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Commentary

Sanger Sequencing: Foundation of Modern DNA Analysis

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DESCRIPTION

Sanger sequencing, also known as chain-termination sequencing, is one of the most influential techniques in the field of molecular biology and genetics. Developed by Frederick Sanger and his colleagues in the 1970s, this method has been instrumental in revolutionizing our understanding of the genetic code and has laid the groundwork for many of the Deoxyribonucleic Acid (DNA) analysis technologies used today. Although newer sequencing techniques, such as Next-Generation Sequencing (NGS), have emerged in recent years, Sanger sequencing remains a foundation in research and diagnostics due to its high accuracy and reliability.

Basics of sanger sequencing

Sanger sequencing works on the principle of selectively terminating the elongation of a DNA strand during replication. The process begins with a DNA template, a short primer that binds to the template, and the enzyme DNA polymerase. DNA polymerase is responsible for synthesizing a complementary DNA strand by adding nucleotides (A, T, C and G) to the growing strand. However, in Sanger sequencing, some of these nucleotides are modified to include Dideoxynucleotides (ddNTPs), which lack the hydroxyl group needed to extend the chain further.

The reaction mixture contains a mixture of normal deoxynucleotides (dNTPs) and a small proportion of ddNTPs, each labeled with a different fluorescent dye. When a ddNTP is incorporated into the growing DNA strand, the elongation process is terminated, producing DNA fragments of varying lengths, each ending with a labeled ddNTP.

These DNA fragments are then separated by size using capillary electrophoresis, a technique where fragments are passed through a gel or a capillary column under an electric field. The fragments are detected by the fluorescence emitted by the ddNTP labels, and the sequence of the DNA can be determined by reading the order of these fragments.

Key features of sanger sequencing

Accuracy and reliability: One of the main advantages of sanger sequencing is its high level of accuracy. The method can reliably generate sequences with fewer errors compared to other technologies, making it particularly useful for applications that require precise sequencing, such as genetic mutation analysis and confirmation of variants found in high-throughput sequencing.

Read length: Sanger sequencing can produce relatively long read lengths, typically ranging from 500 to 1,000 base pairs. This makes it ideal for sequencing smaller DNA regions, such as individual genes or specific regions of interest in a genome.

Cost-effectiveness for small projects: While sanger sequencing may be expensive for large-scale sequencing projects, it remains a cost-effective choice for targeted sequencing or validation of results obtained from other high-throughput methods.

Applications of sanger sequencing

Sanger sequencing has a wide range of applications, both in research and clinical settings. Some key uses include:

Genetic mutations and variant detection: Sanger sequencing is often used for identifying mutations in genes associated with inherited diseases. It is the method of choice for confirming mutations detected through high-throughput sequencing or for sequencing individual genes in clinical diagnostics.

Gene cloning and functional studies: The technique is commonly used for sequencing cloned genes to confirm their identity and study their function.

Forensic DNA analysis: In forensic science, sanger sequencing is utilized for DNA fingerprinting and identifying individuals based on unique genetic markers.

Microbial sequencing: Sanger sequencing is also used to sequence bacterial genomes, identify pathogens and study microbial diversity.

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CONCLUSION

Sanger sequencing, though developed decades ago, remains a vital and widely used tool in genomics. Its accuracy, reliability and ability to sequence long fragments make it indispensable applications, particularly in clinical diagnostics, genetic research and validation studies. While NGS technologies have significantly advanced the field of DNA sequencing, sanger sequencing has continued relevance underscores its foundational importance in genetic analysis. As the field evolves, Sanger sequencing will continue to play a critical role in the study and understanding of genetic material.