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Application of Next Generation Sequencing (NGS) technology in forensic science: A review

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Abstract

One of the most important achievements of Next Generation Sequencing (NGS) is to produce millions of sequences reads in a short period of time, and to produce large sequences of deoxyribonucleic acid (DNA) in fragments of any size. Libraries can be generated from whole genomes or any DNA or RNA region of interest without the need to know its sequence beforehand. In the forensic field, one of the main problems is the limited amount of sample available, as well as its degraded state samples. Next-generation sequencing (NGS) technology, with its high-throughput capacity and low cost, has developed rapidly in recent years and become an important analytical tool for many genomics researchers. New opportunities in the research domain of the forensic studies emerge by using the power of next generation sequencing technology, which can be applied to simultaneously analyzing multiple loci of forensic interest in different genetic materials. Furthermore, next-generation sequencing (NGS) technology can also have potential applications in many other aspects of forensic science such as achieving the simultaneous analysis of the standard autosomal DNA (STRs and SNPs), deoxyribonucleic acid (DNA) database construction, ancestry and phenotypic inferences, monozygotic twin studies, body fluid and species identification, forensic animal, plant and microbiological analyses, mitochondrial DNA, microbiological analysis, epigenetics analysis, MicroRNA analysis, animal and plant DNA analysis and X and Y chromosomal markers. In this study, we review the application of next generation sequencing (NGS) technology in the field of forensic science with the aim of providing a reference for future forensic studies and practice.

Keywords: Forensic applications; Next-generation sequencing; Deoxyribonucleic acid (DNA); Molecular biology

1. Introduction

Molecular biology techniques have rapidly advanced in recent years and keep on moving ahead at a staggering speed, mainly in the area of sequencing. Many of the improvements within these techniques led to Next Generation Sequencing (NGS) development. One of the main things in common with all these NGS techniques is the massive parallel sequencing strategy, where thousands of reads are generated at the same time. The core innovations of next generation sequencing platforms are massive parallel chemical reactions, ultrahigh-resolution optics and computational methods to analyze very short reads. These revolutionary technological advances have drastically reduced the sequencing cost and shortened the turnaround time to merely a few days. Nowadays, science is facing another challenge related to the amount of information which is derived from these technologies, the problem of millions of misleading results. However, these technologies have the potential to launch another revolution in deoxyribonucleic acid (DNA) sequencing and its applications (sequencing single RNA or even protein molecules directly) and the challenge of implementing data storage and processing. Currently, next generation sequencing methodology is mainly applied in biomedical fields, which has made it possible to improve research in the clinical diagnosis of cancer, genetically heterogeneous disorders and common and rare diseases. In forensic science, next generation sequencing (NGS) has been applied into whole mitochondrial sequencing. However, in the cases where a low quantity of deoxyribonucleic acid (DNA) is one of the

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most important limiting factors, next generation sequencing has also obtained great advances, such as in Non-invasive Pre-natal Diagnosis and forensic caseworks. It has long been known that deoxyribonucleic acid (DNA) of the fetus can be found in maternal blood, yet it is generally low in quantity and quality and it is not easy to discriminate fetal from maternal deoxyribonucleic acid (DNA). The enormous power of next generation sequencing (NGS) seems attractive for non-invasive pre-natal testing (Alvarez-Cubero *et al*., 2017).

The recent introduction of next-generation sequencing (NGS) technology, with their high-throughput capacity and low cost, has largely overcome these problems, and these technologies have been applied in various fields of life sciences, including forensics, disease diagnosis, agrigenomics and ancient deoxyribonucleic acid (DNA) analysis (Yang *et al*., 2014). This review article was aimed to provide more information about the application of next-generation sequencing (NGS) in forensic science which will serve as a reference for future frontier research and its application.

Limitation of conventional deoxyribose nucleic acid (DNA) analysis

Conventional Electrophoresis-based analysis has its limitations; 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 deoxyribonucleic acid (DNA) samples, and low-resolution mtDNA and mixture analysis. These limitations of first-generation sequencing make forensic scientists worldwide to explore the usefulness of next generation sequencing (NGS) technology for forensic studies.

2. Application of next generation sequencing (NGS) deoxyribonucleic acid (DNA) technologies in forensic investigations.

The field of forensic science has made significant progress over the years, and one of the latest developments that have revolutionized this field is Next Generation Sequencing (NGS). Next generation sequencing (NGS) has been used to advance forensic investigations in several ways, including the identification of suspects, the determination of ancestry, the analysis of degraded samples, and the discovery of new biological markers. In this article, we will discuss the application of next generation sequencing (NGS) in forensic science.

The application of deoxyribonucleic acid (DNA) technologies in forensic investigations has rendered deoxyribonucleic acid (DNA) analysis an important tool in forensic science. Compared to other fields of life sciences, forensic DNA analysis is confronted with template of low copy number, highly-degraded and contaminated samples, the need for high accuracy and reproducibility, as well as time and cost considerations. Today, the majority of forensic DNA tests employ PCR and capillary electrophoresis (CE)-based fragment analysis methods to detect length variation in short tandem repeat (STR) markers.

2.1. STR analysis

NGS has also been used to discover new biological markers that can be used in forensic investigations. For example, the identification of new STRs and SNPs can provide a more accurate and informative genetic profile of the individual, leading to more precise forensic analysis and identification.

STR analysis is likely to remain the most important and commonly-used genetic technique in forensic science for the foreseeable future. It displays multiple advantages, such as rapid and precise allele determination, low deoxyribonucleic acid (DNA) template requirement, multiplex amplification and fluorescence-based detection, digitized results and utilization of the abundant genomic element.

At present, more than 60 countries worldwide have established forensic deoxyribonucleic acid (DNA) databases based on STRs, and these databases continue to grow rapidly e.g including the Combined DNA Index System (CODIS) utilized in the US. Next generation sequencing technology would therefore significantly facilitate the identification of mixed deoxyribonucleic acid (DNA) samples and analysis of complex paternity cases, and ultimately greatly increase the efficiency and cost-effectiveness of legal cases.

Next generation sequencing (NGS) technology has many potential advantages for STR analysis. These include high throughput, low cost, simultaneous detection of large numbers of STR loci on both autosomes and sex chromosomes, and the ability to distinguish alleles with similar length or digital read count. Next generation sequencing technology would therefore significantly facilitate the identification of mixed DNA samples and analysis of complex paternity cases, and ultimately greatly increase the efficiency and cost-effectiveness of legal cases (Yang *et al*., 2014).

2.2. Mitochondrial genome analysis

Next generation sequencing is a high-throughput sequencing technology that can produce millions to billions of deoxyribonucleic acid (DNA) sequence reads in a single run. This technology has become increasingly popular in forensic science due to its ability to process complex mixtures of deoxyribonucleic acid (DNA) samples, generate highly accurate data, and provide deeper insights into the genetic makeup of an individual. One of the most common applications of next generation sequencing in forensic science is in the analysis of mitochondrial DNA (mtDNA).

Mitochondrial DNA (mtDNA) is a circular genome that is maternally inherited and has a high copy number per cell. The use of mtDNA analysis in forensic investigations has been limited due to its low power of discrimination. However, NGS has allowed for the sequencing of the entire mtDNA genome, which has increased its discriminatory power and the ability to identify individuals with a high degree of accuracy. NGS has also been used to analyze nuclear DNA (nDNA) markers, such as single nucleotide polymorphisms (SNPs), short tandem repeats (STRs), and insertion-deletion polymorphisms (indels). mtDNA has proved to be a useful forensic tool in cases involving low amounts of DNA or wherein the maternal lineage needs to be investigated, due to its characteristics of small size, multiple copies, maternal inheritance, high mutation rate and lack of recombination. Currently, forensic mtDNA analyses usually detect only polymorphisms within a hypervariable region. With the increased application of next generation sequencing technology in various fields, the cost of equipment and reagents has decreased markedly. Parallel sequencing technology, which allows for simultaneous analysis of multiple samples, has also led to cost-effectiveness.

In addition to mtDNA and nDNA analysis, NGS has been used to analyze degraded samples, such as bones, teeth, and hair. Traditional deoxyribose nucleic acid **(**DNA) analysis methods have limitations in their ability to analyze such samples due to the degradation and low quantity of deoxyribose nucleic acid **(**DNA) present. However, next generation sequencing can generate highly accurate data from degraded samples, providing a deeper understanding of the genetic makeup of the sample (Fordyce and Avila-Arcos 2019).

2.3. Y chromosome analysis

Genetic markers on the Y chromosome have assumed a valuable role within forensic molecular biology. Most commonly, Y-STRs are used to unambiguously resolve the male component of deoxyribonucleic acid (DNA) mixtures when a high female background is present, or to reconstruct paternal relationships between male individuals. Using next generation sequencing technology, more than 10 million nucleotides of the Y chromosome were compared between two male individuals who shared the same ancestor 13 generations ago. Four genetic differences were detected, suggesting that Y chromosome sequencing could solve the problem of distinguishing between mixed male samples from the same parent (Yang *et al*., 2014).

2.4. Forensic microbiological analysis

Microbial forensics is a new discipline developed by the Federal Bureau of Investigation (FBI) after the Anthrax attack on 18 September 2001 in the USA. It is based on the fast and accurate detection and identification of microorganisms founded at biological crime, with the aim of tracing the source of the microbe.

Because microbiological terrorist attack could lead to serious consequences, forensic microbiological analysis has been attracting considerable attention Fierer examined the bacteria left by human skin on the surface of contact objects by using next generation sequencing-based metagenomic method and showed that the bacteria left by human skin possess sufficient deoxyribonucleic acid (DNA) information for forensic analysis. All the studies described above demonstrate that next generation sequencing has the advantages of high throughput, multiplexing capability and accuracy, which makes it suitable for rapid whole-genome typing of microbial pathogens during forensic or epidemiological investigation. Rare polymorphisms can be reliably detected by analyzing every base of the genome, thus giving forensic data higher resolution and greater accuracy. It is expected that a high-quality forensic microbial database will soon become a reality and aid in the fast and accurate identification of criminals and biological terrorists.

2.5. Ancestry studies and phenotypic inferences

Another application of next generation sequencing in forensic science is in the determination of ancestry. An individual's ancestry can provide important information about their geographic origin, which can be useful in criminal investigations. NGS has been used to analyze ancestry-informative markers (AIMs) and provide information about the individual's ancestry.

Information embedded within the human genome may provide insights into personal characteristics such as ethnicity, physical and physiological characteristics and age. In forensic studies, characteristics inferred from DNA analysis make

it possible for criminal investigations to evolve from the "passive comparison" into the "active search" stages. For example, in the 2004 Madrid train bombings, source population of the suspects was inferred by using 34 autosomal SNPs related to the ancestry of population. Other studies reported SNPs closely related to colors of the iris and hair with an accuracy of 90%. Klimentidis et al. investigated facial features using DNA test and association analysis and validated their results using facial reconstruction (molecular photo fitting).

2.6. Epigenetic analysis

Deoxyribonucleic acid (DNA) sequencing analysis is a powerful tool in forensic identification. Recently, a number of studies have suggested that epigenetic markers can also have various applications in forensic science. For example, evidence supports that epigenetic markers can be used to distinguish monozygotic (MZ) twins, predict tissue type and accurately determine the age of a deoxyribonucleic acid (DNA) donor.

MZ twin studies continue to be a hot topic in the field of forensic science. As both individuals have exactly the same deoxyribonucleic acid (DNA) sequence, conventional genotyping approaches such as STR, SNP, sex chromosome STR, and mtDNA analyses cannot tell them apart. In 2014, Weber-Lehmann *et al.,* described how identification of extremely-rare mutations by ultra-deep NGS can differentiate between MZ twins, suggesting a solution to paternity and forensic cases involving MZ twins

2.7. MicroRNA (miRNA) analysis

MicroRNA (miRNA) are small, highly conserved non-coding RNA molecules involved in the regulation of gene expression. Although miRNA analysis has become a well-established technique in many forensic laboratories, microRNAs (miRNAs) have only recently been introduced to forensic science. miRNAs are a class of endogenous small RNA molecules 18–24 nucleotides in length. Owing to their small size, resistance to degradation and tissue-specific or highly tissue-divergent expression, they are suitable for forensic body fluid identification, species identification and post-mortem interval (PMI) inference analysis (Alvarez-Cubero *et al*., 2017).

2.8. Animal and plant DNA analyses

Species identification is one of most important components of forensic practice. For example, in some cases of poaching and trading of endangered species, it has been used to provide important information and assist in police investigations. In the food industry, identification of the species present in meat products can be achieved, and in archaeology, human remains can be distinguished from non-human remains.

At present, most deoxyribonucleic acid (DNA) typing methods for species determination are based on polymerase chain reaction (PCR) amplification using species-specific primers for single species. However, forensic scientists are often faced with situations in which no any prior species information is available. The development of next generation sequencing technology has allowed deoxyribonucleic acid (DNA) typing to be used in more projects involving species identification. For example, Cheng et al. recently identified plants and animals in traditional Chinese medicines using a cost-effective and efficient next-generation deep sequencing method (Yang *et al*., 2014).

3. Conclusion

In conclusion, the application of next generation sequencing (NGS) in forensic science has revolutionized the field and provided new opportunities for forensic investigations. The use of next generation sequencing (NGS) has increased the accuracy, sensitivity, and resolution of deoxyribonucleic acid (DNA) analysis, leading to more accurate identification of individuals, plants, animals, microorganisms and the discovery of new biological markers. As this technology continues to evolve, it is likely that next generation sequencing (NGS) will continue to play an important role in forensic science.

Compliance with ethical standards

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Disclosure of conflict of interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the review paper.

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