Sanger Sequencing
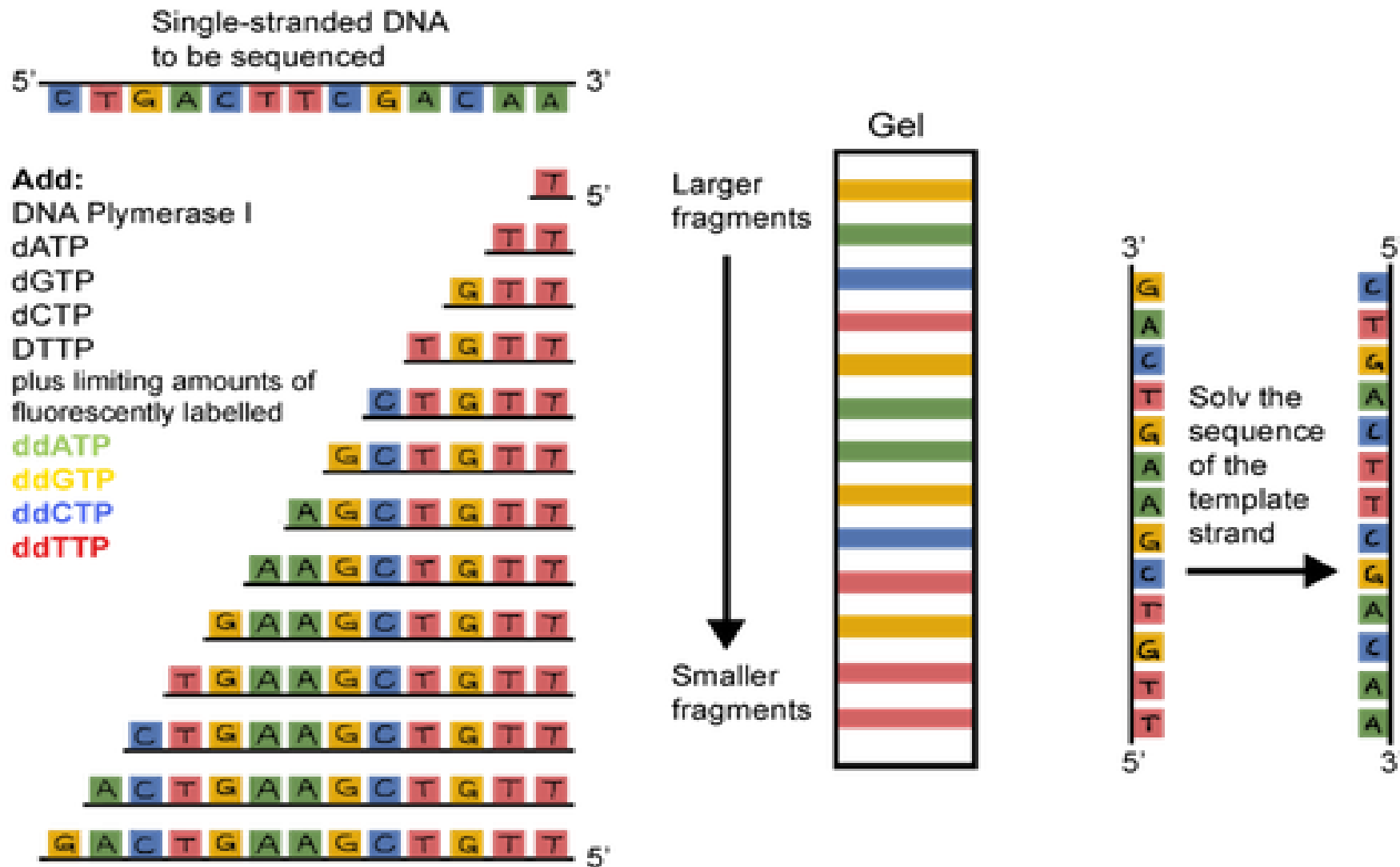
DNA sequencing:

- The term DNA sequencing refers to …..

- Application?

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

1. Maxam – Gilbert sequencing (chemical degradation method).

2. Sanger sequencing (dideoxy chain-termination method).

3. High- throughput sequencing technologies.

Dideoxy Chain Termination DNA Sequencing

Single-stranded DNA to be sequenced

5' C T G A C T T C G A C A A 3'

Add:
DNA Plymerase I
dATP
dGTP
dCTP
DTTP
plus limiting amounts of fluorescently labelled
ddATP
ddGTP
ddCTP
ddTTP

Gel

Larger fragments

Smaller fragments

Solv the sequence of the template strand

Why the reaction terminated by the ddNTPs (dideoxynucleosides) and cannot be continued?

ddNTPs terminate DNA synthesis.

Normal dNTP (extends DNA strand)
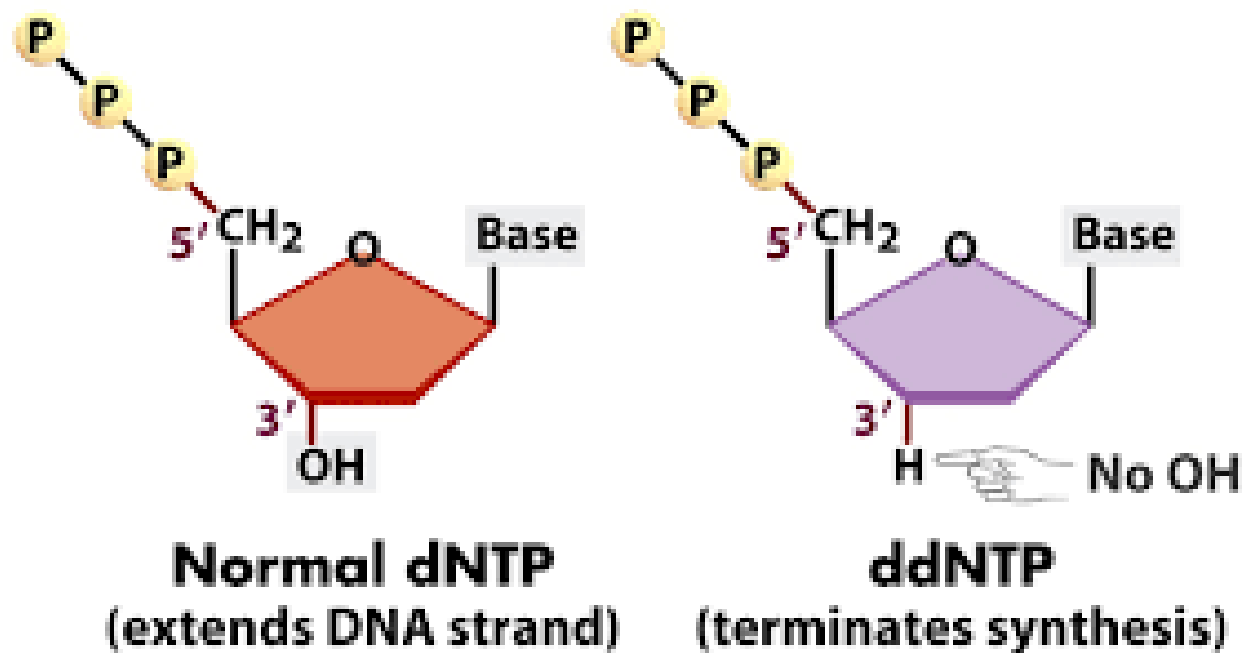
ddNTP (terminates synthesis)

Principle of automated Sanger method:

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

- Different colored DNA fragments are generated.

- Separated **by size** in an electrophoretic gel.

- Color associated with each band is detected with a laser beam.

- The amount of fluorescence in each band is represented as a peak in the computer output.
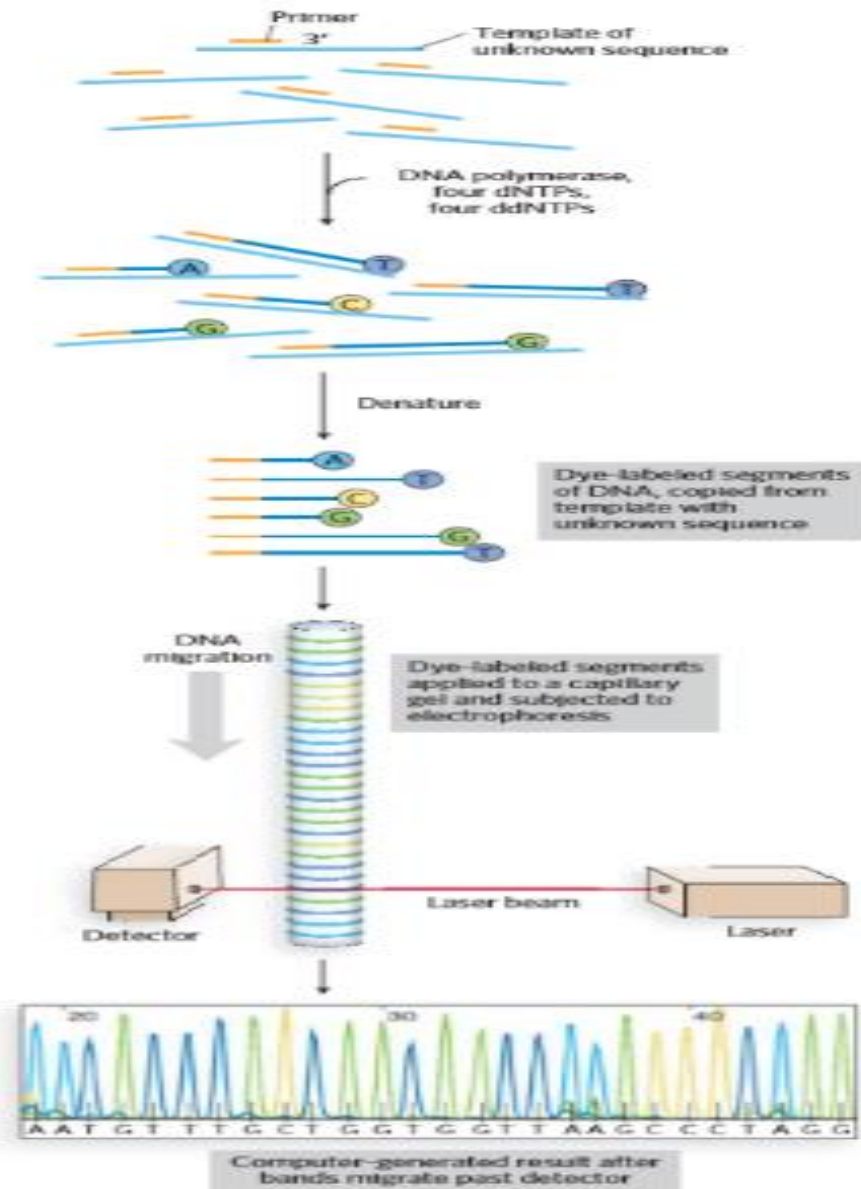
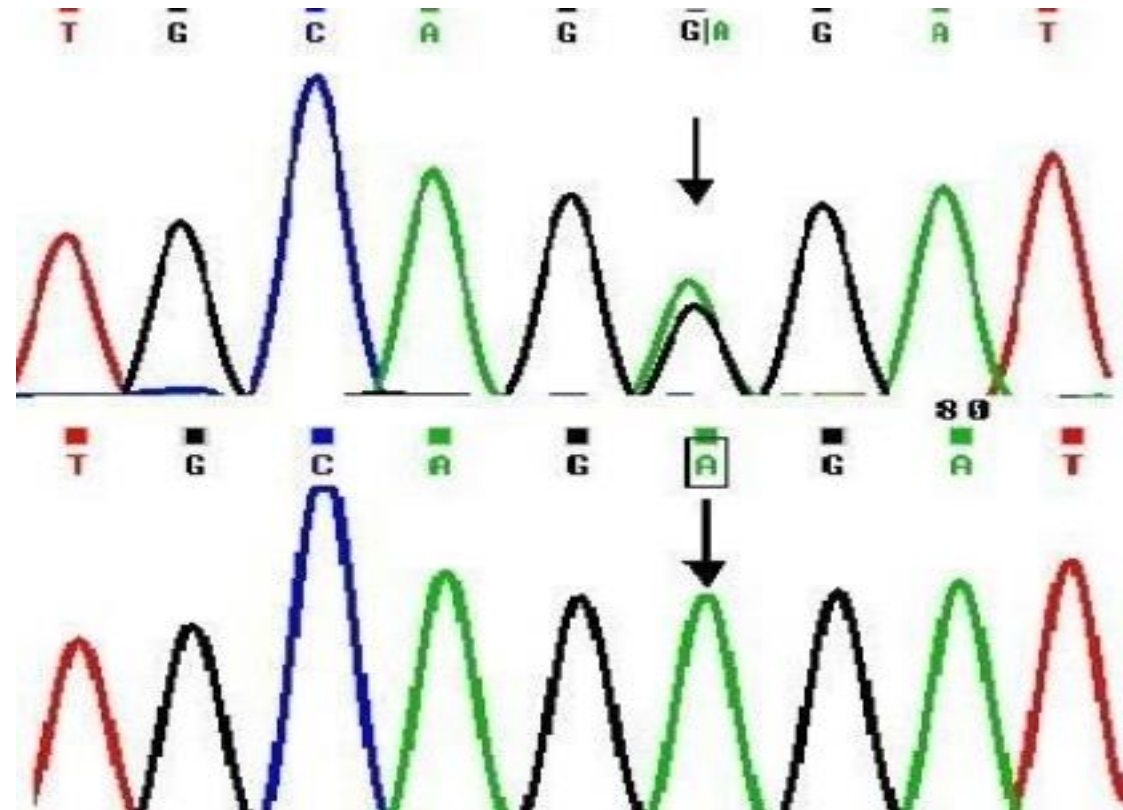# Electropherogram of a Sequencing Reaction
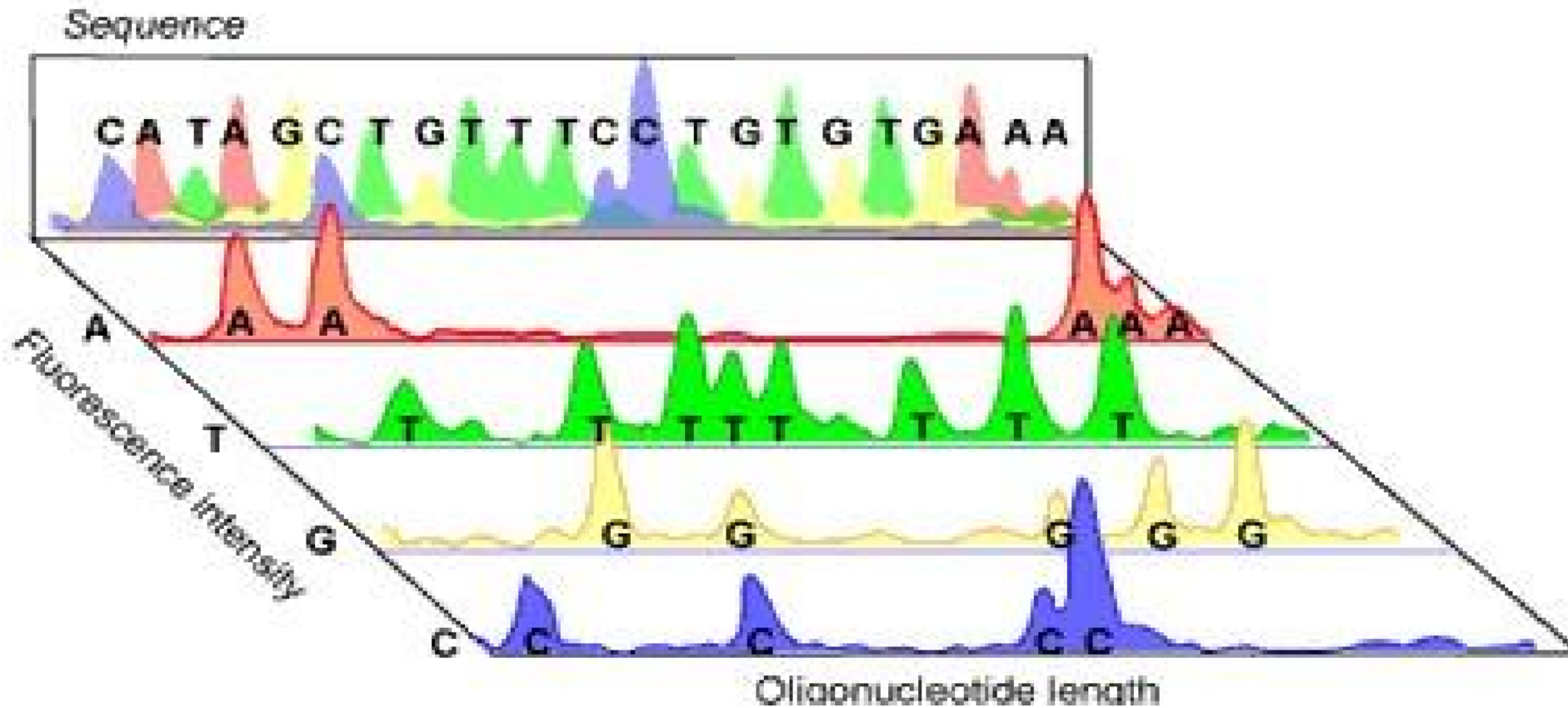
Sanger sequencing workflow:
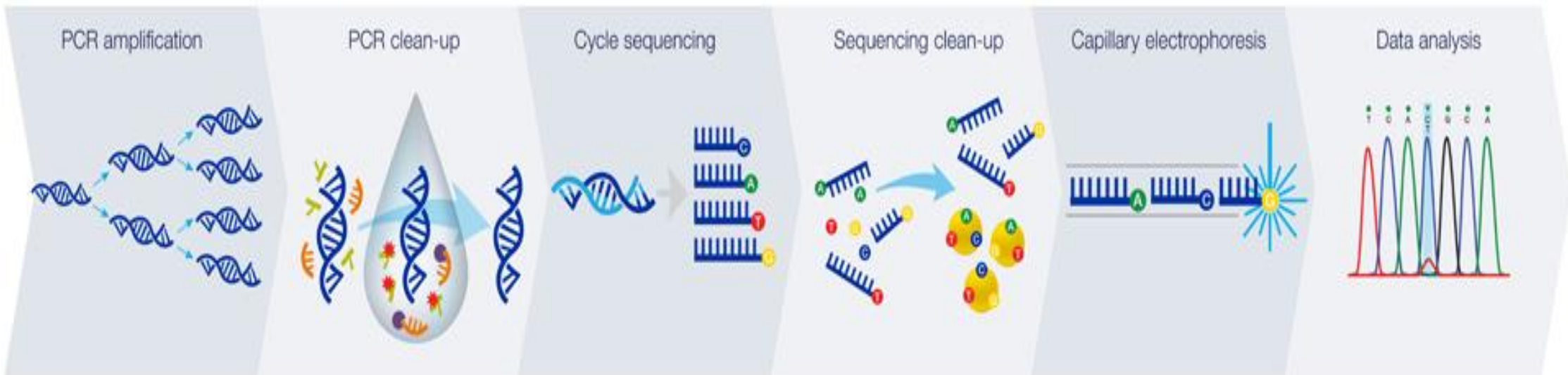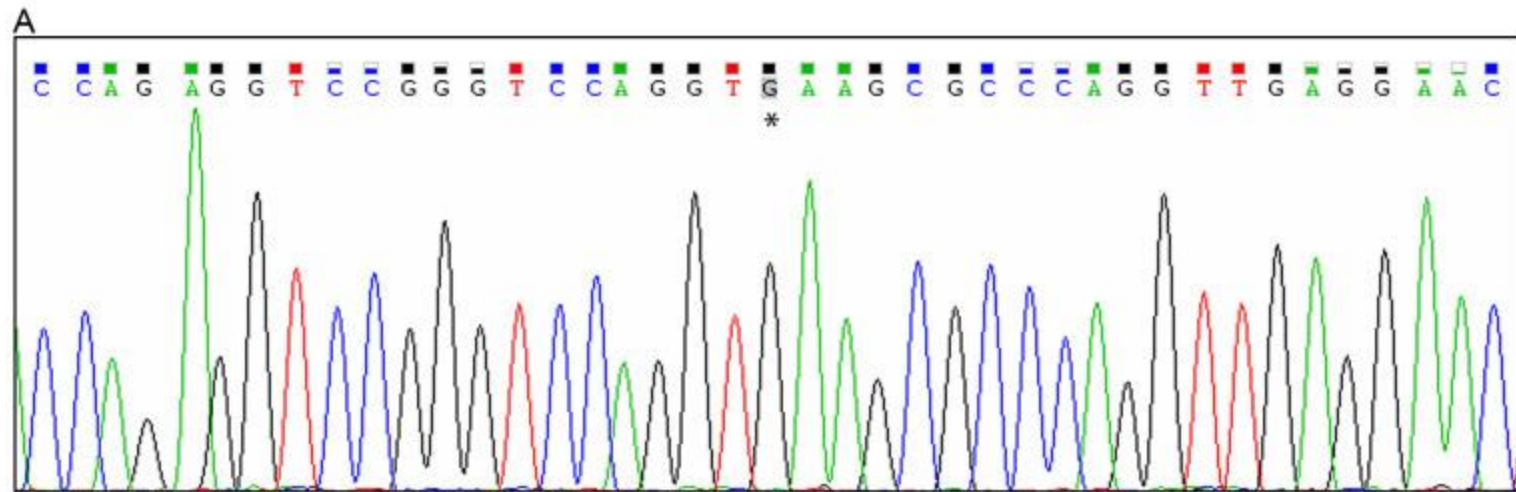
Sequencing results:
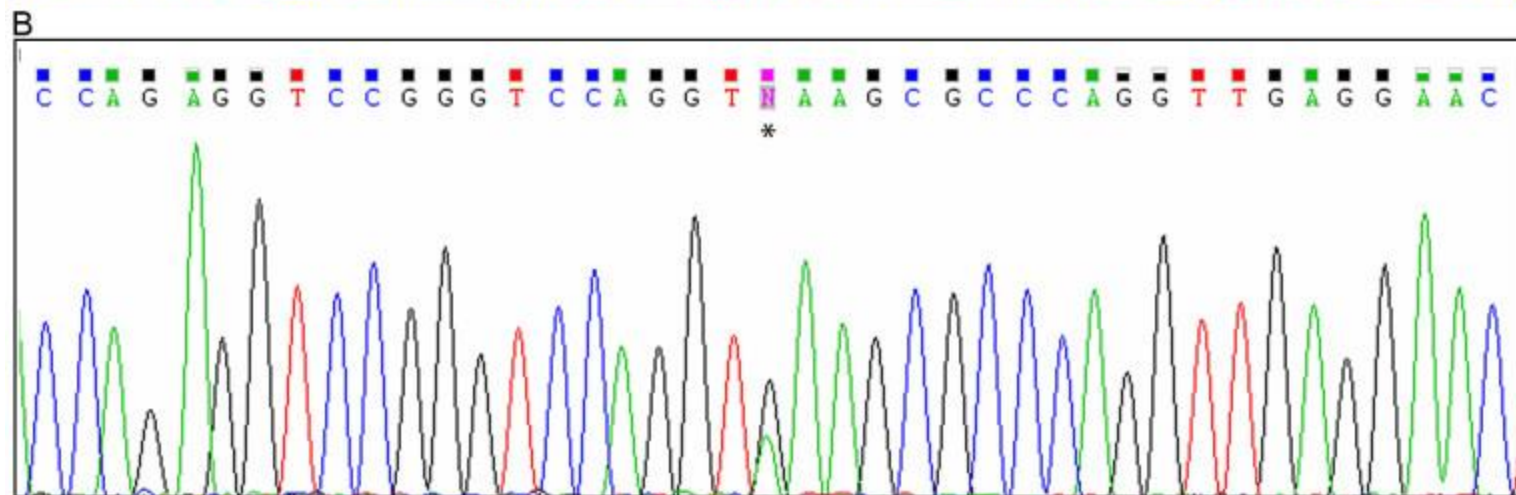
Heterozygous

Homozygous

Sequencing results:

Homozygous

Heterozygous

Sanger sequencing application:

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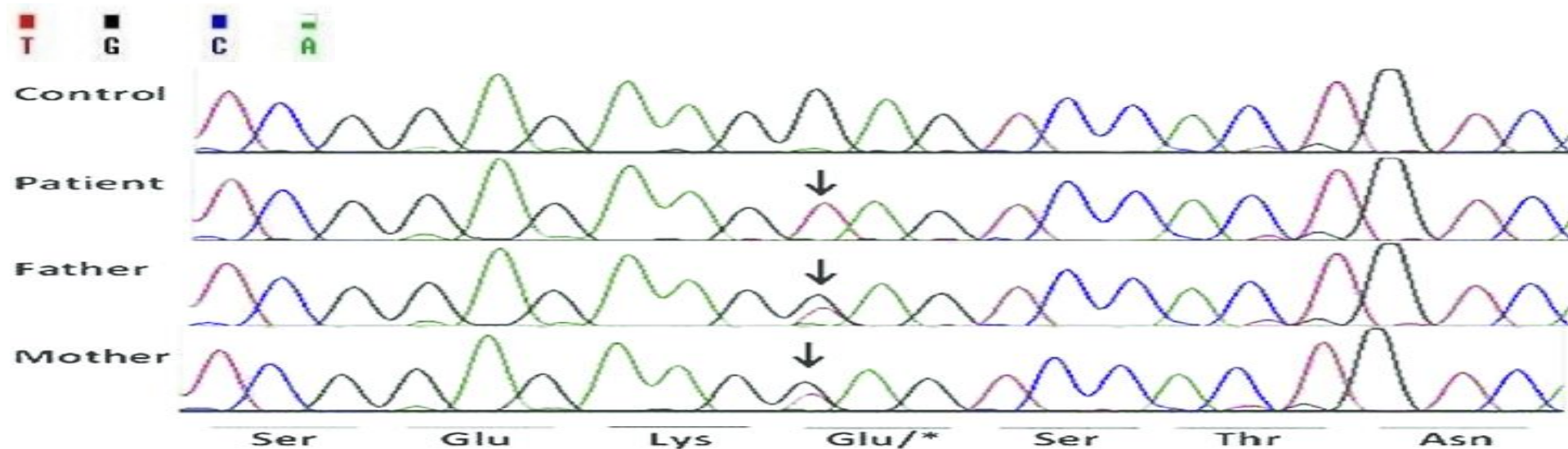3. Mutations detections.

Supporting vedio:

Automated

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

At time 0.13 then 1.13

Manual:

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

Below is electropherogram for exon 7 of the PLCE1 gene (phospholipase C, epsilon1) in a patient and his parents.

1) What is the nucleotide sequence of the patient?
2) What is the difference in the sequence between the patient and his parents in the position indicated by arrows?
3) Does the patient has an identical alleles for the gene? Why?
4) What is the zygosity of the parents genotype?
5) What type of mutation does the patient has?

Home Work:

• **Watch this useful video:**

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

Then explain by your words, how manual sanger sequencing works.