

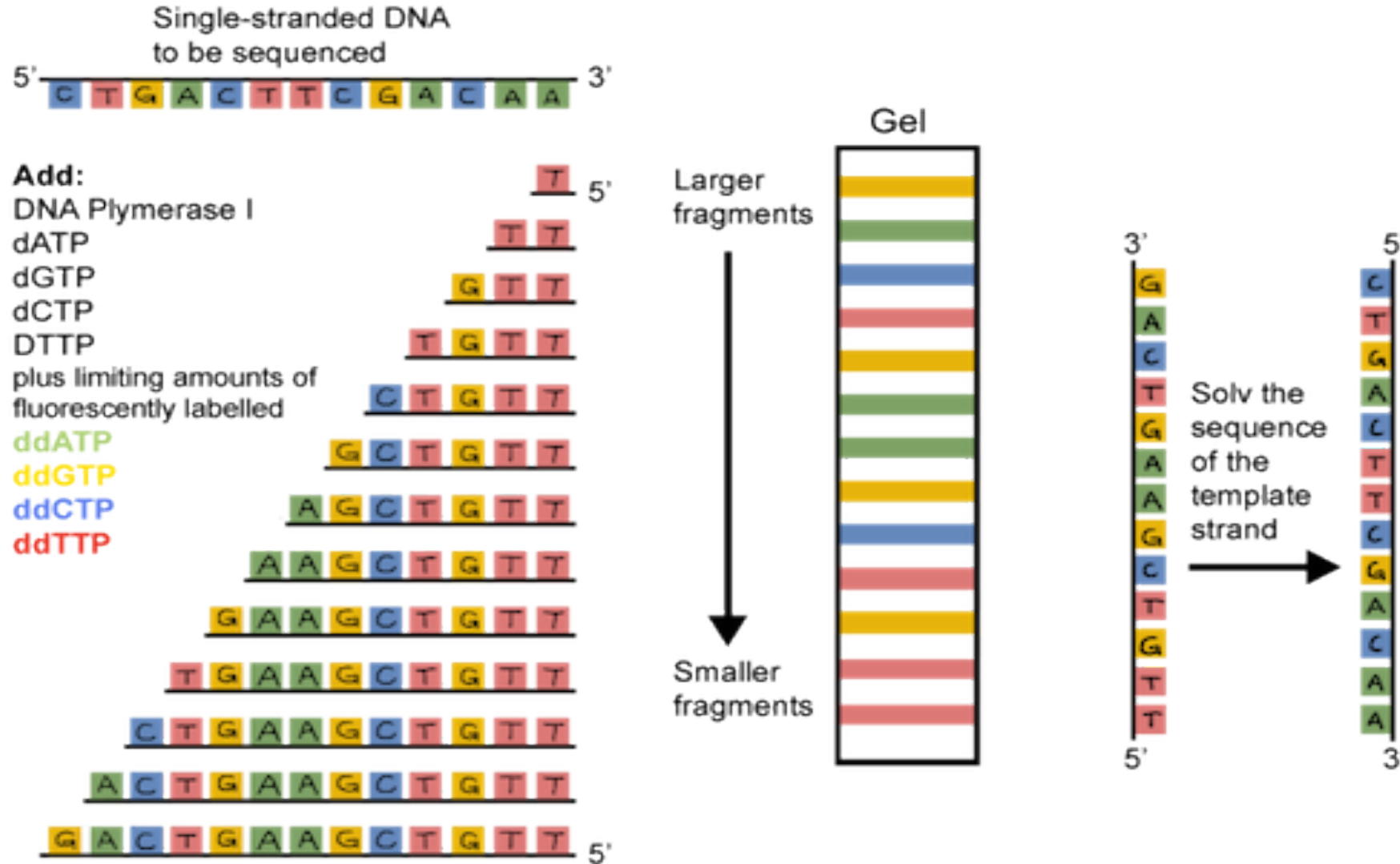
Sanger Sequencing



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



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

ddNTPs terminate DNA synthesis.

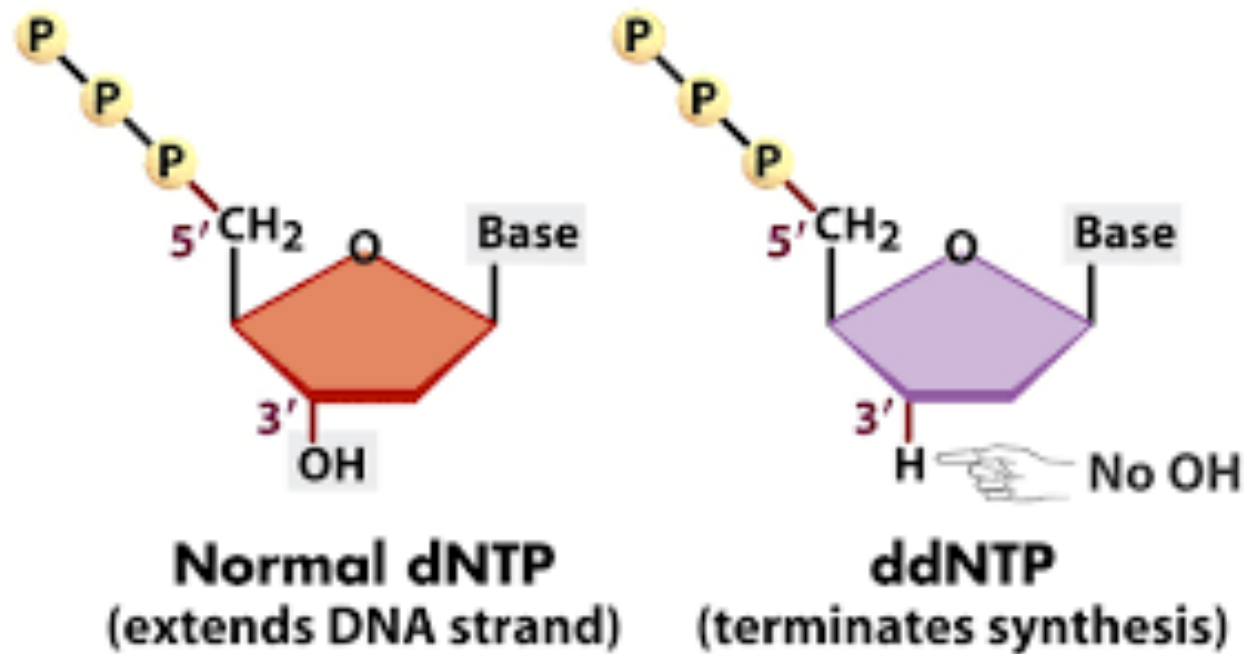
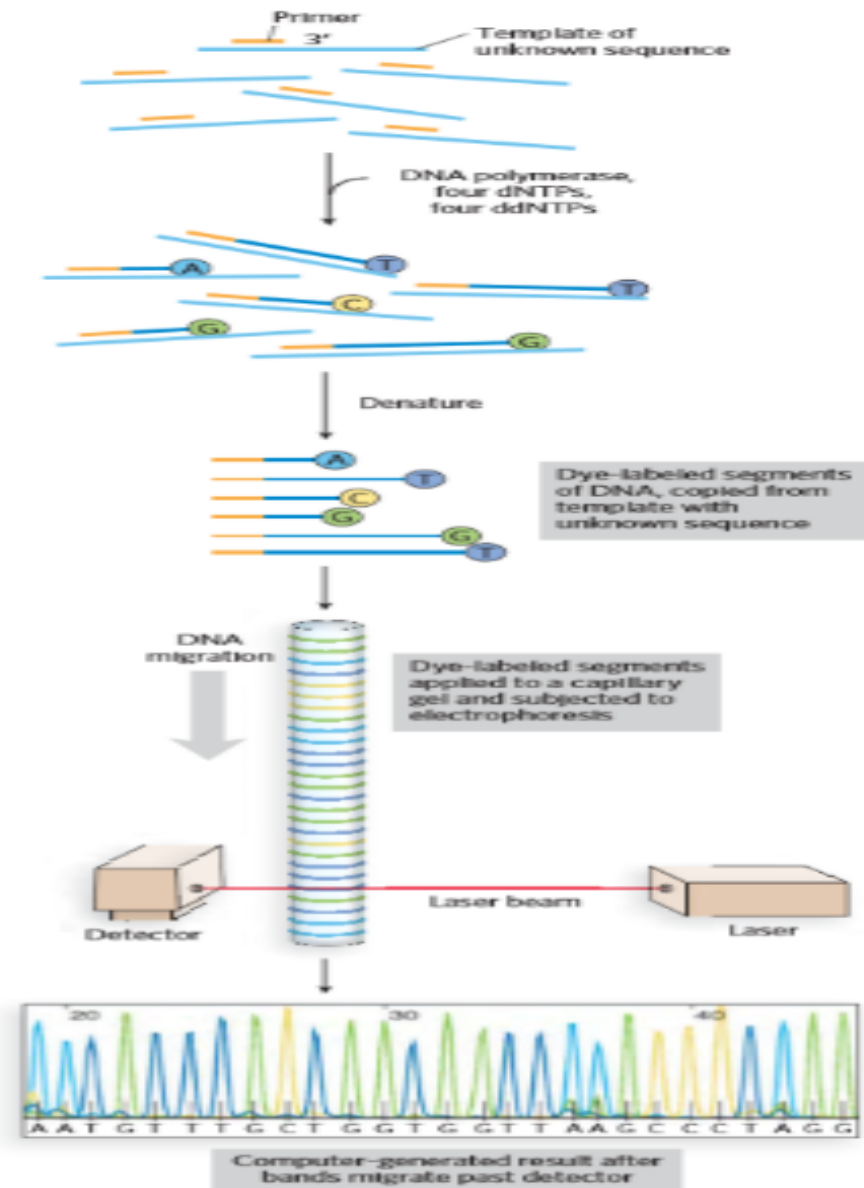


Figure 10-6a Biological Science, 2/e

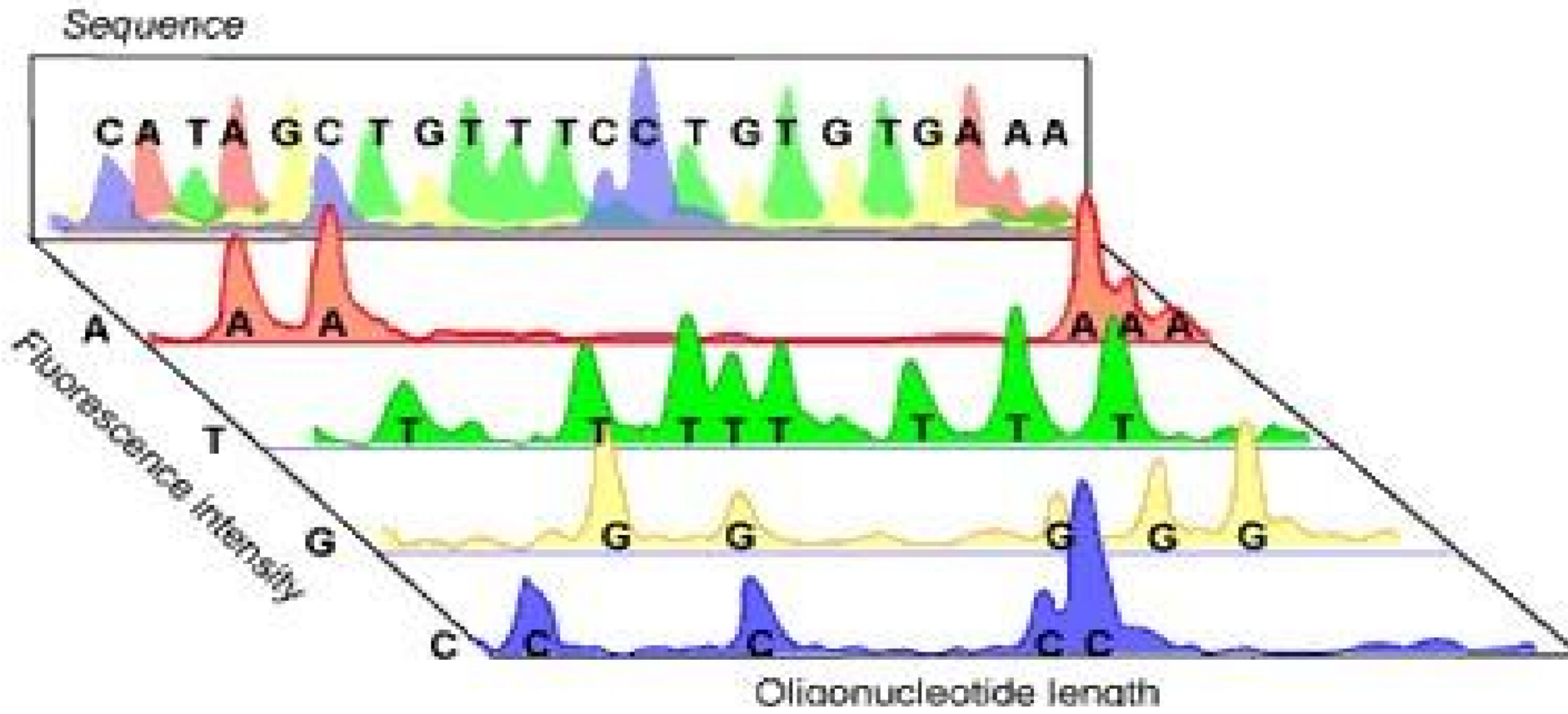
© 2001 Pearson Prentice Hall, Inc.

Principle of automated Sanger method:

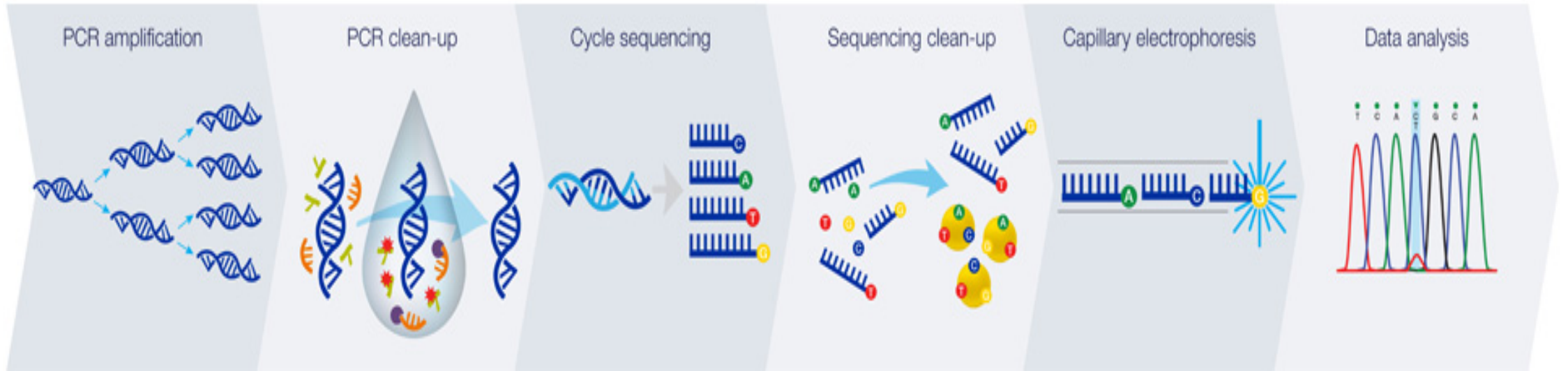
- ddNTPs are tagged with different colored fluorescent dyes (**green, blue, red and yellow**).
- Different colored DNA fragments are generated.
- Separated **by size** in an electrophoretic gel.
- Color associated with each band is detected with a laser beam.
- The amount of fluorescence in each band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



Electropherogram of a Sequencing Reaction

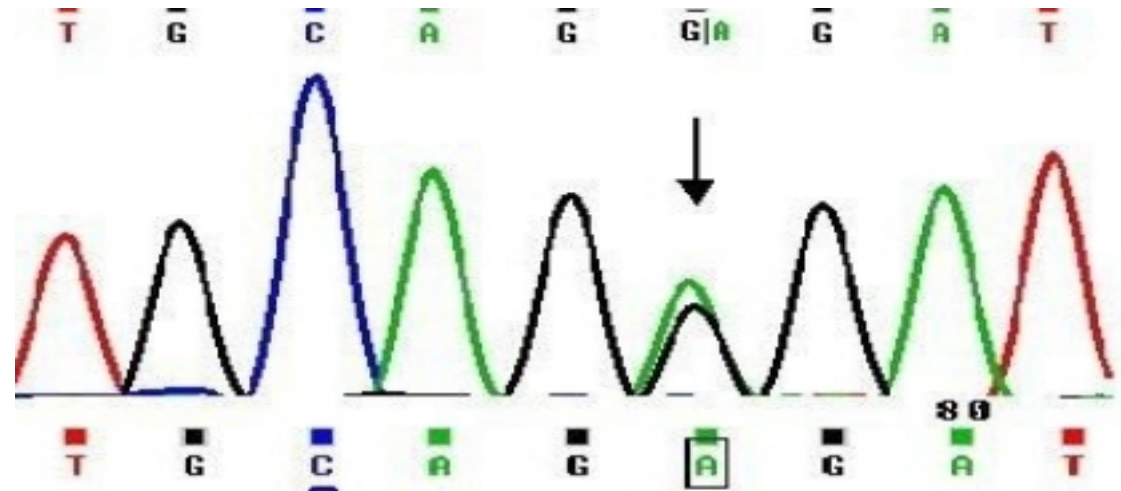


Sanger sequencing workflow:

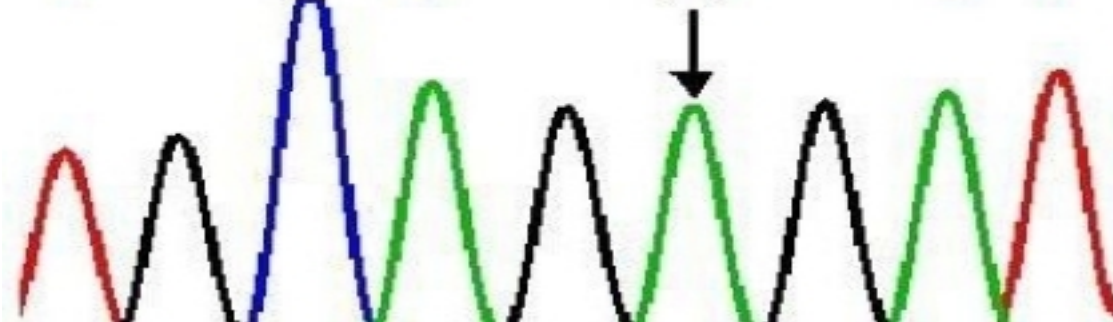


Sequencing results:

Heterozygous

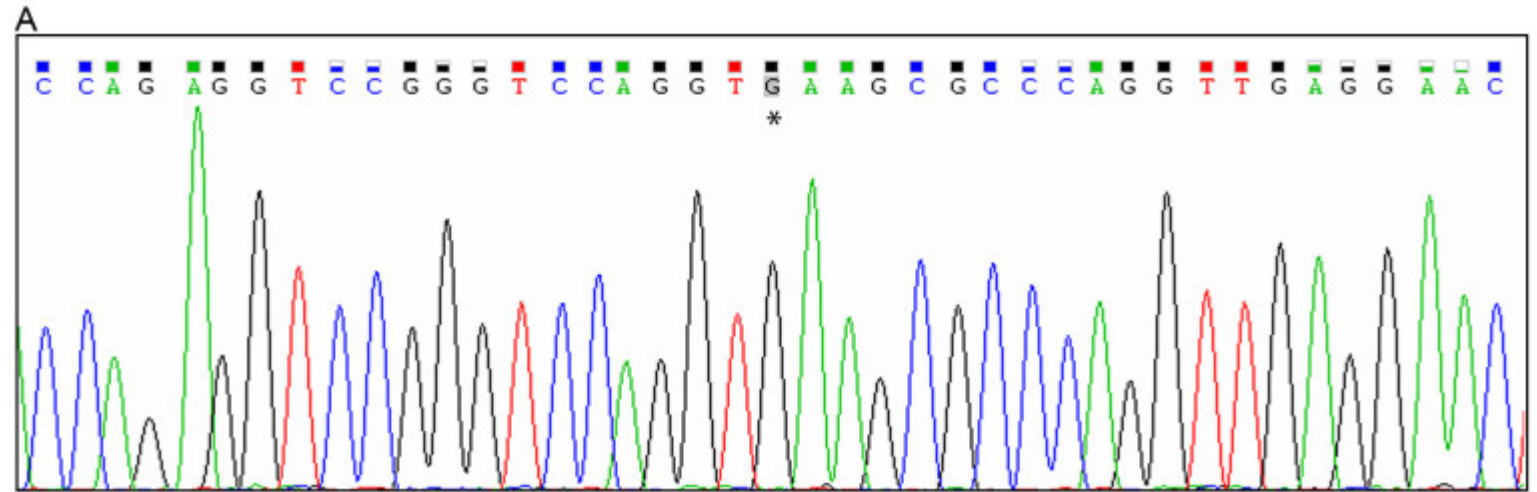


Homozygous

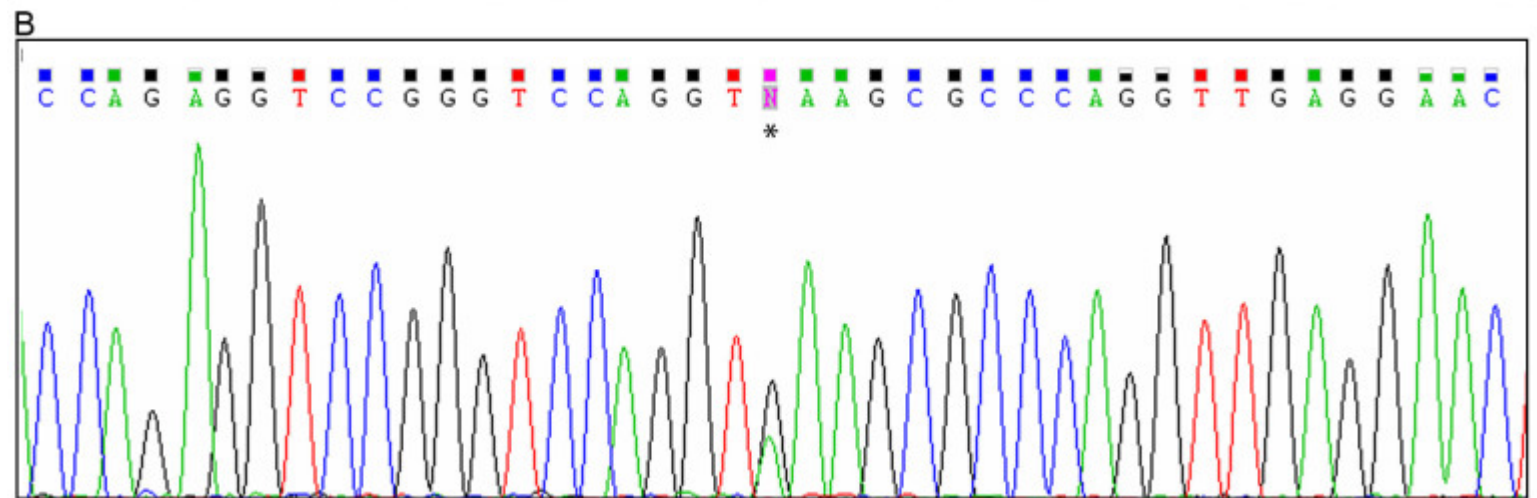


Sequencing results:

Homozygous



Heterozygous





Sanger sequencing application:

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



Home Work:

- Watch this useful video:

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

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