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Opinion Article

DNA Sequencing: Methods and Technologies

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DESCRIPTION

The method of determining the order of the four nucleotide bases (adenine, thymine, cytosine, and guanine) that make up the DNA molecule and convey important genetic information is known as DNA sequencing. The four bases in the DNA double helix bond with a specific partner to form base pairs (bp). Adenine (A) is complementary to Thymine (T), and Cytosine (C) is complementary to Guanine (G). The human genome contains approximately 3 billion base pairs that contain the instructions for creating and maintaining a human being. Because of its based-paired structure, DNA sequences are well suited to store a large amount of genetic information.

DNA sequencing methods

In the 1970s, English biochemist Frederick Sanger discovered Sanger sequencing. The Sanger method is a traditional DNA sequencing technique that employs fluorescent ddNTPs (dideoxynucleotides, N=A, T, G, or C) to prevent the addition of another nucleotide. NGS (also known as massively parallel sequencing) technologies have largely replaced Sanger sequencing due to advantages such as high throughput, cost efficiency, and speed. NGS can simultaneously determine the order of millions of fragments. NGS is a short-read sequencing technique that involves the creation of a small fragment library, followed by deep sequencing, raw data pre-processing, and DNA sequence alignment. Early efforts at gene sequencing were labour intensive, time consuming, and painstaking, such as when Gilbert and Maxam reported the sequence of 24 base pairs using a method known as wandering-spot analysis. Technical advances have automated, sped up, and refined the Sanger sequencing process. Sanger sequencing, also known as the chain-termination or dideoxy method, involves the use of a purified DNA polymerase enzyme to synthesise DNA chains of varying lengths. The inclusion of dideoxynucleotide triphosphates in the reaction mixture is a key feature of the Sanger method (ddNTPs). The 3' hydroxyl (OH) group required to form the phosphodiester bond between one nucleotide and the next during DNA strand elongation is missing in these chain-terminating dideoxynucleotides.

The Sanger process employs four parallel sequencing reactions to sequence a single sample. A single-stranded template, a specific primer to initiate the reaction, the four standard deoxynucleotides (dATP, dGTP, dCTP, and dTTP), and DNA polymerase are all used in each reaction. The polymerase adds bases to a complementary DNA strand to the single-stranded sample template. Then, at a lower concentration than the standard deoxynucleotides, one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP) is added to each reaction. Because dideoxynucleotides lack the 3′ OH group, the growing DNA is terminated when they are incorporated by DNA polymerase. Because four different ddNTPs are used, the chain does not always end at the same nucleotide (i.e., A, G, C, or T). This results in number of varieties.

Applications of DNA sequencing technologies

DNA sequencing reveals the genetic information contained in a specific DNA segment, an entire genome, or a complex micro biome. For evolutionary analysis between species or populations, homologous DNA sequences from different organisms can be compared. DNA sequencing may be used to determine the sequence of individual genes, larger genetic regions (i.e. clusters of genes or operons), full chromosomes, or entire genomes of any organism. DNA sequencing is also the most efficient way to indirectly sequence RNA or proteins (via their open reading frames).

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