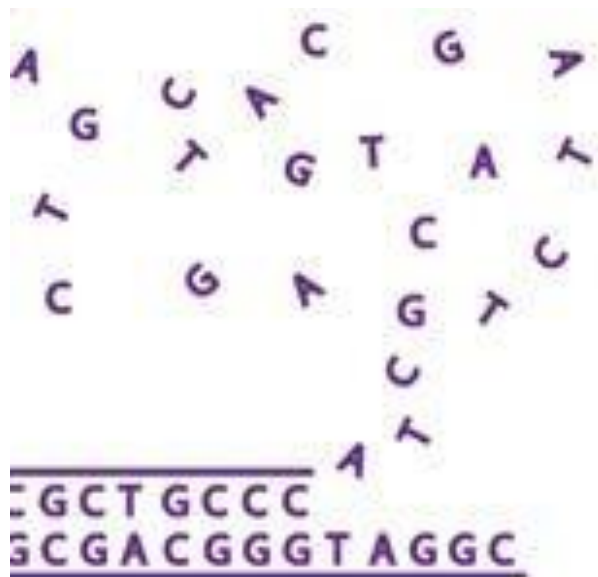


# Sanger Sequencing

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BCH361- Practical



# DNA sequencing:

- The term DNA sequencing refers to .....

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

Maxam – Gilbert sequencing (chemical degradation method).

Sanger sequencing (dideoxy chain-termination method).

High- throughput sequencing technologies.

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

ddNTPs terminate DNA synthesis.

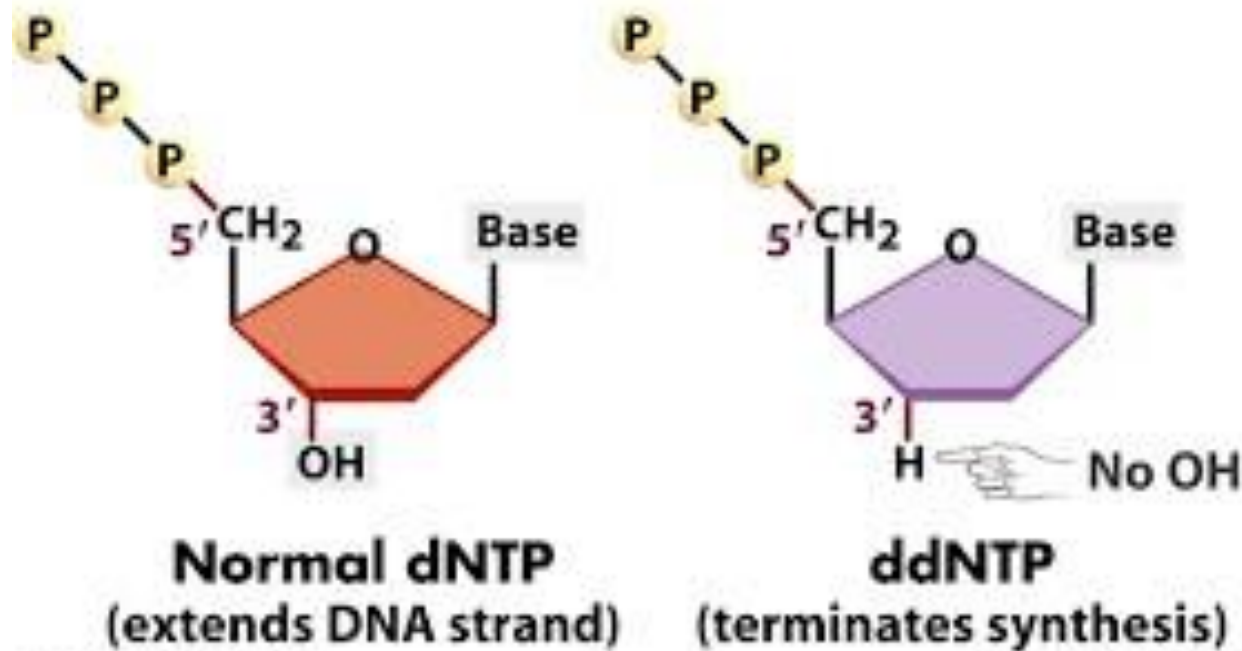
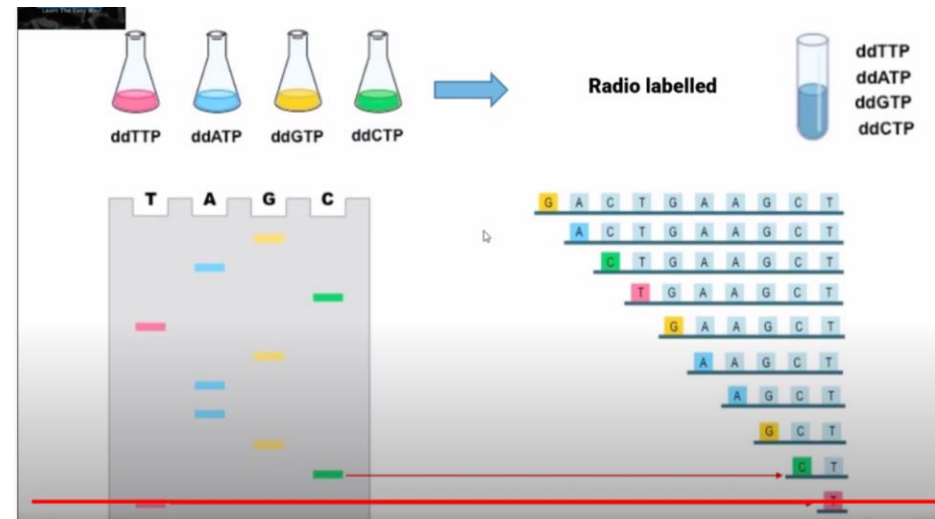
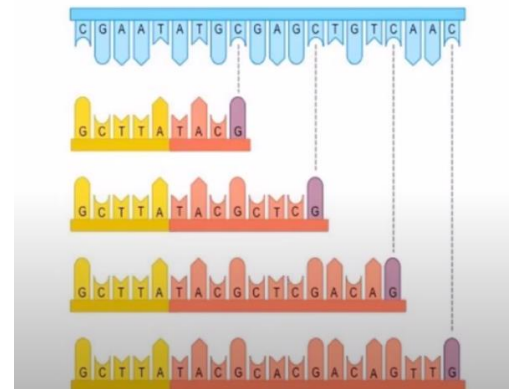
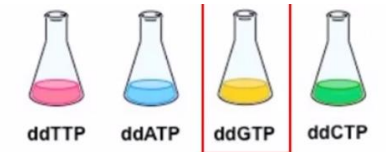
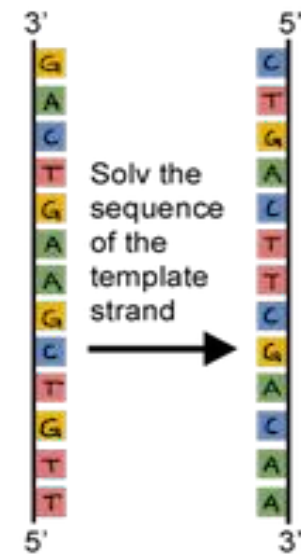
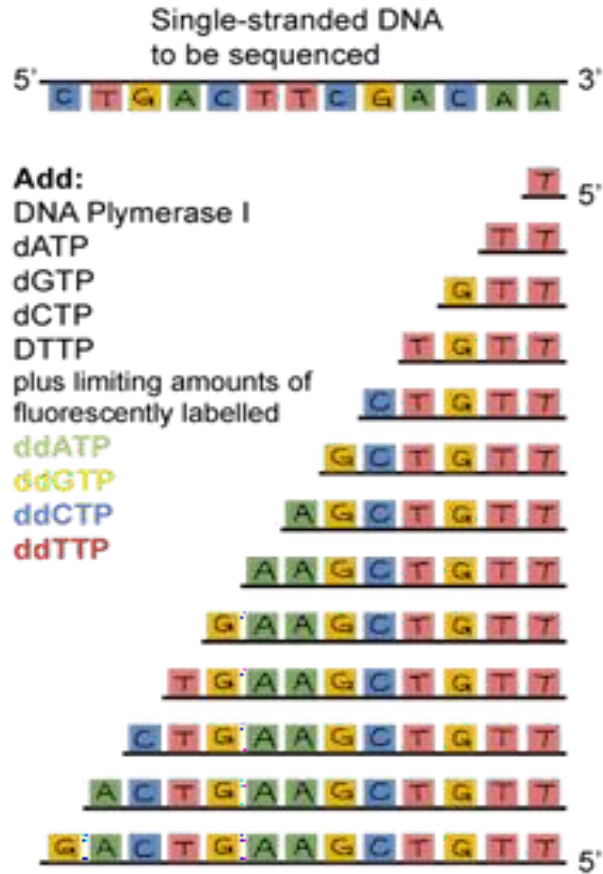


Figure 10-6a Biological Science, 2/e

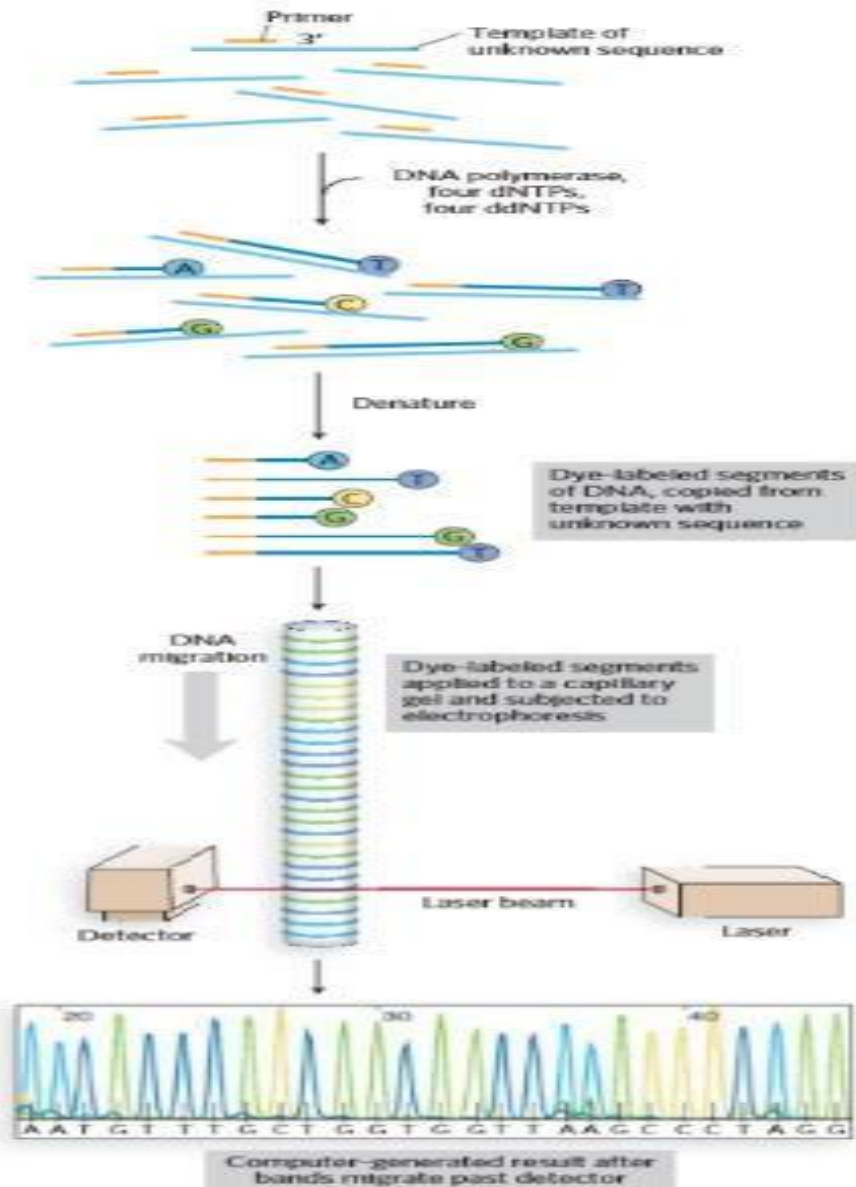
© 2001 Pearson Prentice Hall, Inc.

# Dideoxy Chain Termination DNA Sequencing

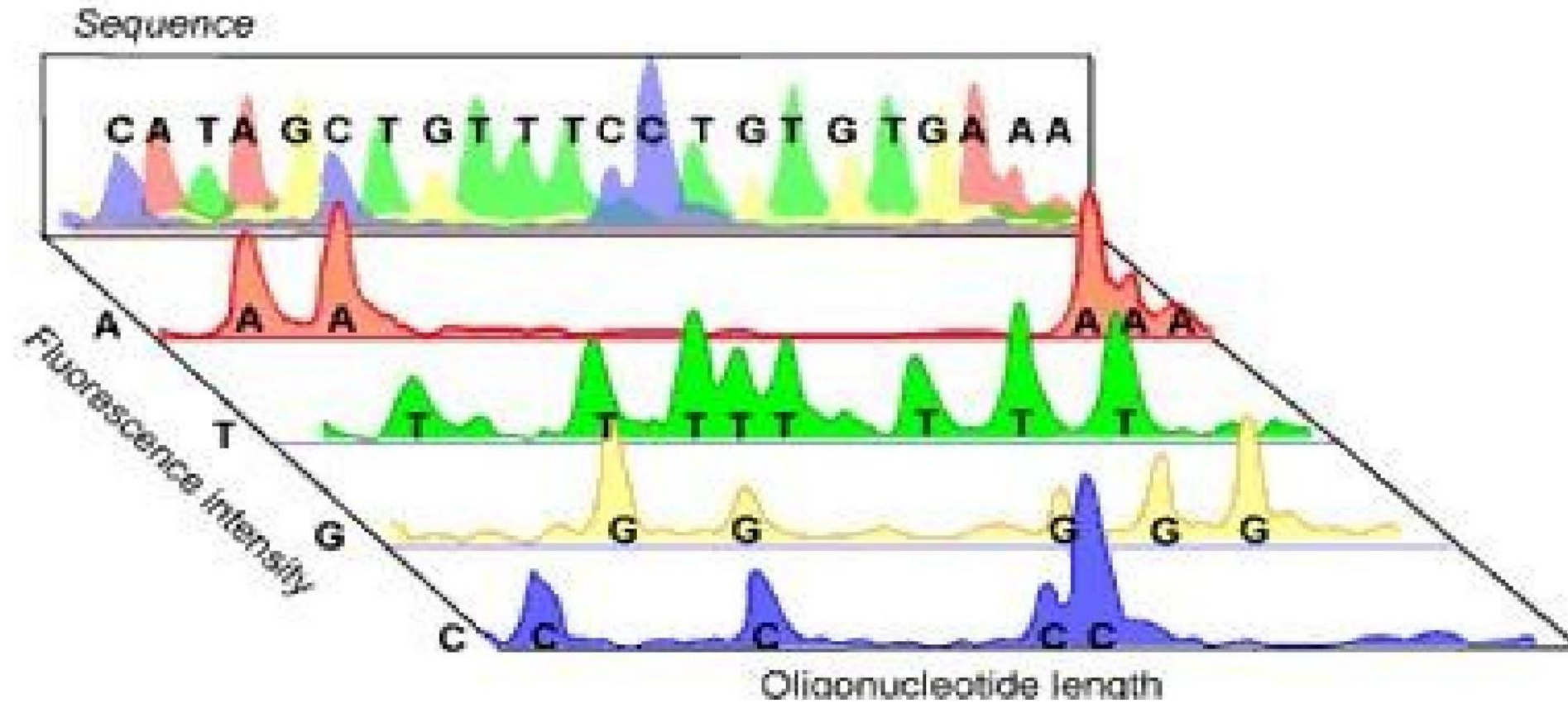


# Principle of automated Sanger method:

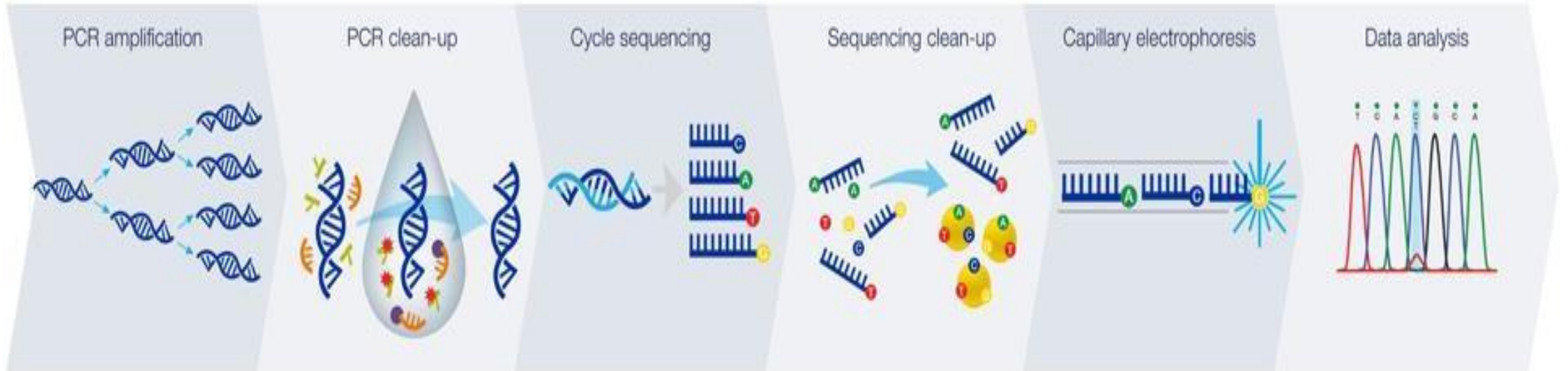
- ddNTPs are tagged with different colored fluorescent dyes (**green, blue, red and yellow**).
- Different colored DNA fragments are generated.
- Separated **by size** in an electrophoretic gel.
- Color associated with each band is detected with a laser beam.
- The amount of fluorescence in each band is represented as a peak in the computer output.



# Electropherogram of a Sequencing Reaction

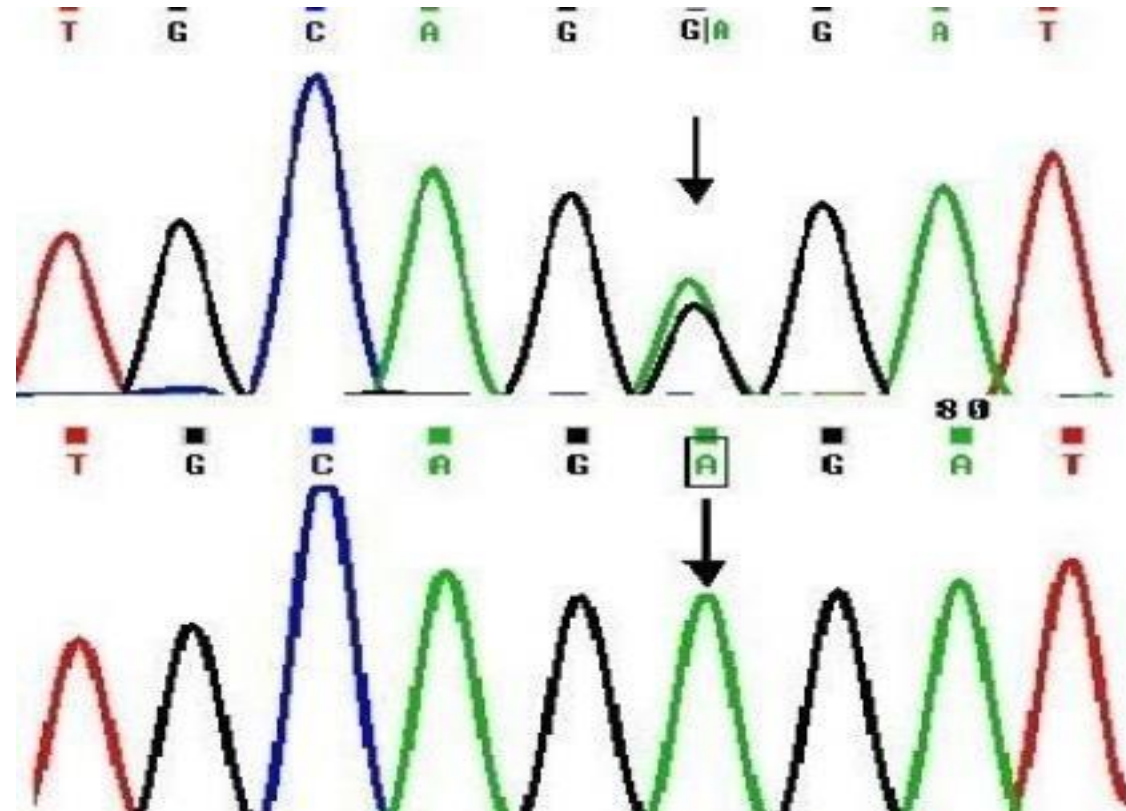


# Sanger sequencing workflow:



# Sequencing results:

Heterozygous

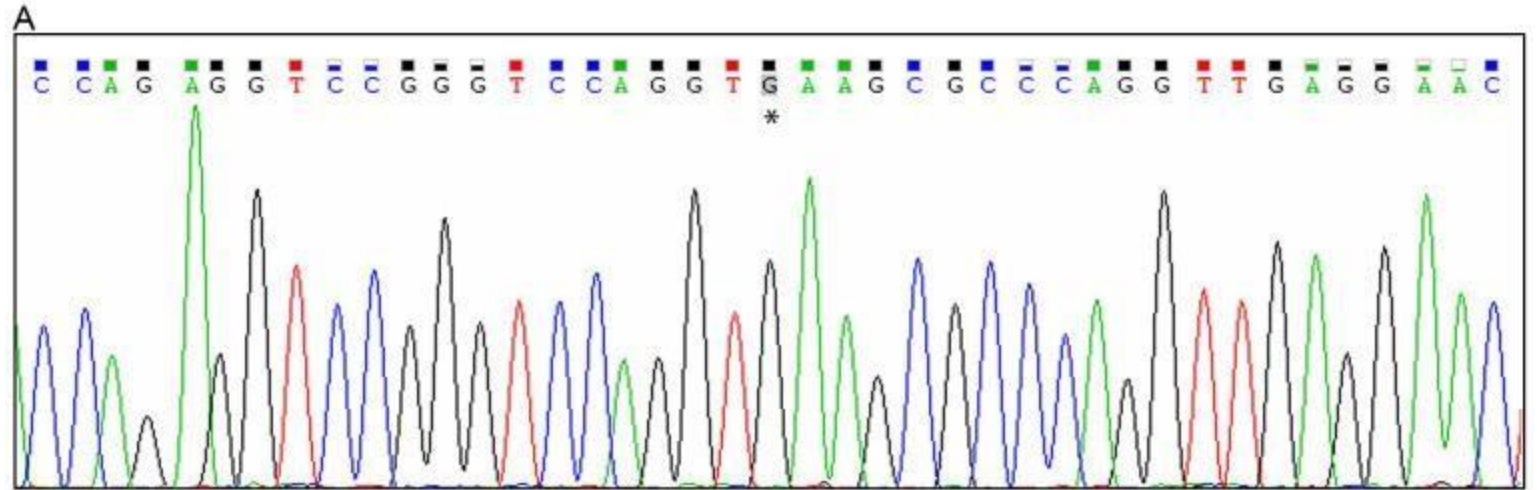


Homozygous

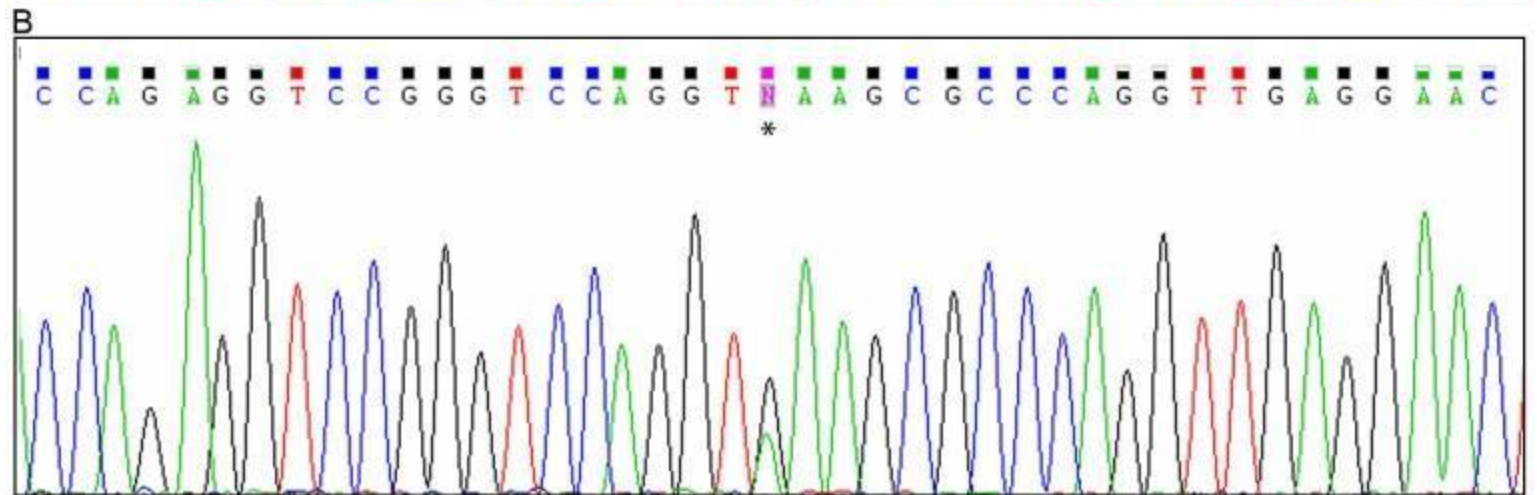


# Sequencing results:

**Homozygous**



**Heterozygous**



# Sanger sequencing application:

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

## Useful videos:

Manual sanger sequencing:

<https://youtu.be/FvHRio1yyhQ>

Automated sanger sequencing:

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

Watch this useful video:

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

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