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Research Article

The Exploration of CRISPR-Cas9 Genome Editing Technology Specifically in the Context of Forensic Science; Advantages, Challenges, And Future Prospects

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Abstract

Forensic science has significantly evolved with advancements in epigenetics, biotechnology, gene editing and DNA profiling. A major breakthrough in this evolution is the CRISPR-Cas9 technology, introduced in the early 21st century, it has revolutionized the field, especially enhanced the precision and accuracy of forensic methods and revolutionized genetic research CRISPR-Cas9 greatly enhancing forensic analysis in areas like DNA fingerprinting, kinship testing and forensic phenotyping. It also improved the accuracy of biological evidence analysis, using techniques such as Single Nucleotide Polymorphism (SNP) analysis and forensic epigenetics. As the field advances, it navigates to complexities of legal and ethical implications, ensuring that forensic science continues to progress with integrity and efficacy. This advancement placed forensic science at the forefront of technological and scientific achievement. This progress marks a significant step forward in the realm of scientific investigation and legal justice.

Keywords: CRISPR-cas9; Forensic Investigation; DNA profiling; Biotechnology; SNPs Analysis; Forensic Science

Abbreviations: CRISR-cas9: Clustered Regularly Interspaced Short Palindromic Repeats; GMOs: Genetically Modified Organisms; DNA: Deoxyribonucleic Acid; SNPs: Single Nucleotide Polymorphisms; STRs: Short Tandem Repeats; PCR: Polymerase Chain Reaction; RNA: Ribonucleic Acid; eDNA: Environmental DNA; gRNA: Guide RNA; GE: Gene Editing; ZFN: Zinc Finger Nucleases; TALEN: Transcription Activator Like Effector Nucleases; CCR5: C-C Chemokine Receptor type 5

Key Points

1. The attractive gene editing technology to improve all the trembling Forensic standards.
2. The development of DNA profiling, DNA fingerprinting with the enlightenment of CRISPR Cas9.
3. Paternity testing, biological evidence analysis and criminal investigation with increasing the standards of testimony through CRISPR Cas9 induction.

Introduction

In recent years, forensic sciences the practice of using scientific methods to investigate crimes and offer testimony in court has made major steps. CRISPR-Cas9 is one of the revolutionary innovations in which forensic scientists are interested [1]. CRISPR-Cas9 was initially created as a tool for gene editing, but it has since found creative uses outside of biology and genetics, overturning the discipline of forensic science [2]. This article introduces the relationship between forensic science and CRISPR-Cas9 while examining its possible implications and the exciting new directions, it might take place in forensic investigations and many others. The gene-editing tool CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats

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and CRISPR-associated with protein 9, is effective at making precise changes to DNA sequences [3]. It was initially identified as a defense mechanism against viral infections in the bacterial immune system [4]. The Cas9 protein can recognize and target particular DNA sequences, resulting in precise DNA breakage and subsequent repair [5]. Cas9 is guided by the tiny RNA molecule guide RNA (gRNA) [6]. The CRISPR-Cas9 system's applications in a wide range of scientific fields have quickly grown because of its exceptional accuracy and adaptability. The use of CRISPR-Cas9 in forensic research has the potential to solve problems and improve current methods [7]. DNA profiling, also known as DNA fingerprinting, is one of the key areas where CRISPR-Cas9 is having a big influence. In forensic investigations, DNA profiling is important for identifying people based on their genetic makeup [8]. The most effective conventional techniques for DNA profiling have been Short Tandem Repeat (STR) analyses [9]. However, CRISPR-Cas9 has several benefits over existing techniques, including improved targeting efficiency, enhanced precision, and increased resolution. Additionally, CRISPR-Cas9 has the potential to transform forensic kinship and paternity tests. Establishing paternity and family ties is essential in many legal matters, including child custody battles and inheritance claims [10]. Forensic scientists may overcome the drawbacks of conventional techniques and provide more precise results, leading to more trustworthy conclusions in such instances, by utilizing the accuracy and efficiency of CRISPR-Cas9 [11]. Phenotyping is the process of inferring physical traits like eye color, hair color, and facial features from a person's DNA. CRISPR-Cas9 can shed light on a suspect's appearance by examining certain DNA markers linked to these attributes [12]. With the use of this information, detectives may reduce the number of prospective suspects and witnesses can give more precise descriptions. Additionally, CRISPR-Cas9 has the potential to improve forensic investigations' examination of biological evidence. The quantity and quality of genetic material that is accessible for examination are constrained by the frequent degradation of DNA samples collected from crime scenes. The enhanced DNA recovery and analysis capabilities provided by CRISPR-Cas9-based approaches allow forensic experts to retrieve important data even from difficult or contaminated material [13]. The deconvolution and interpretation of DNA mixes, which are frequently present in crime scene evidence, can also be improved using CRISPR-Cas9 [14]. Though CRISPR-Cas9's prospective uses in forensic science are exciting, careful examination of ethical and legal issues is required. It's important to carefully consider issues like genetic privacy, technology misuse and the moral consequences of changing someone's DNA. It is important to strike a balance between using CRISPR-Cas9 to enhance forensic investigations and defending people's rights and privacy [15]. In the final analysis, the relationship between forensic science and CRISPR-Cas9 offers a fascinating chance to progress and improve the discipline of criminal investigations. CRISPR-Cas9's accuracy, adaptability, and potentially open up new possibilities for biological evidence analysis, forensic paternity testing, phenotyping, and DNA profiling. To ensure responsible and advantageous usage in the field of forensic science, however, it is important to negotiate the ethical and legal concerns involved with its deployment, as with any strong technology [16].

Methodology

Database Search Terms and Timeline

This review was conducted by performing a systematic literature search on online resources (PubMed, Central database and Google Scholar) until August 2023, by using the following keywords: "(CRISPR Cas9) or (Role of CRISPR in Forensic) or (CRISPR Cas9 applications in forensic sciences) and (Legal and Ethical issues of CRISPR Cas9 in Forensic Sciences) and (Future of CRISPR Cas9 in Forensic Science Investigation).

Inclusion and Exclusion Criteria

The articles which describe CRISPR and their forensic perspectives were included in the given article. The following inclusion criteria were applied: original forensic related articles, case reports, editorials, Research articles, review articles and conference abstract. The following exclusion criteria were adopted: articles are not in English, communications at conference, Clinical studies to remain in the ambit of forensic perspective.

Study Selection

We retrieved almost 130 articles. After excluding all duplicate articles, the reviewers retrieved the abstract and full text of each article independently applying the inclusion and exclusion criteria.

History of CRISPR Cas9

The public and scientific community throughout the world are becoming increasingly aware of CRISPR, particularly since He Jiankui utilized it to tweak the CCR5 gene in two embryos that eventually became twin girls born in 2019. It's feasible that scientists and the general public are aware of CRISPR's potency as a gene editing tool, but they may not be aware of the specifics of how it was discovered or the historic cases that made it well-known in the first place [17]. The cutting-edge gene-editing tool CRISPR-Cas9 enables researchers to precisely alter the DNA of organisms [18]. Biotechnology, agriculture, and other industries might all undergo radical change as a result. The evolution of CRISPR-Cas9 can be briefly summarized below.

Discovery of CRISPR

Early in the 1980s, researchers discovered a peculiar repeating pattern in the DNA of bacteria, which is where the mystery of CRISPR begins. At first, these repeating DNA sequences were thought to be waste or leftovers from viral infections [19]. In 2020, the invention of a CRISPR-Cas9-based genome editing technique won the Nobel Prize in Chemistry, less than ten years after the system's main chemical components were discovered. For the first time in history, two women Emmanuelle Charpentier and Jennifer Doudna were given the Nobel Prize for their important contributions to the science of DNA editing using the CRISPR–Cas9 system, sometimes known as "genetic scissors" [20].

Table 1: Summarizes the data obtained after our literature search.

Engine	Search Strings	Filters
Google Scholar	[[" CRISPR Cas9" OR "CRISPR Forensic" OR " CRISPR Cas B Technology" OR " genetic engineering" OR " gene editing" OR "CRISPR in Molecular Biology" OR " Targeted Gene"]]	2017-2023
	[[" CRISPR Cas9" OR "CRISPR Forensic" OR " CRISPR Cas B Technology" OR " genetic engineering" OR " gene editing" OR "CRISPR in Molecular Biology" OR " Targeted Gene"]]	No Filter
Pub-Med	[[" CRISPR Cas9" OR "CRISPR Forensic" OR " CRISPR Cas B Technology" OR " genetic engineering" OR " gene editing" OR "CRISPR in Molecular Biology" OR " Targeted Gene"]]	2017-2023
	[[" CRISPR Cas9" OR "CRISPR Forensic" OR " CRISPR Cas B Technology" OR " genetic engineering" OR " gene editing" OR "CRISPR in Molecular Biology" OR " Targeted Gene"]]	No Filters

Understanding CRISPR Function

Researchers discovered the CRISPR-Cas system as a bacterial immune system that aids in the defense against viral infections in the late 2000s. The term "CRISPR-Associated Proteins" (Cas) which refers to the repeating DNA sequences and gene editing [21]. Using metagenome data sets or 16S rRNA sequencing data, multiple research projects have shown the uniqueness of the microbiome as a "bacterial fingerprint" to provide a novel technique for the identification of persons involved in crime scenes [22]. A paternal linage marker, the Y chromosome is widely employed in forensic sciences for a variety of tests. This chromosome's genetic material is utilized in genealogy research, anthropological studies, paternity testing, evidence analysis, and studies of human migratory patterns. All these are considered as the apex value of CRISPR Cas9 [23].

Unraveling the Mechanism

In 2012, scientists Jennifer Doudna and Emmanuelle Charpentier worked together to produce a ground-breaking publication that explained the workings of the CRISPR-Cas9 system. They showed that the Cas9 protein could be instructed to target particular DNA sequences and cut them, with the help of RNA molecules [24]. In addition to giving the investigative authorities another weapon for convicting the accused, technology also clears the innocent. It has demonstrated CRISPR Cas9's broad applicability in both criminal and civil situations, including the identification of victims of catastrophic disasters and persons who have gone missing. These days, Forensic DNA fingerprinting is a well-established method for resolving a wide range of criminal cases, including finding the perpetrator in cases of sexual assault, murder, burglary, theft, and many more [16].

Gene Editing Potential

The possibility of CRISPR-Cas9 as a gene-editing tool caused the scientific world to be incredibly excited about Doudna and Charpentier's findings. It provided an easier, adaptable, and affordable replacement for current gene-editing methods [25]. The late 1990s saw the development of the first GE technology. Engineered Meganucleases, ZFN (Zinc Finger Nucleases), TALEN (Transcription Activator Like Effector Nucleases), and CRISPR/Cas are the four genome-editing technologies that have been identified so far. The scientific world has been quite excited about the CRISPR-Cas system due to its benefits over other GE techniques in terms of speed, cost, precision, and efficiency. Compared to TALENs and ZFNs, the CRISPR/Cas approach is simpler and faster, and it is widely used in vitro and in vivo across a range of species [26].

Rapid Development and Applications

Since its original discovery, CRISPR-Cas9 technology has undergone incredible improvement at the international level. Researchers have created other Cas protein variants, including Cas12 and Cas13 are shown in (table1), each having specialized properties [20].

Ethical and Regulatory Issues

The development of CRISPR-Cas9 has brought up significant ethical and governmental issues. The capacity of technology to modify the human germline (sperm, eggs, and embryos) using epigenetics ad gee editing phenomenon has in particular sparked discussions about the morality of changing future generations' genetic background [27]. with the use of the CRISPR/Cas9 system, scientists can modify a variety of genome types with remarkable simplicity. The ultimate goal of this technology is to potentially shield future generations of humans from genetic illnesses. The system presents wide-ranging and revolutionary new opportunities, but these developments have also raised concerns among scientists around the world [28].

Table 2: Different CRISPR Variants and their Functions.

Sr. No	CRISPR Variants	Function	Reference
1	**CRISPR-Cas9**	In forensic investigations, the CRISPR-Cas9 version provides precise DNA editing, enabling the detection of certain genetic variants and alterations like compromised blood samples in collected materials.	[16]
2	**CRISPR-Cas systems for SNP detection**	In forensic research, CRISPR-Cas systems for SNP (Single Nucleotide Polymorphism) detection allow the identification of certain genetic differences at the single nucleotide level, assisting in the identification and profile of individuals.	[32]
3	**CRISPR-based biosensors**	Forensic targets, such as infections or genetic markers, may be rapidly and effectively found in collected samples by using CRISPR-based biosensors that use the Cas proteins to detect and identify particular DNA sequences.	[33]
4	**CRISPR-based DNA methylation analysis**	Using the detection and profiling of DNA methylation patterns, CRISPR-based DNA methylation analysis in forensic science offers epigenetic changes that may be utilized for identification and forensic analysis.	[34]
5	**CRISPR-Cas12a**	Targeted DNA editing and detection are made possible by the CRISPR-Cas12a version, which provides accurate genetic analysis for the identification, profiling, and detection of certain genetic variants or markers that are obtained from forensic evidences.	[35]
6	**CRISPR-Cas12b**	It is A Cas12b-mediated detection (CDetection) strategy, which shows higher sensitivity on examined targets compared with the previos reports of Cas12a-based detection platform.	[36]
7	**CRISPR-Cas13**	The forensic science CRISPR-Cas13 version allows it possible to recognize and identify certain RNA sequences, making it easier to diagnose RNA-based infections and analyze gene expression patterns in forensic investigations.	[37]

Developing Applications

CRISPR-Cas9 has uses in a variety of industries. It has been used to alter the DNA of both plants and animals, potentially advancing farming, developing disease-resistant plants, and improving livestock breeding. In the field of medicine, CRISPR-Cas9 holds promise for the treatment of genetic disorders, the development of precise disease models, and perhaps even the creation of individualized medicines [29]. Target gene loss, inversion, and duplication are possible outcomes of CRISPR/Cas9.

In conclusion, CRISPR/Cas9 technology may be able to effectively modify target genes in humans, making it a promising tool for studying how genes operate in living things [30].

Variation of CRISPR Technology in Forensic

The CRISPR technology has been shown to have enormous potential in a variety of fields, including forensic science [31].

Here are a few CRISPR variations and some forensic science uses they could have shown in Table 2.

Role of CRISPR Cas9 in Different Forensic Disciplines

CRISPR Cas9 in DNA Fingerprint

Genetic engineering has experienced a revolution because of the potent gene-editing technology CRISPR-Cas9. However, it is not utilized directly in the creation of DNA fingerprints [38]. DNA fingerprinting (sometimes referred to DNA profiling or genetic fingerprinting) is a method for identifying and examining a person's particular genetic traits. Short Tandem Repeat (STR) analysis is the technique for DNA fingerprinting that is most often employed. STRs are brief DNA repeat sequences that vary in length from person to person [39]. The procedure entails extracting the DNA sample, using the Polymerase Chain Reaction (PCR) to amplify certain DNA sections, and then examining the amplified fragments to identify the distinctive pattern of STRs. On the other hand, CRISPR-Cas9 is a technique for accurate genome editing [40]. The Cas9 protein is guided by a guide RNA molecule to target particular DNA target sequence, where it causes a double-strand break. The target DNA sequence might change as a result of this break being repaired by the cell's DNA repair system [29]. Despite not being directly engaged in DNA fingerprinting, CRISPR-Cas9 has been used in a number of applications for genetic and genomic research. To explore the function of genes and their involvement in illness, for instance, CRISPR-Cas9 may be used to induce precise genetic alterations in model organisms. Additionally, it may be used to genetically modify crops and animals or establish novel treatment modalities for genetic diseases [41].

In the final analysis, CRISPR-Cas9 is a potent tool for genome editing, however, it is not used directly in the production of DNA fingerprints. DNA fingerprinting uses a variety of methods, including PCR and STR analysis, to pinpoint and examine each person's distinctive genetic traits [25].

CRISPR Cas9 in Forensic Kinship and Paternity Test

There has been research looking at the possible uses of CRISPR-Cas9 technology in normal forensic kinship and paternity testing, even though it is not typically employed in these procedures [16]. To increase the accuracy and efficacy of kinship and paternity testing, these researchers recommend using CRISPR-Cas9 for targeted DNA analysis. The targeted mutation of particular genetic markers to improve their identification is one possible use of CRISPR-Cas9 in kinship and paternity testing [42]. It could be able to enhance the amplification and analysis of these areas throughout the testing process by employing CRISPR-Cas9 to modify the DNA at certain loci of interest. Known genetic variants can be introduced into DNA samples using CRISPR-Cas9 to serve as positive controls during testing [43]. This enhances the testing procedure's accuracy and dependability. It is important to remember that these applications are still in the experimental stage and that CRISPR-Cas9 has not yet been proven to be a reliable tool for forensic kinship and paternity testing. The standard methodologies for these tests continue to be conventional DNA analysis techniques like PCR and STR analysis [44].

CRISPR Cas9 in Forensic Phenotyping

In forensic phenotyping, which includes predicting an individual's physical features or characteristics based on their DNA, CRISPR-Cas9 technology has the potential to be useful. Unlike conventional DNA profiling techniques, which concentrate on using DNA analysis to identify people, forensic phenotyping seeks to reveal more about a person's physical characteristics [45]. The link between genetic variants and physical features may be studied using CRISPR-Cas9. Researchers can employ CRISPR-Cas9 to introduce or edit these variants in model animals to determine their functional impact by focusing on certain genes or genetic markers linked to physical traits. The relationships between certain genetic markers and phenotypic features can be established through these investigations [46]. A prediction model for forensic phenotyping may be developed using the knowledge obtained from such studies. It may be feasible to anticipate a person's physical traits, such as eye color, hair color, or facial features, by examining the presence or absence of particular genetic variations or combinations of variants [47]. It's important to keep in mind that forensic phenotyping is a complicated topic that calls for a lot of study and confirmation. The accuracy and reliability of DNA-based physical characteristic predictions might vary depending on several variables, including the population investigated and the particular genetic markers considered [7]. This field of research is still in its early stages. It is important to note that although CRISPR-Cas9 technology has the potential to advance our knowledge of how genetics affect the physical characteristics, it has not yet become a standard practice in forensic phenotyping. Traditional approaches, such as observation and witness accounts, continue to be the major ways of forensic phenotyping [48]. The discipline is currently continuously investigating and improving methodologies for predicting physical features from DNA. When contemplating the use of CRISPR-Cas9 or any other method in forensic phenotyping, it is important to confer with forensic professionals and follow set norms and standards as with any other forensic application [49].

Utilizing CRISPR-Cas9 Technology Can Significantly Enhance the Investigation of Biological Evidence

While the idea of altering genomes has been around for a while, CRISPR-Cas9 is about to make this a lot more potent reality. The technology is not only accessible and reasonably priced, but it is also very efficient [50]. CRISPR-Cas9 technology offers

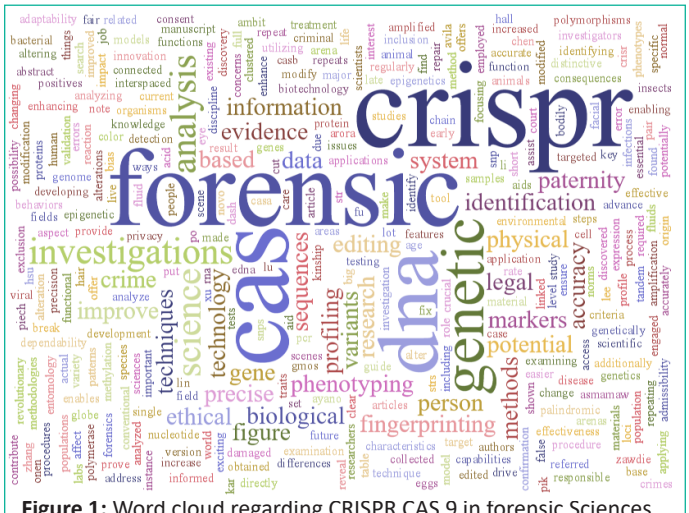

Figure 1: Word cloud regarding CRISPR CAS 9 in forensic Sciences.

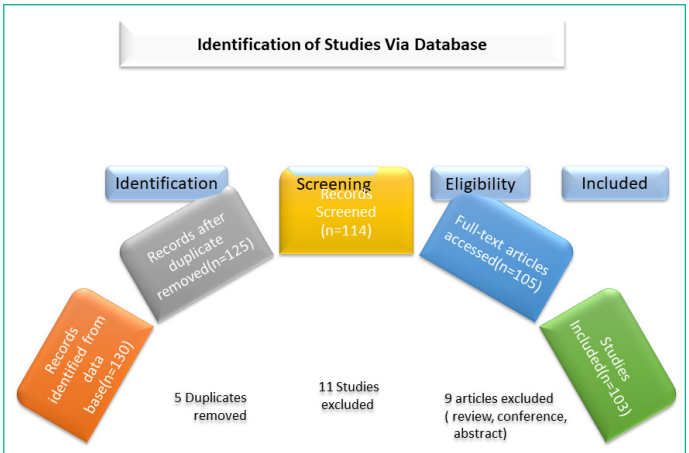

Figure 2: Study selection.

the potential to further develop forensic science's examination of biological evidence (ww 3). Current research examines its uses in building the processing and interpretation of biological evidence, even though it is not yet widely employed in normal forensic investigations [16].

DNA Analysis

CRISPR-Cas9 can help advance forensic investigators' DNA analysis methods. It may be used, for instance, to accurately alter or modify DNA samples to validate and improve the effectiveness of DNA analysis techniques. This can increase sensitivity, decrease false positives or negatives, and increase the overall accuracy of DNA profiling [16]. We present STR-Seq, a next-generation sequencing method that accurately genotypes microsatellites by analyzing over 2,000 STRs in parallel. Utilizing in vitro CRISPR–Cas9-targeted fragmentation, STR-Seq generates distinct DNA molecules that encompass the whole microsatellite sequence [51].

DNA Repair and Reconstruction

DNA forensic evidence from crime scenes is frequently polluted, damaged, or fragmented. Forensic researchers have made significant efforts to automate nucleic acid extraction from a variety of crime scene samples to handle an increasing number of samples [52]. Combining CRISPR-Cas9 with DNA repair enzymes may make fixing and recreating broken DNA sequences easier to fix and recreate broken DNA sequences. The success rate of DNA analysis might be increased with the use of this method, which could assist in recovering important genetic data from damaged samples [53].

DNA Amplification

A primary method for amplifying DNA for examination is the Polymerase Chain Reaction (PCR). By focusing on and altering specific DNA regions, CRISPR-Cas9 technology has the potential to improve PCR by increasing the effectiveness and precision of amplification. Better DNA profile generation may result from doing so, particularly from low-template or damaged DNA sources [54]. Our technique, called Cas9 nickase-based amplification reaction (Cas9nAR), allows us to amplify a target region from genomic DNA at 37 °C indefinitely [55].

Genome Editing for Functional Studies

The CRISPR-Cas9 system allows for precise genetic alterations in living things. Animal or cellular models that simulate certain genetic diseases or mutations relevant to a case might be made using data from forensic investigations [56]. Such models could provide insight into the meaning of certain DNA profiles discovered at crime scenes by helping comprehend genetic variants' functional consequences the functional consequences of genetic variants [57].

Genetic Phenotyping

As previously mentioned, CRISPR-Cas9 can advance the science of forensic phenotyping by examining how genetic differences and physical characteristics interact. This information can help detectives produce composite drawings or make assumptions about a suspect's appearance based on DNA evidence [49]. DNA phenotyping (FDP), enabling the investigators of crime cases to reduce the number of suspects, making their work faster and more precise [58].

Functional Studies

The CRISPR-Cas9 system can help with functional studies including biological data. It enables scientists to alter interesting genes and explore their function in certain biological processes, such as the development of bloodstains or the survival of DNA in harsh environments. These studies can offer insightful perspectives on how to analyze forensic evidence [59].

However, CRISPR-Cas9 shows potential for enhancing forensic investigations, and its routine application in forensic labs is still uncommon. Before reaching its full potential in forensic casework, more study, validation, and standardization are required [60]. To ensure the responsible and ethical application of this technology in the field of forensic science, cooperation between scientists, forensic specialists, and legal authorities is essential [61].

Role of CRISPR Cas9 to Solved Problems and Improve Current Methods in Forensic

With its revolutionary gene-editing capabilities, CRISPR-Cas9 has the potential to improve existing forensic science techniques and address several issues [62]. Here are some potential functions of CRISPR-Cas9 in resolving issues and enhancing present forensic techniques, even if its uses in forensic investigations are still in the early phases of development and require further research [63].

Multiplexing Capabilities

CRISPR-Cas9 makes it possible to alter many DNA targets at once. This feature may be used in forensic applications to analyze numerous genetic markers or DNA regions at once, increasing the effectiveness and throughput of forensic DNA analysis [43]. The identification of multiple infectious diseases, including SARS-CoV-2 and the human papilloma virus, has proven to be sensitive and specific thanks to the recent development of CRISPR diagnostic techniques based on the non-specific trans-cleavage activity of enzymes [64].

Figure 3: The steps illustrating how the use of CRISPR-Cas9 technology can significantly improve the investigation of biological evidence are illustrated in the figure.

SNP Analysis

Single Nucleotide Polymorphisms (SNPs) are base-pair-level genetic differences. SNPs may be identified and analyzed with the use of CRISPR-Cas9, which can yield useful data for forensic investigations. SNPs may be used to identify people, determine their origin, and find certain genetic markers linked to particular populations or characteristics [65]. In forensic DNA analysis, the autosomal STR markers that are examined by multiplex amplification and capillary electrophoresis using fluorescently labeled primers are most frequently utilized. SNP does, however, have several drawbacks when it comes to assessing DNA combinations [66].

Body Fluid Identification

CRISPR-Cas9 can help identify certain bodily fluids discovered at crime scenes. It can help in detecting the origin of the biological material by focusing on and evaluating certain DNA sequences linked to various bodily fluids (such as blood, saliva, semen, or vaginal secretions), giving essential evidence for forensic investigations [67].

Forensic Epigenetics

DNA methylation patterns are an example of an epigenetic change that can contribute to the information available in forensic investigations. In forensic applications that rely on epigenetic indicators, such as age estimate, bodily fluid identification, and others, CRISPR-Cas9 can be used to specifically target and analyze certain epigenetic markings [68].

Accuracy of DNA Profiling Methods

CRISPR-Cas9 can improve the precision of DNA profiling methods including Short Tandem Repeat (STR) analysis. CRISPR-Cas9 can enhance the precision and reliability of DNA profiling data, resulting in more precise forensic identification, by accurately focusing on and amplifying targeted DNA areas of interest [51]. CRISPR-based DNA fragmentation provides an easy way to quickly and effectively enrich tiny targets [69].

Analysis of DNA that has been Damaged or Degraded

CRISPR-Cas9 may be used to fix and restore DNA sequences in DNA samples that have been damaged or degraded [70]. The success rate of DNA analysis in forensic investigations can be increased thanks to this approach, which can assist in recovering priceless genetic information from damaged samples that would otherwise be worthless [71].

Genetic Modification Detection

CRISPR-Cas9 can be used in forensic investigations to identify Genetically Modified Organisms (GMOs). CRISPR-Cas9 can assist in detecting the presence of genetically changed material in samples taken from crime scenes or environmental occurrences by focusing on and analyzing certain DNA sequences linked to genetic changes [72].

Although CRISPR-Cas9 exhibits= potential in forensic applications, more study, validation, and standardization are necessary.

Application of CRISPER Cas9 in Forensic Science

The revolutionary gene-editing technology CRISPR-Cas9 has generated a lot of interest from forensic scientists among other scientific disciplines (Figure 4). CRISPR-Cas9 has the potential to have a variety of effects on forensic investigations, although its main uses are in genetic research and medicines [73]. Several forensic sciences used for CRISPR-Cas9 are listed below Figure 4

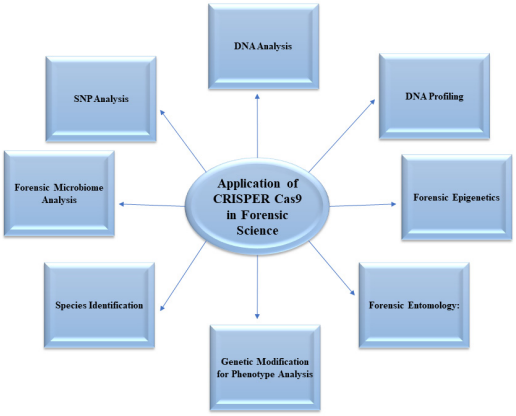

Figure 4: The diagram "presents the sequential steps illustrative of CRISPR-Cas9 application in forensic science, showcasing how this technology can be used to enhance various aspects of forensic investigations," based on the description.

DNA Analysis

Using CRISPR-Cas9, DNA analysis techniques used in forensic investigations can be CRISPR-Cas9, DNA analysis techniques used in forensic investigations can be made more precise and effective [26]. Selecting and snipping exact areas throughout the genome might assist in identifying and studying it might assist in the identification and study of certain DNA sequences. This skill is especially valuable for analyzing genetic variants, mutations, or markers linked to certain features or genetic illnesses [74].

DNA Profiling

DNA profiling, sometimes referred to as DNA fingerprinting, is one of the most important uses of CRISPR-Cas9 in forensics. To establish a distinctive DNA profile for a person, certain sections of a DNA sample may be targeted, amplified, and examined using CRISPR-Cas9. The possible matches or exclusions can subsequently be determined by comparing this profile with samples collected from crime scenes [75].

Genetic Modification for Phenotype Analysis

Using CRISPR-Cas9, researchers may introduce precise genetic alterations into model animals like mice to investigate the impact of certain genes on a range of phenotypes [76]. Forensic investigators may find this knowledge useful when attempting to comprehend the possible influence of genetic differences on an individual's physical traits or behavior [14].

Forensic Epigenetics

DNA methylation patterns are an example of an epigenetic change that might be useful in forensic investigations [77]. These epigenetic changes may be mapped and analyzed with the aid of CRISPR-Cas9, which enables researchers to pinpoint epigenetic markers connected to particular characteristics or environmental exposures. In a forensic setting, this information can be used to assess the potential impact of epigenetics on the observed phenotypes or behaviors [78].

Forensic Entomology

To analyze an insect's behavior, life cycle, or reaction to a particular environmental element, forensic entomologists can employ CRISPR-Cas9 to alter its genes. Forensic investigators can learn more about the time of death or the place of a crime by analyzing the genetic composition of insects collected at a crime scene [79].

Species Identification

CRISPR-Cas9 can help with species identification in situations involving wildlife crimes or illegal animal trafficking. It can offer a dependable and precise way of determining the origin of biological materials obtained at crime scenes by focusing on certain DNA sequences that are exclusive to a given species [16].

Forensic Microbiome Analysis

The microorganisms that live in and on our bodies the human microbiome can offer important forensic evidence. Using CRISPR-Cas9 to analyze and manipulate microbial communities connected to people or crime sites may provide new information to forensic investigations [80].

SNP Analysis

Single nucleotide polymorphism SNP is a difference in a single DNA base pair, may be found with the use of CRISPR-Cas9. SNPs can be employed in forensic investigations to ascertain ancestry and physical characteristics and locate specific genetic markers linked to certain populations or phenotypes [81].

While CRISPR-Cas9 has a lot of potential for forensic research, its actual use in this discipline is still in its early stages. Before these methods may be regularly used in forensic investigations, more study and confirmation are required [29].

Future of CRISPR Cas9 in Forensic Science Investigation

CRISPR-Cas9 has a great deal of promise to improve forensic science research in several ways. Here are some potential uses it could have in the future are shown in (Figure 5).

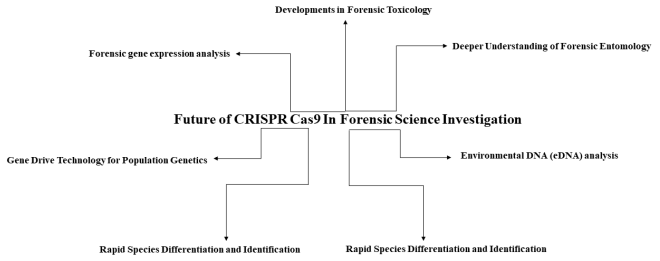

Figure 5: The Figure illustrates the potential of CRISPR-Cas9 in forensic science investigations, showcasing the goal and sequential steps involved in utilizing this technology for advanced forensic analysis.

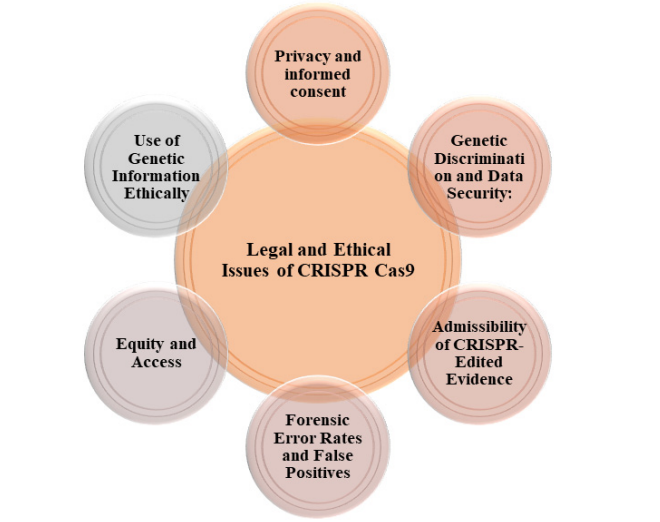

Figure 6: The figure describes the step-by-step procedure and highlights the key issues that arise in applying this technology, providing a comprehensive overview of the legal and ethical considerations surrounding the use of CRISPR-Cas9 in forensic science.

Forensic Gene Expression Analysis

Gene expression patterns can provide details about a person's biological activity, including information on the person's age, tissue types, and exposure to environmental stimuli [82]. Through the alteration and analysis of gene expression in forensic evidence, CRISPR-Cas9 may make it possible to gain a better knowledge of cellular functions and potentially useful indications for investigations [83].

Gene Drive Technology for Population Genetics

CRISPR-Cas9-based gene drive technology can modify or repress particular genes within a population [84]. This technique might be used in the field of forensic science to manage or keep an eye on the populations of mosquitoes and other disease vectors that help spread illnesses like malaria and the Zika virus, which are important for forensic investigations [85].

Rapid Species Differentiation and Identification

CRISPR-Cas9-based techniques may make it easier to quickly identify and categorize distinct species in forensic materials. It could be feasible to easily identify the source of biological material, such as endangered species or unlawful animal products, by focusing on certain genetic markers that are specific to each species [86].

Deeper Understanding of Forensic Entomology

By enabling precise genetic alterations in insects engaged in decomposition processes, CRISPR-Cas9 can contribute to the advancement of the discipline of forensic entomology [79]. The life cycle, behaviors, and development of insects may be better understood as a result, which might improve the calculation of postmortem intervals and other pertinent forensic data [87].

Discovering Genetic Predispositions

The CRISPR-Cas9 system can let researchers examine genetic variants linked to particular features or predispositions important to forensic investigations [20]. Investigating genetic markers connected to aggressiveness, addiction, or other behavioral qualities might be part of this; the results would be useful for creating criminal profiles and comprehending criminal behaviors [88].

Developments in Forensic Toxicology

CRISPR-Cas9 can advance forensic toxicology by precisely altering genes implicated in drug metabolism. This may result in a better knowledge of how medications interact with one another, how metabolism works, and how certain genetic differences affect how a person reacts to poisons or pharmaceuticals [89]. Illegal substance abuse puts the public's health at serious danger and places a heavy financial strain on the healthcare system. Over the past few years, there has been a sharp increase in drug abuse and overdose mortality that is almost epidemic in scope. For law enforcement organizations, smuggling, illegal sales, and drug use have proven to be difficult challenges [90].

Environmental DNA (eDNA) analysis

Genetic material that organisms release into the environment is referred to as environmental DNA (eDNA). It may be used in forensic investigations for things like locating people at crime scenes. CRISPR-Cas9 could make it easier to collect, amp up, and analyze eDNA, enabling more accurate and sensitive genetic material detection and enhancing environmental forensics [91].

It's important to note that CRISPR-Cas9 will need further study, validation, and ethical concerns before it can be used in forensic science investigations. To ensure the appropriate and accountable use of this technology in the forensic sector, regulatory frameworks and norms must be implemented [92].

Legal and Ethical Issues of CRISPR Cas9 in Forensic Science

Several ethical and legal questions are raised by the use of CRISPR-Cas9 in forensic investigations. Here are some important things to think about are shown in (Figure 6).

Privacy and informed consent

The examination and alteration of a person's DNA may be necessary as a result of the usage of CRISPR-Cas9 in forensic investigations but some concerns about privacy and informed consent are raised by this. Adequate safeguards must be in place to guarantee that people are informed about the potential repercussions of having their genetic information analyzed or modified and provide their agreement [93]. Consensus sequencing can be performed by marking each molecule with a unique molecular barcode before making copies, which allows subsequent comparison of these copies or systems whereby copies are physically connected and sequenced together [94].

Genetic Discrimination and Data Security

The sensitive nature of genetic data collected using CRISPR-Cas9 raises questions regarding data security and the possibility of genetic prejudice [95]. Genetic data must be safeguarded to ensure privacy and confidentiality, avoiding unauthorized access or abuse that can result in discrimination in circumstances like job, insurance, or healthcare access [96].

Admissibility of CRISPR-Edited Evidence

Due to concerns regarding the accuracy, dependability, and potential manipulation of genetic evidence, the admissibility of CRISPR-edited evidence in court proceedings may be in doubt [97]. Standardized methodologies, validation procedures, and expert testimony may be required to prove the validity and dependability of CRISPR-edited evidence [98]. In regions of the world where it is legal, the capabilities of DNA databases have been enhanced by familial DNA searches. The need for education and training to enhance the interpretation of complicated DNA profiles is one of the challenges and possibilities that will shape the field of forensic DNA in the future [99].

Forensic Error Rates and False Positives

To ensure accuracy and reduce the possibility of mistakes or false positives, CRISPR-Cas9 procedures must be extensively verified. Off-target effects or unexpected alterations might result, introducing errors and jeopardizing the accuracy of forensic investigation. To keep the forensic process' integrity, certain quality control methods and standards must be established [100]. In addition to mistake rates, accurate and inconclusive rates should be considered when thoroughly evaluating the validity of a forensic procedure. A technique may be highly effective at preventing mistakes, but this does not guarantee that it will also result in the right decisions [101].

Equity and Access

CRISPR-Cas9 should be used in forensic investigations to guarantee fair access and prevent aggravating existing social and economic inequities. The advantages of these technologies should be made available to everyone, regardless of socioeconomic class or geography, and measures should be taken to eliminate unjustified bias or uneven treatment based on genetic information [102].

Use of Genetic Information Ethically

CRISPR-Cas9 raises ethical concerns concerning the proper application of genetic data discovered during forensic investigations. Care should be taken to avoid misuse or injury to individuals in the use of information, kept and disseminated. To control how genetic information gained using CRISPR-Cas9 is used and disseminated, clear rules and moral frameworks must be developed [72]. To ensure the responsible and advantageous use of this technology while defending human rights and societal interests, it is important to address these legal and ethical challenges as CRISPR-Cas9 advances in forensic science [103].

Conclusion

The exploration of forensic standards has further intensified the milieu of world in each aspect. It has profoundly augmented the impacts in biotechnology, plants and animal engineering as well as modifications in the treatment of disease by alteration in Genome. It has also improved criminal investigation system by utilizing de-novo techniques. Gene editing tool has fostered the functionality of DNA fingerprinting or profiling that has solved plenty of mysteries. It can be predicted that relentless development will further modernize the norms of forensic in developing States.

Expert Opinion

With the sufficiently of studies related to forensic has depicted that the discovery of CRISPR-Cas9 has put the world toward highpoint. All the developments like gene editing, biotechnology, DNA profiling and fingerprinting, fluid examination and investigation process are due to this discovery. The future of forensic related standards has been seen as a major contributory factor in each and every aspect of society. The exploration of elementary fields like epigenetics and SNPs analysis have revolutionized the globe. However, it might be anticipated that further innovation will modernize all these techniques.

Ethical Approval

There is no ethical issue.

Competing Interests

All the authors declare no competing interests either financial or personal.

Author's Contributions

U.U.K, M.I and M.N wrote the initial manuscript. M.M.H and A.F constructed the figures and tables, I.U revised the manuscript. A.W revised and finalized the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Conflict of Interest

We have no conflict of interest to declare.

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