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Techniques of Next Generation Sequencing

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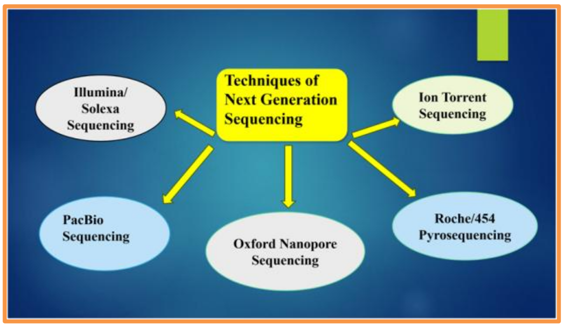
Next-Generation Sequencing (NGS) is an advanced DNA sequencing technology that enables rapid and large-scale analysis of millions of DNA fragments simultaneously. Next-generation sequencing (NGS) has transformed plant breeding by enabling rapid, high-throughput, and cost-effective analysis of plant genomes. It facilitates the identification of genetic variations such as single nucleotide polymorphisms (SNPs), structural variations, and copy number variations, which are essential for marker-assisted selection (MAS) and genomic selection (GS). NGS also supports transcriptome profiling and the discovery of novel genes and regulatory elements, contributing to improvements in crop productivity, stress tolerance, and nutritional quality. This chapter highlights the diverse applications of NGS in plant breeding and outlines the evolution of sequencing technologies from first-generation Sanger sequencing to emerging fourth-generation platforms, emphasizing their role in advancing genomic research and crop improvement.

Keywords: Illumina, PacBio, Marker-assisted selection, Roche/454 Pyrosequencing, Ion Torrent Sequencing

Introduction

Next-Generation Sequencing (NGS) is an advanced DNA sequencing technology that enables rapid and large-scale analysis of millions of DNA fragments simultaneously. It determines the sequence of nucleotides (A, T, G, and C) in DNA or RNA more efficiently and cost-effectively compared to traditional techniques such as Sanger sequencing. This technology has revolutionized fields like genetics, medicine, and plant breeding. NGS plays a crucial role in detecting genetic variations, including single nucleotide polymorphisms (SNPs), structural variations, and copy number variations. These variations are essential for modern breeding strategies such as marker-assisted selection (MAS) and genomic selection (GS). In plant breeding, NGS serves as a powerful high-throughput genomic tool for identifying and characterizing genes and molecular markers linked to important agronomic traits. Additionally, NGS supports the development of reliable and precise marker systems, improving the accuracy of genotype–phenotype relationships. This advancement enhances phenotyping precision and accelerates crop improvement programs. By utilizing high-resolution genomic data, NGS enables breeders to develop crop varieties with better resistance to biotic stresses, improved tolerance to abiotic stresses, and enhanced nutritional quality and yield. Overall, NGS significantly increases the efficiency and precision of plant breeding by enabling faster and more accurate selection of superior genotypes, ultimately contributing to the development of high-performing crop varieties.



Illumina/ Solexa Sequencing

Illumina sequencing, also referred to as Solexa sequencing, is an advanced next-generation sequencing technology that allows fast and precise DNA analysis by simultaneously sequencing millions of DNA fragments. The workflow includes DNA fragmentation, attachment of adapter sequences, formation of clusters on a solid surface, and amplification through bridge PCR, followed by sequencing-by-synthesis using fluorescently labeled nucleotides. This method generates large amounts of reliable data and plays a key role in modern genomics research. It is extensively used for genome sequencing, transcriptome studies, and genetic analysis in various crops. The combination of Illumina with Roche 454 sequencing has been applied to analyze complex genomes such as cotton, while in crops like chickpea and eggplant it aids in studying gene expression and genome mapping. Additionally, approaches like GBS, GWAS, and GS have improved the understanding of genetic diversity in millets, and its applications also include soybean and aloe vera for investigating stress responses, seed development, and transcriptome profiling.

PacBio Sequencing

PacBio sequencing, also referred to as Single Molecule Real-Time (SMRT) sequencing, is a cutting-edge next-generation sequencing technology developed by Pacific Biosciences that allows real-time sequencing of single DNA molecules. A major advantage of this technique is its ability to identify DNA modifications, such as methylation, which play an essential role in gene regulation and epigenetics. It also avoids the need for PCR amplification, thereby minimizing biases and improving data accuracy. When combined with Nanopore sequencing, it has been used to uncover complex chromosomal linkages in tomato. Moreover, PacBio sequencing has been applied in crops like rice to investigate genes associated with abiotic stress, making it highly valuable for plant genomics and functional research.

Oxford Nanopore Sequencing

Oxford Nanopore sequencing is an advanced DNA sequencing technology that differs from conventional methods by analyzing DNA molecules directly, without requiring amplification or synthesis steps. It functions by passing DNA strands through nanopores and measuring changes in ionic current to decode the sequence. There are two primary nanopore systems: biological membrane-based systems that use protein pores like α-hemolysin within lipid membranes, and solid-state nanopores created in synthetic materials such as silicon nitride or graphene. Solid-state nanopores provide benefits like improved scalability, miniaturization, and compatibility with electronic devices. This technology has been extensively used in plant genomics to detect large structural variations, including insertions and deletions, in crops like tomato, rapeseed, maize, and soybean. It has also been integrated with target capture methods to identify specific nuclear loci in polyploid species. Additionally, nanopore sequencing has been applied in transcriptome analysis of strawberry under various treatments and in crops like wheat and tobacco for identifying viral infections and aiding in their management.

Ion Torrent Sequencing

Ion Torrent sequencing is an advanced next-generation sequencing method that employs semiconductor technology to produce rapid, precise, and cost-efficient DNA sequencing data. Developed by Ion Torrent, it detects nucleotide incorporation through changes in pH instead of using fluorescent labeling, which simplifies the sequencing process and improves efficiency. This technology serves as a valuable tool for researchers, clinicians, and scientists in high-accuracy genomic analysis. It has been effectively utilized in genotyping-by-sequencing (GBS), providing a scalable and affordable strategy for analyzing genetic variation. In crops like barley, it has facilitated genome-wide detection of genetic diversity, supporting advancements in crop improvement and genetic studies.

Roche/454 Pyrosequencing

Pyrosequencing is a sequencing-by-synthesis technique that allows rapid and precise identification of DNA sequences by detecting signals produced during nucleotide incorporation. In contrast to traditional Sanger sequencing, which relies on chain termination, Roche/454 pyrosequencing enables the parallel sequencing of millions of DNA fragments in a single run. This method involves DNA amplification and detects nucleotide addition through the emission of light, making it efficient for high-throughput sequencing. It has been widely used in the study of *Miscanthus*, a perennial grass recognized for its potential as a bioenergy crop due to its high yield and low input needs. Through Roche 454 sequencing, researchers have successfully explored its genome, identified key genes, and gained valuable insights into its bioenergy potential.

Conclusion

The diverse range of NGS techniques provides researchers with versatile tools tailored to specific genomic applications. While short-read technologies such as sequencing by synthesis offer high accuracy and throughput, long-read platforms like SMRT and nanopore sequencing enable comprehensive genome assembly and structural variation analysis. The continued evolution of these techniques is driving advancements in genomics, precision breeding, and molecular biology research. So NGS technologies are very useful to the Plant breeders, Researchers and Scientists.

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