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Next Generation Sequencing (NGS)- An Advance Approach to Forensic Science: A Review

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Abstract

DNA typing is a center stage part of modern forensic research. DNA sequencing technologies are using strong instruments that have improved molecular sciences earlier using Sanger or Chain termination sequencing and are continuing to do so using Next Generation Sequencing (NGS). By avoiding the difficulties of the traditional way of sequencing, next-generation sequencing has the possibility to develop and expand molecular utilization in a criminal investigation. By leveraging the possibility of Next Generation Technology (NGS) technology, which might be used to concurrently analyze a lot of loci in a total genomic set on allozymes, medina, and autosomes and bring the latest opportunities in the forensic study arena. Moreover, NGS technology is also expected to have applications in a variety of other areas of investigation. These incorporate DNA data set evolution, family and phenotypic induction, monozygotic twin investigations, determination of bodily fluid and species, and legal creature, plant, and microorganism examinations. The fundamental benefits of NGS contrasted with a regular strategy that it uses at the same time countless hereditary markers with high goal of hereditary information. These advantages might help in addressing a few difficulties like the combination of more than two sample examinations and managing minute degraded samples. This reviewed article is expected to present the Next Generation Sequencing System and its possible applicability in solving crimes and other forensic investigations.

Keywords: Next generation sequencing; DNA; Forensic DNA phenotyping; Microbial DNA

Abbreviation: NGS: Next Generation Sequencing; SMRT: Single-Molecule Real Time; STR: Short Tandem Repeat; CE: Capillary Electrophoresis; DVI: Disaster Victim Identification; PM: Post-Mortem; PMI: Post-Mortem Interval

Introduction

DNA sequencing was dependent on various sequencing methods like Pyro, Sangers, etc. for a long time. But development Biology never stops introducing us to new advanced technologies. NGS is one of the greatest blessings for ultra-modern DNA fingerprinting systems. New relevant markers were added to the existing panel to boost discriminating accuracy and capacity. NGS might open more opportunities. For starters, higher throughput helps forensic laboratories reduce DNA backlog. Second, simultaneous research in NGS can considerably increase throughput and discrimination power. Finally, the cloning aspect of NGS might raise further developments, such as greater sensitivity for difficult-to-analyze combination samples. Finally, as the cost and size of machinery decrease, whole-genome sequencing using a MiSeq/Illumina depending on the sequencing-by-synthesis technique will become viable [1].

Some researchers have lately begun adopting NGS technology for STR testing since alleles of comparable length may be easily separated and computerized reading count might make it much easier to identify mixed DNA samples and analyze complex paternity cases. With its large throughput adequacy and affordable price, NGS technology has addressed most of these challenges, and each method has been applied to a variety of branches in biological sciences, which would include forensic science [2], medical diagnostics [3], Agri genomics [4], and ancient DNA analysis [5]. NGS refers to non-sanger-based strong DNA sequencing. It is possible to sequence millions or billions of DNA molecules concurrently, significantly boosting throughput and reducing the requirement while the cloning of fragment procedure is commonly employed in Sanger or chain termination sequencing.

This includes second-generation sequencing based on loop array sequencing, that could evaluate many samples at once, and third-generation sequencing, that can determine the base makeup of a single DNA. Roche developed "454 DNA Sequencing Technology" in 2005, the world's earliest strong sequencing device based upon pyrosequencing [6]. The first 454 Genome Sequencer could generate about 200,000 110-bp sequences (The maximum read length currently available is 1000 base pairs). In 2010, Ion Torrent, a semiconductor-based sequencer that is faster and less expensive, was introduced. This sequencer doesn't detect sequencing signals using fluorescence, chemiluminescence, or enzyme reactions. This system can now achieve a maximum of 400 base pair read lengths.

All these novel sequencing approaches have resulted in three significant enhancements over traditional technology. First, emerging approaches do not need DNA fragment clones; rather, they depend on cell-free Next Generation Sequencing library creation. Second, they may parallelize thousands to many millions of sequencing processes instead of hundreds. Thirdly, no electrophoresis is required to detect the sequencing result. Because NGS generates a massive unit of reads, complete genomes could be sequenced rapidly, and it became broadly employed in numerous sectors of biosciences. Single molecules can detect as well as the sequence in real-time by third-generation sequencing. In comparison to second-generation sequencing, the most recent single-molecule, real-time (SMRT) technology may produce average read lengths ranging from 5500 to 8500 bp [7].

This current review article aimed to introduce the Next Generation Sequencing System and its possible applicability in solving crimes and other forensic investigations, also this article gives an overview on forensic DNA phenotyping through NGS. NGS could replace all other personal identification techniques soon. NGS should be considered as an advance tool for portrait parle, without any body measurements.

Next-Generation Sequencing Technology's Forensic Application Possibilities

DNA analysis is the most accepted technique in forensic science for individualization. Forensic DNA sequencing is facing new challenges such as a minimum amount of templates, severely damaged and degraded samples, high reliability and reproducibility necessity, and time and cost issues day by day.

Human Origin Markers

STR (short tandem repeat) analysis through NGS

For the foreseeable future, STR typing is expected to be the most significant and widely utilized genetic tool in a criminal investigation. It has several advantages, including speedy and exact allele identification, minimal template DNA demand, multiplex amplification, fluorescent dependent detection, digital data, and the utilization of a plentiful genomic element. Over 60 countries throughout the world have developed forensic DNA databases based on STRs, and these databases are rapidly expanding.

The likelihood of a random match between unrelated individuals increases if statistical analyses are based solely on the 13 routinely used Combined DNA Index System STR markers (i.e., CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11) or 15 markers (13 CODIS loci plus D2D2S1338 and D19S433). To circumvent this, it has been suggested that additional STR markers be included in the present forensic typing test. However, because of the technical constraints of the fluorescent-based CE sequencers now in use, simultaneous detection of additional STR markers would be extremely challenging.

To detect length variation in short tandem repeat (STR) markers, the bulk of forensic DNA testing now uses PCR and capillary electrophoresis (CE)-based fragment analysis methods. The CE-based Sanger sequencing method was utilized to examine specific mitochondrial DNA regions (mt-DNA) [8]. Anyways, there are some limitations to CE-based analysis, such as the inability to analyze multiple genetic polymorphisms in a single reaction using a single workflow, low-resolution genotyping of current markers, loss of useful genomic information from degraded DNA samples, and low-resolution mt-DNA and mixture analysis. Because of the limitations of first-generation sequencing, forensic professionals around the world are investigating the utility of NGS sequencing of the genome for forensic studies. Rapid improvements in next-generation sequencing (NGS) technologies and significant cost reductions in sequencing are providing new opportunities in forensic sciences [9].

Conventional CE-based STR genotyping employs the detection of DNA fragment size. As a result, alleles with the same or comparable length but different sequences cannot be discriminated against. As a result, STR mutations in complicated paternity situations are frequently intractable using typical CE-based STR analysis. The analysis of complex DNA mixes containing DNA from more than one individual presents an extra hurdle for forensic DNA testing. Modern analysis of mixed DNA samples frequently gives poor detection rates and is hence ineffective in criminal investigations.

As soon as the NGS technique was initially presented to genomics, it was unsuitable for STR testing due to the read length being too short in general. The average read length has steadily increased as technology has advanced. Some researchers have recently begun adopting NGS technology for STR testing because alleles of comparable length may be easily separated using NGS technology and digital read count might considerably ease the identification of mixed DNA samples and analysis of difficult paternity cases. Straight Razor, a program that can analyze NGS data for 44 STRs, including 23 autosomal and 21 Y chromosomal

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STRs, was designed by Wars Hauer et al. [10] to evaluate forensic NGS data. Additionally, Van Neste et al. [11] utilized Illumina's MiSeq technology to create a reference allele database to detect the single source and mixed DNA samples; they discovered that most locus genotyping findings were consistent and trustworthy.

Analysis of Y chromosome markers

DNA markers on the Y chromosome have become useful in forensic molecular biology. It is critical to be able to identify males in situations of sexual assault. It became feasible to distinguish closely related individuals in sexual assault situations with many related male perpetrators using rapidly modifying Y-STRs. Also, when a high female background is present, Y-STRs are utilized to resolve the male component of DNA mixes or to reconstruct paternal ties between male individuals. Although, in other applications, such as kinship testing or Disaster Victim Identification (DVI), Y-STRs with lower mutation rates are still preferable [12]. More than 10 million nucleotides of the Y chromosome were compared using NGS technology between two male individuals who had the same progenitor 13 generations ago [13]. Furthermore, Y-SNPs from different geographic areas are found [14,15]. A current NGS study found that Y chromosome sequencing could differentiate between mixed samples of many boys from the same father [12].

Analysis of mitochondrial markers

At low DNA levels or when the maternal connection must be examined, miRNAs is an essential forensic indication [12]. As analysis is limited to the non-coding regulatory region, the discriminating ability of miRNAs is relatively limited [13]. Forensic miRNAs investigations now only discover polymorphisms within a hypervariable area. However, additional polymorphic loci are necessary to strengthen the discrimination power of identification for miRNAs to be utilized as a genetic haplotype marker. As a result, NGS technology has the potential to significantly aid in the investigation of whole mitochondrial sequences. In a parallel investigation, the 454 GS Junior technology was used to explore numerous mitochondrial hypervariable areas, an autosomal STR locus (D18S51), and a Y chromosome STR locus (DYS389I/II).

One of the most difficult difficulties in forensics is interpreting miRNAs mixes. DNA from more than one individual is defined as a mixture sample. Heteroplasmy is a mix of miRNAs genome sequences inside or across cells from different organs belonging to the same individual. Because heteroplasmy influences miRNAs interpretation, knowing the biological basis of heteroplasmy and how to interpret the data is critical [16]. The fundamental reason for not routinely disclosing heteroplasmic variations in forensic instances was the difficulty to properly deconvolute them (based on the Sanger technique). Recent research has demonstrated the sensitivity of NGS in detecting and quantifying heteroplasmic variations and mixture components at extremely low levels (1: 250), which may allow for mixture deconvolution [17].

Analysis of single nucleotide polymorphisms markers

Single nucleotide polymorphisms are single base alterations or insertions/deletions within the genome that account for most of the human genetic diversity. There are four types of single nucleotide polymorphisms: SNPs that test for identification, SNPs that inform about lineage, SNPs that inform about ancestry, and SNPs that inform about phenotype [18]. Several traits, like skin, eye, and hair colour, have an accuracy rate of 80–90%, however, estimating body height has a lower accuracy rate [19]. In these circumstances, entire genome sequencing based on NGS will aid in gathering more information and reliability [12].

Many of the technical issues connected with STRs do not exist for SNPs, therefore replacing them with SNPs might be quite advantageous. SNP profiling from degraded DNA is possible thanks to very small polymerase chain reaction (PCR) amplicons. SNPs are superior for kinship testing and may substitute STRs in such circumstances due to their lower mutation rate compared to STRs, making them more stable when employed as relationship or historical biomarkers. Autosomal SNPs have drawbacks, such as the fact that alleles with multi loci STRs are more variable than bi-allelic SNPs [20]. As a result, SNPs have lesser discriminating strength and detectability of deconvoluting mixes than commonly utilized STR panels. Although employing a greater number of SNPs (20–50 autosomal SNPs) in comparison to STRs (10–15 forensically employed STRs) may compensate for this impact, multiplex genotyping technology may compensate for this effect [18,20].

Analysis of small non-coding RNA/ micro-RNA

Though mRNA sequencing is already a well-established technology in many forensic laboratories, microRNAs (miRNAs) are a relatively new addition to forensic research. Micro-RNAs (miRNAs) are tiny non-coding RNA (ncRNA) molecules that play an important regulatory function in a variety of physiological activities. Mature miRNAs are more stable than mRNAs, which is useful in forensics.

Furthermore, because miRNA is tissue-specific, it is an appropriate biomarker for bodily fluid identification [21]. They are ideal for forensic body fluid identification, species identification, and post-mortem interval (PMI) inference analysis because of their tiny size, resistance to degradation, and tissue-specific or highly tissue-divergent expression [22]. A recent study used a microarray to analyze the expression levels of 718 miRNAs in sperm, saliva, venous blood, menstrual blood, and vaginal fluids [23]. 14 differently expressed miRNAs were

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discovered among them, which might serve as viable candidates for bodily fluid identification. Millions of miRNA sequences may be efficiently evaluated using NGS technology to identify organ- and developmental stage-specific expressions, as well as miRNA expression in distinct disease states, providing a strong tool for forensic investigation.

Analysis of epigenetic markers

In forensics, analyzing tissue-specific DNA methylation is a potential approach [24]. Several studies have recently shown that epigenetic markers may have helped to differentiate monozygotic (MZ) twins [25]. Epigenetic markers have recently been shown to be able to discern tiny changes in epigenetic patterns from 5000 pairs of monozygotic twins using the NGS approach [25,26]. Current genome-wide epigenetic analyses in monozygotic twins demonstrated the ability of this strategy to discover epigenetic alterations associated with complex characteristics [12].

In the domain of forensic research, monozygotic twin studies are still a popular issue. Because both people have the same DNA sequence, traditional genotyping methods such as STR, SNP, sex chromosome STR, and MT DNA studies are unable to distinguish them. In 2014 Weber-Lehmann et al [2], revealed how ultra-deep NGS may distinguish between monozygotic twins by identifying highly unique mutations, suggesting a solution to paternity and forensic situations involving monozygotic twins.

Non-Human Origin Markers

Analysis of microbes/ microbial forensics

It involves microorganism analysis to solve bio crimes and bioterror incidents. Attribution is a high-specificity sample characterization that includes identifying a microbe at several levels, including species, strain, isolate, and even the culture vessel from which the sample came. Microbes are also vital in the process of decomposition in cadavers [27]. Through advanced sequencing, the microbiology of body decomposition may be studied in greater depth. This will aid in understanding the microbial ecology of body decomposition and the use of microorganisms as evidence with an accurate PM interval estimation [28]. Researchers have found that several NGS technology systems can identify biological traces and suspects [12]. It can also detect low-abundance bacteria and distinguish them from others based on genetic fingerprints [27].

Analysis of insects/ forensic entomology

Insects and arthropods are being used to aid medico-legal investigations, such as detecting cases of abuse, corpse movements, and post-mortem (PM) interval estimations. Molecular techniques are critical for species identification; however, next-generation sequencing (NGS) technologies have the potential to expand molecular applications in forensic entomology to include identifications and population assignments [29]. Recent investigations have shown that NGS may successfully identify partial and entire mt genomes from various Diptera of forensic significance. The comparison of complete mitochondrial genomes across multiple species allows for the detection of genetic heterogeneity at various species levels, as well as the exploration of more relevant markers [30].

Other miscellaneous applications

An autopsy is done to determine the cause, manner, and time of death. Sometimes autopsy fails to answer these questions. This type of autopsy is often called a Negative autopsy. Many attempts have been made as part of a trial to carry out and validate molecular autopsy tools in death investigations. Because molecular autopsy is technology-dependent, existing approaches' prices are significant barriers to broad adoption. Analysis for sudden unexplained deaths (SUDs) such as sudden cardiac death (SCD), sudden infant death syndrome (SIDS), or untypical drug-related fatalities are among the molecular investigations that might aid the post-mortem pathologist. These molecular assays provide valuable information that may have medico-legal implications [31,32].

The scientists were able to detect mutations precisely and quickly using next-generation sequencing [33]. Theoretically, cardiac examination for living relatives and molecular autopsy of SUD victims should reveal the aetiology of the disease. The advantages of this technique include determining the cause of death and identifying additional family members who may be in danger [34]. After a thorough investigation, including a complete autopsy, the death circumstances, and clinical history, the sudden unexpected death of a child less than one year of age with the commencement of the fatal attack during sleep looks inexplicable. The failure to uncover new genes in novel pathways that cause SIDS is a limitation of the current technique. By comparing SIDS instances to controls, novel technologies will be used to construct a genetic profile of the sensitive newborn [35].

The burgeoning area of toxicogenomic (the use of gene-expression profiling in toxicology) offers an intriguing method for predicting toxicity and understanding the mechanism of action of the substances under investigation [36]. Because of fast developments in genotyping and the ability of NGS technologies to scan the genome at high resolution, these are quickly growing topics [37]. Apart from these, other advanced techniques like FDP (forensic DNA phenotyping) are one of the well-known undergoing types of research for the purpose of personal identification. Forensic DNA phenotyping can replace other personal identification like anthropometry, 2D or 3D facial reconstruction, superimposition, iris scanner, retina scanner, fingerprints etc. Through FDP DNA scientists claimed that they could be able to make an individual's whole-body structure with every minute detail, only from a piece of DNA. By sequencing the whole genome, they could be able to identify the proteins which are responsible for an individual's body shape, eye colour/shape, hair colour/texture, skin tone, approximate Hight, weight even age also. Next Generation Sequencing shows promising results to take

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a step forward towards PDF. Through NGS forensic analyst could easily done PDF analysis from a fully burnt dead body. Disaster Victim identification will be easier with PDF and NGS.

Advantages of next generation sequencing

For STR analysis, NGS technology offers numerous advantages. High throughput, low cost, simultaneous detection of large numbers of STR loci on both autosomes and sex chromosomes, and the capacity to identify alleles with comparable lengths or digital read counts are just a few of the benefits. As a result, NGS technology will dramatically improve the efficiency and cost-effectiveness of legal cases by allowing for the identification of mixed DNA samples and the analysis of complex paternity cases. Apart from that, NGS can detect anomalies across the whole genome (whole-genome sequencing only), including substitutions, deletions, insertions, duplications, and copy number changes.

Drawbacks of NGS

Some researchers have lately begun adopting NGS technology for STR testing since alleles of comparable length may be easily separated and digital read count could greatly simplify the identification of mixed DNA samples and analysis of complex paternity situations. When the NGS technique was initially presented to genomics, it was not suited for STR testing as the length of STRs is too short to read. The average read length has been steadily growing due to technological advancements. Apart from that, Next generation sequencing also necessitates expensive bioinformatics tools, quick data processing, and enormous data storage capacity. Many institutions may have the financial resources to buy next-generation sequencing technology, but often lack the computing resources and personnel to analyze and clinically interpret the results.

Conclusion

Science will continue to advance if new investigative instruments are developed. New technologies, like NGS, provide several advantages over traditional methods. This prompted the researchers to evaluate these technologies in several forensic disciplines. DNA samples are commonly limited in practical forensic science, and they frequently cannot meet the requirements of concurrently evaluating numerous loci on distinct chromosomes in the mitochondrial genome [38-40]. This may make it difficult to provide enough information, limiting their effectiveness as legal proof. NGS technique not just fits these criteria, but it also has the potential to be used in a wide range of research domains, including DNA database development, ancestry, phenotypic inference, monozygotic twin studies, bodily fluid, and species identification, and forensic microbiological examination. Furthermore, reliability and affordability are crucial factors to consider while selecting the best sequencing platform. The research will continue till that day comes. We anticipate that thanks to NGS technology and forensic scientists' ongoing translational efforts, NGS technology will become a readily accessible standard approach in forensic practice.

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