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Role of Next Generation Sequencing in Biomolecular Sciences: A Review

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ABSTRACT

Next-generation sequencing (NGS) technologies have revolutionized various scientific disciplines, offering high-throughput capacity and cost-effectiveness. This review explores the applications, workflow, advantages, and differences of NGS in diverse fields. NGS has significantly advanced genomics research, clinical diagnostics, personalized medicine, and environmental DNA analysis. The NGS workflow involves DNA fragmentation, library preparation, sequencing, and data analysis, enabling comprehensive genetic analysis. DNA fragmentation methods include sonication and enzymatic digestion, while library preparation involves modifying DNA segments for sequencing adaptors. Sequencing platforms like Illumina and Ion Torrent perform massive parallel sequencing, generating sequence data for bioinformatics analysis. NGS applications span forensic genetics, oncology, agriculture, clinical microbiology, and beyond, showcasing its versatility. The advantages of NGS over conventional methods include high speed, accuracy, and sensitivity in detecting low-frequency variants. NGS platforms offer scalability, rapid data generation.

Keywords: Next-generation sequencing (NGS), high-throughput capacity, environmental DNA analysis, DNA fragmentation, sequencing.

INTRODUCTION

Next-Generation Sequencing (NGS) is a high-throughput DNA sequencing technology that has significantly impacted molecular biology and genetics (Satam *et al.,* 2023). The process involves DNA fragmentation, library preparation, sequencing, and data analysis. NGS platforms like Illumina and Ion Torrent enable parallel sequencing, generating large amounts of data for bioinformatics analysis (Signh *et al.,* 2018).

This technology has been applied in various fields, including forensic genetics for improved DNA profiling, oncology for cancer genomics and personalized medicine, agriculture for plant breeding (Varshney et al., 2012), and clinical microbiology for pathogen detection (Khan *et al.,* 2016; Alghamdi, 2023). NGS offers advantages over traditional sequencing methods, such as high throughput, accuracy, and sensitivity in detecting low-frequency variants (Xu, 2018).

Next-generation sequencing (NGS) technologies have transformed various fields of research and clinical diagnosis due to their high-throughput capacity and cost-effectiveness (Yang *et al*., 2014). These technologies have significantly advanced functional genomics study by enabling comprehensive sequence analysis, DNA studies, and computational biology applications (Morozova and Marra, 2008). In the realm of clinical metagenomics, NGS has transitioned from research settings to clinical laboratories, allowing for in-depth analysis of microbial and host genetic material in patient

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samples (Chiu and Miller, 2019). NGS has had a profound impact on cancer research by facilitating large-scale cohort studies and the analysis of big data, particularly in the context of cancer genomics and artificial intelligence applications (Park *et al*., 2021). The integration of NGS with advanced computational data analysis approaches has revolutionized our understanding of the genomic mechanisms underlying cancer development and progression (Berger and Mardis, 2018).

Moreover, NGS has played a crucial role in the molecular diagnosis of various diseases, including mitochondrial disorders, by enabling targeted sequencing and the identification of disease-causing genetic variants (Mahmud *et al.,* 2022). In personalized medicine and pharmacogenomics, NGS has emerged as a powerful tool for evaluating functional DNA variations associated with a wide range of diseases, from monogenic disorders to complex polygenic conditions (Rabbani *et al*., 2016). The application of NGS in infection prevention has also been highlighted, showcasing its utility in identifying and tracking infectious agents for effective control and management strategies (Kemp and Maiers, 2022). NGS has found applications beyond human health, extending to veterinary infection biology, plant phylogenomics, and environmental DNA research (Borm *et al*., 2016; Yu *et al*., 2018). These diverse applications underscore the versatility and broad impact of NGS technologies across different scientific disciplines. In conclusion, the widespread adoption of NGS has ushered in a new era of genomics research, clinical diagnostics, and personalized medicine. The transformative potential of NGS technologies continues to drive innovation and discovery across various fields, offering unprecedented insights into genetic mechanisms, disease pathogenesis, and environmental interactions.

Next-generation sequencing (NGS) technologies have revolutionized various fields of research and clinical practice by offering high-throughput capacity and cost-effectiveness. In functional genomics research, NGS has enabled comprehensive sequence analysis, DNA studies, and computational biology applications, significantly advancing our understanding of genomic mechanisms (Yang et al., 2014; Morozova and Marra, 2008).

NGS has transitioned from research settings to clinical laboratories, enabling in-depth analysis of microbial and host genetic material in patient samples, and thus transforming clinical metagenomics (Chiu and Miller, 2019). Furthermore, NGS has had a profound impact on cancer research by facilitating large-scale cohort studies and big data analysis, particularly in the context of cancer genomics and artificial intelligence applications (Park et al., 2021; Berger and Mardis, 2018).

In molecular diagnosis, NGS has played a crucial role in identifying disease-causing genetic variants, such as those associated with mitochondrial disorders (Calvo et al., 2012). Personalized medicine and pharmacogenomics have also benefited from NGS, which has enabled the evaluation of functional DNA variations associated with various diseases, from monogenic disorders to complex polygenic conditions (Rabbani et al., 2016).

NGS has also found applications in infection prevention, as it allows for the identification and tracking of infectious agents for effective control and management strategies (Kemp and Maiers, 2022). Beyond human health, NGS has been applied to veterinary infection biology, plant phylogenomics, and environmental DNA research (Borm et al., 2016; Yu et al., 2018)

This review article provides a comprehensive overview of Next-Generation Sequencing

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(NGS) in biomolecular sciences. A systematic literature search was conducted using keywords and search terms related to NGS, genomics, and biomolecular sciences in reputable databases such as PubMed, Scopus, and Web of Science. The review synthesizes the current state of knowledge on NGS, covering its principles, applications, advantages, and limitations. It highlights the transformative potential of NGS in advancing our understanding of biological systems and improving human health. The article aims to provide a detailed understanding of NGS and its role in biomolecular sciences.

WORKFLOW OF NGS

Next-Generation Sequencing (NGS) has revolutionized the field of molecular biology research, enabling the sequencing of entire genomes, transcriptomes, and epigenomes in a single experiment (Barba, 2014). The general workflow of NGS involves several steps, including DNA fragmentation, library preparation, sequencing, and data analysis (Pereira, 2020). he Next-Generation Sequencing (NGS) workflow consists of several key stages, including DNA fragmentation, library preparation, sequencing, and data analysis. DNA fragmentation involves breaking down the DNA into short segments, typically 100-300 base pairs in length, using methods like sonication or enzymatic digestion (Motro and Moran-Gilad., 2017). Subsequently, library preparation involves modifying the DNA segments to add sample-specific indexes and sequencing adaptors, enabling the binding of sequencing primers for parallel sequencing (Li et al., 2015). The prepared library is then loaded onto a sequencer for nucleotide sequence reading, utilizing various NGS technologies like Illumina sequencing-by-synthesis or Ion Torrent semiconductor sequencing (Gaston et al., 2022). The sequencer performs massive parallel sequencing of all DNA segments simultaneously, generating sequence data for analysis (Vanni et al., 2015). The raw sequencing data obtained is processed through bioinformatics pipelines for quality control, read alignment, variant calling, and other analyses (Heeke et al., 2018). These analyses can include gene expression profiling, variant detection, and metagenomic analysis, depending on the research objectives (Periera *et al.,* 2020). The NGS workflow is essential for efficiently and accurately generating comprehensive genomic data, driving discoveries in genomics, transcriptomics, and personalized medicine (Rosenthal et al., 2022). The integration of NGS technologies with advanced computational tools has revolutionized genetic analysis, disease diagnosis, and research applications, offering unprecedented insights into genetic variation and disease mechanisms (Lipsky et al., 2014). Despite the complexity of NGS workflows, advancements in automation, bioinformatics, and high-performance computing have optimized data processing and analysis, bridging the gap between big data and scientific discovery (Dimitrov et al., 2017).

DNA Fragmentation

DNA fragmentation is the process of breaking down the targeted DNA into many short segments, usually 100–300 bp in length. Different methods can be used to achieve this, including mechanical methods, enzymatic digestion, or other methods (Yamamoto and Gerbi, 2018). For example, sonication can be used to break DNA into short segments (Furusawa *et al*., 2014). The short segments relevant to the targeted DNA sequences are pulled out using specific complementary probes of different designs, a method referred to as hybridization capture assay (Schultzhaus *et al.,* 2021). Another method involves polymerase chain reaction (PCR) amplification, in which many pairs of primers are used to amplify the targeted DNA

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segments using PCR (Shahzad *et al.,* 2020). The DNA segments are then used for library preparation.

Library Preparation

Library preparation is the process of preparing the DNA or RNA sample by fragmentation, adapter ligation, and amplification to create a library of DNA fragments suitable for sequencing (Pereira, 2020). Library preparation is a process by which DNA segments are modified so that each DNA sample can have a sample-specific index, such as sample identification, which helps to identify the patient from whom DNA sequencing was performed (Pereira, 2020). This process also allows the sequencing adaptors to be added to the DNA segments (Pereira, 2020). Such modification allows the sequencing primers to bind to all the DNA segments and enables massive parallel sequencing later (Pereira, 2020).

A DNA library is a collection of cloned DNA fragments that represents the entire genome or a specific subset of an organism's DNA (Pereira, 2020). DNA libraries are used in various molecular biology and genetic research applications, including gene mapping, sequencing, and functional analysis (Pereira, 2020).

Sequencing

The prepared library is loaded onto a sequencer, and the sequencing platform reads the nucleotide sequence of each fragment in the library (Pereira, 2020). Different NGS platforms utilize various technologies, such as Illumina sequencing-by-synthesis, Ion Torrent semiconductor sequencing, or PacBio single-molecule real-time sequencing (Pereira, 2020). Massive parallel sequencing is performed using an NGS sequencer (Pereira, 2020). The library is uploaded onto a sequencing matrix in a certain sequencer, and different sequencers have different sequencing matrices (Pereira, 2020). However, the goal is the same, which is to allow massive parallel sequencing of all the DNA segments at the same time (Pereira, 2020). The sequence information generated from such massive parallel sequencing is analyzed using bioinformatics software (Pereira, 2020).

Data Analysis

The raw sequencing data obtained from the instrument is processed through bioinformatics pipelines to remove artifacts, perform quality control, and align the reads to a reference genome or assemble them into a de novo genome (Pereira, 2020). Further analysis can involve variant calling, gene expression profiling, metagenomic analysis, and more, depending on the specific research objectives (Pereira, 2020). NGS has revolutionized many areas of biological and medical research, including genomics, transcriptomics, epigenomics, metagenomics, and personalized medicine (Pereira, 2020). It has facilitated the identification of disease-causing genetic mutations, improved our understanding of complex genetic diseases, enabled the study of gene expression and regulation, and advanced our knowledge of microbial communities and ecosystems (Pereira, 2020). The widespread adoption of NGS has also led to the generation of large-scale genomic datasets, which has prompted the development of new computational and statistical approaches to handle and interpret these data (Pereira, 2020). NGS continues to drive discoveries in various fields of biology and medicine, and its applications are expanding rapidly as the technology evolves (Pereira, 2020).

Applications of NGS

In the field of forensic genetics, Next-Generation Sequencing (NGS) technology has revolutionized DNA sequencing and analysis methods. This innovative technology allows for the concurrent examination of autosomal

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DNA, mitochondrial DNA, and chromosomal markers, significantly enhancing the precision and efficiency of individual identification and profiling in forensic investigations (Álvarez-Cubero *et al*. 2017). The high-throughput nature of NGS has significantly enhanced the efficiency and accuracy of forensic investigations, making it a valuable tool in criminal justice systems worldwide (Børsting and Morling, 2015). In the field of oncology, NGS has played a pivotal role in cancer research and personalized medicine. By combining NGS with advanced computational data analysis, researchers have gained profound insights into the genomic mechanisms underlying cancer development, drug resistance, and the identification of potential therapeutic targets (Berger and Mardis, 2018). NGS has facilitated the accurate and rapid sequencing of whole human genomes, enabling comprehensive genomic profiling for precision oncology and tailored treatment strategies (Bentley *et al*., 2008). NGS has also found applications in agriculture, particularly in plant breeding and genomics. The technology has been pivotal in advancing molecular ecology studies of non-model organisms, providing valuable genomic resources for ecological and conservation genetics research (Ekblom and Galindo, 2010). In the agricultural sector, NGS has revolutionized breeding programs by enabling accurate whole-genome sequencing in crops like strawberries, leading to significant improvements in breeding efficiency and crop quality (Isobe *et al*., 2020). Moreover, NGS has been instrumental in clinical microbiology, allowing for rapid and comprehensive pathogen detection in infectious diseases. By leveraging unbiased NGS approaches, clinical laboratories can identify a wide range of pathogens, aiding in public health surveillance, outbreak investigations, and the diagnosis of bacterial infections (Naccache *et al*., 2014). The use of NGS in diagnosing neuroleptospirosis and periodontitis-related pathogens highlights its clinical relevance and diagnostic capabilities (Wilson *et al*., 2014; Jeong *et al*., 2023).

Advantages of NGS

Next-generation sequencing (NGS) technology offers numerous advantages over traditional sequencing methods, making it a powerful tool with broad applications in various fields. The advantages of NGS include high speed, high throughput, high accuracy, cost-effectiveness, and the ability to generate massive amounts of data efficiently (Ma *et al*., 2017). Compared to Sanger sequencing, NGS enables rapid sequencing of large genomic regions or even whole genomes in a fraction of the time, allowing for comprehensive analysis of genetic material (Ma *et al*., 2017). NGS has revolutionized research in diverse areas such as genetics, oncology, microbiology, agriculture, and clinical diagnostics. In genetics, NGS facilitates the discovery of new disease genes and transforms the clinical diagnosis of inherited diseases by providing comprehensive genetic information (Vinkšel *et al.,* 2021). In oncology, NGS enables genome-wide personalized oncology efforts by identifying actionable variants and guiding precision medicine approaches (Hovelson *et al*., 2015). The technology has also been instrumental in agriculture, enhancing breeding programs and providing valuable genomic resources for ecological and conservation genetics research (Visioni *et al.,* 2023). Moreover, NGS has significant implications in personalized medicine and pharmacogenomics, allowing for the evaluation of functional DNA variations associated with various diseases, from monogenic disorders to complex polygenic conditions (Rabbani *et al*., 2016).

The technology's scalability and versatility have made it indispensable in clinical molecular diagnostics, particularly in cancer

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diagnosis, where it offers enhanced sensitivity in mutation detection and faster workflow speed (Grotta *et al*., 2015). Furthermore, NGS has been pivotal in infectious disease research, enabling the unbiased identification of viral pathogens and revolutionizing our ability to discover emerging pathogens (Wilson *et al*., 2014). The technology's ability to generate massive sequencing data has transformed medical genomics, providing valuable insights into human samples and disease mechanisms (Hosomichi *et al*., 2015). Additionally, NGS has been utilized in resolving serology discrepancies, such as ABO genotyping, by leveraging its high-throughput sequencing capabilities (Wu *et al*., 2018).

Differences between NGS and other sequencing Techniques

Next-generation sequencing (NGS) has transformed genetic analysis by providing numerous advantages over traditional sequencing methods like Sanger sequencing. NGS allows for high-throughput characterization of nucleotide sequences of disease-associated genes, facilitating rapid and efficient sequencing of large genomic regions or entire genomes Pecoraro *et al*. (2020). In comparison to Sanger sequencing, NGS platforms are more effective in analyzing large gene panels, enabling simultaneous screening of multiple genes (Judkins *et al*., 2015). One key advantage of NGS over Sanger sequencing is its capability to detect low-frequency variants and mutations that might be overlooked by traditional methods. NGS technologies can identify low-abundance variants, such as drug resistance mutations in HIV-1, leading to improved patient outcomes and more precise genotypic resistance testing (Tzou *et al*., 2018). Additionally, NGS offers advantages like reduced costs, faster workflow, and enhanced sensitivity in mutation detection, making it a valuable tool for molecular diagnostics and genetic analysis (Singh, 2020).

NGS also provides a more sensitive detection of low-abundance variants, potentially decreasing subjective errors and offering more quantitative and automatable data processing steps compared to Sanger sequencing. The technology's ability to detect low-abundance variants, including those undetectable by Sanger sequencing, enhances its utility in clinical settings for accurate variant calling and genetic analysis (Parkin *et al*., 2020). Furthermore, NGS platforms are well-suited for parental testing due to their capability to detect clinically relevant germline mosaicism, providing valuable insights into genetic inheritance patterns (Brewer *et al*., 2020). Moreover, NGS offers advantages in terms of scalability, rapid data generation, and the ability to analyze tens of thousands of genes simultaneously. The technology's high fidelity of variant calls, combined with its cost-effectiveness and high-throughput sequencing capabilities, has made it a common choice for clinical genetic tests and research applications (Muzzey *et al*., 2019). NGS also allows for the efficient identification of pathogens in clinical microbiology settings, offering a more sensitive and accurate alternative to traditional culture methods for diagnosing bacterial and fungal infections (Chen *et al*., 2020)

Limitations of Next Generation Sequencing

Next-generation sequencing (NGS) has ushered in a new era in genetic analysis by offering several advantages over conventional Sanger sequencing techniques. NGS enables the simultaneous and high-volume analysis of genetic sequences linked to diseases, streamlining the process of sequencing extensive genomic regions or even complete genomes, thereby enhancing the speed and effectiveness of genetic studies (Pecoraro *et al*. 2020). In comparison to Sanger sequencing, NGS platforms are more effective in analyzing large gene panels, enabling simultaneous

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screening of multiple genes (Judkins *et al*., 2015). One key advantage of NGS over Sanger sequencing is its capability to detect low-frequency variants and mutations that might be overlooked by traditional methods. NGS technologies can identify low-abundance variants, such as drug resistance mutations in HIV-1, leading to improved patient outcomes and more precise genotypic resistance testing (Tzou *et al*., 2018). Additionally, NGS offers advantages like reduced costs, faster workflow, and enhanced sensitivity in mutation detection, making it a valuable tool for molecular diagnostics and genetic analysis (Singh, 2020). NGS also provides a more sensitive detection of low-abundance variants, potentially decreasing subjective errors and offering more quantitative and automatable data processing steps compared to Sanger sequencing. The technology's ability to detect low-abundance variants, including those undetectable by Sanger sequencing, enhances its utility in clinical settings for accurate variant calling and genetic analysis (Parkin *et al*., 2020). Furthermore, NGS platforms are well-suited for parental testing due to their capability to detect clinically relevant germline mosaicism, providing valuable insights into genetic inheritance patterns (Brewer *et al*., 2020). Moreover, NGS offers advantages in terms of scalability, rapid data generation, and the ability to analyze tens of thousands of genes simultaneously. The technology's high fidelity of variant calls, combined with its cost-effectiveness and high-throughput sequencing capabilities, has made it a common choice for clinical genetic tests and research applications (Muzzey *et al*., 2019). NGS also allows for the efficient identification of pathogens in clinical microbiology settings, offering a more sensitive and accurate alternative to traditional culture methods for diagnosing bacterial and fungal infections (Chen *et al*., 2020)

Future Prospects of NGS

Future Prospects of Next-Generation Sequencing (NGS): Next-generation sequencing (NGS) technology has rapidly evolved, offering a multitude of applications across various scientific disciplines. The future of NGS holds immense promise and potential for further advancements in research, diagnostics, and personalized medicine. As highlighted in recent studies, the utilization of self-assembling strategies for drug delivery using NGS-derived nanogels showcases the innovative applications of NGS in pharmaceutical sciences Wang et al. (2021). The success of MinION nanopore sequencing technology in neurosurgery indicates a promising future for rapid and accurate gene sequencing in clinical settings (Patel et al., 2018). Additionally, the development of high-throughput NGS strategies has addressed the anticipated future needs for throughput sequencing and cost-effectiveness, paving the way for diverse applications in genomic research (Abdi *et al.,* 2024). In the field of genetic diagnostics, NGS is expected to play a pivotal role in identifying rare genetic disorders and providing insights into disease mechanisms. The application of NGS in plant virology, wildlife biology, and cancer genomics is anticipated to expand, offering valuable contributions to understanding disease pathogenesis and genetic diversity (Barba et al., 2014; Massart et al., 2014; Jamshidi et al., 2015). Furthermore, the integration of NGS with bioinformatics tools and computational analysis pipelines will enhance the efficiency and accuracy of genomic data interpretation, enabling precise diagnosis and treatment strategies (Li et al., 2021; Salzberg et al., 2016). The future prospects of NGS also extend to non-invasive analyses, such as early detection of relapse-determining mutations in cancer patients, and the identification of circulating tumor cells for

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monitoring disease progression (Dietel et al., 2015; Stalke et al., 2017). As NGS technologies continue to advance, the implementation of long-read sequencing methods is expected to revolutionize genetic diagnostics and research in essential tremor genetics and other neurological disorders.

CONCLUSION

In conclusion, Next-generation sequencing (NGS) technologies have revolutionized various fields, including genomics research, clinical diagnostics, personalized medicine, and environmental DNA analysis. The high-throughput capacity, cost-effectiveness, and accuracy of NGS platforms have enabled rapid and comprehensive sequencing of large genomic regions, leading to significant advancements in disease diagnosis, treatment, and understanding genetic mechanisms. The workflow of NGS, from DNA fragmentation to data analysis, has streamlined the sequencing process, allowing for efficient and precise genetic analysis. The applications of NGS in forensic genetics, oncology, agriculture, clinical microbiology, and beyond highlight its versatility and broad impact across different scientific disciplines. The advantages of NGS over traditional sequencing methods, such as its ability to detect low-frequency variants, scalability, and rapid data generation, position it as an indispensable tool for genetic analysis and research. Despite its limitations, NGS continues to drive innovation and discovery in biology, medicine, and beyond, offering unprecedented insights into genetic variation, disease pathogenesis, and environmental interactions. The transformative potential of NGS technologies underscores their pivotal role in advancing scientific research, clinical diagnostics, and personalized medicine, shaping the future of genomic analysis and precision healthcare.

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