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Review Article

What is Next Generation Sequencing?

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Abstract

Next Generation Sequencing (NGS) is a high-throughput technology that enables the rapid and efficient sequencing of millions of DNA fragments simultaneously. Unlike traditional sequencing methods such as Sanger sequencing, renowned for its accuracy and longer read lengths, NGS offers faster, more cost-effective sequencing capabilities by processing large volumes of genetic data in parallel. The standard NGS workflow includes isolation and fragmentation of genetic material, library preparation, amplification, and sequencing via chemical or fluorescent tagging, followed by comprehensive analysis using advanced bioinformatics tools. While Sanger sequencing remains preferred method for applications requiring high precision and long reads, the capacity of NGS to detect rare variants and analyse multiple gene regions simultaneously has made it indispensable in both research and clinical settings. NGS is currently widely implemented across clinical microbiology, food safety, metagenomics, forensic science, pharmacogenetics, and numerous other domains. As NGS technologies continue to advance, their scope of application is anticipated to expand further, making NGS an essential tool in modern genomic research and precision medicine.

Keywords: Bioinformatics, genomic analysis, next generation sequencing, precision medicine.

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INTRODUCTION

Sequencing

Sequencing refers to methods used to determine the sequence of bases in genetic material. Determining the DNA sequence is essential for understanding gene and genome function, detecting mutations and variations, applying it in forensic investigations, and tracing lineage. To achieve this, various sequencing methods have been developed, each presenting its own advantages and disadvantages. First generation sequencing methods include Sanger sequencing and Maxam-Gilbert sequencing methods. Following this, Next Generation Sequencing (NGS) methods that better address limitations and demands were developed, and these are divided into Second and Third Generation Sequencing methods (1,2).

NGS technology

NGS is a technology capable of reading millions of parallel fragments with high speed and efficiency on genetic materials. This technology enables the reading of massive amounts of data in a very short period of time at very low cost. First, the genetic material of interest is isolated and subsequently fragmented into small fragments and converted into a sequencing library. This library is then amplified on a microchip. This allows each genetic fragment to be read independently. Sequencing can be performed through chemical reactions or fluorescent tagging, depending on different platforms. The final data are then analysed using different bioinformatic tools (3,4).

NGS versus Sanger sequencing

Although NGS is very advantageous in terms of cost, parallel reading and speed, Sanger sequencing is still superior in terms of read length (200-600 bp) and accuracy (the read length of NGS is variable depending on the technology used). Due to this advantage, especially when resequencing genomes, Sanger sequencing is preferred over NGS (5). On the other hand, while only one sequence can be sequenced at a time in Sanger sequencing, NGS can sequence millions of sequences in parallel and capture rare variants in deep sequencing. In addition, NGS can analyse many gene regions or genomes simultaneously. It can simultaneously detect a large number of variants, albeit rare, with a low detection limit and high sensitivity (6). Sanger sequencing and NGS are compared in Table 1 below to more clearly demonstrate their pros and cons relative to each other.

Table 1. Comparison of Sanger sequencing vs. NGS (7, 8)

Feature	Sanger sequencing	Short-read NGS (e.g., Illumina)	Long-read NGS (e.g., PacBio, Nanopore)
Read length	200-600 bp	50-300 bp	>10,000 bp (can reach >100 kb)
Accuracy	Very high	High	Moderate-high (improving with updates
Throughput	Low	Very high	High
Cost per base	High	Low	Moderate-high
Sample preparation	Simple	Requires fragmentation	Often less complex
Applications	Targeted regions	Exomes, panels, WGS	Structural variation, full genome, epigenetics

Abbreviation: WGS: whole genome sequencing, bp: base pair, kb: kilobase.

Different types of NGS according to read-length

Recent developments in sequencing technologies have led to the emergence of long-read platforms that address the limitations of traditional short-read systems. Among the most prominent are Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT).

PacBio sequencing is based on Single Molecule Real-Time (SMRT) technology, which allows DNA polymerase activity to be monitored in real time as it synthesises a complementary strand. This enables the generation of long reads from single DNA molecules, typically ranging between 10-25 kilobases, with some protocols achieving even longer reads. These long reads offer significant advantages for *de novo* genome assembly, detection of structural variants, and full-length transcript analysis. Although initial concerns existed regarding error rates, advances in sequencing chemistry and consensus accuracy have significantly improved its reliability for both research and clinical applications (7).

Oxford Nanopore Technologies (ONT), on the other hand, uses a fundamentally different approach. In this system, single-stranded DNA molecules pass through a biological nanopore embedded in a membrane, and the sequence is determined by detecting changes in ionic current. Nanopore sequencing enables real-time, label-free analysis and is known for producing ultra-long reads, with routine read lengths of 20-100 kilobases and some reads exceeding 1 megabase in ideal conditions. Its portable devices, most notably the MinION (a palm-sized, portable nanopore sequencing device by Oxford Nanopore Technologies, enabling DNA/RNA sequencing anywhere in real time), have made sequencing accessible in diverse settings including clinical diagnostics, environmental monitoring, and field-based research (8). Together, these long-read sequencing platforms are reshaping genomics by providing deeper insight into complex regions of the genome and enabling applications that were previously unattainable with short-read technologies.

Applications of NGS technology

Given its many advantages, NGS technology has extensive applications in both research and clinical settings. In clinical microbiology, it enables rapid and precise identification of pathogens, playing an important role in outbreak management and molecular epidemiology (9), prevention of infectious diseases (10), and diagnosis of pathological diseases (11). It is also effective in monitoring and identifying multidrug-resistant microorganisms (12). Additionally, NGS has brought advancements in metagenomic analyses, allowing for direct examination of complex microbial communities from environmental samples, essential for identifying microorganisms that are difficult or impossible to culture in laboratories (13) and the investigation of bacterial ecosystems through bacterial genomes (14). In the field of food microbiology, NGS technology serves as a powerful tool for monitoring foodborne-pathogens and conducting genomic analyses. By comparing the features and performances of different NGS platforms, this technology holds significant potential to enhance food safety through precise tracking of microbial contamination in food supply chain and identifying outbreak sources. NGS also plays an important role in microbial genomics, offering new opportunities to explore bacterial phylogeny and the genetic relationships between species. This technology complements traditional methods, providing high-resolution data that enhances our understanding of the evolutionary history and ecological roles of microorganisms (15). In addition, NGS can be used in the field of pharmacogenetics through Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) based panels (16), studies in forensic medicine (17) and with ancient DNAs (18), molecular genetic diagnosis of diseases (19), personalized diagnosis and treatment considering disease and patient heterogeneity (20) and new areas of application continue to emerge. Although Sanger sequencing is the gold standard in sequencing, NGS continues to develop continuously in line with increasing needs and is becoming more widely employed in numerous areas.

CONCLUSION

NGS has revolutionized genomic research and diagnostics by enabling high-throughput, cost-effective, and parallel sequencing of millions of DNA fragments. While traditional methods such as Sanger sequencing remain valuable for applications requiring long read lengths and high accuracy, NGS provides unmatched scalability and sensitivity, particularly for detecting rare variants and analysing complex genomic regions. Its wide-ranging applications in clinical microbiology, food safety, metagenomics, forensic science, and pharmacogenetics underscore its versatility and transformative potential. As NGS technologies advance, especially with the emergence of long-read platforms, they are expected to further enhance our ability to understand, diagnose, and treat genetic conditions. Moving forward, the integration of NGS with sophisticated bioinformatics and personalized medicine approaches will likely reshape the future of biomedical sciences and public health.

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References

1. Eren K, Taktakoğlu N, Pirim I. DNA sequencing methods: from past to present. Eurasian J Med. 2022;54:47-56.
2. Crossley BM, Bai J, Glaser A, Maes R, Porter E, Killian ML, et al. Guidelines for Sanger sequencing and molecular assay monitoring. J Vet Diagn Invest. 2020;32:767-75.
3. Akintunde O, Tucker T, Carabetta VJ. The evolution of next-generation sequencing technologies. Methods Mol Biol. 2025;2866:3-29.
4. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: an overview. Hum Immunol. 2021;82:801-11.
5. Boluda B, Rodriguez-Veiga R, Sargas C, Ayala R, Larráyoz MJ, Chillón MC, et al. Conventional PCR versus next generation sequencing for diagnosis of FLT3, IDH and NPM1 mutations in acute myeloid leukemia: results of the PETHEMA PCR-LMA study. Cancers (Basel). 2025;17:854.
6. Georget M, Pisan E. Approches diagnostiques basées sur le séquençage à haut débit. Rev Mal Respir. 2023;40:345-58.
7. Han Y, He J, Li M, Peng Y, Jiang H, Zhao J, et al. Unlocking the potential of metagenomics with the PacBio high-fidelity sequencing technology. Microorganisms. 2024;12:2482.
8. Zhang T, Li H, Jiang M, Hou H, Gao Y, Li Y, et al. Nanopore sequencing: flourishing in its teenage years. J Genet Genomics. 2024;51:1361-74.
9. Wilkes RP. Next-generation diagnostics for pathogens. Vet Clin North Am Food Anim Pract. 2023;39:165-73.
10. Kemp M, Maiers M. Application of next generation sequencing in infection prevention. Front Public Health. 2022;10:945595.
11. Rajendhran J, Muthuirulan P, Lakshmanan AP, Sundararaju S. Clinical metagenomics-based diagnostics for infectious diseases. Front Cell Infect Microbiol. 2024;14:1459621.
12. Nazir A, Puthuveettil AR, Hussain FHN, Hamed KE, Munawar N. Endophytic fungi: nature's solution for antimicrobial resistance and sustainable agriculture. Front Microbiol. 2024;15:1461504.
13. Lema NK, Gemeda MT, Woldesemayat AA. Recent advances in metagenomic approaches, applications, and challenge. Curr Microbiol. 2023;80:347.
14. Li F, Liu J, Maldonado-Gómez MX, Frese SA, Gänzle MG, Walter J. Highly accurate and sensitive absolute quantification of bacterial strains in human fecal samples. Microbiome. 2024;12:168.
15. Wang L, Lim DR, Phelan JE, Reyes LT, Palparan AG, Sanchez MGC, et al. Whole genome sequencing analysis of Mycobacterium tuberculosis reveals circulating strain types and drug-resistance mutations in the Philippines. Sci Rep. 2024;14:19602.
16. Enko D, Michaelis S, Schneider C, Schaflinger E, Baranyi A, Schnedl WJ, et al. The use of next-generation sequencing in pharmacogenomics. Clin Lab. 2023;69:8.
17. Pedroza Matute S, Iyavoo S. Applications and performance of Precision ID GlobalFiler NGS STR, identity, and ancestry panels in forensic genetics. Genes (Basel). 2024;15:1133.
18. Hong JH, Fujita H, Kim J, Shin DH. Cost-effectiveness and other considerations for different research techniques applied in ancient DNA analysis. Anat Cell Biol. 2025;58:8-13.
19. Hoffjan S. Next-generation sequencing in human molecular genetic diagnostics. Mol Cell Probes. 2019;45:69.
20. Zech M, Dzinovic I, Skorvanek M, Harrer P, Necpal J, Kopajtich R, et al. Combined genomics and proteomics unveils elusive variants and vast aetiologic heterogeneity in dystonia. Brain. 2025;148:2827-46.