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Short Communication

Brief Look on Different DNA Sequencing Methods

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ABOUT THE STUDY

The method of determining the nucleic acid sequence the order of nucleotides in DNA is known as DNA sequencing. It includes method or technology used for analyzing a set of the four bases: adenine, guanine, cytosine, and thymine. Rapid DNA sequencing tools have transformed biological and medical research and discovery. Modern DNA sequencing technology has assisted in the sequencing of whole DNA sequences, or genomes, of many types and species of life, including the human genome and other complete DNA sequences of many animal, plants, and microbial species. Around 3 billion base pairs make up the human genome, which provides the instructions for the formation and maintenance of a human being. Because of its based-paired structure, DNA sequences are highly adapted to storing a large quantity of genetic data. The mechanism by which DNA molecules are copied, transcribed, and translated is based on complementary base pairing, and the pairing also underpins most DNA sequencing methods. Whole genome sequencing has become viable and advancements in DNA sequencing technologies and methodologies.

A DNA segment, a whole genome, or a complex micro biome can be sequenced to identify the genetic information contained within it.

Sanger sequencing: The chain termination method

The "chain termination method," often known as Sanger sequencing, is a method for determining the nucleotide sequence of DNA. There are three basic steps in it. 1) Chain termination PCR DNA sequence; 2) gel electrophoresis size separation; 3) gel analysis and DNA sequence determination. The user scans all four lanes of the gel at once, from bottom to top, to establish the identification of the terminal ddNTP for each band in manual Sanger sequencing. A chromatogram is the outcome, and it displays the fluorescence peak of each nucleotide along the length of the template DNA. Individual segments of DNA, such as fragments employed in DNA cloning or synthesized through polymerase chain reaction, are still sequenced using Sanger sequencing (PCR).

Next-generation sequencing

NGS often known as high-throughput sequencing, When compared to the traditional Sanger sequencing techniques, which may produce up to 1000 bp of 99.999 % per base accuracies, high-throughput NGS technology, which uses parallel amplification and sequencing, yields shorter read lengths and average raw error rates of 1–1.5%. Library preparation, library amplification, and sequencing are all common steps in typical NGS platforms. RNA or DNA is used as the starting material for library formation (genomic source or PCR-amplified). RNA must be converted into cDNA as of now NGS machines sequence only DNA directly. NGS-based approaches have various advantages, including; It is not necessary to have prior knowledge of the genome or genomic traits while doing; It detects related genes (or characteristics), alternatively spliced transcripts, allelic gene variations, and single nucleotide polymorphisms with single-nucleotide resolution; Signals with a higher dynamic range require less DNA/RNA as input (nanograms of materials are sufficient); higher reproducibility. High-throughput sequencing includes next-generation "short-read" and third-generation "long-read" sequencing methods. Different long-read sequencing methods include Single Molecule Real Time (SMRT) sequencing and Nanopore DNA sequencing whereas short-read sequencing methods contains Massively Parallel Signature Sequencing (MPSS), polony sequencing, pyrosequencing, combinatorial Probe Anchor Synthesis (cPAS), DNA Nano ball sequencing, Microfluidic Systems.

Large-scale sequencing

It is commonly used to sequence very lengthy DNA fragments, such as entire chromosomes. It includes:

De novo sequencing: The term "de novo sequencing" refers to methods for discovering the sequence of DNA that has never been determined before.

Shotgun sequencing: Shotgun sequencing is a way of analyzing DNA sequences with lengths more than 1000 base pairs, up to and including entire chromosomes. The target DNA must be split into random fragments for this procedure to work. Individual fragments can be sequenced using the chain

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termination method and then reconstructed based on their overlapping sections.

CONCLUSION

DNA sequencing using the Sanger method enhanced the efficiency of DNA sequencing, NGS technology was recognized as superior in terms of cost and time. Scientists can deduce which genes and regulatory codes are contained in a DNA molecule using sequence information. For evolutionary studies between species or populations, homologous DNA sequences from various organisms can be compared. DNA sequencing can show alterations in a gene that could cause a disease. It has been employed in medicine for a variety of purposes, including disease diagnosis and therapy, as well as epidemiology investigations.

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