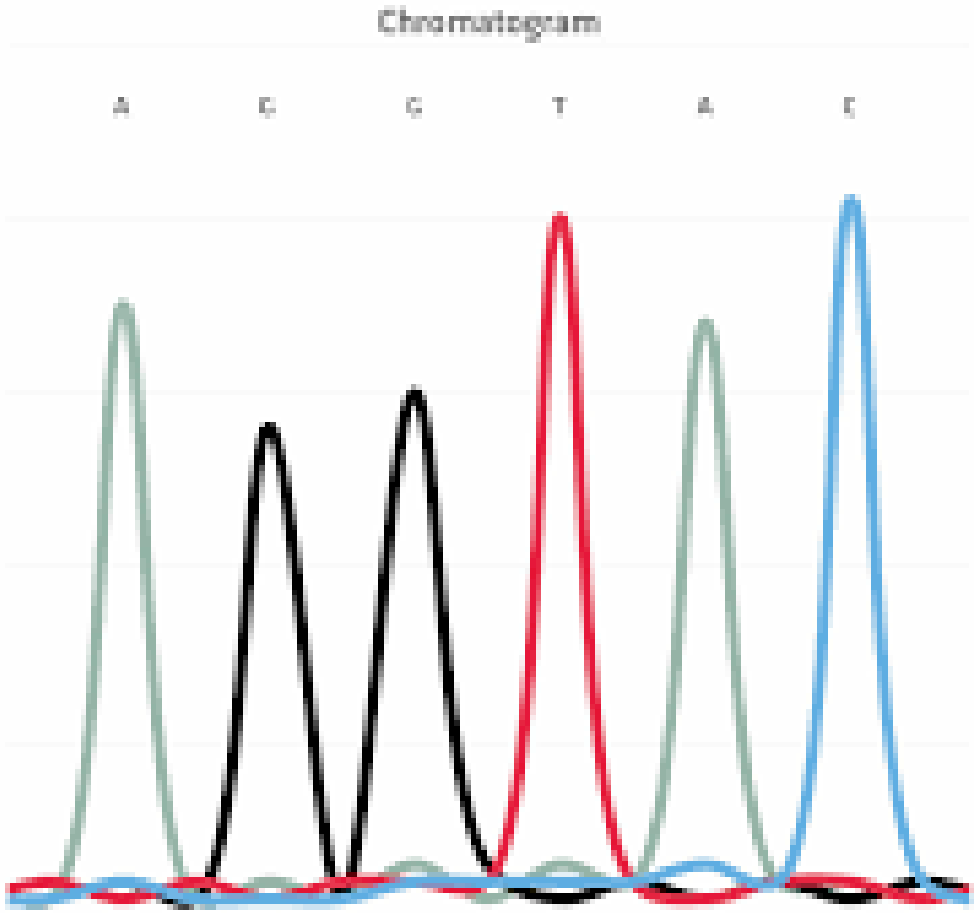
# Sanger Sequencing




*The Gold Standard in DNA Sequencing…*

*When have a doubt just sequence it out…*

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## Outlines:

-Introduction/Background

-Principles/Key Components

-Procedure/Steps

-Applications, Advantages, Limitations, Conclusion

# DNA Sequencing:

-Determining the exact sequence of nucleotides, or bases, in a DNA molecule.

- **A sequencing can be done by different methods including:**

1. Maxam – Gilbert sequencing (chemical degradation method). (1977)
2. Sanger sequencing (dideoxy chain-termination method). (1977)
3. High-throughput sequencing technologies (NGS). (2000)

## -What is Sanger Sequencing?

1-Method to determine nucleotide sequence of DNA.

2-Relies on chain-terminating dideoxynucleotides (ddNTPs).

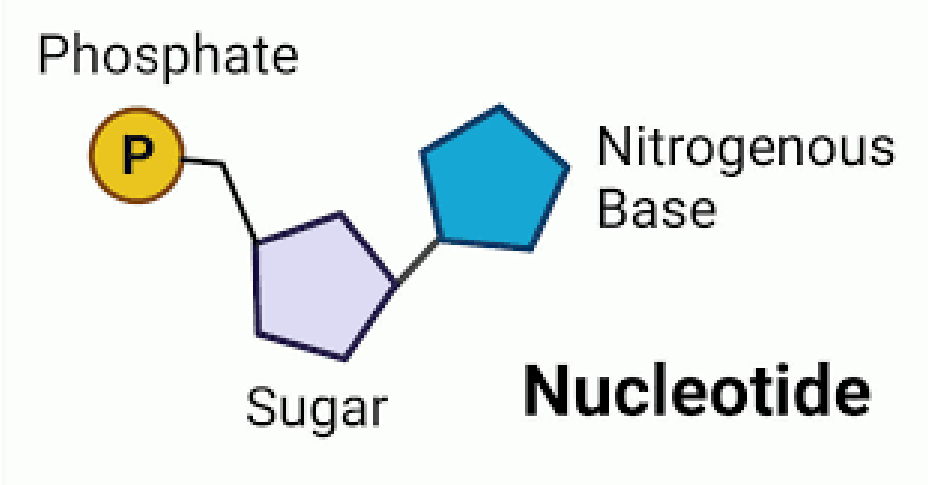
## -Historical Context:

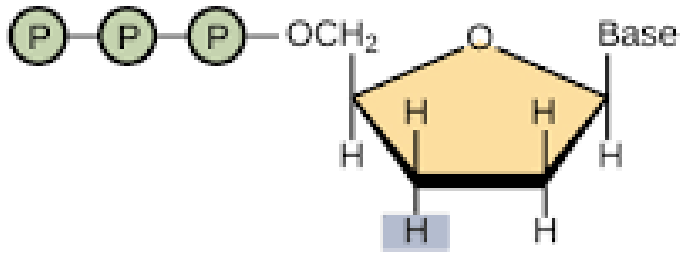
1-Developed by Frederick Sanger (1977).

2-Key role in the Human Genome Project (1990).

## -Significance:

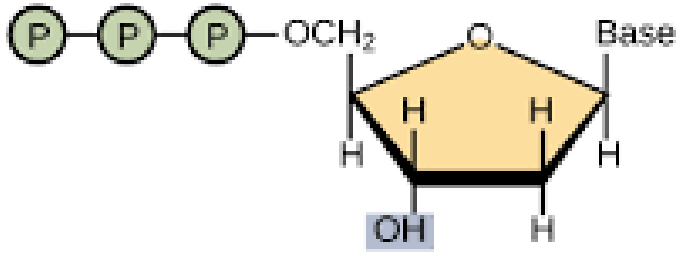
High accuracy; remains a benchmark for validating genetic data.




Dideoxynucleotide (ddNTP)

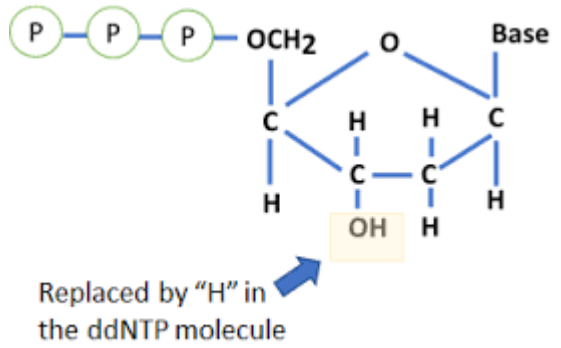
Sanger




Deoxynucleotide (dNTP)

DNA

## Key Principles




**-Chain Termination:**

ddNTPs lack 3'-OH group, halting DNA synthesis.

**-Components:**

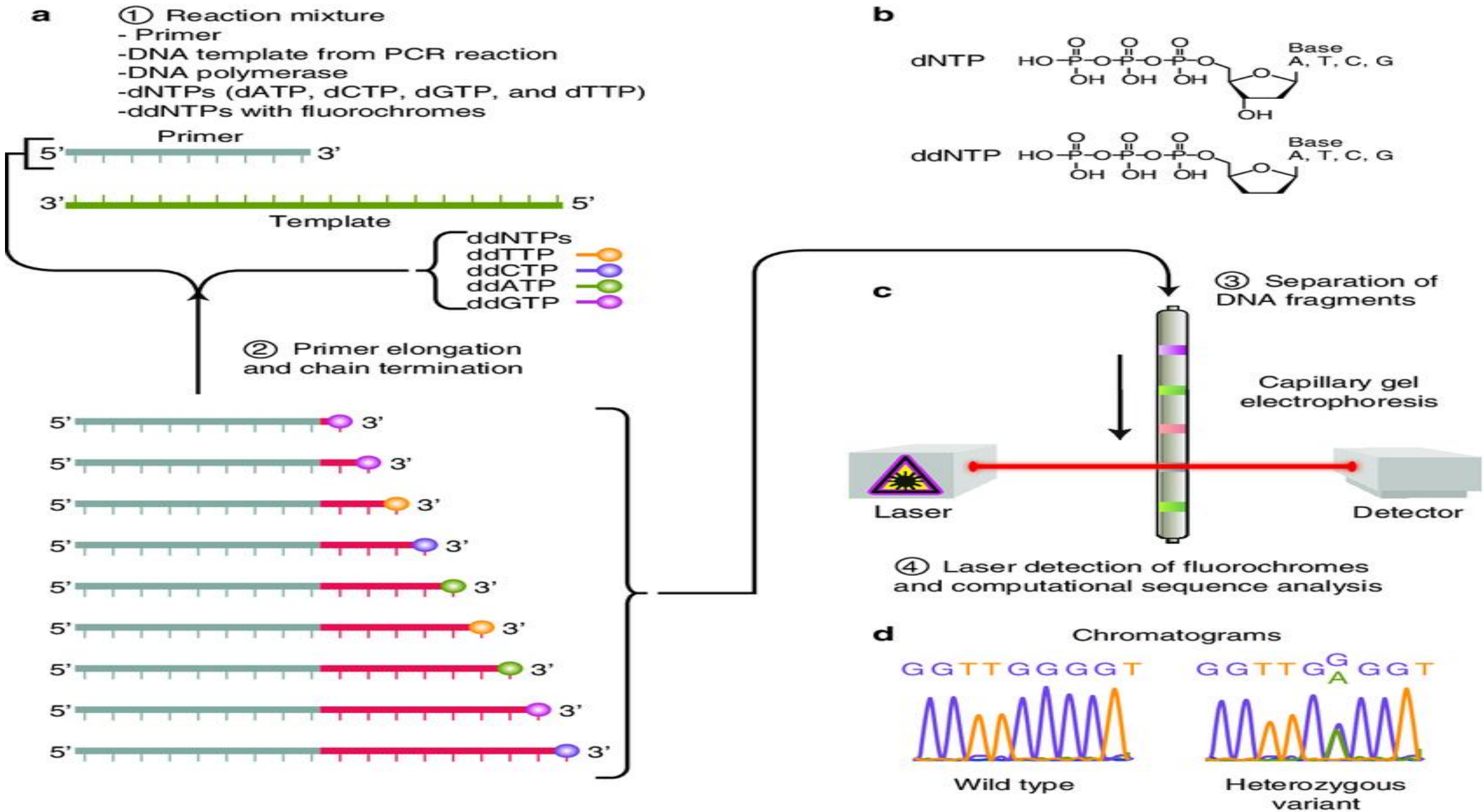
DNA template, primer, DNA polymerase, dNTPs, fluorescently labeled ddNTPs.

**-Detection:**

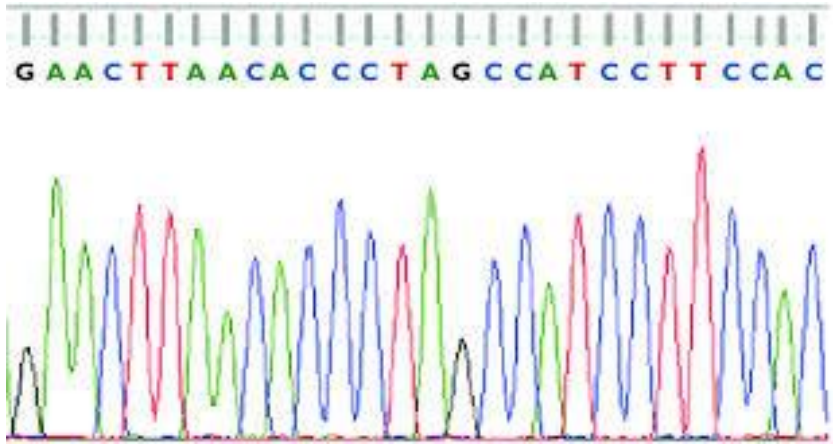
Fluorescent tags on ddNTPs (each base with a unique color). Laser beam

**-Separation:**

Capillary electrophoresis sorts DNA fragments by size.


a
① Reaction mixture
- Primer
-DNA template from PCR reaction
-DNA polymerase
-dNTPs (dATP, dCTP, dGTP, and dTTP)
-ddNTPs with fluorochromes
Primer
5'
3'
3'
5'
Template
ddNTPs
ddTTP
ddCTP
ddATP
ddGTP
② Primer elongation
and chain termination
b
dNTP
ddNTP
Base
A, T, C, G
c
③ Separation of
DNA fragments
Capillary gel
electrophoresis
Laser
Detector
④ Laser detection of fluorochromes
and computational sequence analysis
d
Chromatograms
GGTTGGGGT
GGTTGGGT
Wild type
Heterozygous
variant

# Procedure Overview




**-Template Preparation:**

Isolate target DNA.

**-Cycle Sequencing Reaction:**

Linear amplification with ddNTPs and dNTPs.

**-Denaturation:**

Separate DNA strands.

**-Electrophoresis:**

Fragments separated by size in a capillary tube.

**-Detection & Analysis:**

Laser excites fluorescent tags; software generates chromatogram.

## Applications, Pros/Cons, and Conclusion

**-Applications:**

Genetic disease diagnosis, forensic analysis, microbial ID, mutation detection.

**-Advantages:**

High accuracy (~99.99%), reliable for short reads (up to 1,000 bp).

**-Limitations:**

Low throughput, costly/time-consuming for large genomes.

**-Conclusion:**

Still vital for small-scale projects and validating the (NGS) results.

| | Sanger Sequencing | NGS |
|---|---|---|
| Accuracy | 99.9% | 99-99.9% |
| Cost Effectiveness | < 20 samples | > 20 samples |
| Speed < 20 Samples | Fast | Slow |
| Speed >20 Samples | Slow | Fast |
| Sensitivity | 15-20% | 1% |
| Sample Coverage | 1 Read/Sample (300-850 bp) | Billions of Reads/Sample (Up to 16 Tera bite) |