5 DNA Sequencing

DNA sequencing

DNA sequencing refers to methods for determining the order of the nucleotides bases adenine, guanine, cytosine and thymine in a molecule of DNA. DNA sequencing can be performed by different methods, but there are two main methods are widely known to be used to sequence DNA:

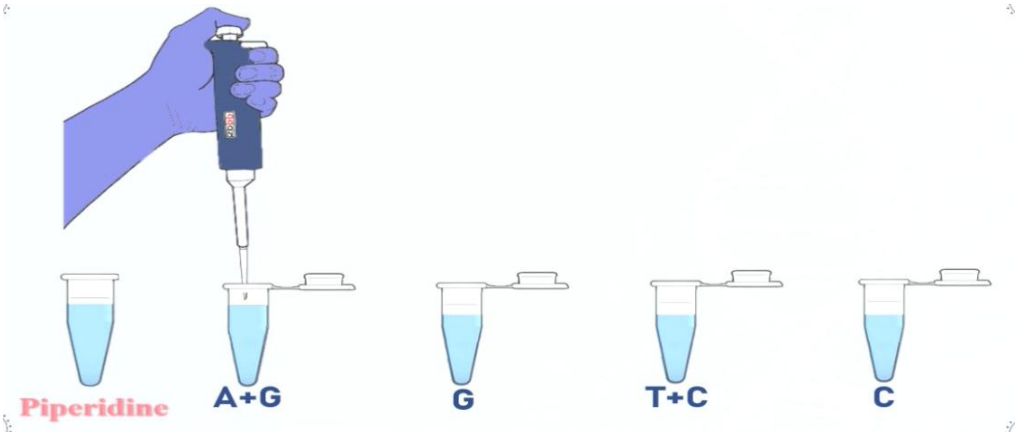
1. **The Chemical Method** (Maxam–Gilbert method).
2. **The Chain Termination Method** (Sanger dideoxy method).

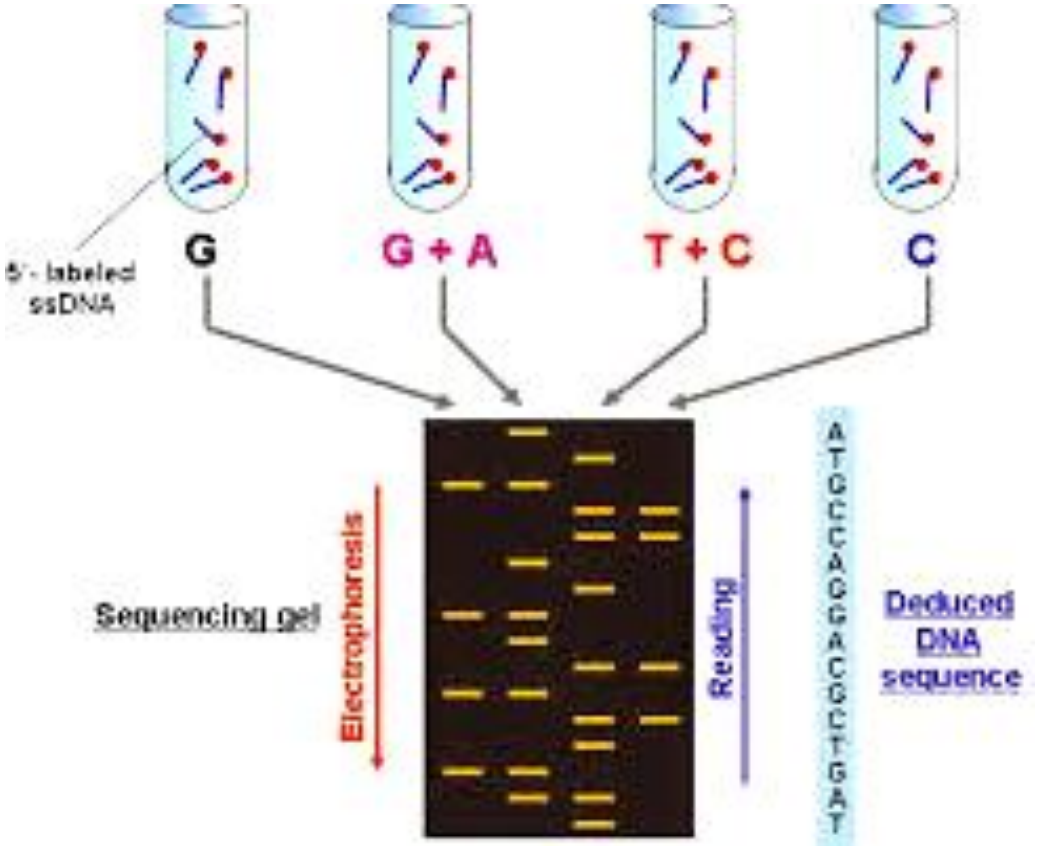
Maxam–Gilbert technique depends on chemical liability of different nucleotide bonds. While the Sanger method interrupts elongation of DNA sequences by incorporating dideoxynucleotides into the sequences. The chain termination method is the method more usually used because of its speed and simplicity.

Maxam – Gilbert sequencing (chemical method)

- In 1976-1977, Allan Maxam and Walter Gilbert developed a DNA sequencing method based on chemical modification of DNA and subsequent cleavage at specific bases.
- The method requires radioactive labeling at one end of the purified DNA fragment that needs to be sequenced.
- Four reactions will be create including radiolabelled nucleotides, each one differ from others. The first one contains (G+A), the second contains (G), the third contains (C), and the last one contains (C+T).
- Chemical treatment generates breaks at small proportions in each one of the four reactions.
- Thus a series of labeled fragments are generated, from the radiolabelled end to the first 'cut' nucleotide in each part of the DNA fragments.
- The fragments in the four reactions are arranged side by side in gel electrophoresis for size separation.

- To visualize the fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each corresponding to a radiolabelled DNA fragment, from which the sequence may be inferred.





Advantages

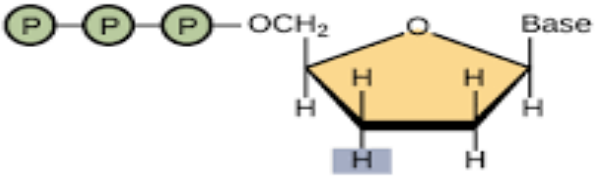
- Purified DNA can be read directly
- Yielding efficiently DNA.
- Can be used to analyze nucleic acid structure and epigenetic modifications to DNA

Disadvantages

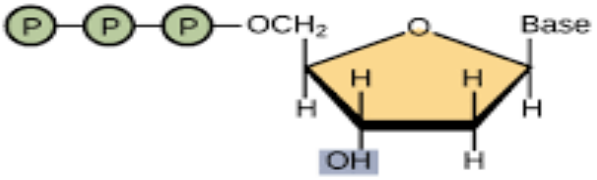
- It requires extensive use of hazardous chemicals.
- It has complex technic.
- It is difficult to sequence more than 500 base pairs.
- It is difficult to DNA kits to this method.

Sanger Method (Chain Termination Method)

The chain terminator method is more efficient and uses fewer toxic chemicals and lower amount of radioactivity than the method of Maxam and Gilbert. The key principle of the Sanger method was the use of **dideoxynucleotide** triphosphates (ddNTPs) as DNA chain terminators, which are lack 3´OH.



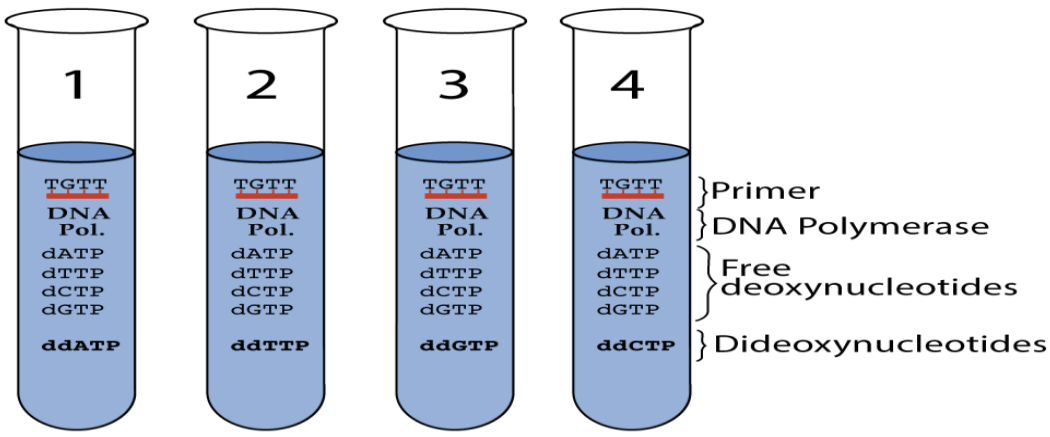
Dideoxynucleotide (ddNTP)



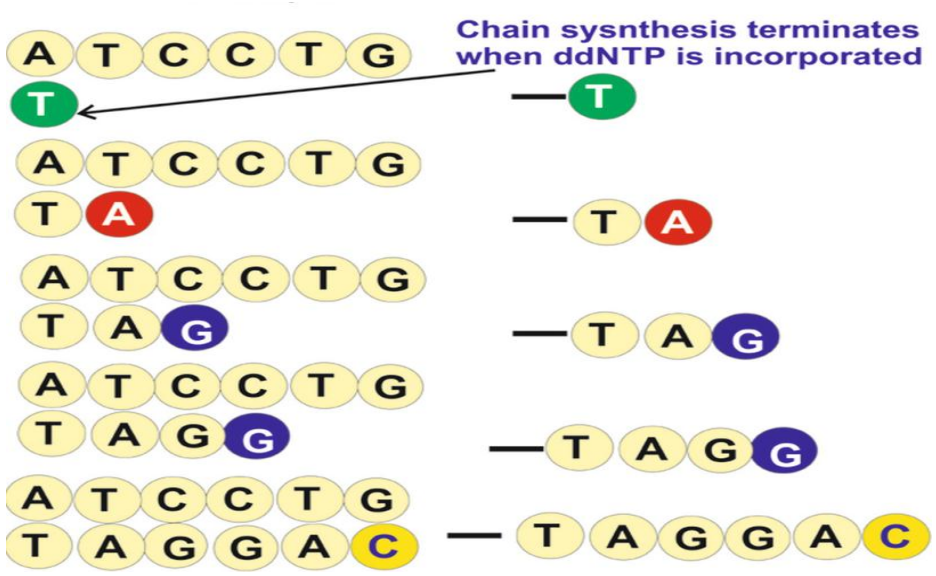
Deoxynucleotide (dNTP)

- The DNA sample is divided into four separate reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP, or dTTP) and the DNA polymerase and DNA primers.

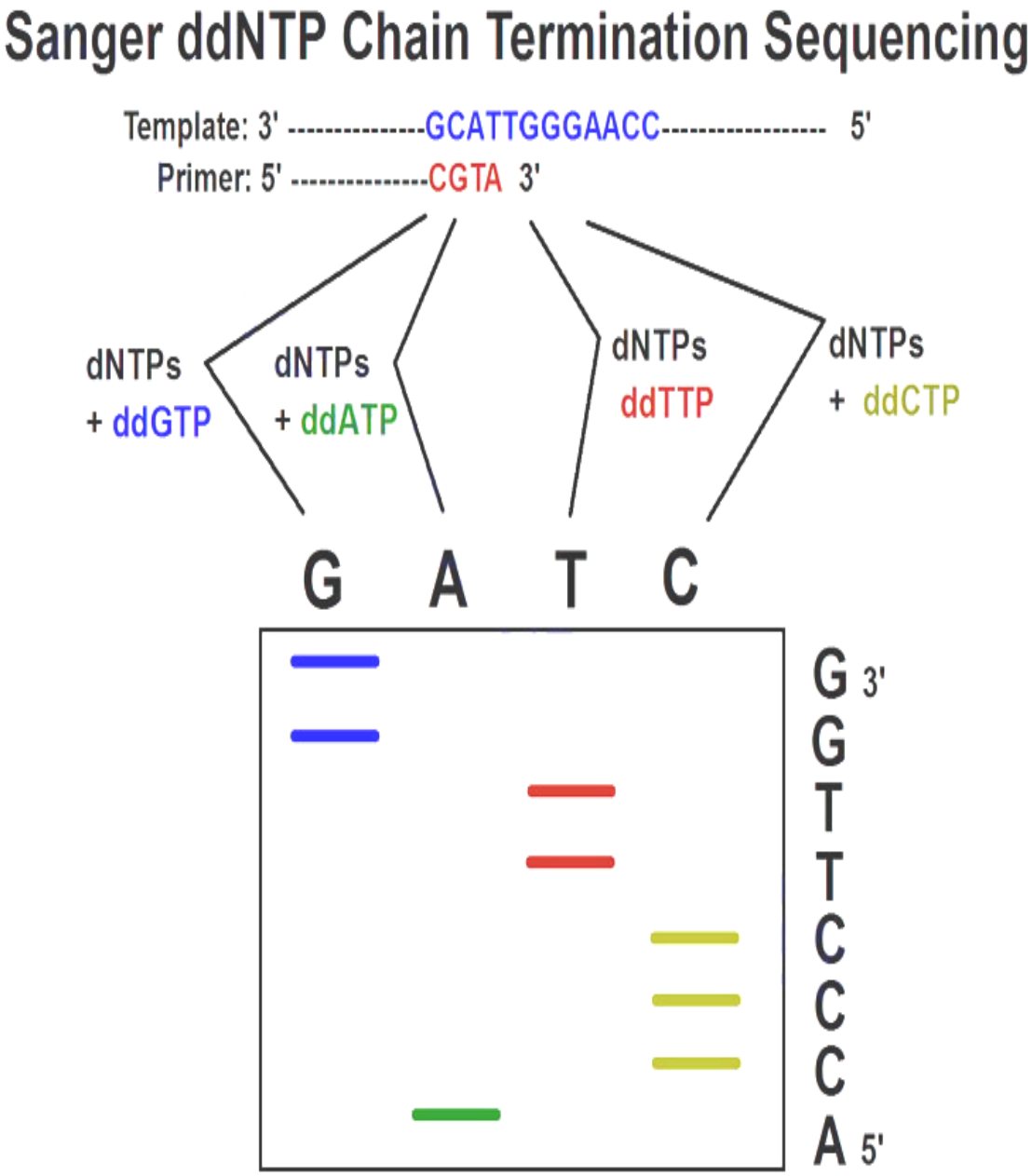
- To each reaction, only one of the four **dideoxynucleotide** (ddATP, ddGTP, ddCTP, or ddTTP) is added, which represents the chain terminating nucleotide.



- When **dideoxynucleotide**s are linked, the reaction in that piece of DNA will blocked because they lacking a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides.



- The newly synthesized and labeled DNA fragments are heat denatured, and separated by size by gel electrophoresis with each of the four reactions run in individual lanes (lanes A, T, G, C).
- The DNA bands are then visualized by autoradiography or UV light and can be read.
- A dark band in a lane indicates a DNA fragment that is result of chain termination after combination of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP).
- The DNA sequence then will be easy to read from bottom to top.



With my best wishes

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