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ORIGINAL ARTICLE

Advancements and Applications of STR Kits in Forensic DNA Profiling: A Comprehensive Review

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Abstract

Short tandem repeat (STR) analysis is a crucial technique in forensic science, providing high-resolution DNA profiling for criminal investigations, paternity tests, and identification of missing persons. This document outlines the key stages of STR analysis, including sample collection and preservation, DNA extraction and quantification, PCR amplification of STR loci, capillary electrophoresis, and data interpretation. Proper handling and storage of forensic samples are essential to prevent DNA degradation and contamination. PCR amplification, using Taq polymerase, enables the replication of STR loci for analysis. Capillary electrophoresis then separates these fragments, generating an electropherogram that is interpreted using specialized software. STR profiling is widely used due to its sensitivity, accuracy, and ability to analyze degraded DNA samples. Despite its advantages, challenges such as DNA mixture interpretation and environmental degradation persist. The continuous advancement of forensic DNA technology, including next-generation sequencing (NGS), aims to enhance STR analysis efficiency and accuracy. This document also discusses various STR kits used in forensic science, their applications, and standard protocols ensuring reliable results. STR analysis remains a cornerstone of forensic DNA testing, playing a significant role in legal and investigative processes worldwide.

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Introduction

Short tandem repeats (STR) profiling is an invaluable tool in forensic science, offering high levels of discrimination and requiring just trace quantities of DNA in order to produce full or partial profiles[1,2]. Short tandem repeats are repeats of units of 3–7 base pairs of DNA, with the average number of repeats for various STR loci among any given population forming a DNA profile, commonly bi-allelic. DNA profiling is an essential methodology in the field of forensic investigations[3]. Over thirty years ago, the first official usage of DNA profiling resulted in the conviction of a convicted murderer. Nonetheless, the forensic science analytical techniques have evolved so much so that DNA profiling developed before the confession was capable of exonerating him[4,5]. As a result of the ever-growing list of applications for DNA profiling in regulatory processes for pharmaceuticals, plants, and organically manufactured agricultural products, the accuracy, effectiveness, and sensitivity have improved significantly[6].

In the context of forensic science, STR kits aid in the expeditious determination of a suspect's DNA profile in addition to identifying potential matches from donations detected at the crime scene[7,8]. From transnational organized crime to theft, homicide, and missing persons, forensic investigations and DNA databases play an important role in solving crime[9]. In recent cross-country investigations, STR profiling has been widely used, as the temporary storage of manufactured goods in bolt hole lock-ups between theft and resale usually happens in another country[10,11]. Such STR data can be obtained from production lines of DNA analysis, objects recovered from crime scenes, and clothing[12].Simultaneously, STR can inform post-bombing recovery teams about unidentified remains in the context of mass disasters and terrorist attacks[13,14].

Background of Forensic Science

Forensic science is instrumental in the detection and investigation of crime. Based on the exchange principle, it is an amalgamation of physical, medical, and biological sciences that are used in law and justice[15,16]. It allows for the determination of physical facts from legal evidence through the application of principles and procedures in the field of science[17]. Precise methods and techniques were developed to conform to the requirements of any court of law for the purpose of scientific investigation and crime detection[18,19]. Forensic study has grown largely since its early applications, but there is an issue with forensic specialists, including law enforcement, legal advisors, and court personnel regarding the validity, reliability, and admissibility of their procedures and techniques[20]. As technology flourishes, the need for a foundation becomes inevitable, in terms of utilizing more practical tools and applications on one hand to enhance one's work as a legal professional and on the other hand to secure one's innocence[21,22].

Forensic science began with the evolution of two fundamental principles. The first principle is the gradual acceptance of the fact that violent crime occurs. The second principle behind the formation of scientific crime detection is the realization and acceptance that an association exists between the suspect and the crime scene[23,24].Forensic science began to formalize and gain recognition during the 1800s, when a deep understanding of analytical chemistry, microscopy, serology, toxicology, and pathology was developed[25].The first applications of DNA were through the use of cells. The forensic use of DNA evidence is no longer just a tool in serious crimes, but has also revolutionized the involvement, liability, and overall success of justice in desired crime policies[26,27].DNA is not deemed to be the complete answer to solving crimes, particularly in cases involving serious pattern crimes, reported crimes, and new offenses. The application of forensic science is not limited to criminal investigations and therefore can be applied to the matters of civil nature mentioned below[28].With the acquisition of greater resources, skills, and a larger customer base, forensic science has expanded. Now, this technique is no longer restricted to the domain of criminal law. It has additional applications in civil disputes. To assist a country's criminal and civil judicial systems, extensive scientific and technical laboratories are set up[29,30].

Overview of STR Kits

Short tandem repeats (STRs) were first discovered in forensic science and then explored in genetic studies on evolutionary biology, population genetics, family studies, and paternity testing[31]. STR analysis has been playing a vital role in personal identification, identity disputes, criminal investigations, and the identification of missing individuals. The highly polymorphic nature, reproducibility, sensitivity, specificity, consilience, and strong signal-to-noise ratios make STRs ideal for most forensic applications[32]. The availability of multiple STR loci and sequence-based sizing information has made it possible for modern forensic kits to be multiplexed to analyze, interpret, and establish consensus profiles in degraded, limited, or mixed forensic biological evidence.

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Although STRs explore abundant features inside the human genome, commercial STR kits are limited, covering less than 1% of the genome[33,34]. Furthermore, future genomic studies should target these potentially informative loci for data mining and application in forensic, genealogical, and anthropological studies. STR is a repetitive sequence that partly, but mostly, obeys the forensic casework laws as X/Y-chromosomal gene markers. STR kits amplify specific DNA regions to identify genetic characteristics[35]. STR kits are specifically manufactured to amplify particular DNA regions. They consist of mixtures of primers that create many copies of a DNA region, resulting in an ameliorating product that can be easily visualized and analyzed. The kit amplifies specific regions of interest for forensic science because these loci are highly polymorphic and display genetic diversity in world populations. Providing a maximum of 24 STR loci, the kit facilitates the amplification of the target loci more quickly and robustly with minimal chances of contamination[36]. Other kits are also being used. Standardization of protocols for STR genotyping is important for accurate scientific research and crime solving[37]. Standard protocols allow the results produced by different groups to be reproducible and reliable. Standardized reagents and consumables provide method reliability, decrease contamination, and allow standardization among authorities. STR profiling data from DNA experts should be standardized. STR kits have crucial importance in forensic science for individualization. STR kits are equally important in genetic diversity studies[38,39].

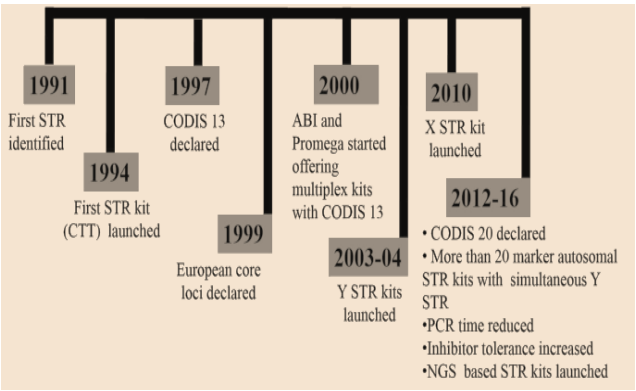

Figure 1; Timeline of key milestones in STR kit development for forensic DNA analysis. It highlights major advancements from the first STR identification (1991) to the launch of the first STR kit (1994), the establishment of CODIS 13 markers (1997), and the introduction of Y-STR (2003) and X-STR kits (2010). Recent innovations (2012–2016) include the CODIS 20 system, improved PCR efficiency, and NGS-based STR kits, enhancing forensic accuracy and speed[40].

Comprehensive Definition and Purpose of STR Kits in Forensic Analysis

Short tandem repeat (STR) kits are specialized tools that are specifically designed for the extraction and amplification of short tandem repeat sequences found within DNA samples[32,40]. The significance of STR lies in its ability to assist analysts in making accurate judgments or conducting in-depth investigations and analyses related to the identification of individuals [33]. The primary purpose of employing STR detection within forensic analysis is to significantly enhance the accuracy and reliability of DNA profiling results[34].Moreover, STR kits have shown substantial promise not only in legal matters but also in various other domains, including paternity tests, kinship analysis, ancestry determination, genetic diversity assessments, and even in fields such as molecular anthropology and archaeology[14,24].The remarkable advantage of utilizing STR is that it enables the rapid differentiation of individuals even within extensive datasets, making it indispensable for disaster victim identification scenarios[44].Furthermore, the comprehensive analysis of systems and perpetrator data has played an immense role in advancing forensic science and improving overall investigative outcomes[45].

STR, which stands for Short Tandem Repeats, is a specific type of repetitive DNA. Due to its unique specificity and the automatic inheritance of its patterns, STR facilitates the distinct identification of individuals through easily recognizable and consistent patterns[46]. This type of genetic marker possesses several notable characteristics, including a high degree of polymorphism, a low mutation rate, and a comparatively short genetic distance among individuals[43]. Research studies have demonstrated that a large number of the various STR loci can be isolated, often resulting in perfect repeated triplets with numerous repeats[14].STRs are distributed throughout the human genome, often with an average interval separating them. Before STRs and other molecular markers can be effectively utilized in practical applications, they must be detected through a process called PCR (Polymerase Chain Reaction), as their resulting output can be extraordinarily complex and intricate. In contrast to standard DNA analysis techniques, analyzing mitochondrial DNA regions or Y-chromosome markers tends to be relatively straightforward when conducted on male and female individuals[48,49,50].

Table 1; Advantages of STRs (Short Tandem Repeats) for Forensic DNA Typing[48,49,50]

Advantage	Description
Abundance of known STRs	Many STR markers are well-documented.
Small size suits for degraded samples	STRs are effective for degraded DNA.
Ease in multiplexing	Multiple STR loci can be analyzed together.
Frequent occurrence throughout the genome	STRs are distributed widely across DNA.
Useful for small quantity of DNA	PCR amplification allows analysis of minimal DNA.
Similarity in allele size	Allele sizes are consistent for efficient comparison.
Highly variable	STR markers are highly polymorphic.

Types of STR Kits

STR kits are classified into two major categories: multiplex and single-plex. Multiplex kits are designed to amplify, detect, and report on multiple STR loci in a single reaction tube[51].There are several multiplexes with different combinations of STR loci, and at different times certain combinations may be more useful for the forensic practitioner than others[52]. Multiplex kits used for forensic examiner casework testing are generally optimized to efficiently analyze forensic samples that may be damaged, contain inhibitors, be of limited quantity, and/or be of mixed origin[53,54]. Essentially, the method combines the amplification of known STR loci with a detection and reporter system. A microfluidic system may be used to assist in the separation of the STR amplicons since their size differences allow them to be resolved by system55.

In contrast, single plex kits typically focus on only one STR locus in the amplification, detection, and reporting systems[56,3]. Historically, single plex investigation of each locus was more prevalent; however, this application is much less feasible for forensic case analysis than a multiplex format[57]. In general, it is believed that multiplex kits are more desirable for the investigative casework conducted by law enforcement agencies and private forensic laboratories, but single plex systems may be used, for example, in academic institutions carrying out research in forensic science or as a confirmation of a frequency in casework should an atypical result be seen when troubleshooting investigations. Essentially, the selection of kit(s) is decided based on the requirements of an investigation[33,34]. Numerous STR kits are commercially available, designed to suit the needs of any crime laboratory interested in adopting STRs for forensic purposes. There are a variety of STR kits, many of which are approved for use in qualifying laboratories. New kits and STR locus combinations continue to evolve and are added as technology matures[41,58].

Table 2: Comparison of Multiplex and Single-Plex STR Kits: Features, Applications, and Limitations

Types of STR Kits		
Category	Description	Applications
Multiplex Kits	Designed to amplify, detect, and report on multiple STR loci in a single reaction tube.	Efficient analysis of forensic samples, including damaged, limited quantity, mixed-origin, or samples with inhibitors.
Single-Plex Kits	Focus on only one STR locus for amplification, detection, and reporting.	Academic research in forensic science or confirmation of frequency during atypical results in forensic casework investigations.
Key Features of Multiplex and Single-Plex Kits		
Feature	Multiplex Kits	Single-Plex Kits
Locus Coverage	Multiple loci in one reaction tube.	Focuses on a single locus.
Efficiency	Highly efficient for forensic casework.	Less efficient for casework, but valuable for specific research or validation.
Forensic Suitability	Optimized for analyzing degraded, inhibited, or mixed-origin samples.	Limited suitability for forensic casework.
Applications	Law enforcement casework, crime labs.	Research institutions, troubleshooting atypical results in casework.
Separation Method	May use microfluidic systems for resolving amplicons by size differences.	Not typically reliant on advanced separation systems.
Advantages and Limitations		
Category	Advantages	Limitations
Multiplex Kits	High throughput; multiple loci in a single reaction; faster and more comprehensive.	More complex design and potential for cross-reactivity.
Single-Plex Kits	Simplicity; focus on individual loci; useful for confirmation and research.	Time-consuming and less practical for large-scale forensic casework.

Commercially Available STR Kits

Forensics is a relatively small area of the world's market, and there are only a few companies that are developing and selling forensic instruments and reagents, including STR kits[51,52]. All instruments and reagents have several advantages, such as ease of use, speed, reliability, efficiency, throughput, the possibility of automation, and compatibility with various sample types. Although created by different manufacturers, all STR kits have the same chemistry and rely on the same science, and thus are capable of yielding similar results[53]. There are many versions of STR kits on the market, from research kits to commercial human identification kits with different capabilities, including options that are specific for casework laboratories, user experience, and applications[54,55].

Several commercially available STR kits on the market provide information about their user experience and applications and the country of the company that made the STR kits[56]. The market generally offers new kits for empowering STR typing for demanding casework applications. STR kits have operational protocols and serve particular forensic applications with case validation and guidelines certified[57]. Some STR kits also display DNA quality and yield faster protocol estimates to help users determine if the DNA sample is suitable for STR profiling, even when the mass is limited, which is very important for dealing with DNA mixtures. Commercially available STR kits already have standards and certifications to guarantee their reliability, performance, and quality[58,59]. The regulations and certifications of several commercially available STR kits are compiled. To overcome the problem of competition between companies that offer various polymorphic DNA analysis systems and forensic science tools, manufacturers must develop their products along with the technology[60]. A summary of several commercially available STR kits and their forensic applications, accuracy, user-friendliness, and distributor regions is provided. Displays of other options and alternatives to kits are possible, depending on the distributor or manufacturer offering the alternatives[61]. User experiences and feedback are discussed, and reviews of the quality and speed of the results, along with some case examples, are used to evaluate the quality and availability of the kits. Case studies with the commercially available STR kits, as well as the applications, benefits, and drawbacks provided by the kits, are also discussed[62]. This discussion is important to help examine and evaluate the STR kits and thus make the right decision in acquiring the appropriate STR kit that can meet users' cases and forensic applications' needs [63].

Table 3; Key Features and Benefits of STR Kits

Feature	Description
Ease of Use	Designed for user-friendly operation.
Speed	Provides faster results for forensic analysis.
Reliability	Ensures consistent and reproducible outcomes.
Efficiency	Optimized for analyzing forensic samples, even with limited DNA mass.
Throughput	Capable of handling multiple samples simultaneously.
Automation Possibility	Many kits are compatible with automated systems for streamlined processes.
Sample Compatibility	Suitable for various sample types, including degraded and mixed-origin DNA.

Table 4; Types of STR Kits and Their Applications

Type	Applications	Examples
Research Kits	Used in academic and research settings for experimental purposes.	DNA quality analysis, validation studies.
Commercial Identification Kits	Designed for human identification in forensic casework.	Crime scene analysis, victim identification.
Casework-Specific Kits	Tailored for casework laboratories to handle specific challenges like DNA mixtures or degraded samples.	High-profile criminal investigations.

Table 5; Comparison of STR Kit Features

Feature	Commercial STR Kits	Research STR Kits
Focus	Forensic applications and casework validation.	Experimental and exploratory studies.
Certification and Standards	Certified with operational protocols and guidelines.	May lack certifications for forensic validation.
Speed	Offers faster protocols for time-sensitive applications.	Speed may vary depending on the research purpose.
User-Friendliness	Optimized for non-specialist use in forensic labs.	Requires technical expertise.

Table 6; Market and Distribution of STR Kits

Aspect	Description
Manufacturers	Few companies dominate the market, each offering unique STR kits.
Geographic Distribution	Kits available globally, with country-specific distributors and regulations.
Alternatives	Custom kits or alternatives are available depending on the distributor or manufacturer.
Competition	Encourages innovation and improvement in technology and kit performance.

Table 7; Casework Benefits of Commercial STR Kits

Benefit	Description
DNA Quality Assessment	Allows users to determine DNA sample suitability for STR profiling, even in limited quantities.
Validation	Certified protocols ensure reliability and performance in forensic applications.
Versatility	Useful for both standard and complex forensic cases involving degraded or mixed DNA samples.
User Feedback	Includes reviews on quality, speed, and usability for forensic practitioners.

Key Components of STR Kits

DNA analysis in the fields of civil and forensic genomics relies on STR markers' ability to segregate alleles from samples containing allelic ladders[64]. Each of the STR kits contains a standard laboratory aliquot setting and quality performance tool[65].One of the most important parts of a kit is the reagents needed for amplification of the STR sequences from the extracted DNA solution. These reagents contain the primers, MgCl, and buffer needed for amplification. Another important part of the kit is the Klenow fragment and Taq polymerase needed for amplification of PCR products. Moreover, reverse sample DNA is needed for the amplification of the allelic ladders[66,67]. Internal Lane standards will provide guidance for capillary analysis while size standards provide guidance for allele calling. Each of the parts listed is important for the amplification of STR sequences. In the laboratory manuals, a kit lists the protocols used in the laboratory for the most effective and efficient analysis of the DNA samples. There are quality control components that are listed in the protocols that are used to validate the results, show the laboratory is running up-to-date standards, and that the results found are accurate. The investigator must follow the validation policies as set forth by the government. Some kits are PowerPlex and Quantifiers. When an individual is looking for the kit, they would need to look to see the parts for each of the kits. The laboratory protocols are described in the user manual for validation and analysis of results to assist in human identification. New protocols look at more complex samples and may have fewer parts or they are already combined. Each of these kits plays a crucial role in the forensic identification process with the need to use a combination of kits. Each lab must work within their own guidelines and run the appropriate protocols for their case[68,69,70,71].

Table 8; Components of STR Kits

Component	Description	Purpose
Reagents	Includes primers, MgCl, and buffer needed for DNA amplification.	Essential for amplifying STR sequences from the extracted DNA solution.
Klenow Fragment	A DNA polymerase fragment used in the amplification process.	Facilitates the synthesis of DNA during PCR.
Taq Polymerase	A thermostable DNA polymerase.	Catalyzes the synthesis of DNA strands during PCR at high temperatures.
Reverse Sample DNA	Reverse DNA strands used in amplification.	Necessary for generating allelic ladders for analysis.
Internal Lane Standards	Guides capillary analysis during electrophoresis.	Ensures proper alignment and measurement of STR fragment sizes.
Size Standards	Provides reference points for allele calling.	Assists in accurately determining allele sizes.

Applications of STR Kits

Several applications of STR include forensic science, covering criminal case resolution, missing persons, and mass disaster victim recovery; civil law cases; anthropological studies; and genetic research for gene association and basic genomic problem solving[8,72]. DNA cases have been solved not only in individual case identifications that have resolved unknowns in mass disasters and human rights issues for missing persons' identifications, but the bodies and remains exhumed during the genocide against Tutsis in Rwanda demonstrate the significance of the original application in forensic sciences[73,74]. Paternity examinations account for the majority of the identification work conducted using DNA tests[75]. Gene mapping markers such as STR

are used in genetic research to assist in gene association testing for human conditions. Research on the Y chromosome STR markers is of anthropological interest because they primarily segregate with surnames. STR testing on the X chromosome can help to resolve unclear mother and maternal child DNA mixtures and to estimate the maternal contribution from complicated samples with multiple contributors[76]. The forensic STR technology was initially developed in the late 1980s, after being released from a Naval Crime Laboratory[77].

The STR technology has universal applicability, especially in forensic science and law enforcement and judicial systems, and it has implications for resolving and preserving law and justice.[3] This technology has applications, though limited to some extent in the undersigned forensic fields, such as medical examiners, in other forensic and non-forensic scenarios outside crime scene investigations[78,33]. The use of STR technology is considered a significant tool in forensic biological associations such as family military and mass disaster victim identifications, missing persons, anti-doping specifications, and wildlife species whenever biological evidence is discovered and stored in forensic files[11,7].STRs are being globally used and appreciated by the global police community[79]. In general, the DNA database initiative is increasingly gaining ground, and technological advancements are evermore on the increase[80].The problems of forensic exclusions, cross-border procedures, data sharing and security, and privacy policy issues are being resolved to best practice standards worldwide[81].

STR kits are used during criminal investigations, both at the scene of a crime and at crime labs when processing biological evidence. There are numerous high-profile cases where law enforcement officials solved crimes by using STR analysis. Currently, numerous arrestees have been connected to their biological evidence left at cold case crime scenes once that evidence has been processed through an STR kit. STR analysis has been used to clear suspects through the elimination of those who are not the source of crime scene DNA or to link arrestees to numerous other crime scenes, giving law enforcement officials additional forensic evidence to solve their cases. The use of STR kits can potentially reopen cold cases by identifying new suspects, eyewitnesses, and developing new leads in those unsolved cases. It is imperative that crime labs and law enforcement in general follow the evidentiary standards set out in their states in addition to following the rules regarding Chain of Custody. The COC guidelines lay out the minimum evidentiary standards that must be maintained in order for the evidence to be introduced in court. At a minimum, basic training for collection, processing, and storage of DNA evidence must be completed by the individual or individuals involved in the processing of evidence. STR profiles are routinely used in courts to help a jury find an individual offender guilty of a crime. STR technology has also been a huge part of the national conversation involving wrongful convictions and has been integral in exonerating people who have been wrongly imprisoned for crimes that they did not commit. STR profiles obtained from crime scenes can help to validate or confirm a confession of an innocent person. The more evidence that directly implicates someone in a crime, the more difficult it becomes for them to challenge their conviction. Police officer access to STR kits can become a hot-button issue because their improper use can lead to lawsuits and wrongful conviction. In order to become familiar with using their STR kit, officers should be trained on it in a classroom setting and become familiar with it before using it for their first forensic submission.

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national conversation involving wrongful convictions and has been integral in exonerating people who have been wrongly imprisoned for crimes that they did not commit. STR profiles obtained from crime scenes can help to validate or confirm a confession of an innocent person[88]. The more evidence that directly implicates an individual in a crime, the more challenging it becomes for them to challenge their conviction[89,90]. Police officer access to STR kits can become a sensitive issue due to the potential for lawsuits and wrongful convictions resulting from their improper use. In order to become familiar with using their STR kit, officers should be trained on it in a classroom setting and become familiar with it before using it for their first forensic submission[91].

Advantages and Limitations

STR profiling techniques have several advantages when compared to other molecular genetic methods[92].As these profile a much greater number of genetic markers on multiple loci throughout the human genome, they are much more discriminating than older genetic marker systems[93,94]. Consequently, this enables the potential for much more precise individual identification[95]. The STR markers are also relatively short in length, thereby conferring additional advantages for forensic analysis in that they are not only extremely stable, but they are also much more easily further amplified using PCR methodology, an essential characteristic given that forensic analyses often involve the analysis of highly degraded DNA[96]. Moreover, the CNVs in some STRs further add to their discriminating potential[97].

However, several important limitations still exist that affect the utility of STR profiling. The major limitation is the requirement for high-quality DNA samples as a reliable source of template DNA[98].This requirement limits the successful amplification and therefore profiling of DNA samples that are highly degraded, such as those present in skeletal tissue or soil-exposed remains, or DNA that is present in very low amounts[99]. Even smaller amounts of DNA present as a mixture of DNA from multiple contributors can reduce the reliability of DNA profiles from one sample readily isolating DNA[100]. Indeed, the requirements for integrity and specificity of DNA samples are also the basis of potential contamination concerns, where low-level mixed DNA is inadvertently amplified and interpreted [101]. It is consequently well recognized that the potential sources of contaminating DNA are varied and complex, and include sample collection and storage, as well as technical contamination within the state of the art in forensic science today[102]. This issue is critically relevant for forensic samples and has instigated considerable research in the development of the relatively new field of forensic DNA sampling, contributing to the investigation of its handling to interpret the potential challenges of DNA contamination[103].

Table 9; summarizing the advantages and limitations of STR profiling

No	Advantages of STR Profiling	Limitations of STR Profiling
1	Highly discriminating due to multiple loci across the human genome.	Requires high-quality DNA samples fo reliable results.
2	Short STR markers make them stable and easier to amplify using PCR.	Degraded DNA or low-quantity samples may fail to amplify properly.
3	STRs are effective for forensic analysis even with degraded samples.	DNA mixtures from multiple contributors can reduce reliability.
4	Copy Number Variations (CNVs) in STRs enhance discrimination potential.	Contamination risks due to improper sample handling and storage.
5	Widely used in forensic science, law enforcement, and genetic studies.	Technical contamination can impact DNA interpretation and profiling.

Recent Developments and Future Directions

In recent years, remarkable new developments in STR technology have improved the sensitivity, accuracy, and effectiveness of forensics[65]. Some of these advancements include kits capable of analyzing up to 27 loci, an increasing number of kits validated for forensic use, and kits capable of improving sensitivity for low template DNA[104,105,16].Next-generation sequencing has been heralded as the third wave of forensic DNA testing and is of great importance to countries and academics[107].STRs can be identified from sequencing data produced using NGS, and this will greatly assist in the detection of inter-kit incompatibilities, the analysis of complex samples, identifying pathogen characteristics, and genetic information[3,108]. Unlike STRs, DNA methylation profiles can be used to identify gender, age, body tissue, cell nature, and may be helpful in determining the potential identity profile. In order to improve the reliability of the interpretation of data and increase the use of DNA methylation, more targeted and less than 5 ng of DNA is required for forensic and more freely available prediction software[109,110].International technological societies are developing functional groups to support the discussion of STR and forensic scientists and the resulting outcomes by maximizing their technological strategy[3,111].The group plans to undertake international harmonization initiatives to guide future forensic techniques in this space. Although STR development in this field is increasing, much further work needs to be undertaken. STR analysis has been used to provide essential evidence for solving crimes, disasters, and issues of parentage[11,111]. Blood and semen-derived samples are routinely used for forensic DNA

casework analysis. Autosomal STR profiling has been used in a majority of forensic laboratories worldwide, although Y-chromosome STR analysis and mitochondrial DNA typing are also performed as necessary for parentage and missing person investigations[112,113]. An immediate application of the resulting scientific knowledge was the development of mini autosomal STR kits and PCR inhibitors removal kits. The current trend is to develop a multiplex reaction capable of amplifying the radically increased number of STR loci in a fast and easy-to-use format[6,114]. Lab-on-chip technology has been combined with a PCR-CE microfluidic device for STR analysis, and this would further enable multiplexing capabilities[115].Non-autosomal STR markers have been introduced, and another kit designed for actively decaying DNA has been released[10].

Procedure of STR analysis

Standard protocols are employed by most forensic laboratories, and while new methods are being tested, they have yet to be widely adopted. First, an evidence sample is collected and preserved with a proper method in order to maintain its integrity. Then, DNA is extracted, and the quantity and quality are assessed. Next, the DNA is amplified using PCR for STR loci. Capillary electrophoresis is used to separate the STR fragments. The electropherograms are read by software and interpreted by experts to generate a DNA profile for future comparison. The steps below summarize the main procedure of STR analysis:

1. Sample Collection and Preservation

The first step in forensic STR analysis is collecting biological samples from crime scenes, ensuring proper reference samples from victims and suspects. Traditional samples include blood, sperm, saliva, and hair, which must be properly stored to prevent degradation. Environmental factors like temperature and moisture can affect DNA quality, so laboratories follow strict guidelines for preservation. STR systems are optimized for long-term storage and forensic casework. Different regions may have varying forensic practices, so laboratories should follow standardized collection guides[115,116,117,118].

2. DNA Extraction and Quantification

The second stage of forensic STR analysis is DNA extraction, with various methods offering different advantages and limitations. DNA quantification follows, assessing the quality and quantity of extracted DNA for further testing. Several kits, both manual and automated, use silica-based technology for DNA isolation. The choice of extraction method depends on the sample type, laboratory equipment, and expertise. While organic extractions yield purified DNA, they may remove inhibitors like humic acid from bone samples[115,116,119].

3. PCR Amplification of STR Loci

Forensic DNA analysis relies on generating detectable STR profiles using polymerase chain reaction (PCR). PCR amplifies specific STR loci from millions to billions of copies, with Taq polymerase being the gold standard due to its high-temperature stability and efficiency. Various PCR instruments and reagents enable high-throughput sequencing with small reaction volumes. Primer design software ensures optimal sequence selection, checking for SNPs, secondary structures, and primer-dimers. Ideal primers are 22-29 nucleotides long, with a melting temperature of 60-63°C and 45-55% GC content[120,121,122].

4. Capillary Electrophoresis

Capillary electrophoresis is widely employed in forensic labs to separate amplified STR products by size. Emerging in the late 1980s and early 1990s, it serves as a high-resolution alternative to traditional gel electrophoresis, enabling simultaneous analysis of multiple loci from STR amplification. While sharing principles with gel electrophoresis, capillary electrophoresis is more objective, provides higher resolution for smaller fragments, and delivers results faster. The primary buffer used is a pH-controlled TBE solution comprising 89 mM Tris, 89 mM Borate, 2 mM EDTA, and 10% glycerol. The separation process utilizes long, narrow capillaries where high voltage is applied at the injection end, allowing negatively charged DNA to migrate towards the positively charged electrode based on size. The injection process attracts DNA molecules into a focused sample band. Forensic samples, such as human blood or semen, require a DNA detection system to visualize fluorescently labeled DNA fragment sizes. The characteristics of the electrophoresis system and sample purification influence peak shapes and resolution, enabling accurate allele scoring. A pooled polymer solution facilitates the simultaneous analysis of STR fragments produced during amplification, resolving best in a 4–7% linear polyacrylamide polymer. DNA electrophoresis runs at high constant voltages, typically ranging from 3.0 to 15.0 kV, with capillaries varying between 50 to 150 microns. Both side and end injection methods offer distinct advantages and limitations, while the electrical power needed for STR fragment separation must be considered in capillary design. Common issues

during runs include sample pull-up, plateaus, peak splitting, and long run times. The resulting data is presented as a computer-generated electropherogram, detailing size determinations and signal strength values in a panel format. Accurate sizing often relies on the allelic ladder technique, where "bin" sizes stem from a calculator influenced by allele size range panels and sample length. Key parameters such as electrical length, color channel, injection time, sample current, and ejection time are critical for effective capillary electrophoresis in STR analysis[123,124,125,126].

5. Data Analysis and Interpretation

STR chemistries create DNA fragment complexes analyzed using various software tools. These applications generate raw data, perform initial STR delineation, and assess data quality for complete STR profiles. Multiple CPUs validate results individually against standards or through consensus from individual call lists. Typically, inter-study consensus of allele calling software is employed, although multiple programs may validate variant calls to enhance accuracy. Common call algorithms utilize (1) a binning algorithm for peak assignment, (2) a peak height threshold for allele calling, and (3) a quality score indicating call confidence. They also incorporate signal intensity balance and reference dye ratio algorithms to assess DNA quality and detect mixed sample contributions. Quality scores may vary by DNA event category, with stutter position data recorded due to software calling tendencies. The call algorithm database confirms unusual peaks and validates small alleles or haplotypes not in the reference database[127,128,129]. Differentiating mixtures from artifacts is essential, evaluated through peak height ratios, peak area ratios, and minor contributor percentages, to robustly interpret STR profiles. Recommendations emphasize assessing allelic peak height and area balance. Low template DNA is particularly challenging, increasing allele drop-out risk and stutter proportions. Evaluating CSF variation in replicates to assess stutter percentages in low templates is advised, alongside peak height and area ratio analysis. The protocols for data analysis and interpretation considerably affect final STR profile conclusions in violent crimes and disaster victim identifications. Standard practices and quality assurance ensure reliable results across laboratories, with specific recommendations for interpreting mechanical mixtures and low template DNA. Strict quality control and validation processes for software and interpretation guidelines have been underscored since their introduction, preceding the development of allele balancing software[130,131].

Conclusion

The primary objective was to present information to national and international audiences about the range of STR kits used in forensic science today. It explored the advantages and disadvantages of a range of STR kits used for various legal agencies and forensic DNA laboratories to help answer various types of casework. It also documented the different software packages used in testing and issues of terminology; the ongoing challenges were discussed, such as mixture analysis and the complexity of stutters. The limitations of the interpretation of certain loci in some populations, issues of privacy and ethical considerations, and the potential strengths and pitfalls of family searching were also presented. The purpose of this summary is to provide an overview of the major points discussed. In conclusion, STR analysis has been used for over 30 years, and today it forms a cornerstone of DNA profiling for forensic identification as it is efficient, robust, and accurate. It has been responsible for both supporting convictions and freeing the innocent and aiding the identification and repatriation of victims of mass disasters or conflict. However, the core technology has not substantially changed, and STR testing is more efficient than nearly all other technologies currently available. Further exploitation of direct sequencing, massively parallel sequencing, rapid, and low-template testing is anticipated; however, challenges over analytical specificity, interpretational thresholds, and mixture analysis still exist. These and the issues of using STR GIS for building stronger cases currently remain a focal point for research and operational development, as does a fresh attempt to understand the rates at which mutations occur in STR loci in different populations. Regular professional development of forensic science practitioners will ensure optimal use of these increased capabilities and a faster initial interpretation of cases where the evidence is found to be abundant, of good quality, and without evidence of acceleration or elevated stutter production. Many other capitalizing uses of STR kits are documented, and these have applications far beyond their original use in criminal investigations. It is therefore crucial that the forensic community begins a process of embracing these new uses and applies them in a responsible, accountable, and transparent manner.

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