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Review Article

Biotechnology in Forensic Science: Advancements and Applications

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Abstract

Background: Biotechnology is a multidisciplinary field based on the expertise of molecular biology, chemistry, biochemistry, chemical and biological engineering, and digital computing. Biotechnology plays an important role in modern forensic science, driving advances in analytical tools and techniques.

This review study provides a brief overview of applications, highlighting advances in forensic biotechnology and key technologies involved in the domains of genomics and DNA analysis, microbial forensics, forensic medicine, and forensic serology. The integration of forensic expertise with technology has increased the accuracy, sensitivity, and efficiency of forensic casework.

Conclusions: This interdisciplinary field extends beyond its usual association with biology to also include chemistry, fingerprint analysis, and toxicology, among others. Continued progress and innovation in this advanced field will further enhance investigative capabilities and facilitate the pursuit of justice.

Abbreviations

VMD: Vacuum Metal Deposition; eDNA: Environmental DNA; FB: Forensic Biotechnology; NFSU: National Forensic Sciences University; PCR: Polymerase Chain Reaction; MPS: Massively Parallel Sequencing; NGS: Next Generation Sequencing; LIMS: Laboratory Information Management Systems; STRs: Short Tandem Repeats; HV1: Hypervariable region 1; HV2: Hypervariable region 2; CE: Capillary Electrophoresis; SNP: Single Nucleotide Polymorphism; CRISPR-Cas: Clustered Regularly Interspaced Short Palindromic Repeats- Cas; BFs: , CT: Computed Tomography; MRI: Magnetic Resonance Imaging; LC-MS: Liquid Chromatography-Mass Spectroscopy; PMI: Post Mortem Interval; Body Fluids; MS: Mass Spectroscopy; HMDB: Human Metabolomic Database, BMRB: Biological Magnetic Resonance Data Bank; ELISA: Enzyme Linked Immuno Sorbent Assay, LAMP: Loop-Mediated Isothermal Amplification

Introduction

Background

Biotechnology is a multidisciplinary field that has emerged by combining biology with technology to create innovative solutions and advancements ranging from health care and agriculture to industrial applications and environmental protection. Logically, the term 'Biotechnology' is derived from two simple words of science, 'Biology' and 'Technology'. Biotechnology integrates expertise and techniques from various fields, including molecular biology, chemistry, biochemistry, chemical engineering, and digital computing [1]. On account of its unique ability to serve and benefit humanity, the last two decades have seen unprecedented growth in biotechnology, opening up previously unimaginable possibilities [2]. Fundamental techniques in biotechnology include a range of methods and processes important for its diverse applications. The sub-disciplines of biotechnology are categorized by different color coding to understand different domains of the field [3] (Table 1).

Forensics, derived from the word "forensic" of Latin origin meaning "of or before the forum", is the application

Table 1: Categorization of Biotechnology Sub-Disciplines by Domain.

Colour	Area
Red	Medicine and human health
Green	Processes improving agriculture
Blue	Marine biotechnology
Yellow	Food and nutrition
Grey	Environmental biotechnology
Gold	Computing science and Bio-informatics
Brown	Biotechnology of dry and dessert areas
Violet	Philosophy, ethics and laws
Black	Biological warfare or bio-terrorism
White	Industrial processes involving microorganisms

of scientific techniques, methods, and principles within the legal and criminal justice systems [4]. This prestigious field "Biotechnology" addresses diverse fields including forensics and its different areas. The application of biotechnology in forensic investigation and analysis emerged as a new branch of forensic science called "Forensic Biotechnology (FB)" [5]. Traditionally, forensic investigations have relied on conventional techniques, although these methods have been invaluable in solving countless cases, they often present limitations in terms of specificity and reliability. Biotechnology tools and techniques are used to uncover important details, such as the presence of specific substances or pathogens at crime scenes, evidence examination and analysis, and much more.

To understand the role of biotechnology in forensic science as "Forensic Biotechnology" a comprehensive and critical evaluation of advancements and applications of biotechnological tools and techniques is conducted. This overview provides insight into the transformative impact of biotechnology on forensic science and highlights its importance in addressing contemporary challenges in criminal investigation and justice. The tools and techniques utilized across various domains of forensic biology, including Genomics and DNA Profiling, Microbial Forensics, Forensic Medicine, and Forensic Serology, are depicted (Figure 1) and extensively discussed in this review paper.

Generally, the application of biotechnology in forensics is related to but not limited to the biology domain only. A research study was conducted on developing fingerprints on advanced polymers using Vacuum Metal Deposition (VMD) to develop new fingerprinting methods to advance the prestigious FB field. VMD accurately detects latent prints on clothing that are difficult to process and detect [3]. While, traditionally relying on basic genetic techniques, recent biotechnology advances in genomics and DNA profiling, transcriptomics, and proteomics have greatly increased the sensitivity and reliability of forensic testing [6]. Isotope analysis using multi-isotope profiles and isoscapes from various tissues to help determine the area of origin, travel history, residence, and diet of the deceased has become a powerful tool in forensic anthropology, aiding human identification [7]. Successful Environmental DNA (eDNA) techniques in biodiversity and conservation efforts can be adapted for forensic use if they address investigative needs and species-specific factors [8]. Numerous institutions in India offer a range of courses in the field of biotechnology. Notably, the National Forensic Sciences University (NFSU), recognized as an Institution of National Importance, currently provides master's and specialization programs specifically focused on FB [9].

Main text

Biotechnology has a profound influence on the scope of forensic science, equipping it with powerful tools and technologies. From genomics to proteomics, advances in biotechnology have significantly increased the accuracy and efficiency of forensic investigations in identifying criminals, exonerating innocents, and uncovering critical evidence in criminal investigations. Continuous research and development improvement of instruments and techniques has increased sensitivity, resolution, reliability, and throughput.

Genomics and DNA analysis

Biotechnological advances play an important role in DNA forensics (Figure 2), enabling the extraction amplification, analysis, and interpretation of DNA evidence. DNA profiling or analysis stands as an important tool in the scope of forensic science and it has evolved significantly over time with the advancements in biotechnology, leading to the emerging and advancement of techniques like Polymerase Chain Reaction (PCR), Massively Parallel Sequencing (MPS) or Next Generation Sequencing (NGS), bioinformatics, etc.

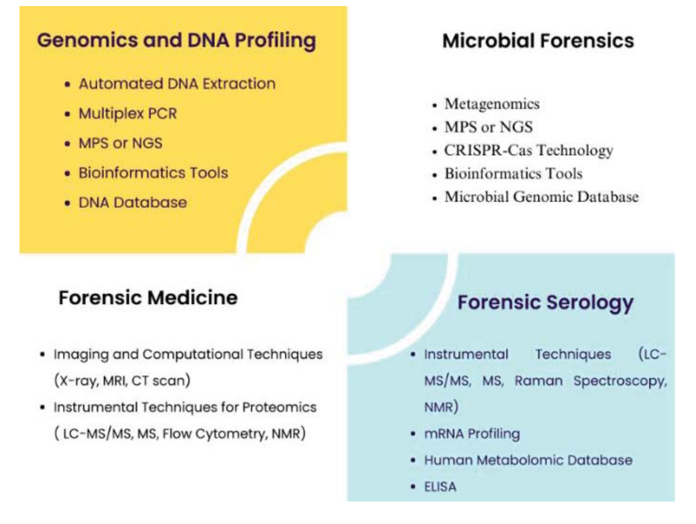

Figure 1: Biotechnological Tools and Techniques in Forensics - Genomic and DNA profiling, Microbial Forensics, Forensic Medicine and Forensic Serology.

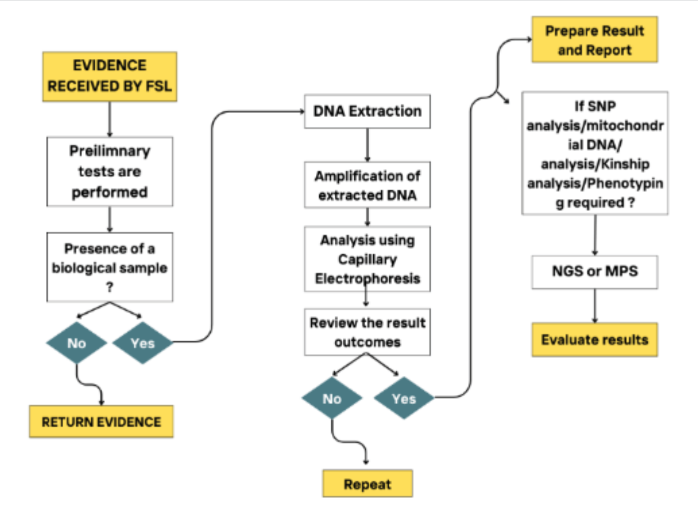

Figure 2: Sequential workflow of forensic Genomics and DNA profiling.

Traditional DNA extraction procedures suitable for forensic samples require multiple sample manipulations and can be time-consuming when processing large numbers of samples. An automated DNA extraction; BioRobot EZ1 workstation based on magnetic bead technology developed by Qiagen Corporation, was a compact and efficient instrument that operates with single-use reagent cartridges and cards with pre-programmed extraction protocol, which is capable of extracting high-quality DNA from up to six samples in less than 20 minutes [10]. The BioMech® NXP platform is specifically designed to process mixed DNA samples from sexual assault cases. This robotic platform provides increased efficiency and speed, reduces the risk of contamination or sample degradation, and ensures standardization of procedures [11]). Qiagen's QIAsymphony SP/AS instrument facilitates the extraction of high-quality genomic DNA, and pathogen DNA/RNA from 1 to 96 samples in 4 batches [12]. It accommodates sample volumes from the smallest forensic samples up to 10 ml, ensuring traceability and compatibility with Laboratory Information Management Systems (LIMS).

Kary Mullis, 1983, conceptualized PCR and developed the technique in 1985 while at Cetus Corporation. Multiplex PCR, first introduced in 1988, has shown the ability to amplify more than 50 loci simultaneously [13]. By the year 2000, with rapid advances in biotechnology, PCR-based DNA testing became a quick and efficient method of choice [14]. This enables analysis even from trace quantities. Multiplex PCR based Short Tandem Repeats (STRs) analysis has been established as the most efficient technique in forensic DNA analysis. A study reported a validated direct PCR amplification protocol for saliva samples using non-direct multiplex STR kits, that is cost-effective, time-efficient, and does not compromise the quality of the DNA profile [15].

Nuclear DNA typing and mitochondrial DNA typing of hypervariable regions 1 and 2 (HV1 and HV2) of 26 decalcified and non-treated bone samples were performed in a study [16]. Future advances in PCR technology of forensic interest include NGS, DNA databasing, and bioinformatics that assist in analyzing complex and vast amounts of data, facilitating connections between different cases [17]. DNA profiling involving specific polymorphic STRs using PCR technique, followed by Capillary Electrophoresis (CE) has been conventionally used in the field of forensics. Some limitations of CE-based fragment-length STR genotyping are that 1) multiplexing of forensically relevant loci is limited to less than 30 [18-20], 2) Partial or inconclusive profiles resulting from disrupted or degraded PCR samples can hinder discrimination and quality assurance [18,21-23], 3) Lower discrimination power compared to sequence-based typing [18]. These limitations may require forensic laboratories to introduce multiple quality assurance programs, workflows, and multiple systems per sample or opt for third-party typing services. With advancements in research and development in biotechnology, NGS has advanced rapidly resulting in state-of-the-art gains in forensic DNA analysis.

NGS has the remarkable ability to read millions of sequences, generating large DNA sequences from fragments of different sizes [24]. Libraries can be generated without prior sequence information which aids in genetic identification and discovery of variations. Forensic applications of the NGS or MPS approach are more targeted, involving initial PCR amplification of a set of target markers [25,26]. The MiSeq FGx™ Forensic Genomics System allows concurrent PCR amplification and sequencing of STR and Single Nucleotide Polymorphism (SNP) loci within a single reaction to maximize operational efficiency and information yield [18].

The advent of NGS has provided a valuable opportunity to streamline SNP typing in forensic applications [27]. Biotechnology advances in DNA analysis techniques have enabled efficient SNP typing to aid in individual identification, ancestry determination, forensic kinship analysis, phenotyping, and mitochondrial DNA analysis [27-31]. Kidd, et al. 2014 developed a panel of 55 SNPs that analyzed 73 populations from around the world. Clustered regularly interspaced short palindromic repeats- Cas (CRISPR-Cas) technology has also shown promising applications in forensic DNA analysis [32].

Manually analyzing complex DNA data can be challenging, time-consuming, and error-prone [33]. Bioinformatics, a sub-discipline of biotechnology, is a rapidly growing field of storing, retrieving, and comparing biological information in the form of databases. A bioinformatics approach that is inseparable from digital approaches could be a means of creating new approaches called cyber-bioinformatics. One limitation of this cyber-bioinformatics may be the potential for increased susceptibility to cyber security threats and data breaches [34].

The field of forensics is increasingly based on bio-molecular data and many countries have established DNA databases [35]. According to a 2008 survey of DNA databases conducted by Interpol in its 172 member countries, DNA profiling in criminal cases is used by 120 countries, 54 countries maintain national DNA databases and 26 countries are planning to establish a national DNA database [36]. Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences (AIIMS), New Delhi has developed India's first identification portal and DNA database of unidentified dead bodies autopsied at their department [37]. A study validated the Loop-Mediated Isothermal Amplification (LAMP) assay for detecting male DNA, which successfully tested samples from 92 sexual assault cases, with performance matching that of Y-STR profiling [38].

Microbial forensics

Microbial forensics emerged with the rise of bioterrorism in the early 2000s [39]. Historically, microbial forensics had a diminutive role in forensic investigations, being almost entirely culturally dependent. Recent advances and cost-effectiveness in biotechnology technologies such as

Microbiome Analysis and Metagenomics, Next Generation Sequencing (NGS) technology, clustered regularly interspaced short palindromic repeats- Cas (CRISPR-Cas) Technology, Bioinformatics Tools and Databases have indeed significantly promoted the development of forensic microbiology in recent years.

Using NGS or what is known as massively parallel sequencing, large genomes can be completely sequenced in a matter of weeks and small genomes such as bacterial genomes can be sequenced within a few days [40]. The ability to sequence mixtures of millions of DNA molecules in a single analytical run has created new opportunities to rapidly and efficiently analyze entire communities of microbes, including many species that cannot be cultured in the laboratory [41]. This makes it a highly reliable technique for signature recognition of microbial species found in various exhibits of forensic concern.

A microbiome-based method was developed in a study for the identification of relevant human body fluids (BFs), including venous blood, menstrual secretions, vaginal secretions, semen, saliva, feces and urine. Their study showed that using this microbiome-based technology all major body fluid samples could be detected and identified with an overall accuracy of 89.9%. Menstrual and vaginal discharge were found to be indistinguishable from each other and were thus classified as "female intimate samples" [42]. Based on a candidate marker for skin microbiome profiling, a novel targeted sequencing method was presented, called hidSkinPlex for forensic human identification. A study analyzed bacterial signatures using 16S rRNA V4-V5 regions in 46 different biological samples from diverse body sites, and was used to identify biological niches and physiological origins can be done [43]. A review work highlighted the main scientific works in relevance to forensics, produced so far that established the potential of using skin microbiome profiling for human identification [44]. Microbiology offers a wide range of forensically relevant applications (Figure 3). Machine learning enhances forensic microbiome analysis by handling complex data using k-nearest neighbors, random forests, and neural networks [45]. A research study combined miRNA and microbial DNA for forensic body fluid identification, achieving 94.6% accuracy using a qPCR-based model, which enabled simultaneous detection of blood, semen, saliva, feces, and vaginal/menstrual secretions, enhancing forensic DNA workflow [8]. Forensic microbiology takes advantage of advanced microbial identification to address iatrogenic infections, antimicrobial resistance, and medico-legal issues [46].

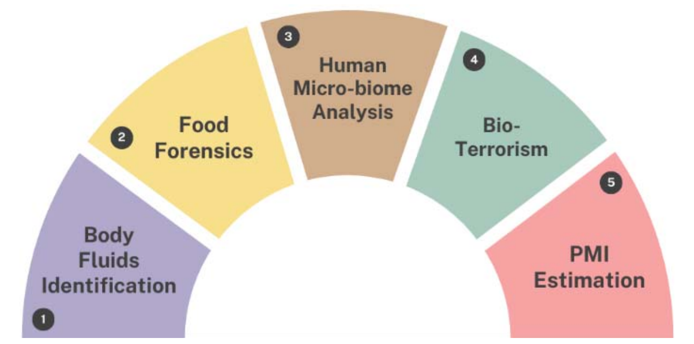

Figure 3: Applications of microbiology in forensics.

Forensic medicine

Biotechnology is in great demand in the field of forensic medicine. Advanced forensic investigation uses modern tools and technologies in investigating civil and criminal cases [31]. Virtual autopsy or virtopsy marks an advanced stage in forensic postmortem investigation using cutting-edge imaging and computational techniques [47]. Modern and advanced imaging technologies like X-ray, Computed Tomography (CT) scans, Magnetic Resonance Imaging (MRI), etc. are non-invasive powerful examination tools to interpret and document the findings during forensic investigation [31,48]. A study showed that CT scanning could potentially detect notable brain swelling and loss of gray-white differentiation in cases associated with cyanide poisoning, a condition that could mimic the natural disease and can make the clinical and forensic diagnosis challenging [49]. Bayesian statistics were applied with CT imaging and suggested that they could be used for the age estimation of the deceased based on costal cartilage calcification [50]. MRI is of vital importance for the diagnosis of natural death and evaluation of traumatic soft-tissue injuries [51]. Imaging techniques play significant in forensic facial reconstruction for Human Identification has been extensively studied in previous review studies [52].

Proteomics, the study of proteins and their functions (Figure 4) indulging advanced biotechnological tools has considerably changed the field of forensic science. According to the existing literature, proteomics offers a wide range of forensic applications to analyze and determine post-mortem remains, body fluids and tissues, bone and nails, and to establish individual identification [53,54]. Proteomics serves as an important alternative in cases where nucleic acids are absent or degraded. Nevertheless, limitations addressed in forensic proteomics, such as the presence of potential contaminants or unknown samples, can be addressed through the application of biotechnological tools and techniques [54].

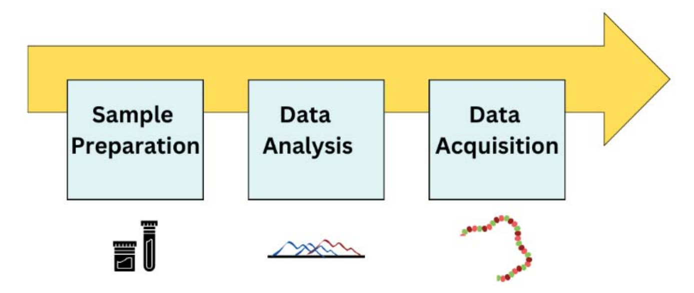

Figure 4: Sequential workflow in proteomics.

Liquid chromatography-mass spectroscopy/mass spectroscopy (LC-MS/MS) analysis of postmortem skeletal muscle samples is a potential method for the identification of biomarkers for Post-Mortem Interval (PMI) estimation by degradation of proteins in a reasonable time frame and is cost-effective [55]. Vitreous humor is the most studied biofluid for postmortem investigation due to its long stability and correlation with antemortem serum composition. A comparative analysis of antemortem and postmortem vitreous humor on animal models to estimate PMI was implemented, and their study validated the applications, limitations, and advances of NMR technology to determine PMI [56].

The rate of DNA degradation in Spleen and brain tissue was analyzed to determine PMI using flow cytometry [57]. The first report on a shotgun bottom-up proteomic methodology leveraging rapid protein extraction and nano-liquid chromatography/high-resolution mass spectrometry for ecchymotic skin testing in forensic pathology [58].

Forensic serology

Accurate identification of biological matrices such as peripheral blood, semen, saliva, menstrual blood, vaginal fluid, etc. is important in forensic casework. Specific or enriched functional identification markers for different Body Fluids (BFs) have traditionally relied on various chemical, enzymatic, or antibody reactions which are not reliable and contemporary with current scientific advances [59]. Advances in proteomic tools and techniques have emerged as an important approach in the investigation and analysis of serological samples. Emerging forensic serology technologies include lateral flow antibody testing for blood and saliva, mRNA-based dual testing for body fluid ID, XRF technology for non-destructive blood/semen detection, and infrared imaging for stain visualization [60].

A Mass Spectroscopy (MS) based proteomics approach was presented in a study, in which body fluids such as blood, saliva, semen, and vaginal fluid as well as their mixtures, were accurately identified and contamination could be easily detected [61]. Gowda, et al. 2015, in their study, identified 67 new metabolites, a third of which were newer than previously reported metabolites in the human serum metabolome database. The peak functions of their resulting spectra were dependent on established literature values, in particular, the Human Metabolomic Database (HMDB), Biological Magnetic Resonance Data Bank (BMRB), and their previous publications on serum metabolome [59]. Thread spray Mass Spectrometry (MS) enables blood analysis directly on textiles without extraction. This method is capable of detecting hemoglobin (α- and β-chains), heme, and lipids, distinguishing human, dog, and horse blood [62]. The specificity of Raman spectroscopy when combined with statistical modeling to develop a specific, reliable, reproducible, non-destructive, and universal approach for the identification of forensically relevant BFs including peripheral blood, saliva, sweat, semen, and vaginal fluid [63]. Figure 5 provides a comprehensive overview of the analysis and examination of forensically relevant body fluids, including conventional and advanced techniques.

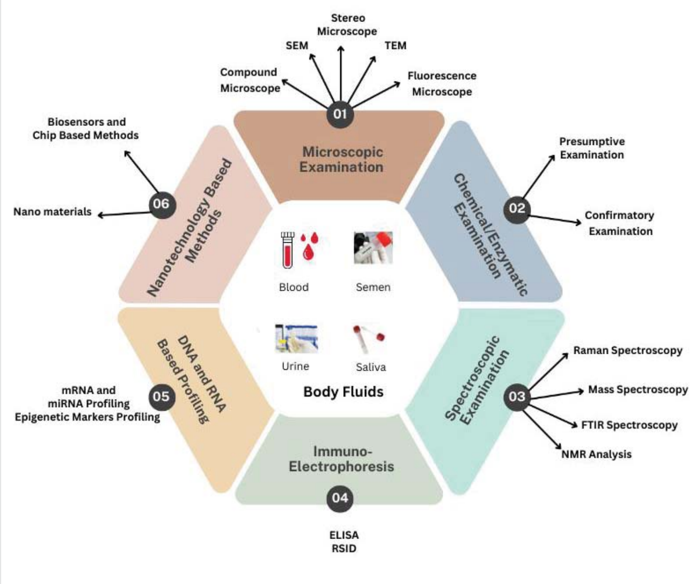

Figure 5: Uncovering clues: Examination of forensically relevant body fluids.

Classification modeling with variable selection was found to be a robust technique, which accurately predicted the identity of 99.9% of the spectra [63]. A multiplex reverse transcription endpoint PCR method was developed, for the identification of BFs including blood, semen, saliva, menstrual blood, and vaginal secretions to investigate the specificity, sensitivity, and suitability of the developed technology. Stains up to 15 months old were found suitable for mRNA profiling. They evaluated 66 target genes, including 6 peripheral blood, 5 menstrual blood, 6 semen, 6 saliva, 4 vaginal secretions, and 6 skin markers. Their research demonstrated the sensitivity, specificity, and ability of the assay to identify BFs in single-source and mixed stains [64]. Biotechnological advances in antibody production and conjugation techniques have led to the development of highly specific antibodies to target antigens found in bodily fluids, which has significantly advanced the specificity and reliability of Enzyme Linked Immuno Sorbent Assay (ELISA). The development of multiplex ELISA testing allowed multiple antigens or proteins in the same sample in one go [65]. The development of microfluidic devices enables rapid, accurate, on-site forensic analysis, serology, human identification, and drug/explosive detection [66]. In a research study that evaluated the detection of blood and semen on various fabrics at 1, 30, and 90 days using immunochromatographic tests, results showed that stain detection could persist for up to three months [67].

Author's perspective

From a biotechnology perspective, advances in genetic analysis, microbial forensics, and instrumental techniques have opened new doors to accuracy in forensic investigations. However, integrating these methods into routine forensic workflow remains a limitation due to cost ineffectiveness and the need for specialized training. Promoting partnerships between biotechnology institutes and forensic laboratories will accelerate innovation adoption.

Being multidisciplinary, biotechnological advancements have revolutionized forensic science by offering cutting-edge technologies for identification and evidence analysis. The rapid pace of biotechnology development poses a challenge in terms of skill enhancement and technology adoption. Collaborative efforts between forensic scientists or experts and biotechnologists are essential to effectively leverage these innovations.

Limitations

One of the major limitations is the high cost of implementing advanced technologies such as NGS and spectroscopy equipment, which can be prohibitive for many forensic-related organizations. Legal acceptability and ethical concerns associated with genetic information also pose barriers to widespread application.

Recommendations

Investment in research, training, and infrastructure is vital to address the challenges facing forensic biotechnology. Establishing standardized protocols for advanced techniques will ensure consistency and reliability in forensic analysis. Encouraging interdisciplinary collaboration will help in the better utilization of emerging technologies.

Future directions

The future of forensic biotechnology lies in the continued integration of artificial intelligence and machine learning with forensic casework to enhance the analysis and interpretation of related pieces of evidence and data.

Conclusion

The advent and application of advanced biotechnology-based methods point toward more accurate and quick forensics analysis and examination that will help to avoid false or inaccurate interpretations and reduce the number of pending cases. The rapid development of biotechnology presents many challenges and ethical concerns that require careful examination. A notable challenge lies in the rapid pace of technological progress, which often outstrips the establishment of adequate ethical frameworks and regulatory measures. Additionally, biotechnology approaches in genomics and the rise of DNA analysis in forensics raise privacy and consent concerns. Access to genetic information raises questions about the rights of individuals, particularly when data can be accessed or shared without explicit consent. The solution lies in implementing strong regulatory frameworks that prioritize individuals' rights to privacy and autonomy. Translating emerging biotechnology into practical applications for crime investigation and justice requires continued research and development efforts aimed at increasing sensitivity, accuracy, and efficiency in the field of forensics by collaborating between interdisciplinary teams including scientists, engineers, and forensic experts.

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