# DNA SEQUENCING

# - Objective:

- To determine the order of the nucleotides in a given DNA sample.

# - Introduction:

***In 1977, two separate methods for DNA sequencing were developed, they are:***

1. Sanger method; chain termination method (Commonly used method).

2. Maxam and Gilbert method; chemical degradation method.

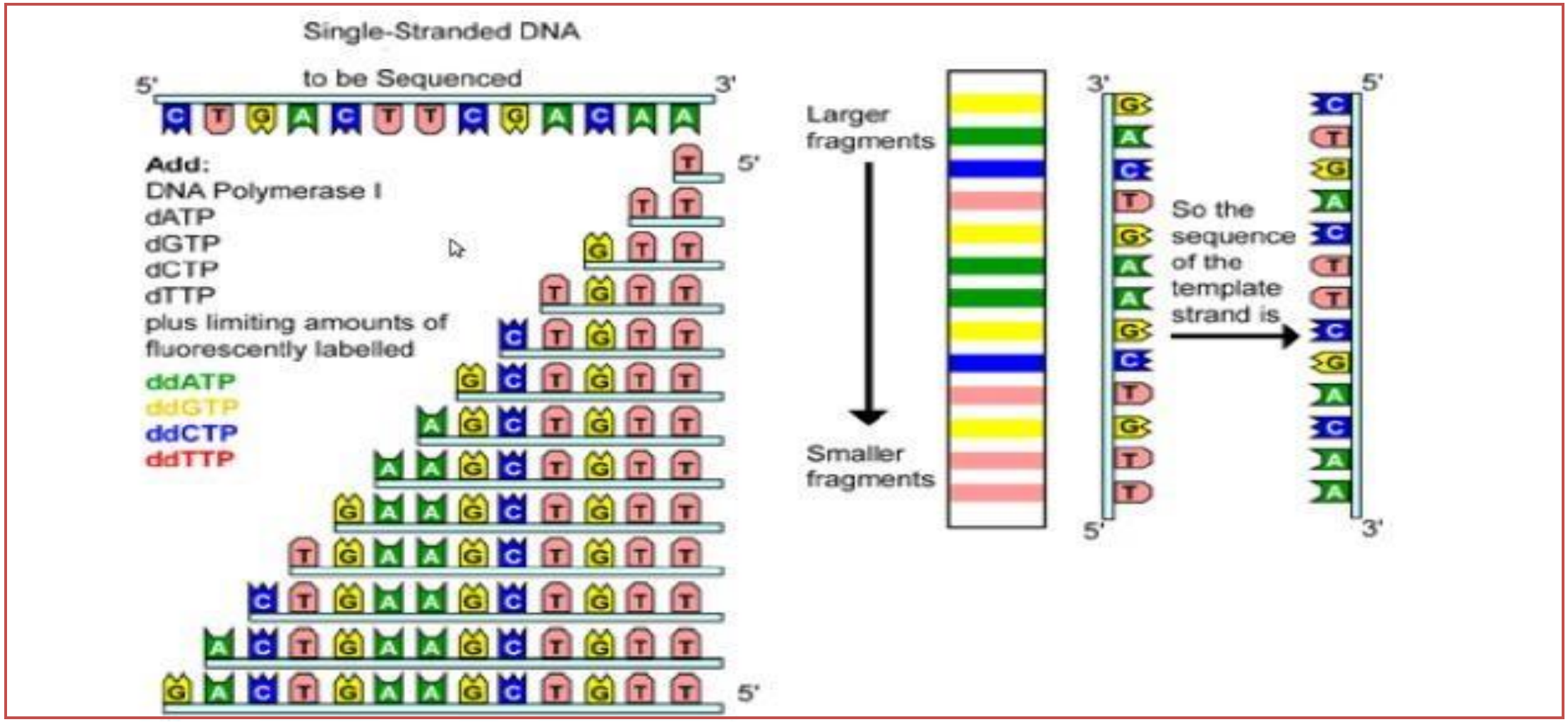



**Figure 1:** **Chain Termination DNA Sequencing**

## - Automated Method:

- In this method, **cycle sequencing**, the dideoxynucleotides -not the primers- are tagged with different colored fluorescent dyes, thus all four reactions occur in the same tube and are separate in the same lane on the gel.

As each labeled DNA fragment passes through the bottom of the gel, a laser reader detect the fluorescence of each fragment (blue, green, red or yellow) and compiles the data into an image

**(Figure 2).**

**- This video shows the principle of DNA Sequencing by sanger method.**

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

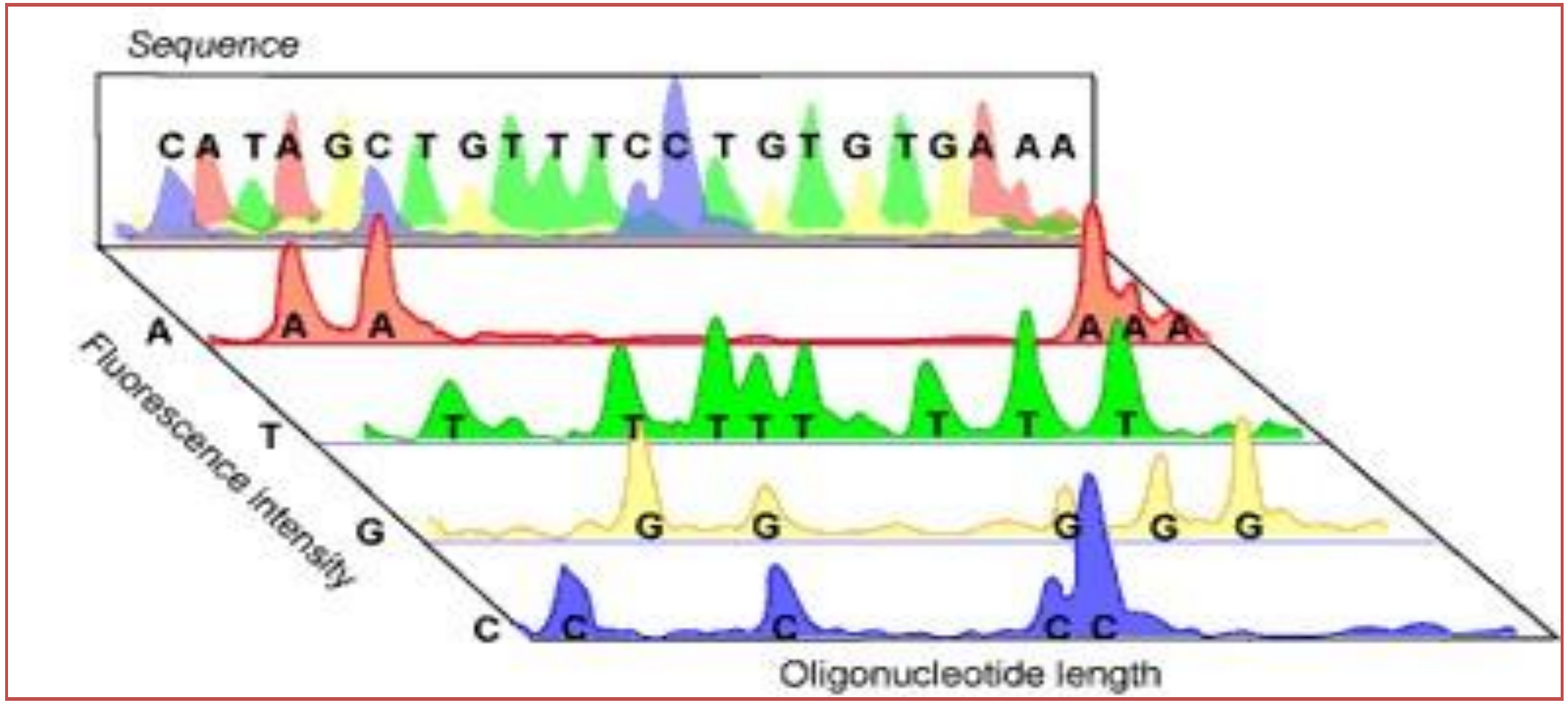



**Figure 2: An Electropherogram of a Sequencing Reaction**

## - For Sequencing:

**A) Purification of the PCR product.**

**B) Sequencing Reaction.**

**C) Post reaction cleanup.**

- The post sequencing reaction product needs to be purified for removal of excess dye terminators and unused primer by using ethanol precipitation protocol.

# - Applications of DNA sequencing:

- Sanger sequencing supports a wide range of DNA sequencing applications.

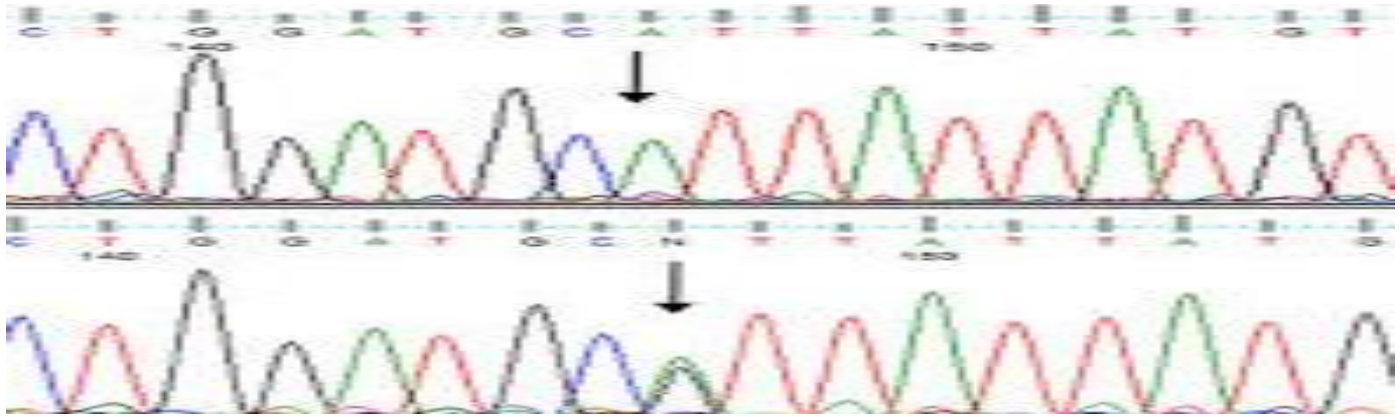
1. Single nucleotide polymorphism (SNP) detection.

2. Single-strand conformation polymorphism (SSCP).

3. Mutations detections.

# - Sequencing Results:

**Heterozygote**



**Homozygote**