Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam
Volume 22 No. 1, June 2025 DOI 10.31851/sainmatika.v22i1.17388
https://jurnal.univpgri-palembang.ac.id/index.php/sainmatika

A Mini Review of Short Tandem Repeat (STR) as DNA Profiling Markers in Forensic Biology

Antonia Anindyanari Paramartastri Nitisara, Laurentia Henrieta Permita Sari Purba*
*e-mail: laurentiapermita@staff.ukdw.ac.id

Biology Study Program, Faculty of Biotechnology, Duta Wacana Christian University, Indonesia

ABSTRACT

The use of forensic biology methods plays an important role in handling criminal cases such as sexual violence, murder, and assault to enhance the quality of criminal investigation. These methods use polymorphic genetic markers such as short tandem repeat (STR) or microsatellites to identify individuals based on the DNA profile of the cell nucleus left on the evidence. STR markers are considered suitable for forensic analysis due to their ability to work with degraded DNA and the high heterozygosity, providing strong discrimination capabilities. The DNA profiling process involves collecting evidence samples, DNA extraction, PCR amplification, capillary electrophoresis, and comparison with national or international DNA databases. All processes follow standards to ensure accurate results. Challenges in STR analysis include the need for skilled analysts, protocol updates, database expansion, and ethical considerations regarding genetic data. Improvements in the forensic system will enhance law enforcement effectiveness and strengthen public trust in the future of forensic science.

Keywords: criminal cases, forensic biology, DNA profiling methods, genetic material, short tandem repeat

INTRODUCTION

Indonesia is categorized as one of the countries with a high crime rate. Indonesia ranks 20th out of 193 countries on the global crime index (Ocindex, 2023). The increasing crime index in Indonesia is not accompanied by an improvement in the country's readiness to address criminal issues. This is evident from a decrease in the resilience score by 0.08, which measures a country's ability to handle crime (Ocindex, 2023). Forensic science is a valuable approach for enhancing resilience scores by utilizing new technologies to solve crimes for uncovering crimes (Roux *et al*., 2021). In general, forensic science generally involves the application of scientific knowledge and methodology to solve criminal cases within the legal system(Shen & Vieira, 2016). Various crimes, like rape, murder, assault, drug abuse, and rioting, can leave genetic material, known as deoxyribonucleic acid (DNA) (Halim *et al*., 2022). This genetic material is used for identifying victims or suspects (Neves & Zieger, 2023).

DNA profiling is a widely used method in forensic biology (Prinz *et al*., 2007). DNA profiling is used to identify individuals by analyzing biological traces found at crime scenes, such as blood, hair, nails, and sperm. The DNA profiles of the victim and suspect are compared to reference profiles for identification purposes. The DNA profiles of the victim and suspect are compared to = reference profiles (Hänggi *et al*., 2023). Reference profiles usually come from individuals related to the victim or suspect. Before analyzing an individual's DNA profile for a case, genetic material in biological samples must undergo DNA extraction and amplification. Then, the DNA

p-ISSN 1829 586X
e-ISSN 2581-0170

fragment DNA was amplified using polymerase chain reaction (PCR) technology to increase the copy number (Garibyan & Avashia, 2013). Proper handling and treatment of samples is crucial for maintaining DNA quality and quantity to ensure accurate analysis results (Adouani *et al*., 2023). The workflow for evidence examination, biological sample collection, DNA extraction, amplification, capillary electrophoresis, and DNA profile analysis must adhere to forensic matrix conservation standards (Jickells & Negrusz, 2008). This mini review focuses on the development of methods, technologies, and markers used in DNA profiling, particularly short tandem repeat (STR) profiling, for forensic purposes. This mini review aims to explore alternative technologies for advancing forensic biology in Indonesia.

METHODS

The method used in this study literature review by collecting and analyzing a total of 68 journal articles or scientific papers. Articles were retrieved using the Google Scholar search engine and various repositories, such as PubMed, Springer, Elsevier, ScienceDirect, and MDPI, which are indexed in SINTA and Scopus. This literature review also utilized several eBooks related to DNA profiling methods. To enhance the accuracy and credibility of this mini review, international statistical databases, namely the Genetic Discrimination Observatory and the Global Organized Crime Index (Ocindex), were used to examine the distribution of forensic databases, crime rates, and the percentage of criminal case resolutions in different countries. Several keywords were used to obtain relevant literature, including "DNA profiling", "forensic development", "criminal cases in Indonesia", "STR typing", "DNA extraction methods for DNA profiling", and others.

DISCUSSION

DNA Profiling in Forensic Biology

DNA profiling from biological samples has been widely used in solving criminal cases since around the 1980s. DNA profiling methods have rapidly improved in speed and sensitivity, enabling analysis of diverse sample types (McCord *et al*., 2019). DNA profile databases help identify suspects and evaluate DNA evidence from crime scenes (Butler, 2015; Roewer, 2013). Several genetic markers that can be used for DNA profiling, along with their characteristics, are presented in Table 1 (Jaiswal & Nayyer, 2023).

Table1. Various types of genetic markers used in DNA profiling

DNA profiling marker	Characteristics
Restriction Fragment Length Polymorphism (RFLP)	A molecular marker is specific to a single-clone / enzyme restriction combination (Jarcho, 2000). Uses restriction enzymes to cut DNA at unique restriction sites (Hashim & Al-Shuhaib, 2019).
Variable Number Tandem Repeat (VNTR)	Consists of short nucleotide sequences repeated consecutively (10-12 nucleotides) with repetitions ranging from 10 to 100 times (Panneerchelvam & Norazmi, 2003).
Short Tandem Repeat (STR)	Composed of short nucleotide sequences repeated consecutively (4-6 nucleotides) (Lu *et al*., 2021). Refers to 13 specific autosomal STR loci used for forensic identification, including paternity testing.
Single Nucleotide Polymorphism (SNPs)	DNA sequence variations are caused by a change in a single nucleotide (high-resolution detection of genetic variation) A, T, G,

DNA profiling marker	Characteristics
typing.	and C within the whole genome sequence (Garcia *et al*., 1996).
Y-*chromosome analysis*	Analysis of regions on the Y chromosome for sex differentiation and paternal lineage tracing (Vuylsteke *et al*., 2007).
mtDNA analysis	DNA found in mitochondria is inherited through the maternal lineage (Garcia *et al*., 1996). Commonly present in hair, bone, and teeth samples (Silva *et al*., 2007).

Although genetic markers for DNA profiling analysis are quite diverse, the STR marker has been widely used in forensic science (Wyner *et al*., 2020). STR (microsatellites) markers were first utilized in forensic cases in the United Kingdom in 1994 (Kimpton *et al*., 1994). Since then, STR typing has been recognized as the "gold standard" for human identification in forensic applications (Goodwin *et al*., 2007; Kimpton *et al*., 1994). This marker is suitable for the amplification process because of its simple repeat sequences. At the same locus, the tetra-nucleotide sequence (CTAG) differs among individuals (Figure 1).

AGTC-CTAG-CTAG-CTAG-CTAG-CTAG-AGTC-AGTC-AGTC-AGTC- *human* 1

AGTC-CTAG-CTAG-CTAG-CTAG-CTAG-CTAG-AGTC-AGTC-AGTC- *human* 2

AGTC-CTAG-CTAG-CTAG-CTAG-CTAG-CTAG-CTAG-AGTC-AGTC- *human* 3

Figure 1. STR tetra-nucleotide (CTAG) with different lengths at the same locus (modified from Udogadi *et al*., (2020))

STR markers exhibit alleles with regular spacing and variations in four nucleotide bases (quadruplex) (Wyner *et al*., 2020). DNA profiles created with STR markers are commonly used in paternity and maternity testing (Tamaki *et al*., 2009), kinship testing (Allen *et al*., 2007), the identification of rape perpetrators, and the identification of disaster victims (Yoshida *et al*., 2011). Forensic DNA profiling is conducted by amplifying non-coding regions to create a unique genetic profile for identification purposes, specifically 16-27 STR markers using PCR techniques. DNA profiling was performed using data from capillary electrophoresis (CE) analysis. The results are displayed as peaks in an electropherogram and compared to the national criminal DNA database (Glynn, 2022). Key features of an STR system are high heterozygosity, consistent repeat units, distinct alleles, and reliable amplification. The number of STR markers analyzed varies by country. The United States uses the Combined DNA Index System (CODIS) Core 20 STRs, the United Kingdom and Ireland use DNA-17, Europe uses the European Standard Set (ESS), and Indonesia uses an STR kit with core markers compatible with international profiles (Yudianto *et al*., 2022). Table 2 outlines the pros and cons of utilizing STR markers in DNA profiling. STR markers are essential in the criminal justice system, aiding forensic scientists in re-evaluating old cases that were previously considered closed due to lack of evidence (Udogadi *et al*., 2020).

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Table 2. Advantages and disadvantages of STR fragment length in forensic DNA profiling (Jordan & Mills, 2021)

Advantages	Disadvantages
Fast (capillary electrophoresis) and simple, high sensitivity (~0.1-2 ng DNA) and specificity, accurate	Deconvolution of DNA mixtures and contamination is challenging
Highly reproducible	The presence of inhibitors and PCR artifacts (stutter, allelic drop-out, background noise) can interfere with result analysis.
High discrimination power, high multiplex capacity, codominant alleles	Difficulties in analyzing highly degraded DNA or low template DNA samples
Standardized across forensic laboratories and widely available commercial STR kits	Less effective in distinguishing closely related individuals (e.g., siblings with shared genetic markers)
Requires a small amount of DNA (limited biological samples) for amplification	Dependence on databases for comparison
Genetic profile databases and allele frequency data allow statistical comparison (Butler, 2015; Canturk *et al*., 2014; Udogadi *et al*., 2020)	Ethical and privacy concerns; potential misuse of genetic information complicating the application of STR profiling in forensic science (Butler, 2015; Canturk *et al*., 2014; Vidaki & Kayser, 2018)

Workflow of DNA Profiling Using STR Markers in Forensics

Technological advancements have made forensic genetics one of the most effective solutions for analyzing human genetic variations (Jaiswal & Nayyer, 2023). STR-based DNA profiling (Chiu *et al*., 2021) enhances system sensitivity, improves result productivity, and ensures compatibility with computer-based databases (Manjunath *et al*., 2011). The workflow scheme for DNA profiling using STR markers, from sample collection to data analysis, is illustrated in Figure 2. Biological samples obtained from evidence at the crime scene (CSI) are analyzed to determine the DNA profiles of both victims and suspects in each case. Crime scene protocols are crucial for guiding sample collection and determining sample suitability for analysis (Sultana & Sultan, 2018). Each sample should be handled based on its unique characteristics. Properly collecting and storing samples is essential to maintain the accuracy and reliability of forensic evidence (Budowle *et al*., 2006; Starnbach *et al*., 1989).

The first step in DNA profiling after sample collection is DNA extraction. The principle of DNA extraction involves cell lysis to release DNA. A detergent solution or lysis buffer is used to clean and purify the extracted DNA. The addition of proteinase is also essential for removing proteins bound to DNA. The lysis procedure sometimes requires the use of heat and agitation to accelerate enzymatic reactions and improve lipid solubility (Rapley & Whitehouse, 2007). There are various DNA extraction methods, one of which is organic extraction using phenol-chloroform (Bukyya *et al*., 2021), then Chelex extraction (Genetics, 2024) or chelating resin-based methods (Walsh *et al*., 2013), as well as the use of commercial kits. Some examples of commercial forensic DNA extraction kits include the PrepFiler™ Forensic DNA Extraction Kit, the PrepFiler™ Forensic DNA Isolation Kit for blood samples on colored fabrics, the PrepFiler BTA™ Forensic DNA Extraction Kit for bone and tooth samples, and the Prep-n-Go™ Buffer for buccal cell (swab) or fresh

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e-ISSN 2581-0170

blood samples (Badu-Boateng *et al*., 2018; Lasota, 2014; Mundotiya *et al*., 2023). The selection of a DNA extraction method should be adjusted based on the characteristics of the sample type.

The next step after DNA extraction is amplification, which increases the concentration of DNA fragments in vitro (Martin & Linacre, 2020). The principle of PCR is enzymatic DNA synthesis using two oligonucleotide primers that hybridize to opposite strands of the DNA template (Chauhan, 2018). The PCR reaction involves multiple cycles (Saiki *et al*., 1988), each consisting of denaturation, annealing, and elongation phases (Bukyya *et al*., 2021). Amplification involves using the product from one cycle as a template for the next cycle, resulting in a rapid increase in the number of target copies with each cycle (Youngest *et al*., 2022). Designing specific primers is a challenge in PCR amplification for DNA profiling (McDonald *et al*., 2024). STR markers are regularly assessed in multiplex reactions with 16 or more markers in a single tube (Elkins, 2015). In the PCR technique, the primers need to function specifically within the reaction. Automated software tools are used to design the primers. PCR primers are necessary for amplifying and targeting specific DNA regions.

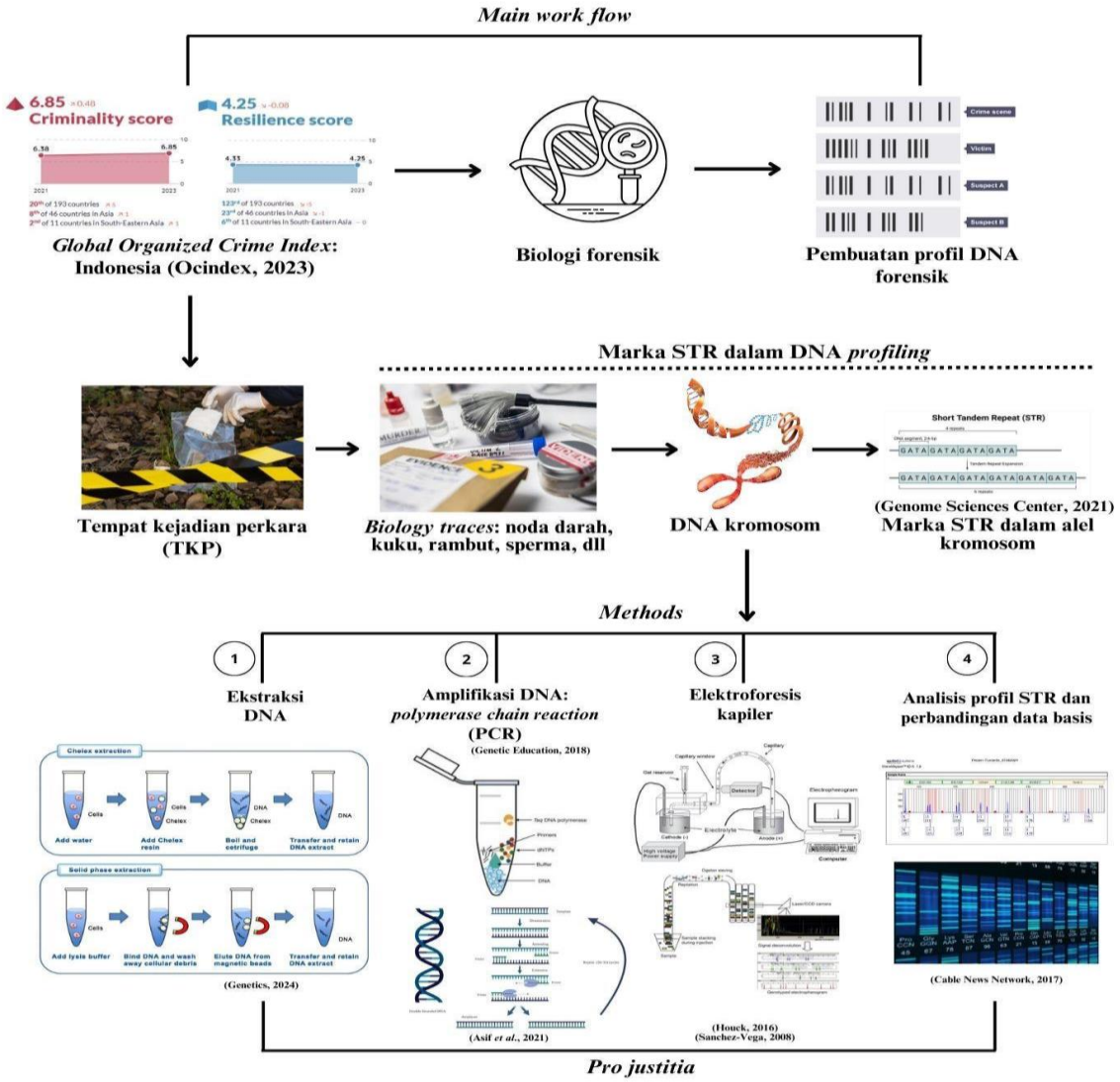

Figure 2. Workflow scheme for DNA profiling using STR markers

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Several parameters must be considered when designing a primer (Asif *et al*., 2021; Dieffenbach *et al*., 1993), including:

(1) The optimal primer length should range between 14-28 bp for standard PCR and 28-34 bp for multiplex PCR.
(2) The optimal GC content of the forward and reverse primers should be between 40% and 60%.
(3) Repetitive sequences, such as TTTTT, and consecutive dinucleotide repeats like ATATATATAT should be avoided, as they may cause primers to bind to non-specific or non-target DNA.
(4) The most crucial parameter is the melting temperature (Tm), which should be between 52°C and 60°C, with a maximum difference of 5°C between the forward and reverse primers.
(5) Additionally, hairpin loop structures and primer-dimer formation should be prevented to maintain reaction specificity and stability.

After the PCR process is completed, the capillary electrophoresis (CE) method is used to analyze the amplification results, which are represented by peaks and relative fluorescence unit (RFU) values for each STR locus. CE has become a more promising approach as it replaces the earlier requirement of manually pouring gel and loading DNA samples (Houck, 2016). Capillary electrophoresis (CE) automatically analyzes DNA using a small sample for injection and detection, enabling easy reanalysis if necessary. (Sanchez-Vega, 2008). CE technology uses fluorescence detection and attaches fluorescent dyes to the non-reactive 5' end of each primer or primer pair (Giusti & Adriano, 1993). CE instruments require proper sample injection standards and adequate temperature control to ensure the efficient separation of DNA fragments in DNA profiling, based on the length of STR regions (Smith & Nelson, 2003).

Several software programs are used to process data from capillary electrophoresis (CE) to generate STR genotypes. Universally, the primary functions of these software programs include controlling electrophoresis conditions, regulating the wavelength of light detected by the charge-coupled device (CCD) camera, and determining the sequence of data processing steps. The output of these data collection programs consists of "raw data", represented as relative fluorescence units (RFU) on the y-axis and the number of data points collected on the x-axis (Butler *et al*., 2004). Specific programs such as GeneMapper™ID, GeneScan, and Genotyper are required to convert raw data into visual representations containing STR genotype information.

Challenges and Future Directions of Forensics in Indonesia

Forensic technology in developing countries like Indonesia faces challenges that impede the adoption of advanced forensic methods. The challenges in Indonesia are mainly due to limited resources and infrastructure, as well as the absence of a comprehensive national genetic database. Without a comprehensive and standardized database, interpreting DNA profiling results accurately becomes challenging (Jordan & Mills, 2021). Currently, Indonesia still relies on the universal global DNA database, the Combined DNA Index System (CODIS). Since the establishment of the UK National DNA Database (NDAD) based on CODIS, many countries worldwide have started developing their national DNA databases

p-ISSN 1829 586X
e-ISSN 2581-0170

(Uberoi *et al*., 2024). As the use of DNA databases increases, investigators are adding more DNA profiles to national databases. Data indicates that 9 out of 84 countries have forensic databases with DNA profiles from over 5% of their population (Genetic Discrimination Observatory, 2023). According to the forensic map (Figure 3), Indonesia does not have a national DNA database in place. (Uberoi *et al*., 2024). Indonesia, the world's largest archipelagic country with over 17,000 islands and a diverse population of around 300 ethnic groups, needs to address this issue (Farhaeni & Martini, 2023). Indonesia is creating a national DNA database that reflects its diverse population, positioning the country to join the global network of DNA profiles.

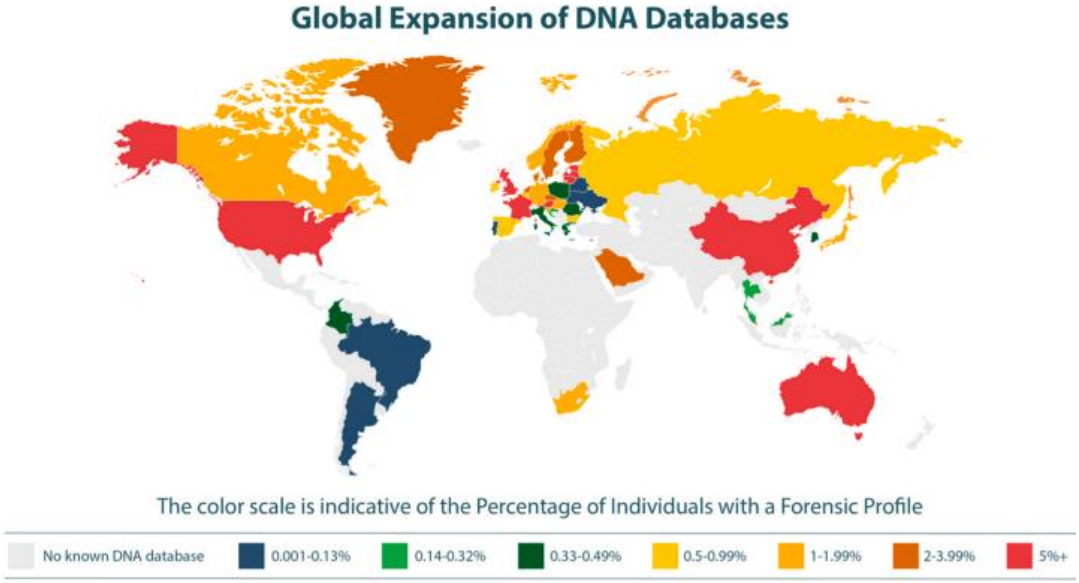

Figure 3. Expansion map of forensic DNA profile databases (Genetic Discrimination Observatory, 2023)

DNA profiling in forensic science has rapidly advanced in a short time. Advancements in forensic science are tied to breakthroughs in molecular biology technology (Jordan & Mills, 2021). Various genetic analysis techniques, such as STR analysis, Sanger sequencing, SNapShot, and capillary electrophoresis-single strand conformation polymorphism (CE-SSCP), have been used to analyze DNA samples. STR marker-based PCR analysis is now widely used in solving criminal cases in many countries. Investing in training and education for forensic professionals is essential for mastering the latest technologies and improving the quality of forensic services. Massively Parallel Sequencing (MPS) or Next-Generation Sequencing (NGS) is a cutting-edge DNA sequencing technology that plays a vital role in forensic sampling. Metagenomic sequencing enables investigators to analyze low-biomass environmental DNA (eDNA) samples, such as those from soil, water, and air.

Forensic scientists use Next-Generation Sequencing (NGS) to solve different types of criminal cases, such as (1) Maternal lineage investigations using Mitochondrial DNA (mtDNA) identification; (2) Identifying male DNA in contaminated samples with Y-chromosome STR analysis; (3) Investigating poisoning cases through non-human DNA analysis; (4) Tracing ancestry and conducting paternity analysis (Hajibabaei *et al*., 2011; Phillips

p-ISSN 1829 586X
e-ISSN 2581-0170

et al., 2009; Tang & Huang, 2010; Van Geystelen *et al*., 2013).

CONCLUSION

DNA profiling using STR markers is crucial in forensic science, providing accurate identification in criminal cases. The standard workflow includes sample collection, DNA extraction, PCR-based amplification, and capillary electrophoresis analysis. Recent advancements, particularly next generation sequencing (NGS), offer promising opportunities to enhance forensic capabilities in Indonesia. A key challenge is the absence of a national DNA database. Indonesia currently relies on the Combined DNA Index System (CODIS), which lacks specificity for local applications. Establishing a national database would improve suspect identification, support population diversity studies, and aid forensic research. With investments in technology, training, infrastructure, and public awareness, Indonesia can strengthen its judicial system and enhance it through advanced DNA profiling.

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