identification of degraded human remains. However, unrelated individuals can have identical mtDNA types and maternally related individuals can have similar profiles. Thus, mtDNA is generally less discriminating than autosomal STR profiling [8, 57, 59].

mtDNA → Good for degraded samples, maternal-lineage studies.

Y-Chromosome analysis

The Y chromosome is passed down thru the paternal line and is found mostly in men. Y-STRs and Y-SNPs are therefore useful for the study of male genetic material [30].

Y-STR analysis is especially useful in sexual-assault cases where male and female DNA are mixed and in paternal-lineage and male kinship cases. However, individuals with the same paternal ancestry may have similar Y-chromosomal profiles, limiting the discrimination

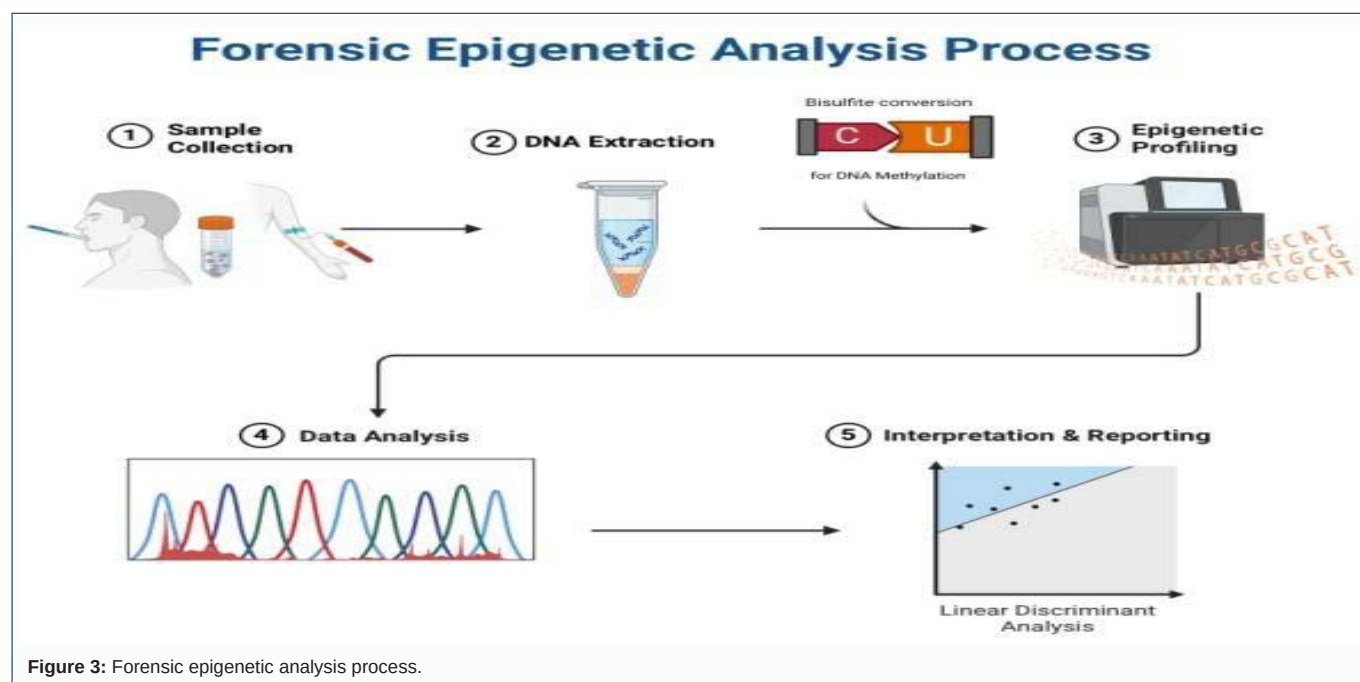


Figure 3: Forensic epigenetic analysis process.

power of individuals [55, 30].

Y-chromosome analysis → Male DNA and paternal line information.

Next-Generation sequencing/massively parallel sequencing

Next-Generation Sequencing (NGS), also known as Massively Parallel Sequencing (MPS), is a major breakthrough in forensic genomics. Traditional STR analysis only provides the length of the fragment, whereas MPS can identify the actual sequence of the genetic markers [6, 52].

MPS can evaluate several types of markers simultaneously, including:

STRs, SNPs, mtDNA, Y-chromosome markers

Variants associated with ancestry and phenotype

This allows for the provision of sequence level information and increased molecular detail from forensic samples. Targeted MPS shows great promise for complex and degraded samples, but there are some issues with cost, data analysis, validation, standardization and interpretation [6, 7, 52, 56].

MPS/NGS → Genomic information with high resolution sequencing.

Role of genomics in multi-omics forensics

The first molecular layer of the multi-omics approach is genomics. Genomics mainly provides information about genetic identity and variation but other OMICS technologies can provide complementary information [6, 13-17].

Genomics → Who helped?

↓

Epigenomics → Age/tissue specific information

↓

Proteomics → Characteristics of body fluid/tissue

↓

Microbiomics → Associations with microbes/environment

↓

Integration of multi-omics → Holistic biological characterization

Recent advances suggest that forensic genetics is entering an omics era, in which genomic information is being increasingly complemented with epigenomic, transcriptomic, proteomic and microbiome-based approaches [6,13-17, 56].

Forensic genomics is still the basis of human identification but, together with other molecular layers, it can give a wider picture of biological evidence and aid in the reconstruction of the crime scene [6,13-17, 56].

Epigenomics in Forensic Biology

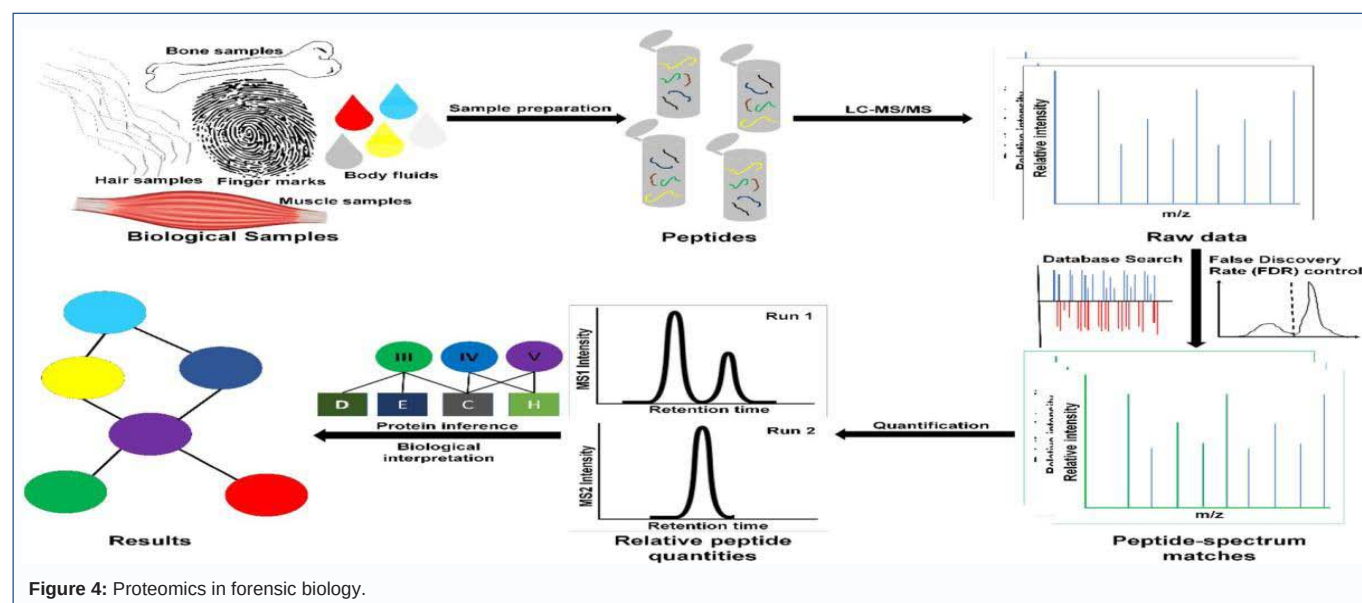
Epigenomics is the study of molecular modifications that affect gene activity without altering the DNA sequence. DNA methylation is the most studied epigenetic marker in forensic biology because methylation patterns can vary between tissues and change with age, giving information that goes beyond conventional DNA profiling [3, 24-26]. The general process of forensic epigenetic analysis is illustrated in Figure 3.

DNA methylation

DNA methylation is the addition of a methyl group to the cytosine residue, most commonly at CpG sites. Tissue and individual specific methylation differences can be used to characterize forensic biological samples. These patterns may help to differentiate between different body fluids and tissues and may give information not available from conventional STR profiling [3, 24-26].

Age estimation

Specific CpG sites undergo DNA methylation changes with chronological aging. These age-associated markers form the basis



of epigenetic clocks that can be used to estimate the biological or chronological age of an unknown contributor [24-26]. Recent studies are improving methylation-based age prediction models, but accuracy can depend on tissue type, population and other factors [24-26].

Tissue identification

DNA methylation patterns can be characteristic for different tissues and body fluids. Thus, methylation markers can potentially be used to identify whether a forensic sample was coming from blood, saliva, semen, vaginal material, buccal cells or other tissues [3, 24-26]. This may be especially useful for crime-scene reconstruction and interpretation of biological stains [24-26].

Environmental influences

Environmental and lifestyle factors such as age, diet, smoking, stress, environmental exposures and other biological conditions can affect DNA methylation. For forensic applications, temperature, humidity, UV exposure, microbial activity and sample storage may also affect stability or interpretation of methylation markers [3, 24-26]. Therefore, forensic epigenetic assays need to be developed and validated taking into account environmental effects [37-42].

Role of epigenomics in multi-omics forensics

In forensic multi-omics, epigenomics is a significant second layer of molecules. Genomics provides information about the genetic identity, epigenomics provides information about changes in gene regulation, especially methylation of DNA, without changing the DNA sequence. The combination of these two layers may lead to a more complete characterization of forensic biological evidence [3, 6, 13-17, 24-26].

Epigenomics role in multi-omics forensics can be summarized as:

- **Genomics + Epigenomics:** Genomic markers can help establish identity on an individual basis, DNA methylation markers may provide information on age, tissue type and biological characteristics [3, 6, 24-26].
- **Epigenomics + Proteomics:** Changes in DNA methylation can influence gene expression and the production of proteins. Thus, the combination of methylation and protein profiles may contribute to

the understanding of the biological origin of a forensic sample [3, 4, 24, 53].

- **Epigenomics + Microbiomics:** The host epigenetic modifications and microbial communities may interact with each other. Together their analysis may provide information about the biological and environmental context of a sample [32-35].

- **Crime-scene reconstruction:** Epigenetic information can help to identify the type of biological material and the approximate age of its contributor, supplementing genetic identification [3, 24-26].

- **Limited or degraded evidence:** Epigenetic markers may give additional information where conventional DNA profiling alone yields limited information [3, 24].

Proteomics in Forensic Biology

Proteomics is the study on a large scale of proteins in a biological sample. Proteins are important in forensic biology because their makeup can reveal information about the source and type of biological evidence. Proteomic analysis especially MS-based proteomics has been studied for body-fluid identification, tissue identification and protein biomarker discovery [4, 27-29, 31, 53]. The application of proteomics in forensic biology is illustrated in Figure 4.

Protein biomarkers

Protein biomarkers are proteins or peptides specific to a given biological material. Different combinations of proteins are found in different body fluids and tissues. This means that these molecules can be used as molecular markers [27-29, 31, 53].

For example, blood contains hemoglobin, saliva contains α -amylase, and semen contains semenogelin. Instead of relying on a single marker, multiple protein biomarkers may be used to enhance the reliability of biological material identification [27-29, 31].

Body-fluid identification

Proteomics can be used to identify the source of body fluids in a forensic stain. Mass-spectrometry analysis can identify characteristic proteins or peptides from blood, saliva, semen, vaginal fluid, and urine [4, 27-29, 31].

Table 1: Body fluids with their protein biomarkers.

Body fluids	Example protein biomarkers
Blood	Haemoglobin
Saliva	α -Amylase
Semen	Semenogelin, PSA
Vaginal fluid	Cornulin, involucrin
Urine	Uromodulin

Examples of representative protein biomarkers associated with different body fluids are summarized in Table 1.

Proteomic approaches are especially promising as they allow the simultaneous analysis of multiple proteins, which can improve the discrimination of biological fluids and support the analysis of complex samples [27-29, 31, 53].

Tissue identification

Different tissues express characteristic sets of proteins. Therefore, proteomic profiles may be used to identify the tissue of origin of an unknown biological sample [4, 53].

Proteomic studies have explored the identification of tissues such as:

- Muscle
- Skin
- Brain
- Bone
- Hair

This information can be used to complement DNA profiling by giving information about the source of the biological material, as opposed to just identifying who contributed it [4, 53].

Mass spectrometry

Mass Spectrometry (MS) is one of the key analytical technologies used in forensic proteomics. A common approach is to use Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) [4, 53].

A simplified workflow is:

```

Forensic sample
↓
Protein extraction
↓
Enzymatic digestion → peptides
↓
Liquid chromatography
↓
Mass spectrometry (LC-MS/MS)
↓
Protein/peptide identification
↓
Forensic interpretation

```

MS can give information about the mass and sequence of peptides, which can then be used for forensic interpretation [4, 53].

Role in multi-omics forensics

Proteomics adds a functional molecular dimension to genomics and epigenomics:

Genomics → Who?

Epigenomics → age/tissue specific information?

Proteomics → What proteins/body fluid/tissue are there?

Microbiomics → What are the microbial/environmental associations?

Thus, integrating proteomic data with genomic and other omics data may allow for a more complete interpretation of forensic biological evidence. Recent research explicitly identifies the potential of proteomics to move from source attribution to the reconstruction of forensic events in combination with other types of evidence [4, 6, 13-17, 53].

Microbiomics in Forensic Biology

Microbiomics is the study of the composition and genetic features of microbial communities associated with humans and their environment [32-35, 60]. The advances in DNA sequencing and metagenomics have opened up the possibility to investigate microbial signatures as potential forensic evidence. Microbiomics can offer additional information to traditional human DNA profiling but most applications are still in the research and validation phase [32-35, 60]. The potential applications of microbiomics in forensic biology are illustrated in Figure 5.

Skin microbiome

The skin microbiome is the collection of microorganisms living on human skin. Its composition differs from person to person and from site to site [32-34]. Therefore, microbial signatures passed from skin to objects thru contact have the potential for human association and trace evidence analysis. Microbial profiles of skin can be used to link individuals to objects or locations, but environmental variation and temporal changes are major limitations [32-35].

Skin microbiome → Possible connection with individual and contact

Oral microbiome

The oral microbiome is a diverse community of bacteria that live in the mouth and saliva. Oral microbial communities may differ from other body sites and may help in identifying or characterizing saliva-associated forensic evidence [32-35]. Thus, the oral microbiome analysis can be a complement to the classical DNA analysis, especially when the investigation of biological traces and contact between persons and objects [32, 33].

Saliva-related and biological trace data → Oral microbiome

Vaginal microbiome

The vaginal microbiome consists of distinct microbial communities, frequently dominated by *Lactobacillus* species. Differences in genital microbial communities have been studied as a way to identify biological material and to detect sexual contact or microbial transfer [32-35]. This may be especially relevant to forensic investigations of sexual-contact evidence. However, such evidence needs to be interpreted in light of individual variation and factors

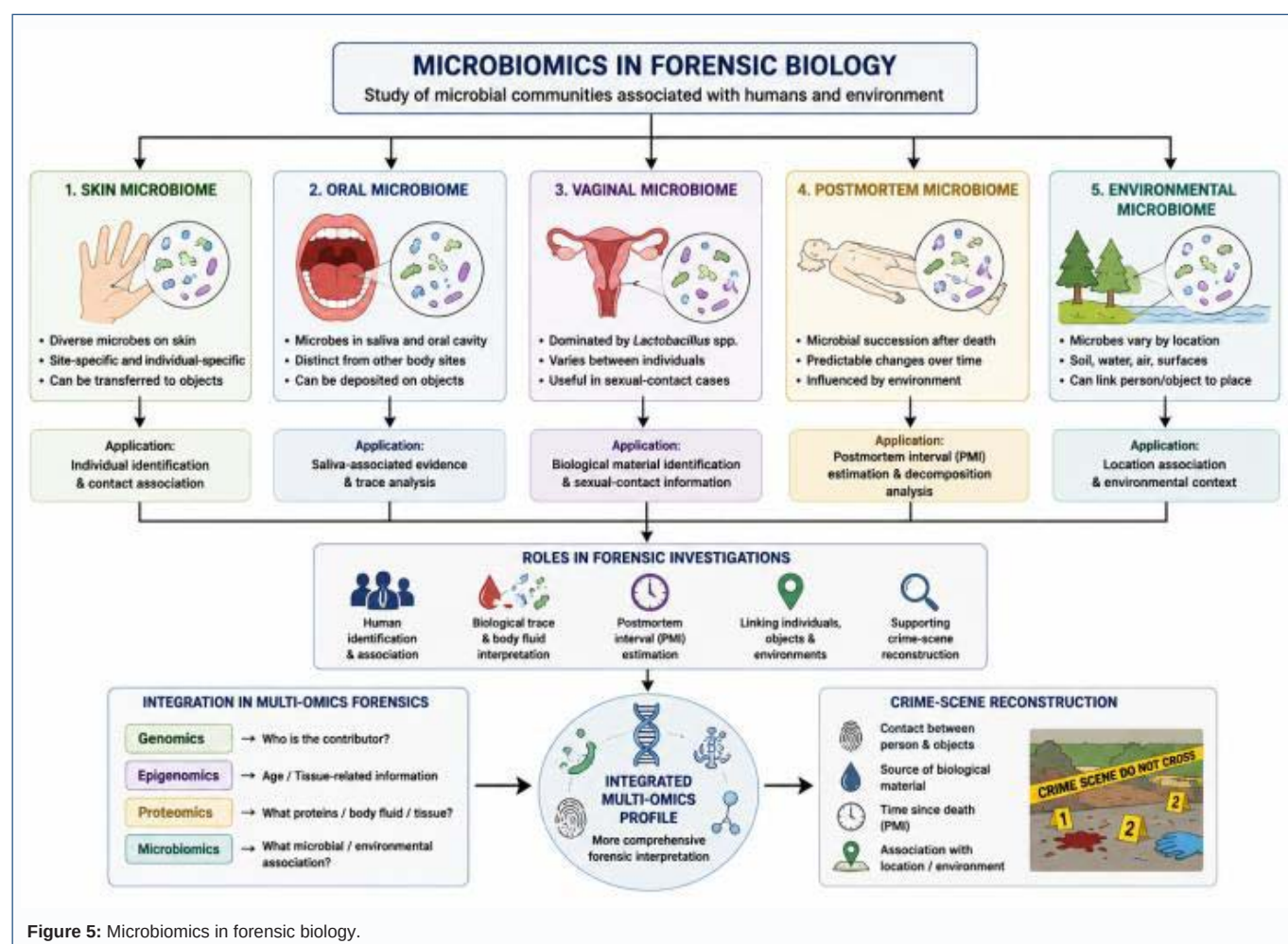


Figure 5: Microbiomics in forensic biology.

influencing the microbiome [32-35].

Vaginal microbiome → Possible biological-material and sexual-contact information.

Postmortem microbiome

Postmortem After death, shifts in microbial communities are caused by changes in physiological conditions of the body. The microbial communities associated with decomposition are sometimes called the thanatobiome. The estimation of the Postmortem Interval (PMI) is being studied by looking at predictable changes during decomposition [33-35].

Recent research describes microbial succession as a possible “microbial clock” but accuracy can vary substantially with environmental conditions, body location, temperature, soil and other factors [33-35].

Microbiome: a potential source of PMI and decomposition information.

Microbiomics in crime-scene reconstruction

Microbial evidence could be useful in crime scene reconstruction providing information about contact, location, biological material and decomposition. Microorganisms can be transferred between people, objects and environments, leaving traces of microbes that may complement conventional evidence [32-35, 60].

For example:

Person → Skin microbiota → Object

Person → Biological trace → Oral/vaginal microbiome

Body → Postmortem microbial succession → Decay/PMI

Environment → Environmental microbiome → Possible connection to location

Microbiomics may then help reconstruct relationships between individuals, objects and places eventually [32-35, 60]. Current research, however, stresses that the evidence from microbiomes should be considered as a supplement to, not a substitute for, traditional forensic DNA methods [32-35].

Role of microbiomics in multi-omics forensics

The fourth molecular layer of the multi-omics framework of your review is microbiomics.

Genomics → Who?

↓

Epigenomics → Age / tissue info?

↓

Proteomics → What biological material?

↓

Microbiomics → How are microbes associated with the

environment?



Integrated Multi-Omics → Comprehensive forensic interpretation

Therefore, microbiomics can provide environmental and microbial context to the genetic, epigenetic and protein information obtained from a forensic sample [6, 13-17, 32-35, 60].

From Single-Omics Analysis to Multi-Omics Analysis

Single-omics approach, where one type of molecular information is studied in isolation, is often characterized in traditional molecular biology [13-17].

For instance:

Genomic → DNA

Proteins → Proteomics or Microorganisms → Microbiomics

But biological systems are very interconnected. Genetic variation can change gene regulation. Gene expression influences protein output. Proteins are part of biochemical pathways. The host is in continuous communication with microorganisms and the environment [13-17, 32-35].

"Consequently, the individual analyzes of these molecular layers may miss the connections between them.

Multi-omics aims to study these relationships by integrating datasets from different molecular domains [13-17]. Reviews of multi-omics integration highlight that integration of genomics/metagenomics, transcriptomics, proteomics and metabolomics can provide a more holistic understanding than individual datasets, although data compatibility, experimental design and computational integration remain major challenges [13-17].

Multi-Omics Workflow in Forensic Biology

A generalized multi-omics workflow for forensic biological evidence can be summarized as follows:

Step 1: Collect biological evidence

Biological material such as blood, saliva, semen, hair, skin cells, bones or tissue are recovered at the crime scene [1, 6, 32-35].

Step 2: Preparation and Handling of Sample

The sample is sufficiently documented, preserved and prepared for molecular analysis with minimal contamination and degradation [37-42].

Step 3: Creation of molecular datasets

Various analytical techniques are used depending on the research or forensic question [13-17].

For instance:

- DNA sequencing → Genomics
- DNA methylation analysis → Epigenomics
- RNA sequencing → Transcriptomics
- Mass spectrometry → Proteomics
- Microbial sequencing → Microbiomics

Step 4: Quality control

The datasets generated were evaluated for:

contamination, degradation, quality of sequencing, reliability of analysis, missing data, and technical variation [37-42].

Step 5: Bioinformatics processing

Large sets of molecules are processed by computational pipelines. This can include sequence alignment, variant detection, methylation analysis, protein identification, microbial taxonomic classification and statistical analysis [13-17].

Step 6: Integrate data

Statistical, computational or network-based approaches are used to integrate different molecular datasets [13-17].

Step 7: Interpretation

The combined information is examined in light of the forensic question [6, 13-17].

Step 8: Forensic conclusion

The results are combined with traditional forensic evidence and suitable statistical interpretation to establish their evidentiary significance [37-42, 43-45].

Integration of Multi-Omics Approaches in Forensic Biology

The combination of genomics, epigenomics, proteomics and microbiomics shifts the paradigm from single marker analyzes to more complex molecular analyzes of forensic biological evidence. Each omics layer gives different but complementary information: genomics mainly contributes to individual identification, epigenomics can provide age and tissue-related information, proteomics can assist in body-fluid and tissue identification and microbiomics can provide microbial and environmental information [6, 13-17, 24-29, 31-35, 53, 60, 96]. Therefore, the combination of these layers can provide a wider interpretation of a biological trace than a single approach [6, 13-17].

Complementary role of different omics layers

The four main approaches can be summarized as follows (Table 2):

The merging of these data sets can enhance information retrieved from limited forensic samples but can also enhance interpretation when conventional evidence is incomplete or complex [6, 13-17]. Nevertheless, integration of multi-omics data is technically and computationally challenging [37-42]. The key information and forensic significance of the major omics layers are summarized in Table 2.

Workflow of multi-omics integration

For example, a generic forensic multi-omics workflow can be:

Table 2: Omics layer with their key information and forensic significance.

Omics layer	Key information	Forensic significance
Genomics	DNA variation	Individual identification, kinship
Epigenomics	DNA methylation	Tissue and age-related information
Proteomics	Proteins and peptides	Identification of body fluids/tissues
Microbiomics	Microbial communities	Populations Contact and environment information

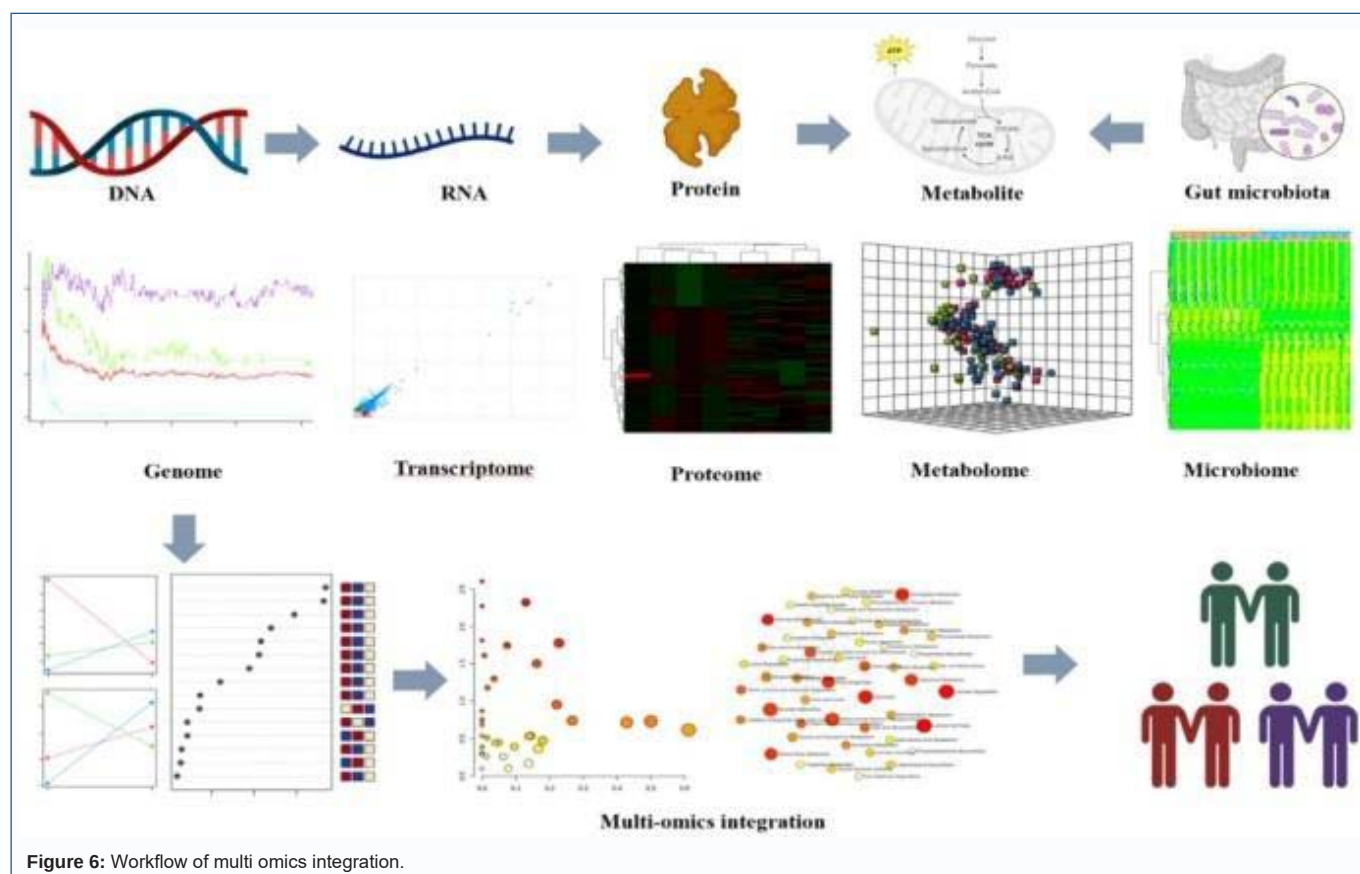


Figure 6: Workflow of multi omics integration.

Biological evidence collection



Sample processing and extraction



Genomic + Epigenomic + Proteomic + Microbiomic analysis



Quality control and pre-processing



Bioinformatic and statistical integration



Integrated molecular profile



Forensic interpretation

The omics datasets have different formats and biological characteristics. This calls for computational methods to normalize, compare and integrate the data [13-17]. Modern multi-omics studies use statistical, network and machine learning approaches for this purpose [13-17]. The overall workflow for multi-omics integration in forensic biology is illustrated in Figure 6.

Applications of Multi-Omics in Forensic Investigation

The multi-omics approach may extend forensic investigations

by integrating information from genomics, epigenomics, proteomics and microbiomics. Rather than depending on one biological marker, several molecular layers can be studied to gain information about the individual, biological material, age, tissue, microbial environment and circumstances of the evidence. These approaches have recently been reviewed as an emerging component of modern forensic genetics [6, 13-17]. However, most applications require further validation before use in routine casework [6, 13-17, 37-42]. The major applications of multi-omics approaches in forensic investigation are summarized in Figure 7.

Human identification

Genomics, particularly the analysis of STR and SNP, is the primary molecular method for human identification. Multi-omics can supplement genomic identification with epigenetic, protein and microbial data [2, 6, 18-23].

Do not add or omit any information. Do not add any other text to the output. For example:

Genomics → Profiling an individual's DNA

Epigenomics → Age-Related Info

Proteomics → Biological/tissue properties

Microbiomics → Individual/contact-specific microbial signatures

This combination could be especially helpful for difficult identification cases, such as unidentified remains and missing-person investigations, where multiple independent lines of biological evidence might be needed [6, 13-17].

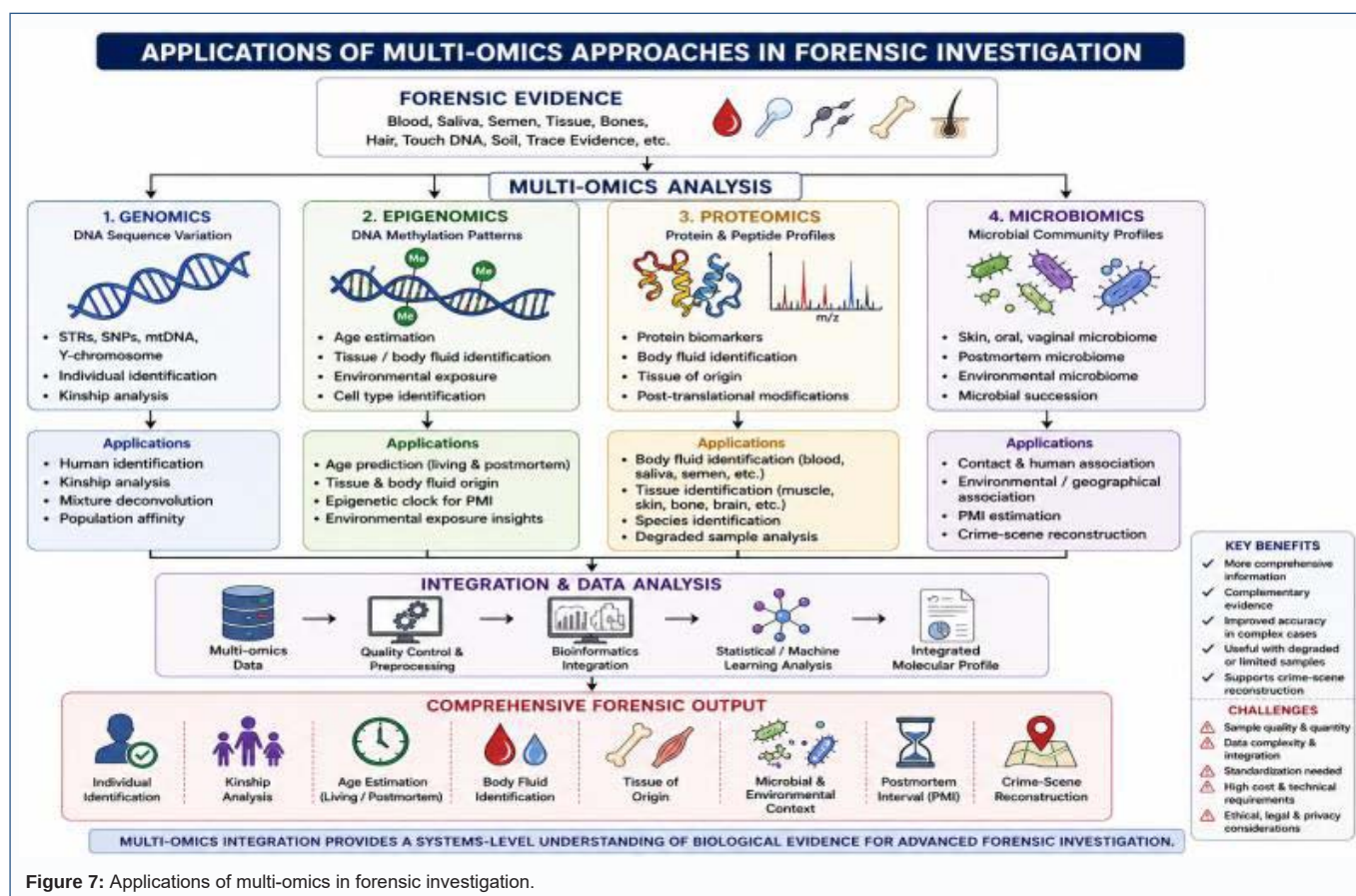


Figure 7: Applications of multi-omics in forensic investigation.

Body-fluid and tissue identification

Traditional DNA profiling can identify who has contributed biological material, but generally cannot tell what type of body fluid the DNA originated from.

Multi-omics may overcome this limitation by integrating:

- DNA markers for identification of contributors
- DNA methylation for tissue/body fluid identification
- Protein Biomarkers for Body Fluid Identification
- Microbial Signatures for More Biological Context [3, 4, 24-29, 31-35].

Mass-spectrometry-based proteomic approaches are being explored for blood, saliva, semen and other biological fluid identification [4, 27-29, 31, 53].

Age estimation

Changes in DNA methylation at certain genomic locations have been linked to chronological aging. Therefore, epigenomic information is integrated with genomic information for the age estimation of an unknown contributor [24-26].

The addition of molecular information from proteins and other omics layers may improve age predictions. Multi-omics approaches are also being used to estimate postmortem age-at-death and related forensic questions [3, 4, 24-26, 33-35].

Genomics → Identity

Epigenomics → Aging information

Crime-scene reconstruction

One of the most promising potential applications of multi-omics is the reconstruction of crime scenes.

Complementary information can be provided by different molecular layers [6, 13-17, 36]. The forensic information that may be obtained from the different molecular layers is summarized in Table 3.

The combination of these datasets may assist investigators in developing a more comprehensive interpretation of the biological evidence deposition at a scene [6, 13-17, 32-36].

Postmortem Interval (PMI) estimation

Estimating Postmortem Interval (PMI) is an important forensic problem. Environmental and biological variation can affect traditional methods [6, 13-17].

Multi-omics approaches can study postmortem changes at several molecular scales, including:

- DNA and epigenetic modifications

Table 3: Molecular layers with their forensic information potential.

Molecular Layer	Forensic information potential
Genomics	Who supplied the biological material?
Epigenomics	Age/tissue related characteristics
Proteomics	What body fluid/tissue is it?
Microbiomics	Association with contact/environment
Integrated analysis	Relation between people, biological material or environment

- RNA breakdown
- Protein breakdown
- Metabolic changes.
- Microbial succession [6, 13-17, 33-35]

Changes in the microbiome are especially promising because microbial communities change during decomposition. Integrated multi-omics approaches to PMI estimation are also being explored in recent research [33-35, 60].

Identification of environmental and geographical associations

Microbiomic data can provide clues about the environmental or geographic origin of biological evidence because microbial communities differ according to location and environmental conditions [32-35, 60].

Microbial profiles from soil, dust or objects could potentially contribute to geolocation or association of evidence with a particular environment. Microbial genomic methods are explored for human identification, geolocation, and cause-of-death studies.

Microbial evidence may provide another environmental layer for crime-scene interpretation when combined with human genomic and epigenomic information [32-35, 60].

Identification of contact and trace evidence

Microorganisms are constantly being transferred between people, objects and environments. Microbial signatures obtained from items such as clothing or personal items may therefore be a potential source of information about contact or association [32-35].

For instance:

Person → Skin microbiome → Item → Crime scene

Genomic analysis can detect human DNA but microbiomic analysis might give more information about microbial transfer. But such applications are promising but still affected by environmental variation and need further validation [32-35, 37-42].

Identification of unknown or degraded evidence

Forensic evidence can be exposed to heat, moisture, microorganisms, sunlight and other environmental conditions that cause degradation.

Such evidence may provide complementary information from different omics approaches:

Degraded sample

↓

Genomic analysis → DNA ID

↓

Epigenomic analysis → Methylation info

↓

Proteomic analysis → Protein biomarkers

↓

Microbiomic analysis → Microbial signatures

↓

Integrated forensic interpretation

Therefore, employing various molecular approaches could enhance the amount of useful information extracted from little evidence. The appropriateness of each technique, however, is conditional on the quality and validation of the samples [6, 13-17, 37-42].

Identification of human remains and missing persons

Multi-omics approaches may be especially useful in unidentified human remains and missing-person investigations. Genomics, epigenetics, proteomics and microbiomics can provide additional leads for investigation when traditional methods of identification such as fingerprints, dental records or adequate nuclear DNA are not available [6, 13-17, 57, 59]. Let's look at an example:

Genomics → Genetic identity / kinship

Epigenomics → Information related to aging

Proteomics → Tissue/biological features

Microbiomics Environmental/post-mortem information

These approaches can together help in developing a more complete biological profile of an unidentified individual [6, 13-17, 32-35].

Advantages of Multi-Omics Approaches in Forensic Biology

Multi-omics approaches integrate information from different molecular layers, such as genomics, epigenomics, proteomics and microbiomics. In forensic biology, this combination may provide a more complete picture of biological evidence than analysis of a single molecular layer. Recent forensic literature suggests that omics-based approaches are widening the scope of information that can be retrieved from human biological traces [6, 13-17].

Comprehensive biological information

The major benefit of multi-omics is that different molecular technologies generate different kinds of information from the same biological evidence.

- Genomics → identity genetic
- Epigenomics → Age and tissue information
- Proteomics → characteristics of body fluids and tissues
- Microbiomics → Microbiome and environmental associations

The combination of these layers can yield a more complete molecular profile of a forensic sample [3, 4, 6, 13-17, 24-29, 31-35].

Improved human identification

The conventional STR profiling is very successful for individual identification but additional omics layers may provide supplementary information. Genomic information can establish identity, whereas epigenetic, protein and microbial profiles may give further characteristics of the biological material or its context [1, 2, 5, 6, 18-23].

Such a tool can be especially useful in complex forensic investigations and cases of unidentified remains [6, 13-17, 57, 59].

Multiple questions from a single biological sample

A major potential benefit is the ability to answer several forensic

questions from limited evidence.

For instance:

- Who's that guy? → Genómica
- What is the person's age? → Epigenomics (en)
- What bodily fluid or tissue is present? → Proteomics/Epigenomics
- What microbial or environmental association exists? → Microbiomics

Thus, multi-omics can move forensic analysis beyond simple DNA matching toward comprehensive biological characterization [3, 4, 6, 13-17, 24-29, 31-35].

Better characterization of biological evidence

Multi-omics could help to characterize the source and nature of the biological material. Protein and epigenetic markers can be used for body-fluid or tissue identification while microbiomic profiles could help provide additional information about contact or environmental associations [3, 4, 24-29, 31-35, 53, 60].

This additional information can help improve interpretation of biological stains and trace evidence.

Potential use with complex and degraded samples

Forensic evidence can be damaged by environmental conditions, including DNA and other biomolecules. Different molecular layers may react differently to degradation. Therefore, the integration of available genomic, epigenetic, protein and microbial information may improve the amount of useful information obtained from a challenging sample [6, 13-17, 37-42].

However, this should be regarded as a potential advantage, since the performance of individual omics techniques is highly dependent on the quality of samples and the specific forensic application [37-42].

Improved crime-scene reconstruction

Multi-omics may contribute to the reconstruction of events by combining information on:

- The person connected with evidence
- Type of biological material
- The contributor's biological characteristics or age
- Associations Microbial/Contact
- Environmental or post-mortem changes [6, 13-17, 32-36]

Thus, integrated molecular information may contribute to assist investigators in understanding the relationship between people, biological evidence, objects and environments [6, 13-17, 32-36].

Discovery of new forensic biomarkers

Omics technologies enable the simultaneous analysis of thousands of molecular features. This allows the identification of new DNA variants, methylation sites, proteins and microbial signatures that could be used as future forensic biomarkers [3, 4, 6, 13-17, 24-29, 31-35, 53, 60].

Untargeted omics methods have already proven to be successful for the identification of relevant biomarkers in forensic genetics and biological trace analysis.

Increased reliability through complementary evidence

If independent molecular layers provide corroborating information, they can improve the overall interpretation of a forensic sample.

For example:

DNA profile → Person B

Methylation profile → Uniform age range

Protein pattern → Blood

Microbiome → Appropriate contact/environmental association

These results may provide a stronger overall biological interpretation than any one dataset alone. However, the evidential value of each result must be assessed independently using appropriate validation and statistical methods [6, 13-17, 37-42].

Advancement toward personalized forensic profiles

Multi-omics can generate very detailed molecular profiles of individuals and biological traces. This could allow forensic scientists to one day get information on identity, ancestry, age, tissue origin, body fluid and environmental context from the same evidence [6, 13-17, 19-26, 32-35].

This development has been recently described as part of the transition toward a 'omics era' of forensic genetics [1, 6].

Support for future forensic research

Multi-omics also opens up possibilities for research into:

- DNA phenotyping forensic
- Age determination
- Estimation of postmortem interval
- Forensic Microbiology
- Identification of Body Fluids
- Human remains identification
- Crime scene reconstruction
- Novel biomarkers discovery

Therefore, multi-omics will be an important direction for the future development of forensic biology [37-42].

This development has been recently described as part of the transition toward a 'omics era' of forensic genetics [6, 13-17, 33-35, 37-42, 60].

Limitations and Challenges of Multi-Omics in Forensic Biology

While multi-omics approaches provide a more comprehensive view of forensic biological evidence, their application in routine forensic casework is still ongoing. The integration of genomics, epigenomics, proteomics and microbiomics poses challenges such as sample quality, data complexity, standardization, costs, interpretation and legal acceptance. Recent forensic literature has suggested that omics technologies hold great promise, but many applications require additional validation before they can be considered standard forensic methods [6, 13-17, 36-42, 61-71].

Limited and degraded forensic samples

Forensic samples are often small, degraded or affected by

environmental conditions such as heat, moisture, sunlight and microorganisms. A single sample may not contain enough DNA, proteins and microbial material for reliable analysis of all omics layers [6, 32-36].

This is particularly important as multi-omics analysis may require sufficient material for a number of analytical techniques. Therefore, a destructive analysis and limited sample quantity may constrain analysis simultaneously [13-17, 37-42].

High data complexity

The number of molecular variables produced by multi-omics is very large. For example, one sample can produce thousands of measurements of the genome, epigenome, proteome and microbiome [13-17, 37-42].

The number of variables can be far greater than the number of forensic samples, which can pose statistical problems such as overfitting and false correlations. This can lead to apparently strong relationships that are not biologically meaningful [13-17, 37-42].

Difficulty in data integration

Different omics technologies produce data that differ in:

- Formats
- Scales
- Sizes
- Properties of measurement
- Probability distributions [13-17, 37-42]

For instance, genome sequencing data cannot be simply combined with proteomic or microbiome data without proper preprocessing and normalization. Thus, one of the key challenges of multi-omics research is the integration of these heterogeneous datasets [13-17, 37-42, 61-71].

Batch effects and technical variation

Differences in: may influence outcomes

- Laboratory instrument reagents
- Sample preparation
- Date of experiment
- Sequencing platform
- Pipeline for Data Processing

These differences can result in batch effects, where technical variation is confused with biological variation. Recent multi-omics studies still show that batch-effect correction and harmonization are still important challenges [43-45].

Lack of standardized protocols

Forensic laboratories need very standardized and reproducible methods. However, the multi-omics technologies currently use a wide range of extraction methods, instruments, databases, analytical pipelines and statistical methods [13-17, 37-42].

Without widely accepted protocols, it is difficult to compare results between laboratories and to establish consistent forensic standards [37-42].

Requirement for large reference databases

Reliability of forensic interpretation requires appropriate

reference populations and databases. Epigenomic and microbiomic analyzes can differ by age, tissue, population, lifestyle, geographical location and environmental conditions for methylation patterns and microbial communities [3, 24-29, 72-80].

Thus, we require large and diverse reference datasets to assess whether an observed molecular pattern is truly informative for forensic purposes [13-17, 37-42].

Environmental and biological variation

Biological evidence is intrinsically variable. Environmental conditions may affect microbial communities, DNA methylation and protein composition [15, 16, 39-42, 45-53].

For example, a person's microbiome may differ according to place, time, contact with the environment and personal factors. Thus, a microbial signature found at a crime scene may not be unique to a person [60, 72-80].

This variability can make interpretation difficult and reduce the reliability of predictive models [32-35, 72-80].

High cost and specialized equipment

Multi-omics analysis usually requires sophisticated instruments such as:

- Next generation sequencing platforms
- Mass Spectrometry
- Sophisticated molecular biology tools
- High Performance Computing Systems

Smaller forensic laboratories may struggle with comprehensive multi-omics analysis due to the cost of equipment, reagents, maintenance and skilled personnel [13-17, 37-42].

Computational and bioinformatic requirements

Specialized knowledge is needed to analyze multi-omics datasets in:

Bioinformatics Statistics Data Science Genomics Proteomics Microbial.

Big data needs massive computing storage and processing power. It can be difficult to select the right algorithms and interpret their outputs, particularly if different analytical methods yield different results [17, 37-42, 54, 63-71].

Validation and reproducibility

Prior to implementation of multi-omics methods in forensic casework, they need to be extensively validated for:

- Accuracy
- Precision
- Sensitivity
- Specificity
- Replicability
- Reliability
- Environmental effect
- Population Differences

Many omics-based forensic applications are still in research or

development stage and not yet universally accepted routine methods [6, 36-42, 54, 61-71].

Ethical and privacy concerns

Traditional STR profiling can be expanded to molecular profiling which provides much more information. Genomic and epigenomic data may potentially reveal information related to the ancestry, phenotype, biological characteristics or family relationships [6, 19, 20, 47-51, 81-84].

So, the increased amount of information generated by multi-omics leads to important questions about:

- Confidentiality
- Data storage
- Consent
- Databases access
- Use of genetic data for secondary purposes
- Proper bounds of forensic inference

These issues must be considered in tandem with technological development [47-50, 81-84].

Legal and courtroom acceptance

For forensic evidence to be admissible in court it must be both scientifically and legally acceptable. Many of the multi-omics methods are relatively new and questions may arise regarding their:

- Scientific credibility
- Error rates
- Standardization
- Interpretation
- Population Databases
- Reproducibility [37-45, 47-50, 81-84]

Hence, extensive validation and clear reporting standards will be needed for wider courtroom acceptance [37-42, 43-45].

Ethical, Legal and Social Considerations of Multi-Omics in Forensic Biology

The combination of genomics, epigenomics, proteomics and microbiomics can reveal much more biological information than traditional forensic DNA profiling. But the increased amount of information also raises important Ethical, Legal and Social (ELS) issues. These concerns are particularly salient because genomic and epigenomic data may reveal information about the individual and their biological relatives, while microbiomic information may reveal information about health, lifestyle or environmental exposure. Thus, the responsible application of multi-omics technologies must strike a balance between criminal investigation, individual rights, privacy and the public interest [47-51, 81-84].

Ethical considerations

Privacy and confidentiality: Multi-omics analysis can produce very detailed biological profiles. Genomic and epigenomic datasets, unlike a standard forensic STR profile, can contain information beyond simple identification, potentially including ancestry, phenotype-related features, and biological relationships [6, 47, 81-84].

Forensic laboratories must therefore ensure:

- Molecular data safe keeping.
- Restricted database access.
- Sufficient anonymization or pseudonymisation.
- Prevent data from leaking to unauthorized parties.

Data deletion or retention policies that are well defined [48, 82, 83].

Genetic privacy is important because genetic information can reveal information about biological relatives, not only the person from whom the sample was taken [47, 48, 82, 83].

Informed consent: Consent is particularly important when biological samples are collected for research or when samples originally collected for one purpose are analyzed using new technologies [47-50].

Where appropriate and legal persons should be informed of:

- The purpose of collecting samples.
- Types of molecular analysis that might be performed.
- Possible future applications of the data.
- Procedures for data storage and sharing.
- The potential dangers of genetic information [48-50, 82, 83].

However, the legal basis for collection and analysis for forensic casework may be different than that of the usual research consent requirements. This distinction should be clearly delineated in the applicable law and laboratory policy [48-50].

Genetic surveillance: Large forensic databases of genomic information may raise concerns about genetic surveillance. The more molecular information collected, the greater the chance that forensic databases will be used for purposes other than those originally intended [47-50, 81-83].

Purpose limitation is therefore a major ethical principle, meaning that information collected for forensic identification should not automatically be used for unrelated purposes. Ethical debates on forensic genetics often focus on the balance between the prevention of crime and privacy, dignity, justice and proportionality [47-50, 82, 83].

Risk of discrimination and stigmatization: Some multi-omics methods may provide information on ancestry, phenotype or biological traits. If this information is misunderstood or misinterpreted, it might lead to discrimination or stereotyping certain individuals or groups [47, 50].

Forensic scientists should therefore clearly separate:

Scientific probability ≠ Surety

Predictions based on genomic, epigenomic or microbiomic markers should not be treated as definitive characterizations of an individual [47, 50].

Legal considerations

Legal authority for sample collection: Blood, saliva, semen, hair, tissue or other biological material must be collected in accordance with applicable laws and procedures of the jurisdiction [48, 49].

Legal structures should clearly lay out:

- Who can authorize collection of biological samples.
- Under what circumstances samples can be collected.
- Which offences justify particular forensic analyses.
- How long samples and molecular data may be retained.
- Who can access forensic databases [48, 49, 82, 83].

Because legislation differs considerably between countries, multi-omics forensic practices must operate within the specific legal framework of the jurisdiction [48, 49].

Admissibility of new forensic technologies: Compared to conventional STR profiling, the use of multi-omics technologies is relatively recent. Methods need to be properly validated scientifically and evaluated for: Before results are used as evidence in court.

- Precision
- Sensitivity and specificity
- Reproducibility
- Error rates
- Population change
- Statistical models reliability [37-45]

Thus, a new molecular signature should not be considered legally conclusive simply because the results are statistically significant [47, 50].

DNA phenotyping and genetic genealogy: Advanced genomic analysis has the potential to provide information about ancestry or externally visible characteristics. Genetic relationships can also be used to generate investigative leads through investigative genetic genealogy [19, 20, 49, 50, 81]. These techniques have raised significant legal questions concerning database access, consent, relatives who never provided samples themselves and appropriate limits on law-enforcement searches [49, 82, 83].

Such methods require clearly defined rules concerning:

- Which cases can use the technology.
- Which databases can be searched.
- Whether relatives can be indirectly implicated.
- How investigative leads should be confirmed.
- How long derived information can be retained [49, 82, 83].

Data protection and security: Multi-omics generates huge amounts of sensitive biological data. Legal structures should deal with data security, access control, retention, sharing and destruction [48, 82, 83].

This is especially important as genomic data is effective in identifying and can show relationships between family members [47-50, 81-83].

Social considerations

Public trust: The successful implementation of forensic technologies is dependent on public acceptance. Overcollection or poorly regulated use of biological information can erode public confidence in forensic institutions [47, 48].

Clear policies explaining why samples are taken, how they are analyzed and how long information is kept can help build public trust [47, 48].

Impact on families and relatives: The genetic information is not completely individual. DNA can tell you something about a person's biological relatives [47-49, 82, 83].

Therefore, multi-omics analysis may unintentionally affect family members who have never provided biological samples. This issue is particularly important for forensic genetic genealogy and familial searching [49, 82, 83].

Equality and representation: It is necessary to develop and validate forensic prediction models on diverse populations. If reference databases are heavily based on certain populations, the prediction accuracy might vary among populations. This could potentially lead to unequal investigative results. Researchers should therefore focus on representative datasets and the performance of forensic models on different populations [20, 24-29, 72-80].

Misinterpretation by the public and media: Multi-omics results can be technically challenging. If the science only shows an association or probability, statements like "DNA indicates the person was there" or "the microbiome identifies the person" can be misleading [47, 50].

Forensic scientists should communicate their findings in scientifically appropriate terminology and clearly describe uncertainty and limitations.

Balance between public safety and individual rights: The fundamental social question is:

How much biological information should society allow law enforcement agencies to collect in exchange for the possible benefits to be derived from investigating crime?

Multi-omics might improve investigations; however, its usage should be proportional to the purpose of the investigation. Forensic genetics repeatedly comes to an intersection of public safety and individual privacy [47-50, 81-83].

Future Perspectives of Multi-Omics in Forensic Biology

Multi-omics is anticipated to be an important area of future forensic biology, as it allows the integration of information from genomics, epigenomics, proteomics, microbiomics and other molecular approaches. Research is moving beyond traditional DNA identification to obtain more information about biological traces, such as tissue type, age, time of deposition, postmortem changes and environmental associations. However, most multi-omics applications are still under development and require further validation before they come into routine casework [36-42, 54, 61-71].

Development of integrated forensic profiles

In the future, it could happen that forensic investigations may have a combined molecular profile from a single biological sample.

For example:

Genomics → Individual identification ↓ Epigenomics → Age/tissue information ↓ Proteomics → Body fluid/tissue characteristics ↓ Microbiomics → Contact/environmental information ↓ Integrated profile → Comprehensive forensic interpretation [3, 4, 24-29, 31-35, 53, 60].

Such integration may provide investigators with a much greater amount of information than conventional STR profiling alone [36, 61-63].

Advanced sequencing technologies

The increasing application of Massively Parallel Sequencing (MPS) and, in some research applications, whole genome sequencing, is predicted to extend the scope of genetic information that can be obtained from forensic samples [2, 18-23, 52, 58].

Future sequencing approaches may probe different types of markers simultaneously and provide sequence level information for STRs, SNPs, mitochondrial DNA and other genomic regions [2, 18-23, 52, 58].

Improved DNA methylation-based age estimation

Forensic age estimation is expected to be increasingly influenced by epigenomics. Future research will focus on identifying more robust age-associated methylation markers and develop models that are applicable across tissues, populations and environmental settings [24-29, 37-42].

More advanced models can estimate the age of an unknown contributor when standard DNA profiling is unable to provide this [24-29].

Advancement of proteomic forensics

Mass-spectrometry-based proteomics could be more useful for identification of body-fluid and tissue. Future studies may be able to identify larger panels of protein biomarkers instead of individual proteins [27-29, 31, 53].

Faster and more sensitive mass spectrometers could also make proteomic analysis more feasible for forensic laboratories. Recent reviews emphasize proteomics and multi-omics as emerging tools for forensic identification and cause-of-death inquiries [53, 61-63].

Development of microbiome-based forensics

Microbiomics might yield new forms of forensic evidence – microbial signatures. Potential future applications include:

Human/contact link

Body Fluid Characterization

Environmental correlations

Geographic information

Estimation of the postmortem interval Studies on decomposition [72-80, 60].

Microbial succession is highly promising for estimating the postmortem interval, but environmental variability is a major obstacle.

Improved postmortem interval estimation

One promising future application is the combination of multiple molecular changes after death.

Genomics + Epigenomics + Proteomics + Microbiomics + Other Omics

Could provide additional insight into the biological alterations that occur during decomposition [33-35, 53, 60, 61-63].

In 2026, a review proposed that the integration of multiple

omics layers could improve the accuracy and reliability of PMI estimation. The future research directions were discussed including standardization, machine-learning models, and portable technologies [54, 61-71].

Integration with artificial intelligence and machine learning

Multi-omics datasets are very large and complex. Artificial Intelligence (AI) and Machine Learning (ML) could therefore be relevant for detecting patterns that are not easy to detect with conventional statistical methods [54, 61-71].

Potential future applications include:

- Classification of biological specimens
- Age determination
- PMI prediction
- Biomarker identification
- Microbiomes classification
- Integration of multiple omics datasets [54, 61-71]

However, before AI-generated forensic conclusions can be trusted in legal investigations, they will need transparent models, proper validation and explainability. Recent researches on machine-learning based PMI models have indicated the future direction [37-42, 54, 61-71].

Portable and rapid multi-omics technologies

Another important direction for the future is the development of portable analytical systems which can be used closer to the crime scene.

Miniaturized sequencing, microfluidics, biosensors and portable mass-spectrometry technologies could potentially reduce the time between evidence collection and molecular analysis. Recent studies of forensic microbiome technologies emphasize the potential of combining microbiome analysis with biosensors, microfluidics and portable detection systems for rapid or on-site analysis [54, 60, 64-71].

Spatial multi-omics

Another future development is spatial multi-omics which combines molecular information with the location of cells or molecules within a tissue.

This could help researchers understand in forensic applications eventually:

- The location of particular molecular markers in tissue
- Changes in Decomposition
- Relationships between tissue and microbial communities
- Biological evidence spatial patterns [54, 61-71]

This is still an emerging research area and will need a lot of technological development before it can be used routinely in forensic work [37-42, 54, 61-71].

Expansion from human identification to crime-scene reconstruction

The future of forensic multi-omics may transcend the question “Who is the person?”

Instead, the integrated molecular analysis may help answer:

Who?

What biological material?

How old are you?

Where at?

When? And

what did you do?

Such as:

- Genomics → Who provided the DNA?
- Epigenomics – How old/tissue do you estimate it to be?
- Proteomics - “What biological material is there?”
- Microbiomics → Associations with contact or environment?
- Integrated analysis → What does the synthesis of the evidence tell us about the event? [36, 53, 54, 60-71].

This could push forensic biology toward more complete reconstruction of crime scenes [36, 53, 54, 60-71, 85, 86].

Conclusion

Emerging and promising direction in forensic biology are multi-omics approaches which provide the possibility of obtaining complementary information from different molecular domains. The integration of genomics, epigenomics, proteomics and microbiomics has the potential to extend forensic investigation beyond conventional DNA profiling by providing information on individual identification, age estimation, tissue and body-fluid identification, postmortem changes, microbial associations and crime-scene reconstruction [1, 2-36, 46, 51, 53, 55-57, 59-62, 72-80, 84-95]. Recent advances in forensic genetics indicate that the omics-based approaches are already expanding the scope of information that can be retrieved from biological traces [6, 31, 46, 61, 84, 87, 88, 92].

Genomics still provides the basic foundation for human identification with STRs, SNPs, mitochondrial DNA, and Y-chromosome analysis, and massively parallel sequencing further broadens the genetic information that can be extracted from forensic samples [1, 2, 5, 7-12, 18-23, 30, 51, 52, 55-59]. Epigenomics adds another molecular layer especially for age estimation and tissue/body-fluid identification [3, 24-29, 31, 94]. Proteomics offers possibilities for identifying proteins and biomarkers of biological fluids, tissues and postmortem changes [4, 32-35, 53, 62, 89, 93, 96, 97]. Microbiomics provides data on microbial communities that may be useful in contact evidence, environmental association, and postmortem interval studies [60, 72-80, 86, 91, 95].

The complementarity of multi-omics is especially powerful. Instead of just asking “Whose DNA is present?”, an integrated approach might ultimately help address multiple forensic questions simultaneously: Who is related to the evidence? What is the biological material? What can be inferred about the contributor? What environmental or microbial information is contained in the sample? And what can the combined molecular evidence tell us about the circumstances of the event? This more thorough strategy may be especially useful in complex cases, unidentified human remains and crime scene reconstruction [6, 13-17, 36, 54, 61-71, 84, 85, 89, 92, 93].

Nevertheless, the transition from research to routine forensic casework remains challenging. Challenges to be overcome include limited and degraded samples, high dimensional datasets, challenges in integrating heterogeneous omics data, batch effects, lack of standardized protocols, population variation, computational requirements, cost and need for extensive validation. Finally, the evidential value of multi-omics results needs to be carefully assessed to avoid overinterpretation and incorrect weighting of related molecular findings. Current reviews stress that although the potential is large, many omics applications need to demonstrate scientific reliability, robustness and courtroom admissibility [13-17, 37-45, 63-71].

As forensic technologies provide more detailed biological information, ethical, legal and social considerations will also become more important. To maintain public confidence in forensic science will require safeguarding genetic privacy, prudent management of molecular databases, appropriate data retention, avoidance of discrimination, and transparent communication of uncertainty [51, 47-50, 81-83].

Future integration with artificial intelligence, machine learning, single-cell analysis, spatial omics, advanced sequencing, and portable analytical technologies may further enhance the capabilities of forensic biology. Multi-omics approaches are also explored for other applications such as postmortem interval estimation. This could take advantage of the integration of multiple levels of molecules to obtain a more comprehensive view of postmortem biological changes [33-36, 52, 54, 58, 61-71, 84, 86, 89-95].

References

1. Jobling MA, Gill P. Encoded evidence: DNA in forensic analysis. *Nat Rev Genet.* 2004; 5: 739-751.
2. Jobling MA, Gill P. Encoded evidence: DNA in forensic analysis. *Nat Rev Genet.* 2004; 5: 739-751.
3. Vidaki A, Kayser M. Recent progress, methods and perspectives in forensic epigenetics. *Forensic Sci Int Genet.* 2018; 37: 180-195.
4. Parker GJ, McKiernan HE, Legg KM, Goecker ZC. Forensic proteomics. *Forensic Sci Int Genet.* 2021; 54: 102529.
5. Butler JM. *Fundamentals of Forensic DNA Typing.* Academic Press. 2010.
6. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet.* 2026; 27: 170-186.
7. Virkler K, Lednev IK. Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic Sci Int.* 2009; 188: 1-17.
8. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten.* 1900; 27: 357-362.
9. Butler JM. *Fundamentals of Forensic DNA Typing.* Academic Press. 2010.
10. Jobling MA, Gill P. Encoded evidence: DNA in forensic analysis. *Nat Rev Genet.* 2004; 5: 739-751.
11. Butler JM. Short tandem repeat typing technologies used in human identity testing. *Biotechniques.* 2007; 43: ii-v.
12. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 1987; 155: 335-350.
13. Santiago-Rodriguez TM, Hollister EB. Multi 'omic data integration: a review of concepts, considerations, and approaches. *Semin Perinatol.* 2021; 45(6): 151456.

14. Huang S, Chaudhary K, Garmire LX. More is better: recent progress in multi-omics data integration methods. *Front Genet.* 2017; 8: 84.
15. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet.* 2018; 19: 299-310.
16. Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, et al. Multi-omics factor analysis—a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol.* 2018; 14: e8124.
17. Veenstra TD. Omics in systems biology: current progress and future outlook. *Proteomics.* 2021; 21: E2000235.
18. Butler JM. *Fundamentals of Forensic DNA Typing.* Academic Press. 2010.
19. Butler JM, Coble MD, Vallone PM. STRs vs. SNPs: thoughts on the future of forensic DNA testing. *Forensic Sci Med Pathol.* 2007; 3: 200-205.
20. Kayser M, Branicki W, Parson W, Phillips C. Recent advances in forensic DNA phenotyping of appearance, ancestry and age. *Forensic Sci Int Genet.* 2023; 64: 102870.
21. Budowle B, Allard MW, Wilson MR, Chakraborty R. Forensics and mitochondrial DNA: applications, debates, and foundations. *Ann Rev Genomics Hum Genet.* 2003; 4: 119-141.
22. Jobling MA, Gill P. Encoded evidence: DNA in forensic analysis. *Nat Rev Genet.* 2004; 5: 739-751.
23. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet.* 2026; 27: 170-186.
24. Vidaki A, Kayser M. Recent progress, methods and perspectives in forensic epigenetics. *Forensic Sci Int Genet.* 2018; 37: 180-195.
25. Frumkin D, Wasserstrom A, Budowle B, Davidson A. DNA methylation-based forensic tissue identification. *Forensic Sci Int Genet.* 2011; 5(5): 517-524.
26. Freire-Aradas A, Phillips C, Lareu MV. Forensic individual age estimation with DNA: From initial approaches to methylation tests. *Forensic Sci Rev.* 2017; 29: 121-144.
27. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet.* 2018; 19: 371-384.
28. Kader F, Ghai M, Olaniran AO. Characterization of DNA methylation-based markers for human body fluid identification in forensics: a critical review. *Int J Legal Med.* 2020; 134: 1-20.
29. Gonzalez-Romero R, Suarez-Ulloa V, Ibañez O. A survey on forensic age estimation through DNA methylation: recent progress and future directions towards legal age estimation. *Legal Med.* 2026; 84: 102888.
30. Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints'. *Nature.* 1985; 318: 577-579.
31. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet.* 2026; 27: 170-186.
32. Parker GJ, McKiernan HE, Legg KM, Goecker ZC. Forensic proteomics. *Forensic Sci Int Genet.* 2021; 54: 102529.
33. van Steendam K, Ceuleneer MD, Dhaenens M, Van Hoofstat D, Deforce D. Mass spectrometry-based proteomics as a tool to identify biological matrices in forensic science. *Forensic Sci Int Genet.* 2013; 127(2): 287-298.
34. Duong A, Park JM, Lim HJ, Lee H. Proteomics in forensic analysis: applications for human samples. *Appl Sci.* 2021; 11: 3393.
35. Noor Z, Ahn SB, Baker MS, Ranganathan S, Mohamedali A. Mass spectrometry-based protein identification in proteomics—a review. *Brief Bioinform.* 2021; 22: 1620-1638.
36. Procopio N, Bonicelli A. From flesh to bones: Multi-omics approaches in forensic science. *Proteomics.* 2024; 24: e2200335.
37. Santiago-Rodriguez TM, Hollister EB. Multi 'omic data integration: A review of concepts, considerations, and approaches. *Semin Perinatol.* 2021; 45: 151456.
38. Huang S, Chaudhary K, Garmire LX. More is better: recent progress in multi-omics data integration methods. *Front Genet.* 2017; 8: 84.
39. Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, et al. Multi-omics factor analysis—a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol.* 2018; 14: e8124.
40. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet.* 2018; 19: 299-310.
41. Muller E, Bamberger T, Borenstein E. Navigating multi-omic integration methods for human microbiome research. *Nat Microbiol.* 2026; 11: 1153-1167.
42. Chetty A, Blekhan R. Multi-omic approaches for host-microbiome data integration. *Gut Microbes.* 2024; 16: 2297860.
43. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. *Nat Rev Genet.* 2010; 11: 733-739.
44. Choi JM, Chae H. A unified framework for correcting batch effects and integrating multi-omics data. *Sci Rep.* 2026; 16: 12341.
45. Baker M. Batch effects in high-throughput biological data and their implications for data integration. *Trends Biotechnol.* 2017.
46. Ramazan A, Burak T, Mahmut ŞY, Halit CA, Necdet S. Omics era in forensic medicine: towards a new age. *Turk J Med Sci.* 2020; 50: 1480-1490.
47. Williams R, Wienroth M. Social and ethical aspects of forensic genetics: A critical review. *Forensic Sci Rev.* 2017; 29(2): 145-169.
48. White TJ, Lee SB. *Forensic Genetics, Ethics, Privacy, and Public Policy.* Oxford University Press; 2020.
49. McEwen J, Pino N, Raphael A, Renna K, Boyer J, Brody LC. Investigative genetic genealogy: Ethical, legal, and social issues and directions for future research. *Forensic Genomics.* 2021; 1(3): 91-98.
50. Slabbert N, Heathfield LJ. Ethical, legal and social implications of forensic molecular phenotyping in South Africa. *Dev World Bioeth.* 2018; 18(2): 171-181.
51. Budowle B, van Daal A. Forensics and the new genetics—societal and ethical implications of emerging technologies. *Forensic Sci Int Genet.* 2010; 5(1): 147-153.
52. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. *Nat Rev Genet.* 2023; 24: 494-515.
53. Parker GJ, McKiernan HE, Legg KM, Goecker ZC. Forensic proteomics. *Forensic Sci Int Genet.* 2021; 54: 102529.
54. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics data integration, interpretation, and its application. *Bioinform Biol Insights.* 2020; 14: 1177932219899051.
55. Kaye DH. Blood testing in forensics: a review. *J Forensic Sci.* 1960; 5(4): 400-415.
56. Harris H. Applications of isozyme systems in the identification of human biological material. *Adv Human Genet.* 1971; 2: 55-92.
57. Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. *Nature.* 1985; 314: 67-73.
58. Mardinoglu A, Palsson BØ. Genome-scale models in human metabologenomics. *Nat Rev Genet.* 2025; 26: 123-140.
59. Butler JM. The mitochondrial DNA analysis for forensic purposes. *Forensic Sci Rev.* 2005; 17(1): 3-22.
60. Robinson JM, Pasternak Z, Mason CE, Elhaik E. Forensic applications of microbiomics: a review. *Forensic Sci Int Genet.* 2021; 11: 608101.
61. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet.* 2026; 27: 170-186.

- 170-186.
62. Procopio N, Bonicelli A. From flesh to bones: Multi-omics approaches in forensic science. *Proteomics*. 2024; 24: e2200335.
63. Santiago-Rodriguez TM, Hollister EB. Multi 'omic data integration: A review of concepts, considerations, and approaches. *Semin Perinatol*. 2021; 45: 151456.
64. Huang S, Chaudhary K, Garmire LX. More is better: recent progress in multi-omics data integration methods. *Front Genet*. 2017; 8: 84.
65. Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, et al. Multi-omics factor analysis—a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol*. 2018; 14: e8124.
66. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet*. 2018; 19: 299-310.
67. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
68. Procopio N, Bonicelli A. From flesh to bones: Multi-omics approaches in forensic science. *Proteomics*. 2024; 24: e2200335.
69. Santiago-Rodriguez TM, Hollister EB. Multi 'omic data integration: A review of concepts, considerations, and approaches. *Semin Perinatol*. 2021; 45: 151456.
70. Huang S, Chaudhary K, Garmire LX. More is better: recent progress in multi-omics data integration methods. *Front Genet*. 2017; 8: 84.
71. Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, et al. Multi-omics factor analysis—a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol*. 2018; 14: e8124.
72. Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R. Forensic identification using skin bacterial communities. *Proc Natl Acad Sci USA*. 2010; 107: 6477-6481.
73. Schmedes SE, Woerner AE, Budowle B. Forensic human identification using skin microbiomes. *Appl Environ Microbiol*. 2017; 83: e01672-17.
74. García MG, Pérez-Cárceles MD, Osuna E, Legaz I. Impact of the human microbiome in forensic sciences: a systematic review. *Appl Environ Microbiol*. 2020; 86: e01451-20.
75. Johnson HR, Trinidad DD, Guzman S. A genomic blueprint of the human microbiome. *Forensic Sci Int Genet*. 2016.
76. Schmedes SE, Sajantila A, Budowle B. Expansion of microbial forensics. *J Clin Microbiol*. 2016; 54: 1964-1974.
77. Robinson JM, Pasternak Z, Mason CE, Elhaik E. Forensic applications of microbiomics: a review. *Forensic Sci Int Genet*. 2021; 11: 608101.
78. Zhang J, Liu W, Simayijiang H, Hu P, Yan J. Application of Microbiome in Forensics. *Genomics Proteomics Bioinformatics*. 2023; 21: 97-107.
79. Franceschetti L, Lodetti G, Blandino A, Amadasi A, Bugelli V. Exploring the role of the human microbiome in forensic identification: opportunities and challenges. *Int J Legal Med*. 2024; 138: 1891-1905.
80. Kalanjali Y, Isukapatla AR. Postmortem microbiome dynamics: Review of forensic microbial clock. *J Forensic Legal Med*. 2026; 117: 103024.
81. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
82. Erlich Y, Narayanan A. Routes for breaching and protecting genetic privacy. *Nat Rev Genet*. 2014; 15: 409-421.
83. Naveed M, Ayday E, Clayton EW, Fellay J, Gunter CA, Hubaux JP, et al. Privacy in the genomic era. *ACM Comput Surv*. 2015; 48(1): 6.
84. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
85. Dahal A, McNevin D, Chikhani M, Ward J. An interdisciplinary forensic approach for human remains identification and missing persons investigations. *WIREs Forensic Sci*. 2023; 5: e1484.
86. Zar MS, Bibi A, Akhtar MS. Unravelling time after death: A comprehensive review of multi-omics approaches in postmortem interval estimation. *Forensic Sci Int*. 2026; 379: 112779.
87. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
88. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
89. Procopio N, Bonicelli A. From flesh to bones: Multi-omics approaches in forensic science. *Proteomics*. 2024; 24: e2200335.
90. Ramazan A, Burak T, Mahmut ŞY, Halit CA, Necdet S. Omics era in forensic medicine: towards a new age. *Turk J Med Sci*. 2020; 50: 1480-1490.
91. Zar MS, Bibi A, Akhtar MS. Unravelling time after death: A comprehensive review of multi-omics approaches in postmortem interval estimation. *Forensic Sci Int*. 2026; 379: 112779.
92. Kayser M. Forensic genetics in the omics era. *Nat Rev Genet*. 2026; 27: 170-186.
93. Procopio N, Bonicelli A. From flesh to bones: Multi-omics approaches in forensic science. *Proteomics*. 2024; 24: e2200335.
94. Gerra MC, Dallabona C, Cecchi R. Epigenetic analyses in forensic medicine: future and challenges. *Int J Legal Med*. 2024; 138: 701-719.
95. Zar MS, Bibi A, Akhtar MS. Unravelling time after death: A comprehensive review of multi-omics approaches in postmortem interval estimation. *Forensic Sci Int*. 2026; 379: 112779.
96. Shehata TP, Alex S, van Lierop SNC, Blom MJ, van de Wetering-Tieleman J, Prust N, et al. An integrated proteomic workflow for body fluid classification and single amino acid variant identification. *Forensic Sci Int Genet*. 2026; 81: 103343.
97. Alex S, Shehata TP, Gergely AI, de Puit M. Proteomics in forensics: from source attribution to reconstruction of events. *Sci Justice*. 2025; 65(6): 101320.