

The first techniques used to identify and sequence DNA genomes included several key methods, with one of the most significant being Sanger sequencing, also known as the chain termination method. While newer methods, such as next-generation sequencing (NGS), have largely supplanted Sanger sequencing due to their speed and cost-effectiveness, Sanger sequencing remains a fundamental technique for validating results and sequencing smaller DNA fragments. Here's an overview of Sanger sequencing:

Sanger Sequencing Description: Developed by Frederick Sanger and his colleagues in the 1970s, Sanger sequencing revolutionized DNA sequencing by allowing researchers to determine the precise order of nucleotides in a DNA molecule.

Principle: Sanger sequencing utilizes modified DNA synthesis to terminate the elongation of the DNA strand at specific nucleotides.

DNA Synthesis and Termination: The DNA polymerase extends the primer by adding complementary nucleotides.

Fragment Separation: The resulting fragments are then separated by size using capillary electrophoresis, where shorter fragments travel faster through a gel or capillary.

Detection and Analysis: The fragments are detected, and the order of the nucleotides is determined based on the fluorescent labels on the ddNTPs. When a ddNTP is incorporated instead of a normal dNTP, the synthesis terminates because ddNTPs cannot form a bond with the next nucleotide.

Significance: Sanger sequencing was the first method used to sequence entire genomes, including the human genome as part of the Human Genome Project.

DNA Template Preparation: The DNA sample to be sequenced is denatured to create single strands.

- o Normal deoxynucleotides (dATP, dTTP, dCTP, dGTP).
- o A small proportion of fluorescently labeled dideoxynucleotides (ddNTPs), which lack a 3'-OH group.

Reaction Mixture: A mixture is prepared containing:

- o The single-stranded DNA template. This results in a collection of DNA fragments of varying lengths, each ending with a ddNTP. The sequence of the DNA can then be reconstructed from the order of the detected fragments.
- o A primer (short piece of DNA complementary to the template).
- o DNA polymerase enzyme.

2.3.4.5.