Sanger Sequencing

Objectives

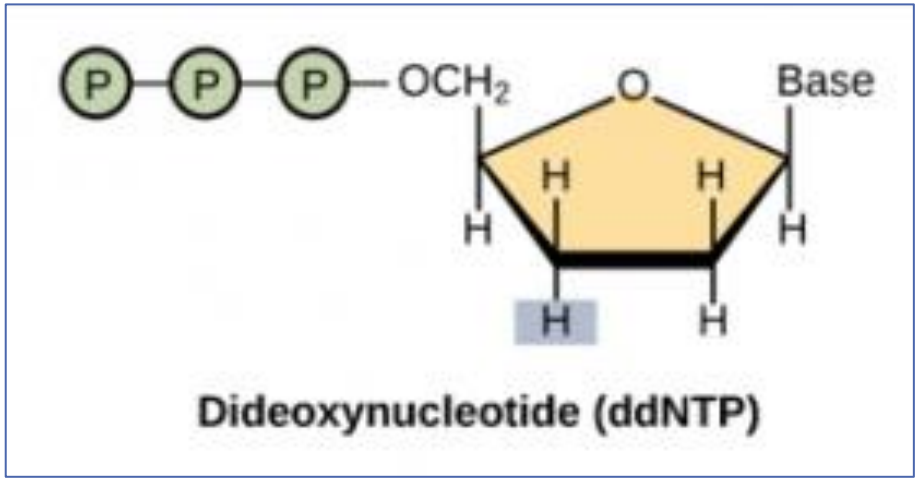
Get familiar with the technique.

Sanger sequencing, also known as the “chain termination method”.

* Developed by Frederick Sanger and colleagues in 1977. ➔ English biochemist

Principle:

This method is based on termination of polymerase reaction using labeled dideoxynucleotide (to terminate the new synthesized nucleotides). In order to get different size strands, According to resultant sizes, the sequence of the DNA is determined (from small to large size in 5’ to 3’ direction).

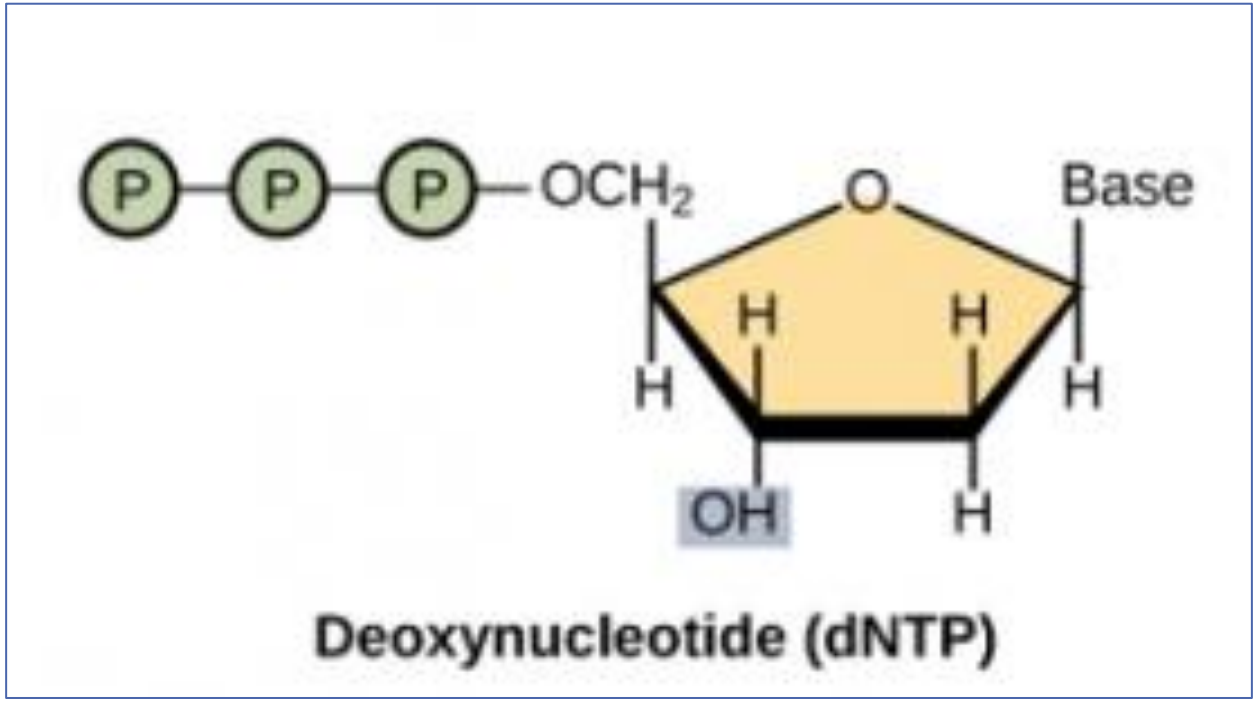


Dideoxynucleotide (ddNTP)

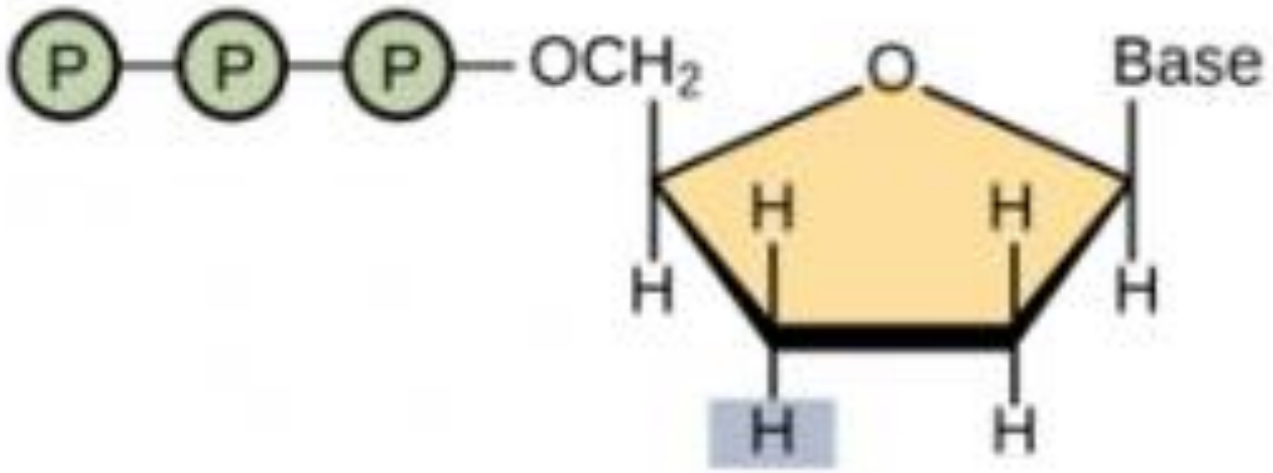
So, Sanger sequencing, which is based on the principle and biochemistry of DNA replication, follows two basic steps:

1.PCR using fluorescent chain-terminating dideoxynucleotides (ddNTPs).

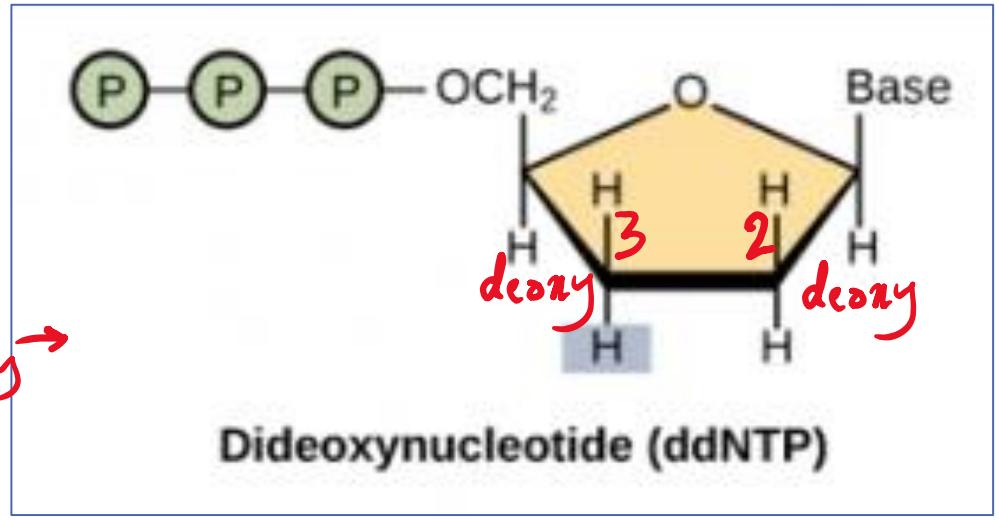
2.Size separation and analysis by capillary electrophoresis.



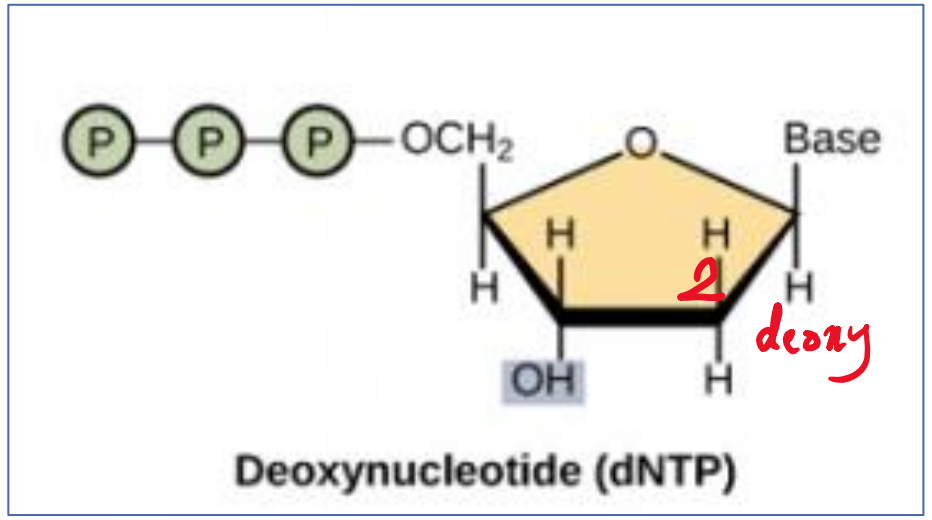
Deoxynucleotide (dNTP)



Dideoxynucleotide (ddNTP)



The polymerase can not extend the synthesis reaction and terminate the synthesis, Because of the absence of 3'OH in (ddNTP).



The polymerase can extend the new synthesized strand by adding complementary nucleotide.

Image credit: "Whole-genome sequencing: Figure 1," by OpenStax College, Biology).

Sanger Sequencing components:

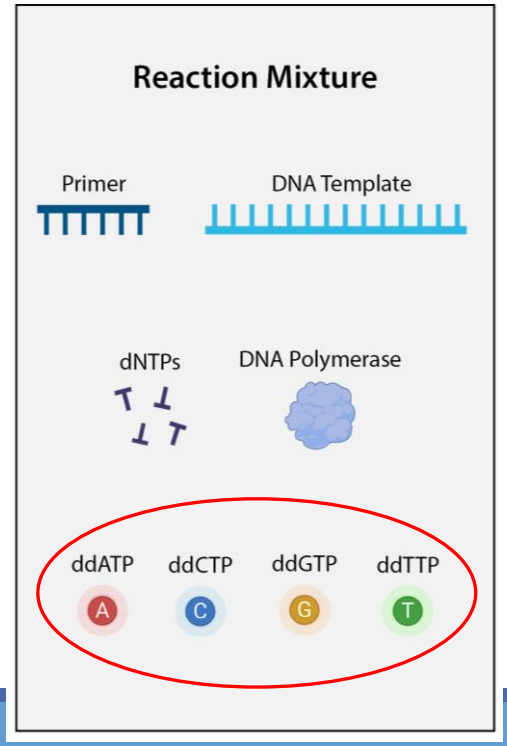
(1) DNA template.

(2) Primers.

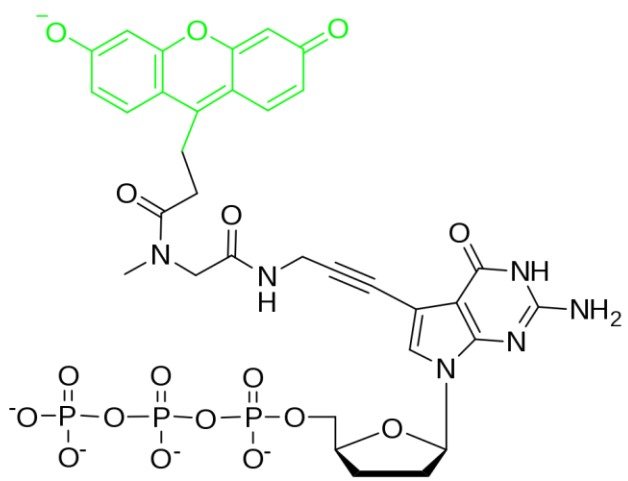
(3) Deoxynucleotide triphosphates (dNTPs). (dNTPs: A, G, C, and T)

(4) Thermostable DNA polymerase.

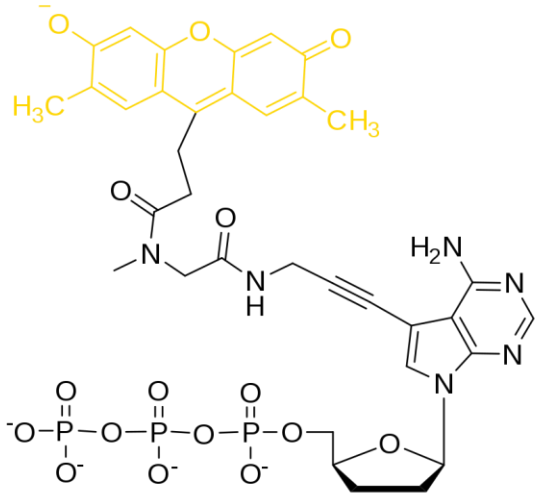
(5) Dideoxynucleotide triphosphates (ddNTPs) (ddNTPs: ddATP, ddGTP, ddCTP, and ddTTP) labeled with a distinct fluorescent dye.



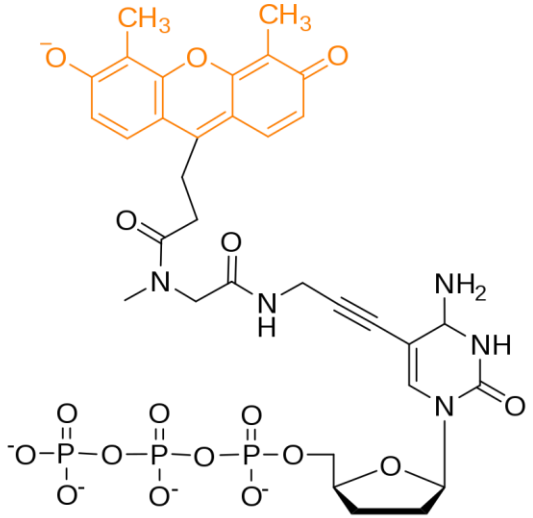
wikipedia



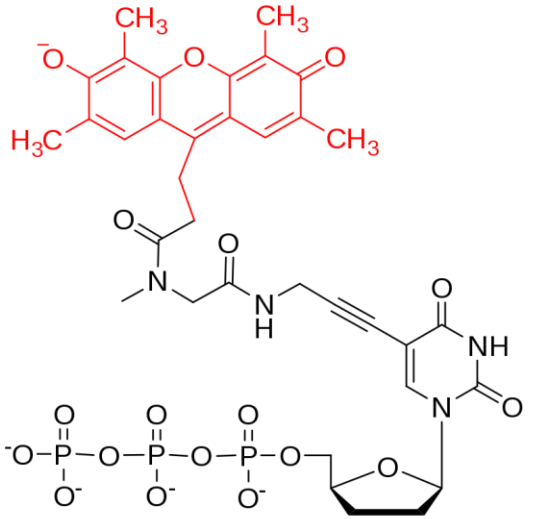
G-505



A-512



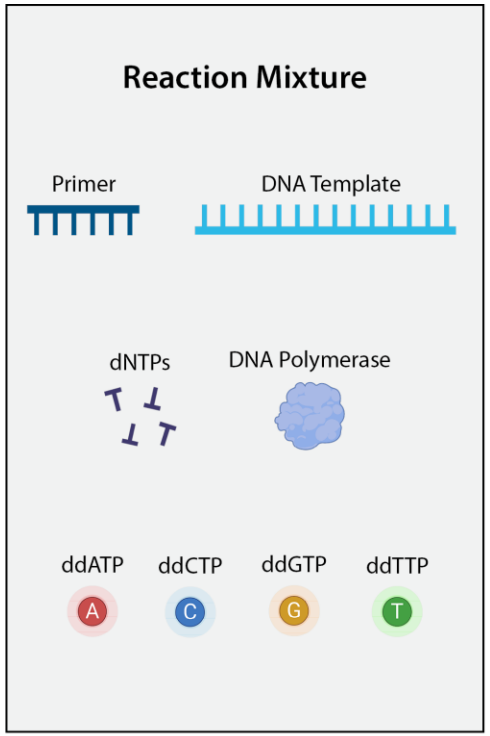
C-519

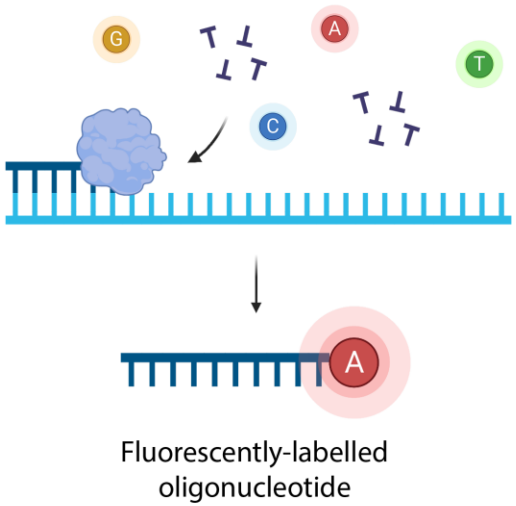


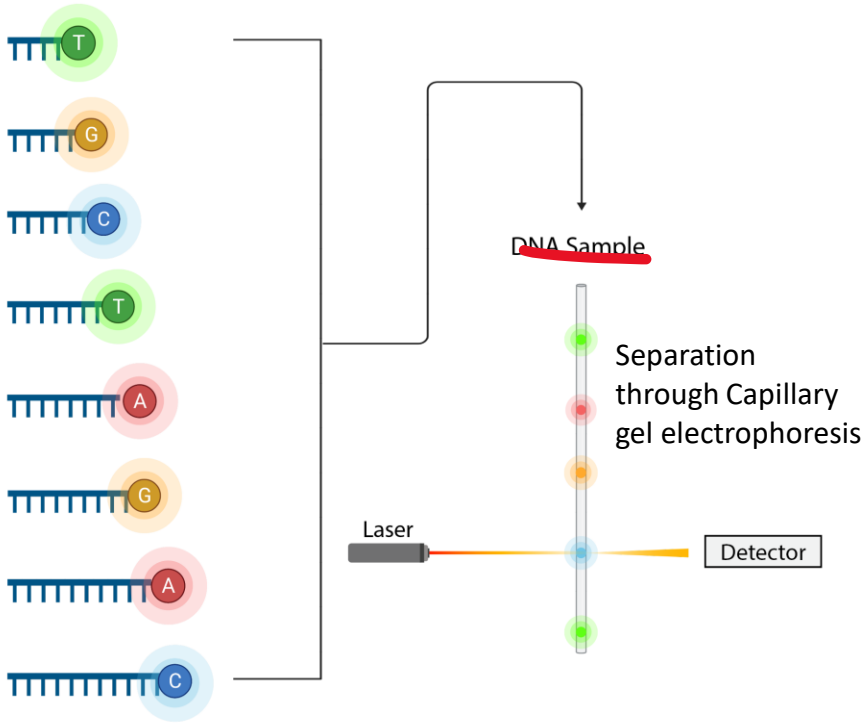
T-526

How Does Sanger Sequencing Work?

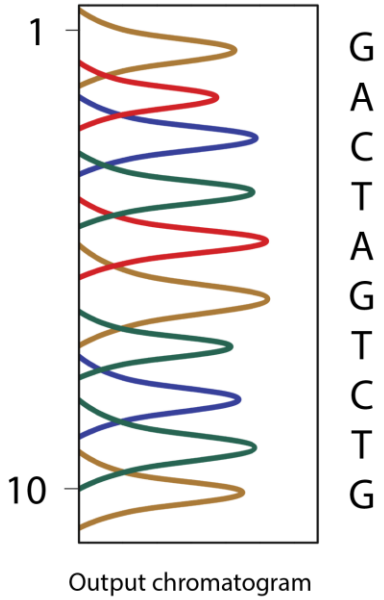
Sanger Sequencing







computational sequence analysis



Output chromatogram

1. Chain-termination PCR using fluorescent ddNTPs
2. Size separation and sequence analysis using capillary gel electrophoresis and fluorescence detection

https://www.aatbio.com/catalog/sanger-sequencing
https://www.sciencedirect.com/topics/medicine-and-dentistry/sanger-sequencing

How Does Sanger Sequencing Work?

1- ddNTPs are tagged with different colored fluorescent dyes (green, blue, red and yellow).

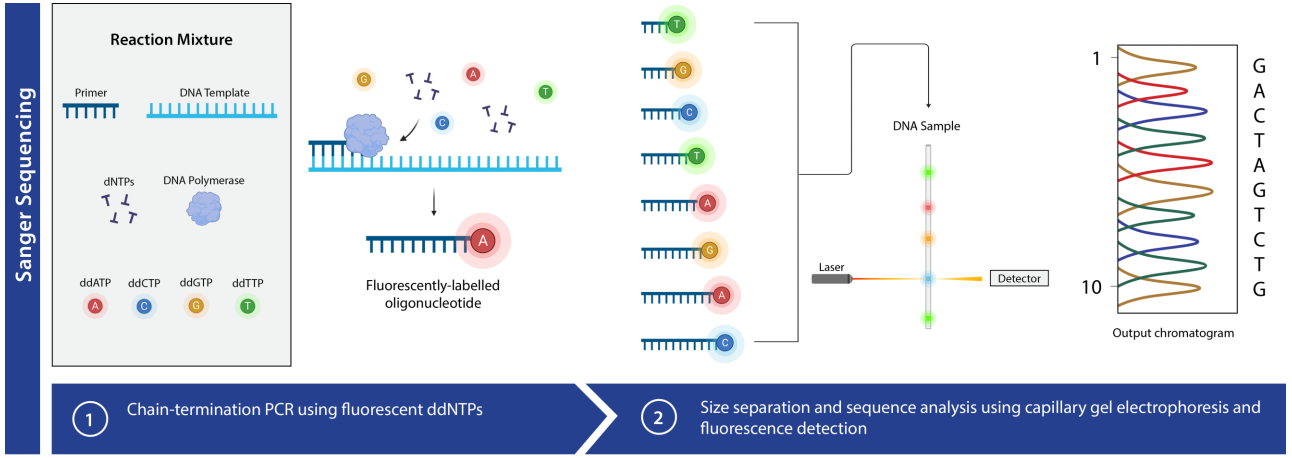
2- Different colored DNA fragments are generated (different sizes of strands).

3- Separated **by size** in an electrophoretic gel (the capillary gel).

4- Color associated with each band is detected with a laser beam. and a computer detects the resulting light emitted.

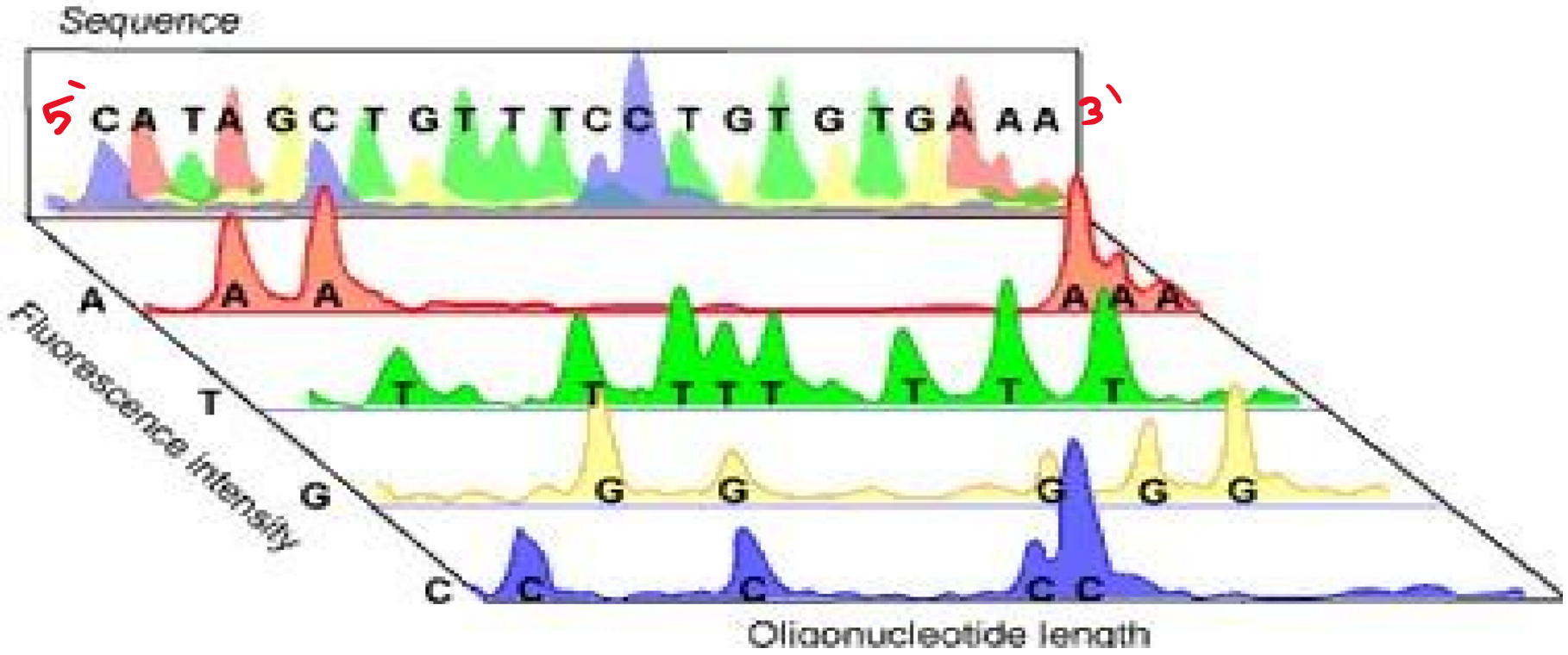
5- The amount of fluorescence in each band is represented as a peak in the computer output.

-The light emitted can be directly tied to the identity of the terminal ddNTP. The output is called a chromatogram.



Electropherogram of a Sequencing Reaction

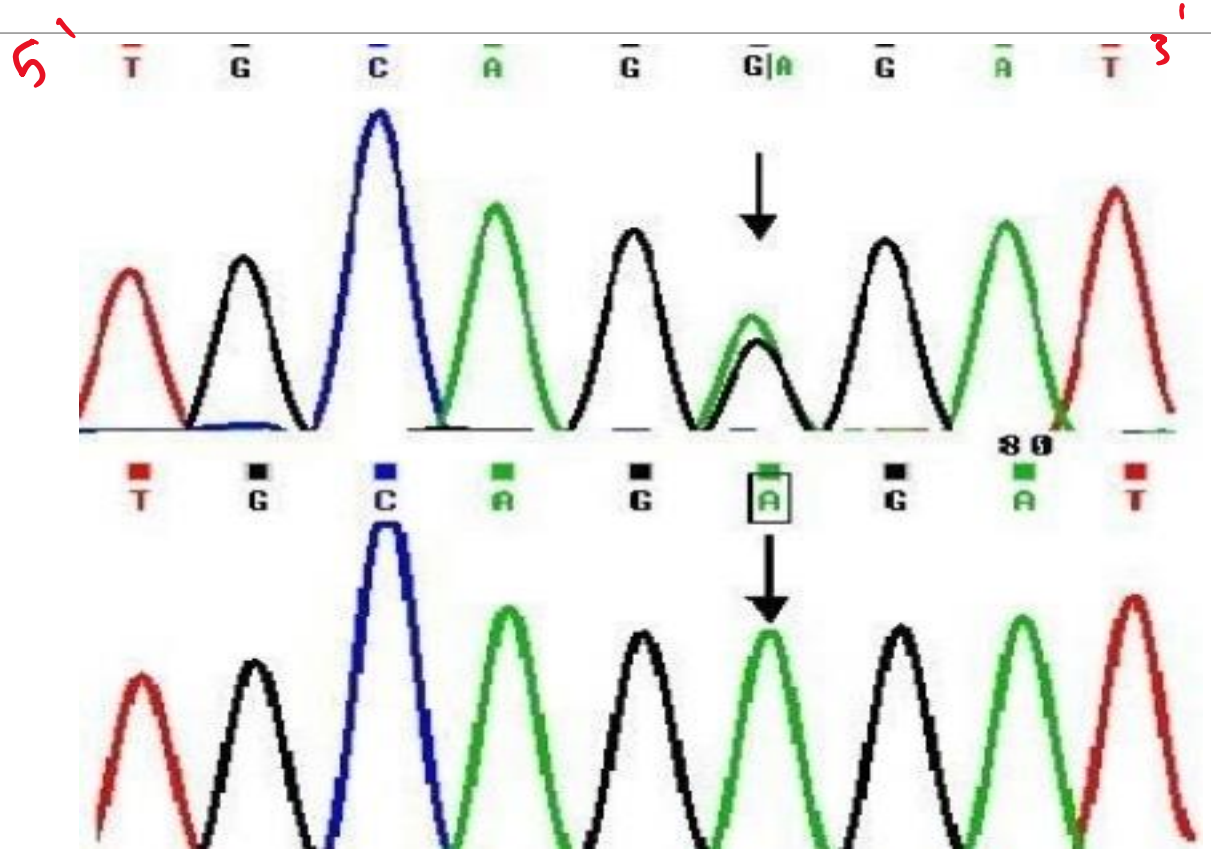
by reading the gel bands from smallest to largest, we can determine the 5’ to 3’ sequence of the original DNA strand.



Sequencing results:

Heterozygous

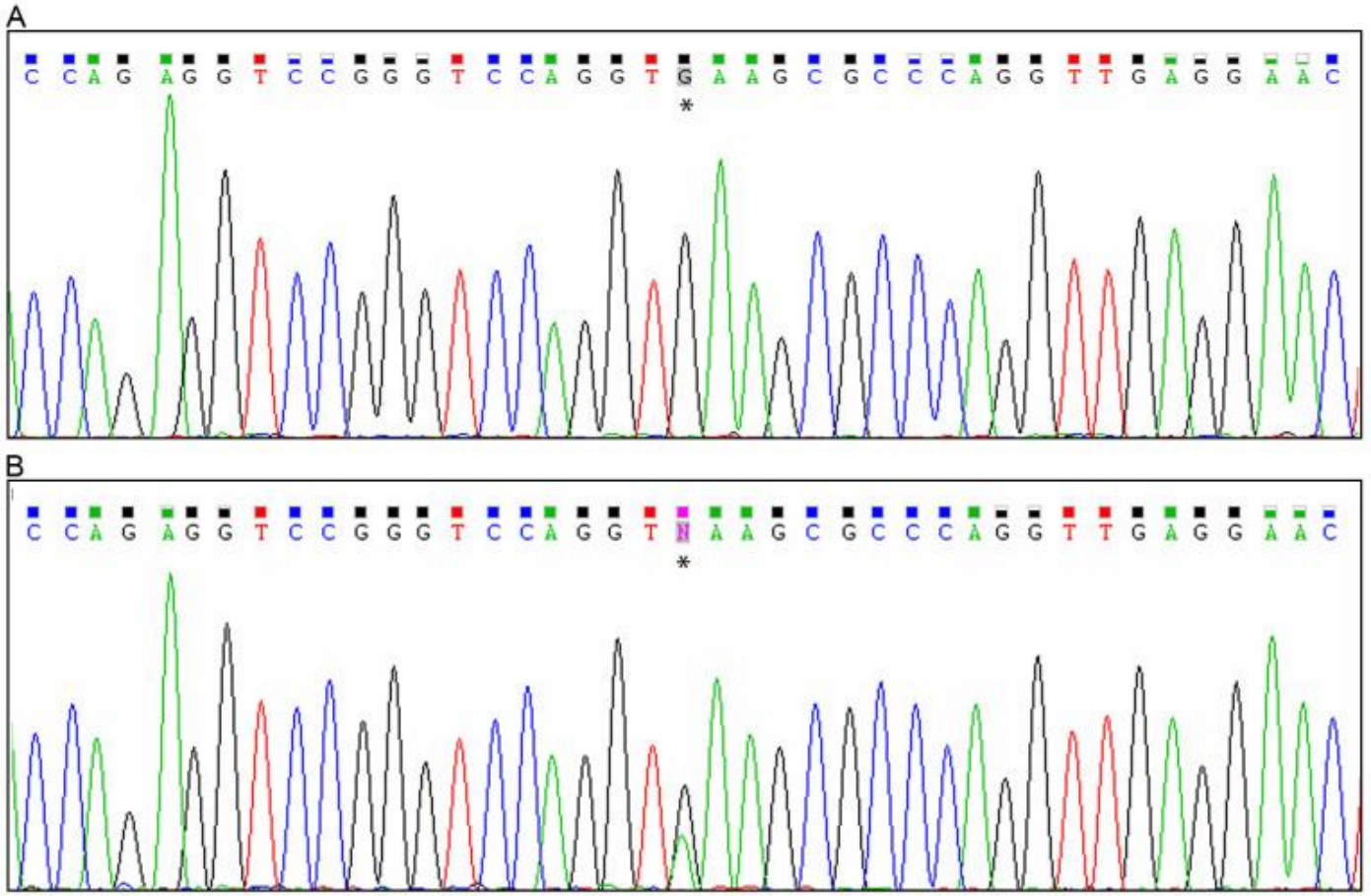
Homozygous



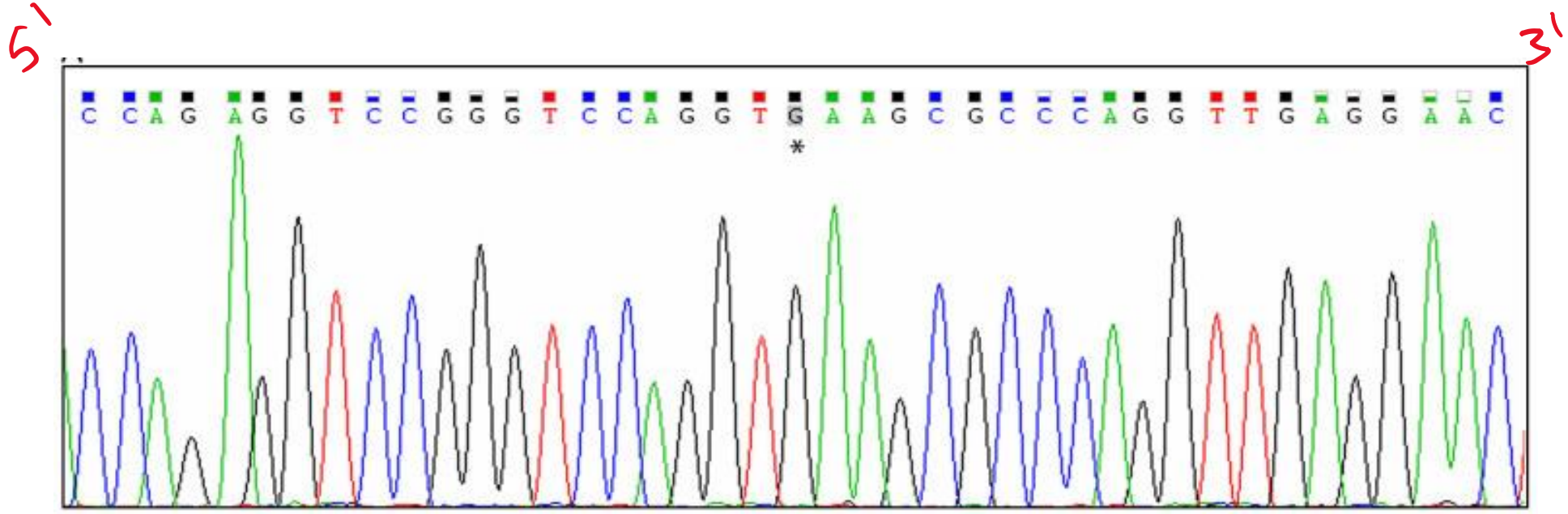
Sequencing results:

Homozygous

Heterozygous

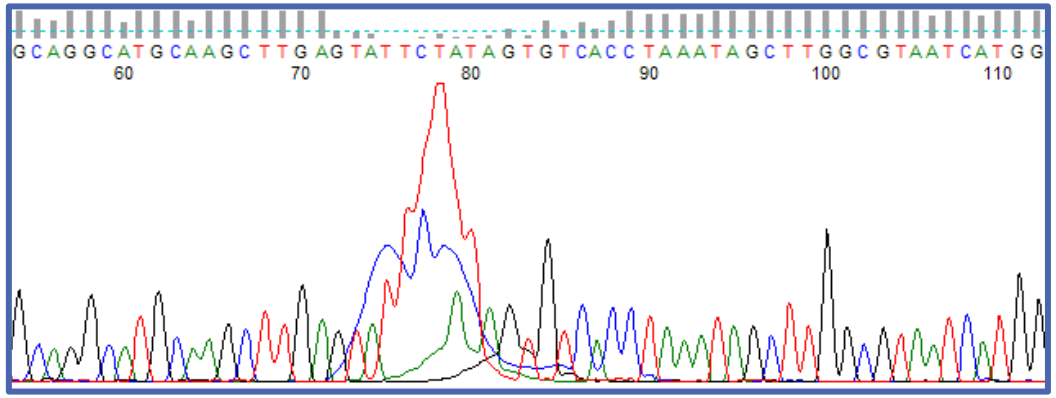


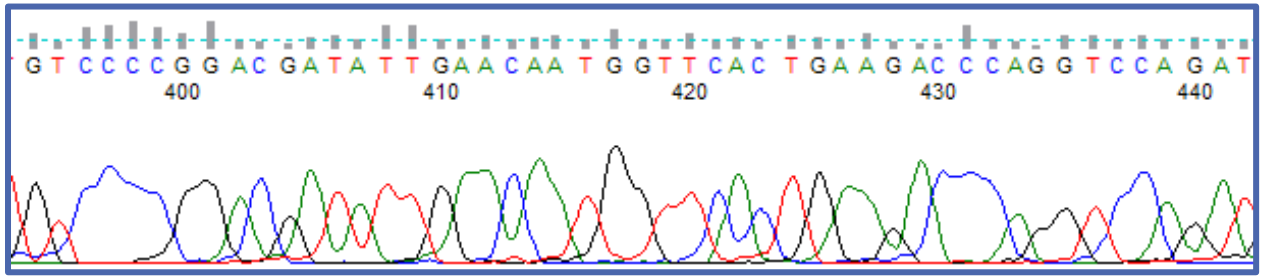
Sequencing result:

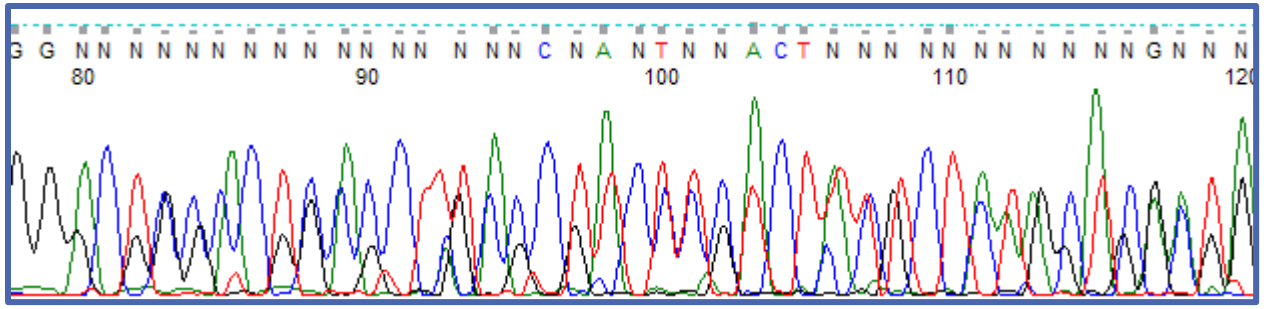


Each peak represent one nucleotide.

Bad Sequencing result:

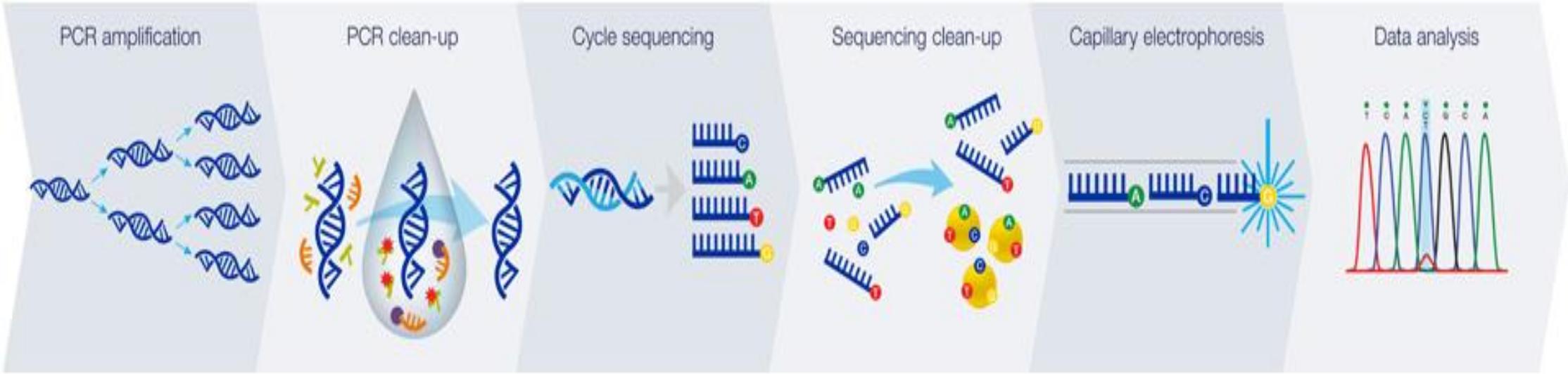






https://www.nucleics.com/DNA_sequencing_support/DNA-sequencing-troubleshooting.html

Sanger sequencing workflow:



https://www.youtube.com/watch?v=ksMuuz٦qNtI&list=PL٠nShjWmjPadEQI٠_٩NyvNcHGadnb٦JZd&index=١٥

Sanger sequencing application:

1. Single nucleotide polymorphism (SNP) detection.
2. Single-strand conformation polymorphism (SSCP).
3. Mutations detections.

Supporting video :

Automated

https://www.youtube.com/watch?v=wdS3j0TgbjM

At time 0.13 then 1.13

Manual:

https://www.youtube.com/watch?v=AI4CnG5Jp4s

Methods of DNA sequencing:

1. Maxam – Gilbert sequencing (chemical degradation method).
   (https://www.youtube.com/watch?v=cl2s-ZMmcbc&t=166s)
2. Sanger sequencing (dideoxy chain-termination method).

3. High- throughput sequencing technologies (Next Generation Sequencing (NGS)).
   (https://www.youtube.com/watch?v=WKAUtJQ69n8&t=17s)