

Introduction to DNA Sequencing (Sanger's Method)

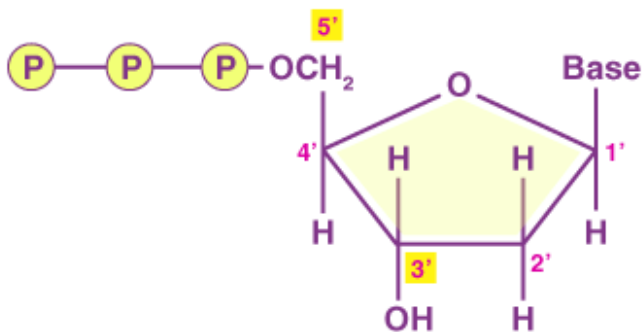
DNA sequencing is the process of determining the exact **order of nucleotides** (A, T, G, C) in a DNA molecule. One of the first and most famous methods for sequencing is **Sanger sequencing**, developed by **Frederick Sanger in 1977**. It is also called the **chain termination method** because it stops DNA synthesis at specific points, allowing scientists to "read" the sequence.

How Sanger Sequencing Works (Simple Steps)

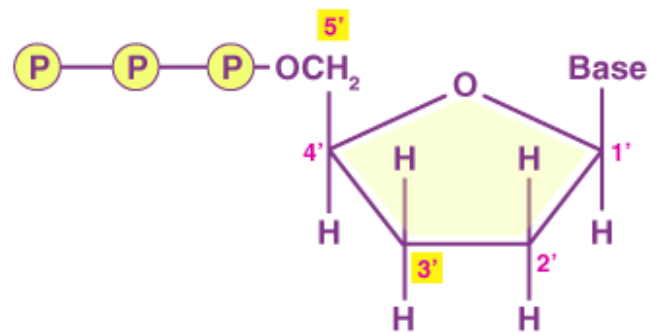
1. Copying the DNA

- A small piece of DNA (the template) is copied using a special enzyme called **DNA polymerase**.
- To do this, scientists mix:
 - The **template DNA** (the one to be sequenced).
 - Normal **nucleotides (dNTPs)** – A, T, G, and C.
 - **Primer** – A short DNA piece that starts the copying process.
 - **Dideoxy nucleotides (ddNTPs)** – Special, modified nucleotides that **stop** the DNA from growing further.

STRUCTURAL DIFFERENCE BETWEEN DNTP AND DDNTP



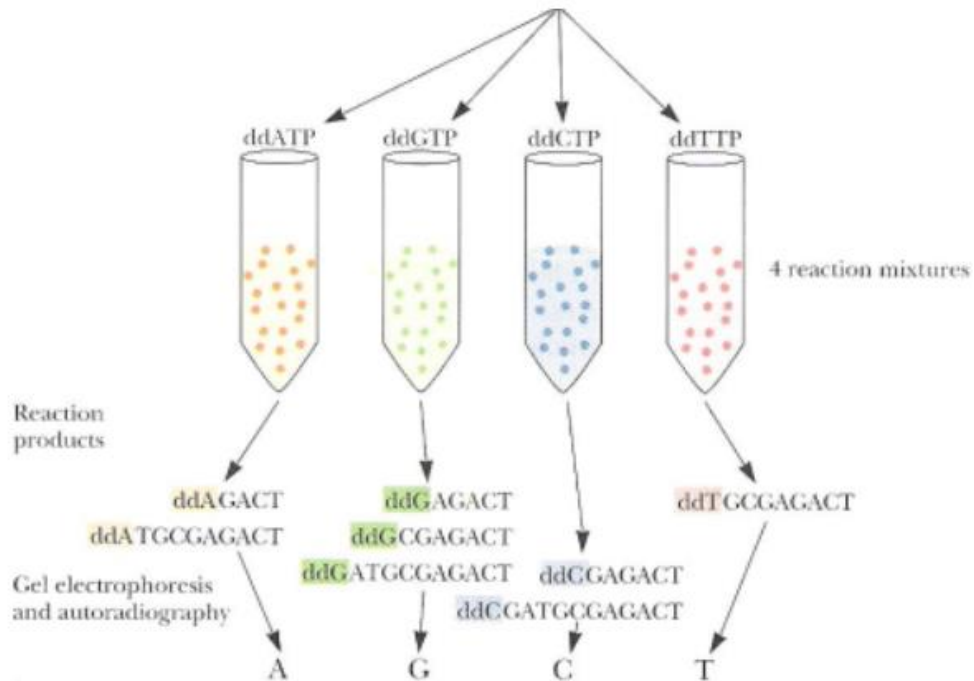
dNTP-deoxynucleotide



ddNTP- dideoxynucleotide

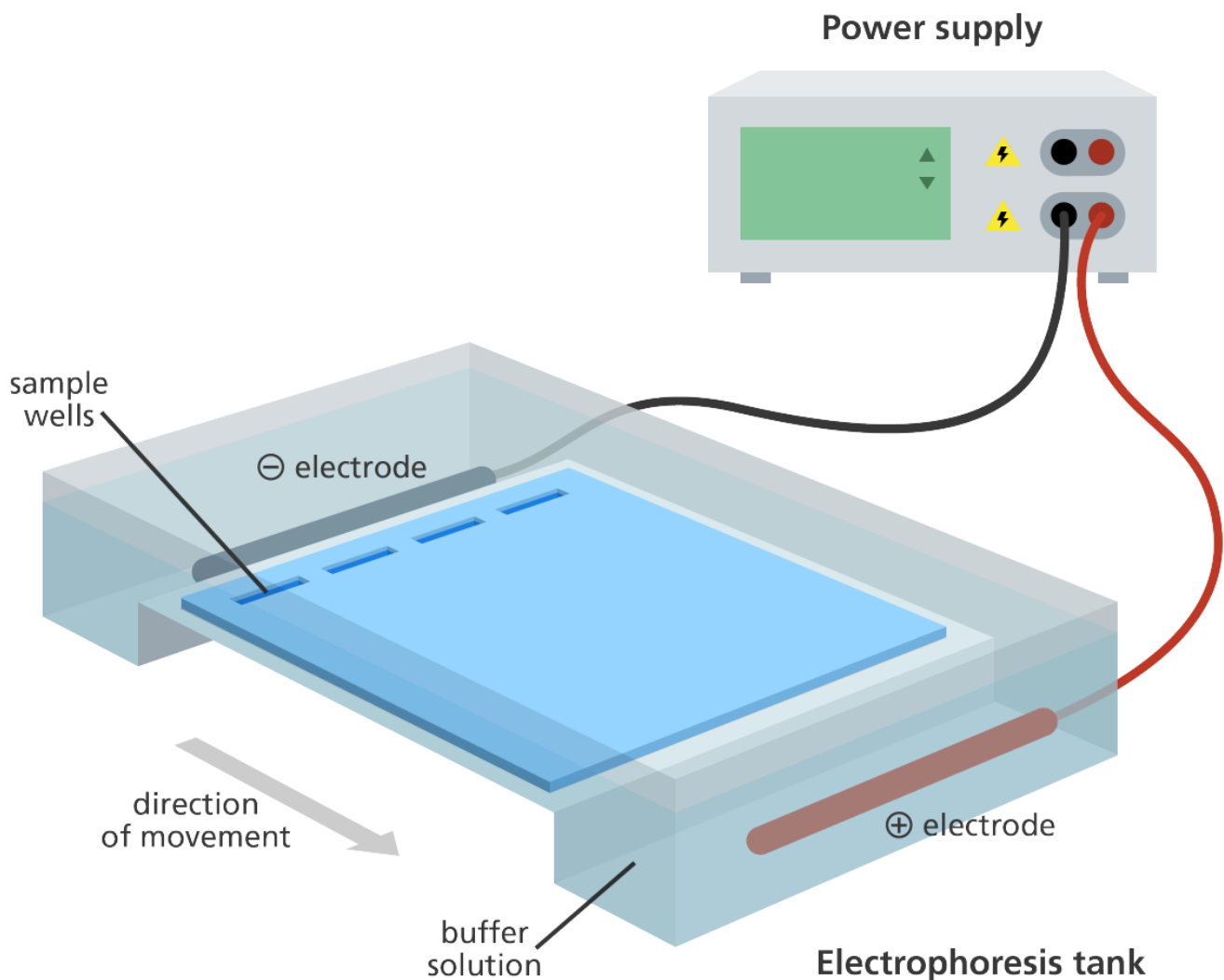
2. Randomly Stopping DNA Copying

- When a **ddNTP** is added to the new DNA strand, it **stops growing** because it lacks a 3'-OH group needed for elongation.
- Since ddNTPs are added randomly, the process creates **many DNA fragments of different lengths**.



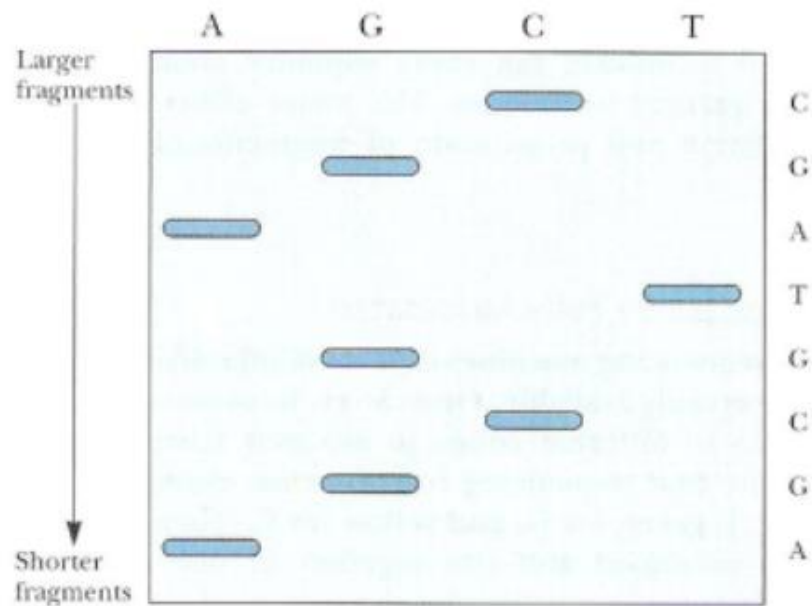
3. Sorting DNA Fragments by Size

- The mixture is run through a gel (**gel electrophoresis**) or a capillary system.
- Smaller fragments move **faster**, and larger ones move **slower**.



4. Reading the DNA Sequence

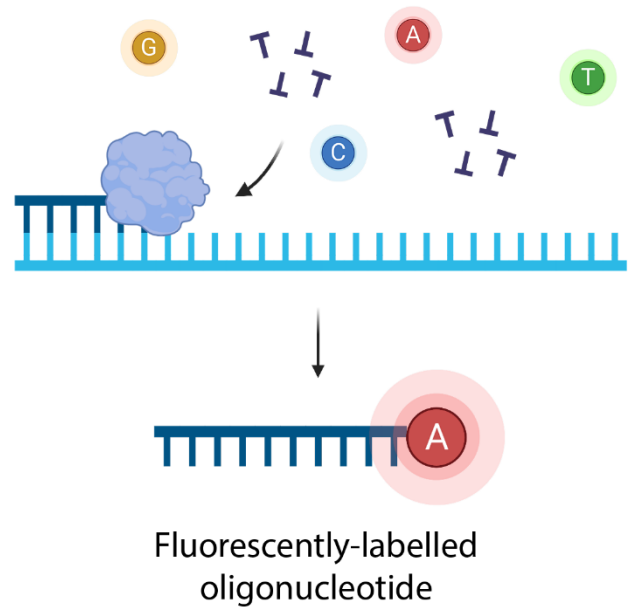
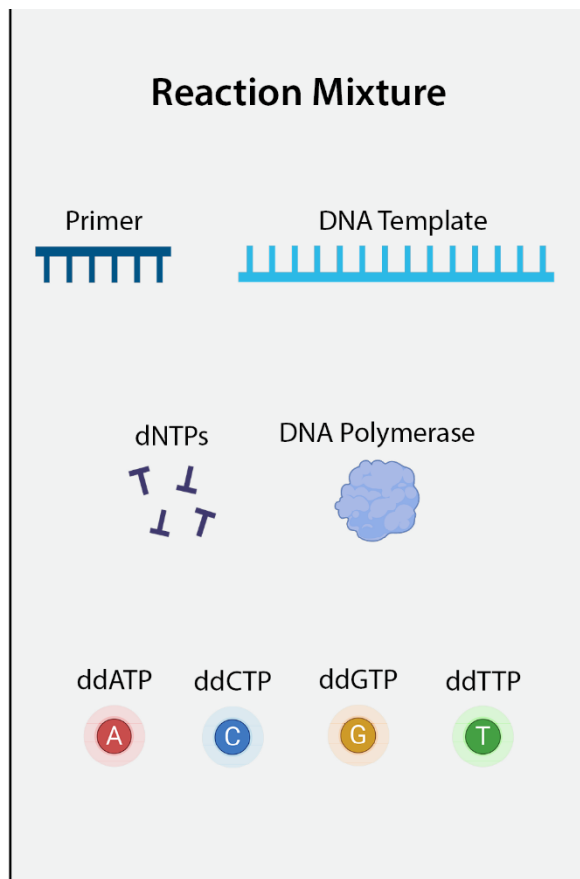
- Each ddNTP is labeled with a **fluorescent dye** (different color for A, T, G, and C).
- A laser detects the color of each fragment, helping computers reconstruct the DNA sequence.



Why Sanger Sequencing is Important?

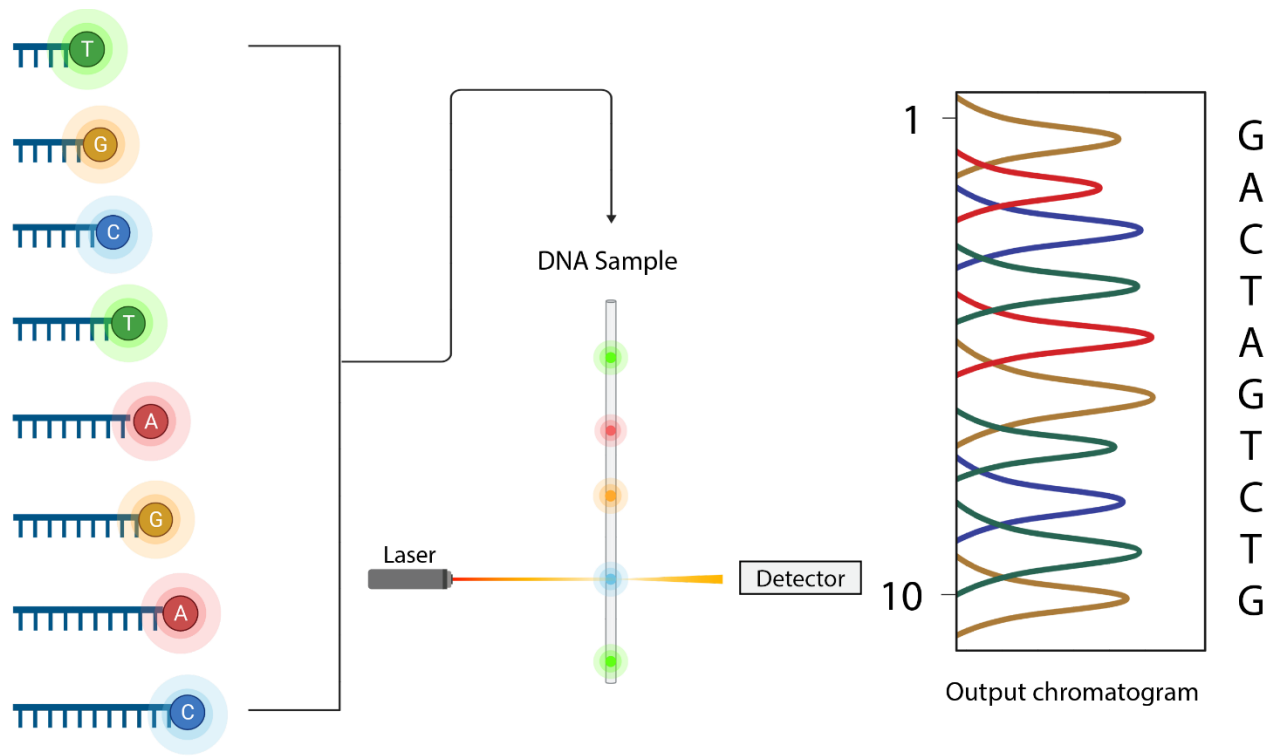
- It was the first method used to **sequence entire genomes**, including the **human genome project**.
- It is still used today for **small-scale sequencing** (e.g., sequencing a single gene).
- It is highly **accurate and reliable**.

Sanger Sequencing



1

Chain-termination PCR using fluorescent ddNTPs



2

Size separation and sequence analysis using capillary gel electrophoresis and fluorescence detection