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Advancements and Current Trends in Next-Generation Sequencing (NGS) Technology

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ABSTRACT

NGS represents a ground-breaking technology facilitating swift, high-throughput sequencing of DNA or RNA molecules. NGS techniques empower the simultaneous parallel sequencing of millions to billions of DNA or RNA fragments, a capability unmatched by traditional Sanger sequencing, which is constrained by scalability and speed. The versatility of NGS platforms has expanded the scope of genomics research, enabling investigations into rare genetic diseases, cancer genomics, microbiome analysis, infectious diseases, and population genetics. Furthermore, NGS has paved the way for the advancement of targeted therapies, precision medicine strategies, and enhanced diagnostic methods.

Keywords: Genomics, Genes, Sequencing, Next-Generation

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INTRODUCTION

The emergence of NGS has catalyzed a paradigm shift in genomics research, providing unmatched capabilities for analyzing DNA and RNA molecules with high throughput and cost-effectiveness.[1] In contrast to traditional Sanger sequencing, which faces constraints in scalability and speed, NGS techniques facilitate the simultaneous parallel sequencing of millions to billions of DNA fragments.[2] This revolutionary technology has rapidly driven advancements in genomics across a wide range of fields. NGS enables the rapid sequencing of millions of DNA fragments simultaneously, offering comprehensive insights into genome structure, genetic variations, gene expression profiles, and epigenetic modifications.[3] The adaptability of NGS platforms has broadened the horizon of genomics research, facilitating investigations into rare genetic diseases, cancer genomics, microbiome analysis, infectious diseases, and population genetics. Furthermore, NGS has fostered the development of targeted therapies, precision medicine strategies, and enhanced diagnostic techniques.[4]

DIFFERENT PLATFORMS USED IN NGS

Several platforms are used for NGS, including Illumina (Solexa), Ion Torrent (Proton), PacBio (SMRT), and Oxford Nanopore. Each platform has its unique sequencing chemistry and capabilities, catering to different research and diagnostic needs **(Table 1)**.[5]

Key Steps Involved in NGS

- Library Preparation: DNA fragmentation, adapter ligation, and amplification.
- Sequencing: High-throughput sequencing of millions of DNA fragments in parallel.
- Data Analysis: Alignment, variant calling, and interpretation of sequencing data.
- Data Interpretation: The results are interpreted in the context of the research question or clinical application.[6]

NGS in Diagnostics

- Genetic Disease Diagnosis: Identification of genetic mutations causing diseases, such as cystic fibrosis, sickle cell anemia, muscular dystrophy, etc.[7]
- Cancer genomics for personalized treatment strategies: NGS helps to characterize the molecular profiles of tumors, guiding therapeutic decision-making and predicting treatment responses **(Table 2).**[8]

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Table 1. Different Platforms Used in NGS

Illumina	• Reversible terminator chemistry • Short read lengths (generally up to a few hundred base pairs) • Well-suited for: WGS (Whole Genome Sequencing),WES (Whole Exome Sequencing),RNA Seq and Target Seq
Ion Torrent	• Semiconductor technology • Short read lengths (generally up to a few hundred base pairs) • Commonly used for: Target Seq, Amplicon Seq, Small Genome Seq
PacBio	• Single-molecule, real-time (SMRT) sequencing technology • Generates long read lengths (thousands of base pairs) • Ideal for: De novo genome assembly, detection of structural variants, and characterization of complex genomic regions
Oxford Nanopore	• Nanopore-based technology • Offers long read lengths (tens of kilobases) • Suitable for rapid sequencing in various applications, including whole-genome sequencing, metagenomics, and field-based sequencing

Table 2. NGS panels &associated candidate genes

Lung Cancer Panel	EGFR, ALK, ROS, RET, ERBB2, NTRK, BRAF, KRAS, NRAS, HRAS, MET, PIK3CA
Endometrium Cancer Panel	POLE, P53, MLH1, MSH2, MSH6, PMS2
Colorectal Cancer Panel	EGFR, ALK, ERBB2, ERBB4, FGFR1, FGR2, FGR3, MET, DDR2, KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAPK21, STK11, NOTCH1, CTNNB1, SMAD4, FBXW7, TP53
Glioma Panel	IDH1, IDH2, MGMT, EGFR, TERT
Myeloid Leukaemia Panel	ABL1, BRAF, CBL, DNMT3A, CSF3R, HRAS, IDH1, IDH2, CKIT, NPM1, FLT3, JAK2, MPL, CALR, ASXL1, ETV6, CEPBA, CEPBB, PDGFRA, PDGFRB

- Hereditary cancer gene panels: The NGS-based hereditary cancer gene panel is a diagnostic tool used in clinical genetics to identify mutations or variants in genes associated with an increased risk of developing hereditary cancers. These panels typically include a selection of genes known to be involved in various hereditary cancer syndromes, such as BRCA1 and BRCA2 in hereditary breast and ovarian cancer syndrome, and TP53 in Li-Fraumeni syndrome. Examples of such panels are the Hereditary Breast and Ovarian Cancer (HBOC) Panel, exclusively to identify the risk of breast and ovarian cancer, and the Hereditary Cancer Panel contains 32 genes, 154 genes, etc.[9]
- Infectious Disease Testing: NGS-based approaches are used for the detection and characterization of pathogens, including bacteria, viruses, fungi, parasites, and antimicrobial resistance genes, aiding in the diagnosis and surveillance of infectious diseases.[10]
- Pharmacogenomics for drug response prediction: NGS facilitates pharmacogenetic testing to identify genetic variants influencing drug metabolism, efficacy, and toxicity, thereby minimizing adverse drug reactions and improving therapeutic outcomes.[11]
- Non-Invasive Prenatal Testing (NIPT): NGS-based NIPT analyzes cell-free fetal DNA in maternal blood to screen for common chromosomal abnormalities, such as trisomy 21 (Down syndrome), trisomy 18, and trisomy 13.[12]
- HLA Typing: NGS has revolutionized Human Leukocyte Antigen (HLA) typing, a critical component of transplantation and immunogenetics.[13]

ADVANTAGES OF NGS

- High throughput and scalability.
- Cost-effective compared to traditional sequencing methods.
- Rapid turnaround time.
- Ability to sequence multiple samples simultaneously.
- Enables comprehensive analysis of complex genomic regions.[14]

LIMITATIONS OF NGS

- Short-read lengths may limit the ability to resolve repetitive or complex genomic regions accurately.
- Error rates can be higher in certain regions.
- Bioinformatics expertise is required for data analysis and interpretation.
- Initial setup costs can be substantial.[14]

SUMMARY

NGS stands as a formidable tool employed in genomics research. NGS can sequence millions of DNA fragments simultaneously, furnishing comprehensive insights into genome structure, genetic variations, gene activity, and alterations in gene behavior. Recent developments have prioritized faster and more precise sequencing, cost reduction, and enhanced data analysis. These advancements offer great potential for unveiling novel insights into genomics and enhancing our comprehension of diseases and personalized healthcare.

END NOTE

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