Chapter 13

Introduction to Sanger sequencing

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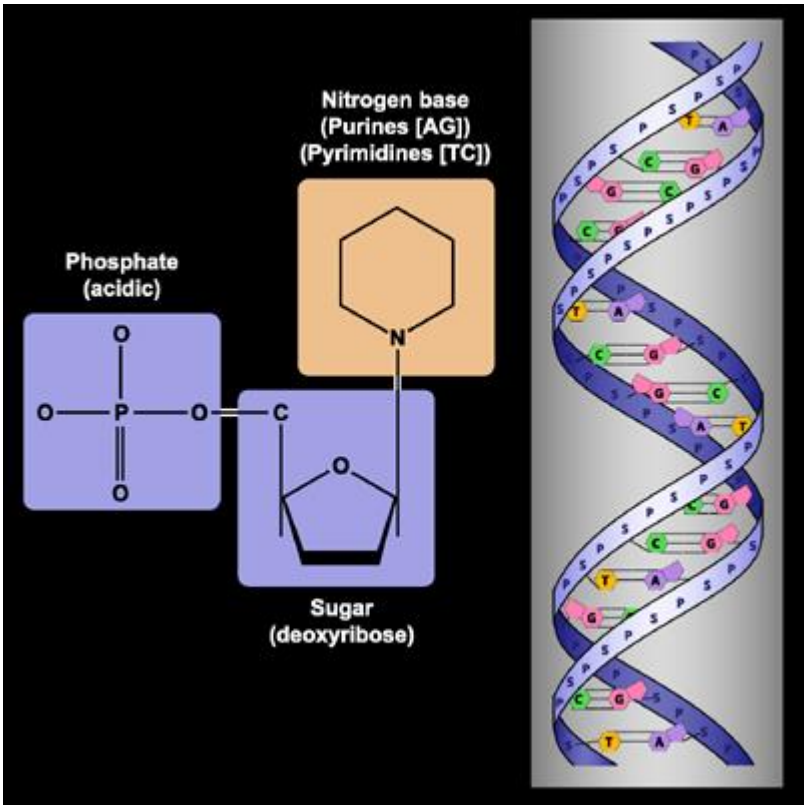
DNA is the cell's genetic material, storing cell data as a nucleotide which contains 1. Phosphate group, 2. Deoxyribose sugar 3. A carbon-nitrogen ring (Base). Based on the base present, the nucleotide can be of 4 types: Adenine, Guanine, Cytosine, and thymine. In this adenine and guanine, double rings of carbon and nitrogen are called purines. The cytosine and thymine have a single ring of carbon and nitrogen called pyrimidine bases. DNA is double-stranded. The chains are attached by hydrogen bonds. A purine always bonds with a pyrimidine. Adenine bonds with thymine, and Cytosine bonds with guanine. One chain of double strands is complementary to the other.



Sequencing determines the order of the four chemical building blocks - called "bases" - that make up the DNA molecule. The sequence tells about the kind of genetic information of the particular region. It can code for a gene or protein or sometimes a non-coded region. Sequence data can also tell about the changes of the original sequence, which caused mutation related to diseases.

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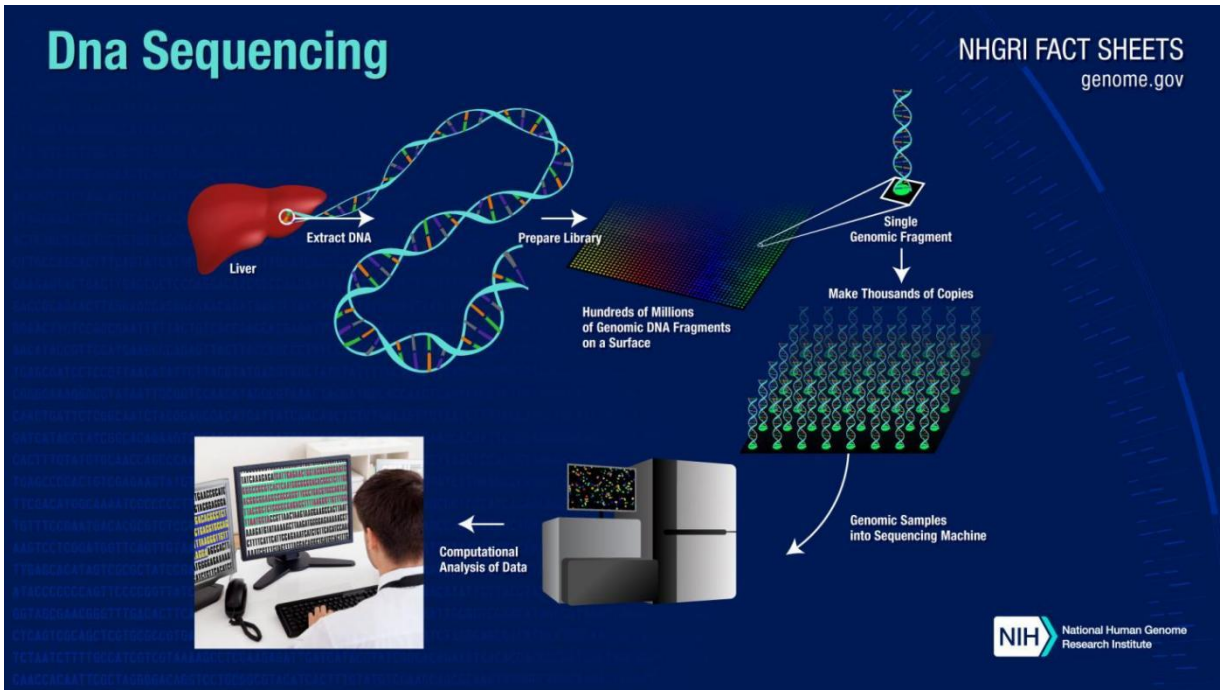
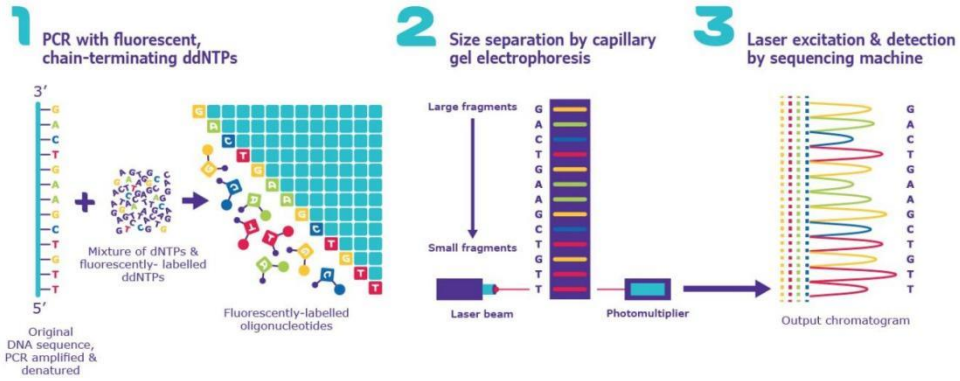


Sanger sequencing, also known as the "chain termination method, "is a method for determining DNA nucleotide sequences. The technique was developed by Nobel Laureate Frederick Sanger and his colleagues in 1977, hence the name the Sanger Sequence. The Sanger sequencing is considered the gold standard for validating DNA sequences. Even with advanced sequencing methods, the Sanger sequencing was performed to determine the purity of the sequence. The regions are divided into less than 1000 bp regions and sequenced using Sanger sequencing. The human genome project made the total human genome into more minor sequences and walked through multiple rounds. The fragments were aligned to assemble the sequences.

Steps in Sanger sequencing



Sanger sequencing has 3 main steps

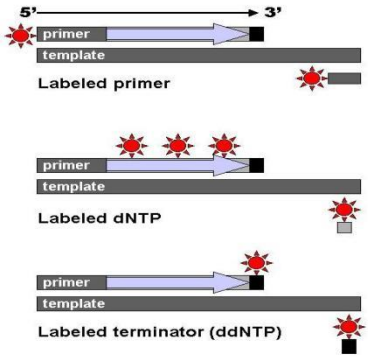
There are three main steps in Sanger sequencing.

1. PCR with Florescent chain terminating dNTPs

2. Size separation by capillary gel electrophoresis

3. Laser excitation and detection, gel analysis

1. PCR with Florescent chain terminating dNTPs



In Sanger sequencing DNA to be sequenced is used as a template for the PCR. It is similar to the standard PCR; instead of normal DNTP, it is

supplemented with modified DNTP. The modified dNTP s have deoxyribonucleotides. The DNA polymerase adds dNTP to the growing DNA strand in the extension steps. There the phosphodiester bond is formed between two nucleotides. A free OH group is necessary for the nucleotides to continue this chain. Since the modified nucleotide lacks the OH group, the chain will be terminated in the step. So it is called the chain termination PCR. Whenever the modified dNTP joined, it terminated the chain formation. So millions of oligonucleotide copies form by chain termination. In manual Sanger sequencing, four PCR reactions are set up, whereas, in automated Sanger sequencing, all four DNTP have unique fluorescent probes.

Gel electrophoresis for size separation

The DNA sample to be sequenced is loaded in gel electrophoresis after PCR. DNA will move to the positive electrode. Because the oligonucleotides are small, they will move according to their size. The smaller the fragment faster the movement. The resultant gel has oligonucleotides arranged from more minor to larger. All nucleotides will be run separately from the 4 PCR reactions in manual Sanger sequencing. In automatic Sanger sequencing, the gel is run in single gel electrophoresis.

Determination of Sequence through gel analysis

The gel analysis is done through software in automated Sanger sequencing. It is done manually in the manual Sanger sequencing. In manual sanger sequencing, if the bottom of the band is found in the column corresponding to DGTP, it is G in the first place. Same way next one will be read. In automatic sequencing, the computer reads the gel and identifies the corresponding DNTP.