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Research Article

Exploring the Role of Short Tandem Repeats (STR) in Forensic Biotechnology: Challenges and Innovations

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Abstract

Short tandem repeat (STR) typing remains a crucial tool in forensic DNA profiling worldwide. This technology not only helps in convicting criminals and proving wrongful convictions but also establishes essential links to actual perpetrators of crimes and can act as a deterrent to potential offenders. DNA profiling has also allowed forensic scientists to re-examine closed cases due to insufficient evidence. To conduct this review, an extensive electronic literature search was performed using databases such as PubMed, Science Direct, Google Scholar, and Google Search. All relevant works, including reviews, retrospective studies, observational studies, and original articles, were thoroughly reviewed. This paper explores the challenges and perspectives associated with using STRs in forensic investigations. STRs have high polymorphism, Mendelian inheritance, and the ability to analyze multiple loci in one reaction, making them essential tools in forensic science. Additionally, their suitability for analyzing degraded or traces DNA samples enhances their usefulness in criminal investigations and paternity testing. This abstract summarizes the current landscape of STR applications in forensic biotechnology, highlighting the challenges researchers face and the promising features that drive innovation in this field.

Keywords

DNA Profiling, Short Tandem Repeat, Crime Scene, Forensic, DNA Marker, Incest

1. Introduction

Since its introduction in 1987, the analysis of DNA from biological evidence in criminal cases has had a profound impact on forensic investigations. Over the past three decades, significant advancements have been achieved in the discrimination power, speed, and sensitivity of DNA profiling methods, along with notable improvements in the analysis of more complex samples [1]. In forensic science, microhaplotypes have proven useful applications for individual identification, mixture recognition [2], and ancestry inference [3]. In our opinion, compound biomarkers, consist of two or more variants located within a small region, can be considered generalized microhaplotypes. These include single nucleotide polymorphisms (SNPs) that are closely linked to short tandem repeats (STRs) (SNP-STR), insertion and deletion polymorphisms (indels) that are closely linked to STRs (DIP-STR), indel polymorphisms that are closely linked to SNPs (DIP-SNPs), and several indel polymorphisms that are tightly linked (multi-indels) [4, 5]. DNA profiling, is the gold standard for resolving forensic cases and providing precise identification of victims and suspects in

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some cases. It continues to be a valuable tool in cases with multiple pieces of evidence [6].

Among various markers, short tandem repeats (STRs) are the most widely used multiallelic markers in forensics worldwide. Due to their high polymorphism and ability to effectively distinguish between individuals, STRs have been adopted as reference loci for the Combined DNA Index System (CODIS) and have also enabled the global implementation of National DNA Databases (NDNADs) [7]. This markers have various important applications, including molecular diagnosis, population studies, linkage analysis, identity testing, forensics, and cases involving paternity and kinship [8].

STRs are consisting of repeat units that are 2 to 7 bp in length. They are found extensively throughout the human genome [9] and exhibit significant variability among individuals in a population. STR typing continues to be the primary workhorse in forensic DNA analysis although other genetic markers could be used for specific applications [10]. While this method has proven valuable over the years, it does have its limitations. In CE, it is only possible to multiplex more than 5 loci in a single assay by employing different fluorescent labels in the PCR and using non-overlapping PCR fragment lengths for STRs with the same fluorescent label. As a result, most commercial assays have a PCR fragment range of 80 to 500 bp [11].

The usefulness of STR markers has increased due to their short size, polymorphic nature, ease of amplification, the development of the multiplex system, and rapid analysis. As a result, STR markers have become an essential part of most DNA analysis laboratories [12]. However, there are challenges in forensic DNA analysis using STR markers, such as degradation, low copy numbers, and mixed sources [10]. The aim of the review on the application of STRs in forensic biotechnology is to provide a comprehensive analysis of both the challenges and the promising features associated with their use in forensic science.

2. DNA Profiling Markers

The methodologies and technologies used in Forensic DNA profiling differ in their ability to distinguish between two individuals and the speed and sensitivity of the results obtained. In recent years, there has been a significant improvement in the speed of performing forensic DNA profiling [13]. Ever since Sir Alec Jeffreys discovered a reliable and modern methodology for generating genetic profiles, which can be used to pinpoint individual identification in criminal investigations, there have been significant advancements in DNA profiling methodology and technology. These advancements have greatly improved the speed and specificity of generating highly discriminating profiles [14].

Currently, there are several molecular technologies being used for DNA profiling. These include, but are not limited to, the Restriction Fragment Length Polymorphisms (RFLP), variable number of tandem repeat sequences (VNTR), and short tandem repeat (STR), which is considered the gold standard for generating profiles to be stored in the DNA database [13]. SNPs are the other most common type of genetic variation in the human genome and are particularly valuable for studying human evolutionary history over long time scales [15]. In contrast, indels occur less frequently. Additionally, indel markers are almost non-existent in commonly used forensic STRs, such as the expanded CODIS core loci STR set and the Extended European Standard Set (ESS) [5]. For these reasons, SNP-STRs effectively combine the benefits of both SNPs and STRs. They provide more information than DIP-SNPs and SNP-SNPs, have a greater number of candidates than DIP-STRs, and offer the potential for deeper insights into population genetic processes [16].

Short Tandem Repeat (STR)

STRs are short sections of DNA, usually 2-6 nucleotides long, which are repeated in a consecutive manner at a specific location and accounts at least 6.77% of the human genome and exhibit high levels of genetic variation [17]. The lengths of STR can be altered during DNA replication due to slippage events on misaligned strands, errors in DNA repair during synthesis, and the formation of secondary hairpin structures. This makes STR lengths relatively unstable and prone to frequent mutations, which contribute to genetic variation in human populations. Compared to SNPs in non-repetitive contexts, STRs have a much higher mutation rate. Short tandem repeats are widely distributed in the human genome, its predominance in the noncoding region of the genome. Within this distribution, STRs are mostly found in the noncoding regions, but can also slightly be found in the coding [18].

The wide distribution of microsatellite and the uniqueness with location specificity necessitated a uniform and simple nomenclature for the human identification. For instance, the STR marker designated as “D3S1266”, D represents DNA, 3: Chromosome 3 on which the STR locus locates, S: STR, and 1266: the unique identifier [19]. STRs are preferred markers in human identification analysis for several reasons. Firstly, they are highly polymorphic, which means they have a high capacity to differentiate between individuals. Secondly, they can be rapidly and easily analyzed using PCR-based technology and capillary electrophoresis automated fluorescent detection. Lastly, STRs have the ability to generate multiple DNA profiles simultaneously, and they are particularly useful for degraded DNA samples due to their short amplicon lengths [20]. Due to the highly polymorphic nature of STRs, the higher the number of STRs loci used for the purpose of identification, the greater the discriminating power [19, 21]. In the forensic settings, STRs are suitable marker for identification purpose owing to the following factors; suitability for easy amplification by PCR, its variable nature of short repeat sequences among individuals, small in size which gives it the advantage for degraded samples, low mutation rate and high power of discrimination [22]. In light of this, the introduction of an amplification technology linked to STRs method of DNA profiling resulted to the availability

of appropriate vigorous systems for the establishment of an effective and efficient DNA database [23, 24]. STR markers are highly variable, yet they are stably inherited from parents to children. This makes them effective for human identification. Currently there are twenty CODIS Core loci use for forensic DNA and human identification analysis and testing. These loci include CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, FGA, TH01, TPOX, vWA, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045. These loci are commonly used due to their high heterozygosity, discriminatory power, clearly defined repetitive units, and the ease of amplification and detection using commercial kits [25].

3. Applications of STR in Forensic Science

Short tandem repeat (STR) markers are repetitive nucleotide sequences found in the non-coding regions of the human genome. These sequences have various applications, including molecular diagnosis, population studies, linkage analysis, and identity testing. Additionally, they are commonly used in forensics, crime scene investigation, paternity testing, and kinship cases [8]. The technique of DNA fingerprinting originated many years ago with the introduction of restriction fragment length polymorphism (RFLP). In the 1990s, PCR methodologies replaced the RFLP method due to their ability to amplify DNA, giving them a distinct advantage [26]. The usefulness of STR markers has been greatly enhanced by their short size, polymorphic nature, ease of amplification, and the development of the multiplex system. Their rapid analysis has also contributed to their popularity in DNA analysis laboratories [12]. Research findings have shown that using a greater number of STR loci for typing increases the discrimination value [21]. This is because the probability of finding two individuals from a random population with the exact same number of repeat units for all the analyzed STRs is extremely rare [27]. These repeat units can vary in size from person to person without affecting the individual's genetic health [28]. For instance, at the same locus, a tetranucleotide repeat sequence (represented by CTAG) will differ among individuals, as shown in Figure 1. Person 1 has 5 repeats, person 2 has 6 repeats, and person 3 has 7 repeats. However, the challenges of degradation, low copy numbers, and mixed sources present significant obstacles in forensic DNA analysis with STR markers [10].

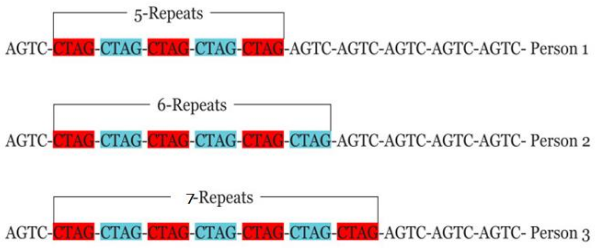

Figure 1. *Showing tetra-nucleotide (CTAG) short tandem repeats (STRs) of varying lengths located at the same locus.*

3.1. Parenthood Testing

Parentage testing (PT) is important for various purposes such as finding missing persons [29], identifying victims of disasters [30], resolving inheritance disputes [31], and handling immigration cases [32]. Also, it's a crucial tool in the field of forensic genetics, with applications in various areas. It is important to compare the main differences between the PT and the maternity test (MT). In the case of the PT, the involvement of the mother's genotype enhances the identification of the biological father [33]. However, if maternal data is not available, the test may yield inconclusive results. Inserting markers based on STR sequences and mitochondrial DNA sequence variations has been found to improve the efficiency of paternity testing. Specifically, markers linked to the analysis of sex chromosomes (X and Y) have shown to provide greater efficiency compared to markers linked to autosomal chromosomes [34]. This is because of the inheritance pattern of the X chromosome, where the daughter receives an unchanged paternal X chromosome. Consequently, markers on the X chromosome have a high power of exclusion [35]. The higher exclusion power of X-STRs is due to the difference in the number of alleles compared to autosomal alleles in male individuals [36]. Currently, a study was conducted on various parental cases using STR markers as listed in the table below.

Table 1. *The paternity case profiling by STR.*

No	Sample	Researches	Reference
1	Blood	Early non-invasive prenatal paternity testing by targeted fetal DNA analysis	[37]
2	Blood & swabs	Analysis of data and common mutations encountered during routine parentage testing in Zimbabwe	[25]
3	Swab	Zygotic-splitting after in vitro fertilization and prenatal parenthood testing after suspected embryo mix-up – a case report	[38]

No	Sample	Researches	Reference
4	Swabs	Confirmation of paternity despite three genetic incompatibilities at Chromosome 2	[39]
5	Swabs	Circulating Cell-Free Plasma DNA in Noninvasive Prenatal Paternity Testing with Short Tandem Repeats (STRs)	[40]
6	blood and oral mucosa	Uniparental disomy of chromosome 21: A statistical approach and application in paternity tests	[41]

3.2. Kinship Testing

Kinship testing, which assesses genetic relatedness, has found extensive application in practical scenarios like disaster victim identification and resolving inheritance disputes. Various genetic markers, such as short tandem repeats (STRs), single nucleotide polymorphisms (SNPs), small insertion/deletion polymorphisms, and microhaplotypes, are employed for kinship testing [42]. Different kinship cases were set by the STRs as listed below table. Its which are considered the most widely used genetic markers for kinship testing, are the preferred option globally [42].

Table 2. *Kinship case profiling by STR.*

No	Sample	Researches	Reference
1	Buccal swabs	Pairwise kinship testing with a combination of STR and SNP loci	[43]
2	Buccal swabs	Determining the effects of genetic linkage when using a combination of STR and SNP loci for kinship testing	[44]
3	Blood	Comparative evaluation of autosomal STRs and X-chromosome STRs as a complement of autosomal STRs in kinship testing in Southern Han Chinese	[45]
4	Whole blood	Pairwise kinship testing with microhaplotypes:	[46]
5	Blood	Evaluation of DNA Polymorphisms for Kinship Testing in the Population of Saudi Arabia	[47]
6	Buccal swab samples	Complex kinship analysis with a combination of STRs, SNPs, and indels	[48]

3.3. Incest

Child sexual abuse is a global public health concern, which the WHO considers a silent health emergency [49]. Victims of incest often remain silent about their situation due to feelings of embarrassment, guilt, and fear. As a result, cases of incest are rarely reported. Additionally, it is well known that families often try to cover up instances of incest [50, 51]. In many instances, DNA profiling of autosomal STR loci can effectively be used in solving criminal and paternity cases involving males [52]. However, when the potential fathers in question are close blood-relatives, the exclusion power of autosomal STRs is significantly reduced, and ChrX (Chromosome X) STRs may be more suitable [53]. For instance, if two alleged fathers are father and son, ChrX markers would be more effective than autosomal STRs since father and son do not share any X-chromosomal alleles. In certain criminal paternity investigations, the high rate of homozygosity exhibited by the child may raise suspicions of an incestuous situation [20, 51].

Table 3. *Incest case profiling by STR.*

No	Sample	Researches	Reference
1	Blood	Analysis of aborted fetal material using autosomal STR markers in forensic cases of	[54]

No	Sample	Researches	Reference
		sexual assault	
2	Rinses and swabs	Evaluation of usefulness of further Y-STR analysis in sexual assault cases on PSA positive samples resulting in female autosomal STR profiling	[55]
3	Rinses and swabs	Evaluation of sexual assault evidence collection kit: Comparison of the success rate of STR analysis of swabs versus rinses	[56]
4	Body fluid	Enhancing the sexual assault workflow: Development of a rapid male screening assay incorporating molecular non-microscopic sperm identification	[57]

3.4. Crime Scene Investigation

Blood stains, semen, and other biological traces, as well as the victim's body, are frequently discovered at crime scenes and can all serve as valuable evidence. Forensic scientists analyze this evidence to connect the case to the suspect in custody by matching trace evidence to the suspect's DNA profile [58]. In cases where no suspect can be identified, DNA samples from the crime scene can be compared to profiles in a National DNA Database for a match. If a match is found, an investigation can be opened on individuals with matching profiles [58, 59].

The forensic identification of a human body is especially difficult in mass disaster scenarios [60]. Because sample degradation poses challenges for DNA amplification and lowers the success rate of STR profiling [61]. Therefore, it is crucial to collect and preserve biological material carefully for successful DNA analysis in forensic cases [62]. Until now, free DNA evaluation has mainly concentrated on assessing the amount of free DNA obtained from tissue samples preserved in various solutions and its ability to produce complete downstream STR profiles. This evaluation is mostly conducted using fragment analysis through capillary electrophoresis (CE) [63].

In 2009, a mother and daughter were murdered. The initial DNA analysis did not identify a suspect. In 2019, a match was finally found in the national Y-STR database, which led investigators to a person with a criminal record. By broadening the search to 60 Y-STR loci, an exact match was discovered with the crime scene evidence [64]. A married couple was found murdered in a village near Kicevo, with their bodies corded. DNA analysis from the crime scene, including blood and rope debris, was performed using a Qiagen mini kit and a PCR amplification kit. The DNA from the rope matched an unknown male, but a search of the Macedonian forensic database yielded no results. Five years later, blood found at a church burglary matched the DNA of a suspect. This suspect's profile also matched the DNA from the double murder case, thereby solving the long-standing investigation [58].

A 35-year-old man was shot dead in his backyard in an urban neighborhood. Police found a handgun, gloves, and shirt sleeves at the crime scene. An autopsy revealed a gunshot wound from the recovered handgun. DNA from the gloves and sleeves led to a matching profile in CODIS. The alleged shooter was charged with felony murder and attempted robbery. He received a sentence of 65 years in prison [65]. Overall, STR DNA profiling is a crucial tool in forensic science used worldwide to confirm suspects and solve cases. However, in developing countries, this technology is not yet widely available.

4. Challenges in STR Analysis

Despite its usefulness in various fields such as forensic science, genetic research, and paternity testing, STR analysis is accompanied by several challenges. These challenges encompass the complexity of alleles, stutter peaks, allelic dropout, PCR inhibition, mixture interpretation, as well as considerations regarding the quality and quantity of DNA, and data interpretation [66]. Low levels of template DNA, equivalent to approximately 15 copies of diploid cells/genomes (˜100 pg of DNA), can lead to the inability to identify heterozygous alleles. Additionally, stochastic effects can disturb the balance between different STR profiles across loci [67, 68]. Furthermore, the failure to detect a specific allele, known as allele dropout, can result in falsely identifying an individual as homozygous. Conversely, the presence of extra alleles or allele drop-in can indicate contamination events. A stutter peak, which is a by-product of PCR that is usually one repeat unit shorter than the actual allele, can occur due to DNA strand-slippage during the PCR process. This can potentially impact the accurate interpretation of STR data [69]. This often leads to a stronger signal (peak) for di- and tri-nucleotide STR repeat motifs compared to tetra- and pentanucleotide motifs. Such an artifact can have a significant impact on the analysis of mixed DNA profiles and affect their interpretation. This is especially true when the peaks from the minor donor are located in the stutter range position of alleles from the major donor [70]. Additionally, interpreting profiles from mixed samples with more than two contributors can be challenging. This is due to both the potential presence of numerous alleles in the profile and the fact that these profiles are often low-level, with complicating features like allele drop-out/drop-in and heterozygous imbal-

ance [67]. To aid DNA analysts in interpreting complex profiles, multiple probabilistic genotyping software have been developed [71].

5. Future Perspectives

The feature perspective of STRs encompasses their variability, utility in genetic mapping and forensic science, clinical implications in genetic disorders, technical aspects of their analysis, and their role in evolutionary studies. These features collectively make STRs powerful tools for forensic applications in recent decades. However, there are several disadvantages to STR testing. Primarily, the individual biostatistical efficiency of commonly used STR markers is limited, specifically in measuring exclusion power in paternity trio cases [72]. Additionally, the significant mutation rates of STR markers, which can be as high as 0.05 pose significant problems, particularly when the mother is not available for matching. Another major challenge of autosomal STR profiling is dealing with samples from multiple perpetrators. In a typical single-source profile, two peaks corresponding to alleles are generated at a single genetic marker (STR locus) with a heterozygous nature. Therefore, analyzing mixtures becomes crucial, yet reliable techniques for common forensic applications are still lacking. To improve the reliability of STR DNA profiles, increasing the number of markers and using a balanced mix of simple, compound, and complex, as well as bi-, tri-, tetra-, and pentanucleotide repeat STR markers, may be effective. Additionally, incorporating a combination of autosomal STR markers, Y chromosome STR markers, and SNP markers as well as NGS can enhance the usefulness of the DNA typing technique in forensic applications.

6. Conclusion

Short Tandem Repeats (STRs) play a vital role in forensic biotechnology because of their high polymorphism and compatibility with PCR. This has greatly impacted human identification and forensic investigations by allowing for precise matching of biological evidence to individuals. As a result, they are used in criminal cases, paternity tests, and missing persons inquiries. STRs are able to produce reliable results from small or degraded DNA samples, making them particularly valuable in difficult forensic situations. Their wide variability among populations enhances their ability to differentiate between closely related individuals. However, challenges exist, such as interpreting complex DNA mixtures and the possibility of genotyping errors, requiring the use of advanced statistical models and strict quality control measures. Continuous research and technological advancements are crucial to address these challenges and ensure the accuracy of forensic analyses. By overcoming these obstacles, STRs will remain a critical tool in supporting justice and providing closure for families worldwide.

Abbreviations

STR	Short Tandem Repeated
CE	Capillary Electrophoresis
NGS	Next Generation Sequencing
SNP	Single Nucleotide Polymorphism

Conflicts of Interest

The authors declared no conflicts of interest.

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