DNA sequencing

By

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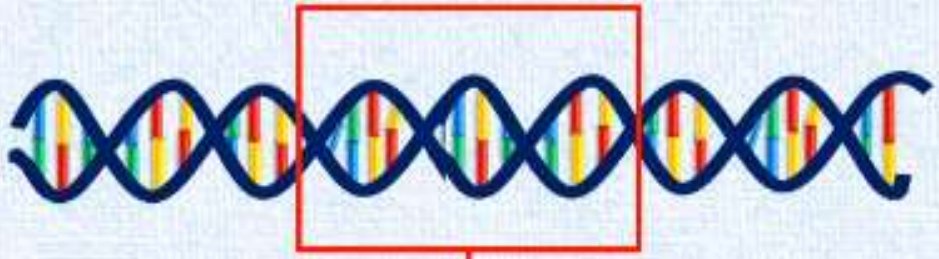
DNA sequencing

The technique by which the **precise order of nucleotides** in a DNA segment can be determined.

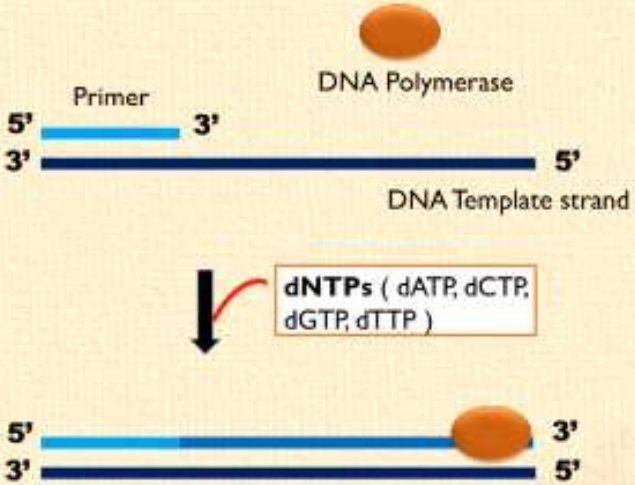


5' ATGCACTTGATC 3'

3' TACGTGAACTAG 5'

Sanger Sequencing

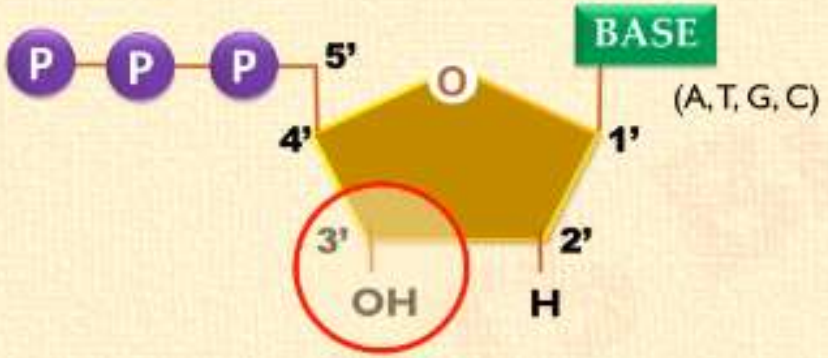
- Developed by **Frederick Sanger** and colleagues at University of Cambridge, 1977
- Involves *in vitro* DNA synthesis
- Based on the principle and biochemistry of DNA replication



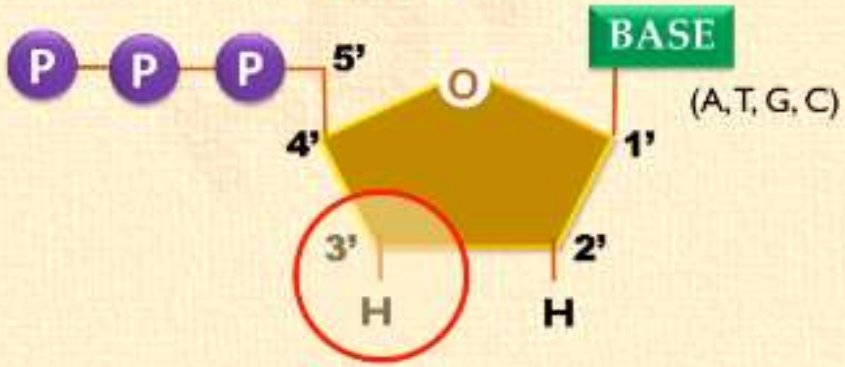
Primer is essential for DNA synthesis

Primer provides **initial 3' hydroxyl** (3'OH) **group** to form phosphodiester bond with the incoming dNTP.

Sanger DNA sequencing technique makes use of modified deoxynucleotides known as **DIDEOXY**NUCLEOTIDES.



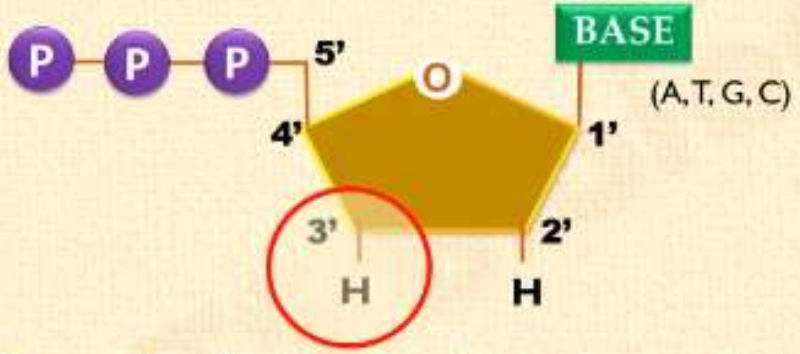
Deoxynucleotide (dNTP)



Dideoxynucleotide (ddNTP)

When a ddNTP is added in a DNA synthesis reaction…..

DNA synthesis will TERMINATE with the incorporation of ddNTP.



ddNTPs are also known as chain terminating nucleotides.

Sanger Sequencing is also known as chain termination method or dideoxy DNA sequencing.

Sanger Sequencing

ddNTP (small amounts)

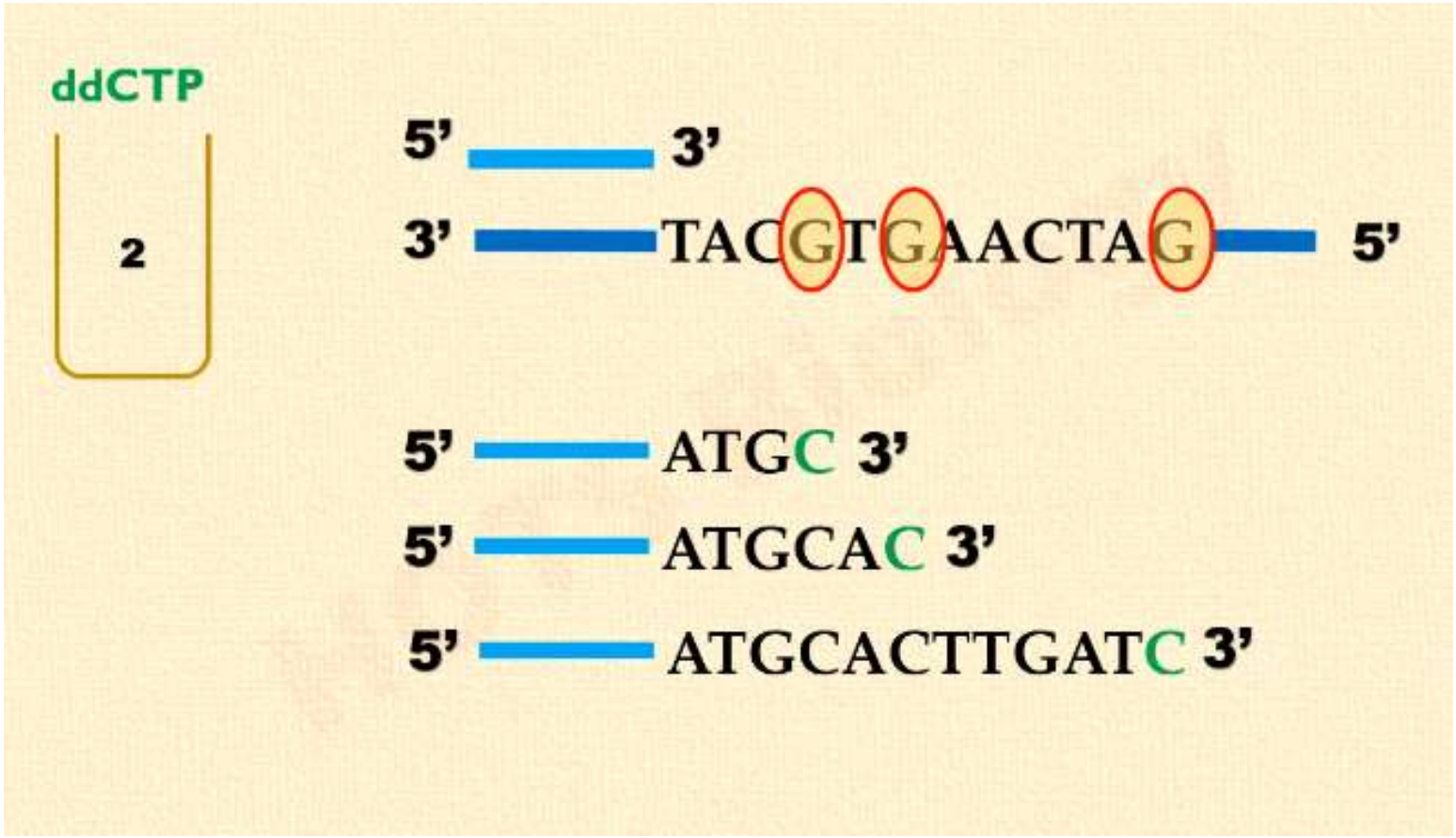
Common reaction components

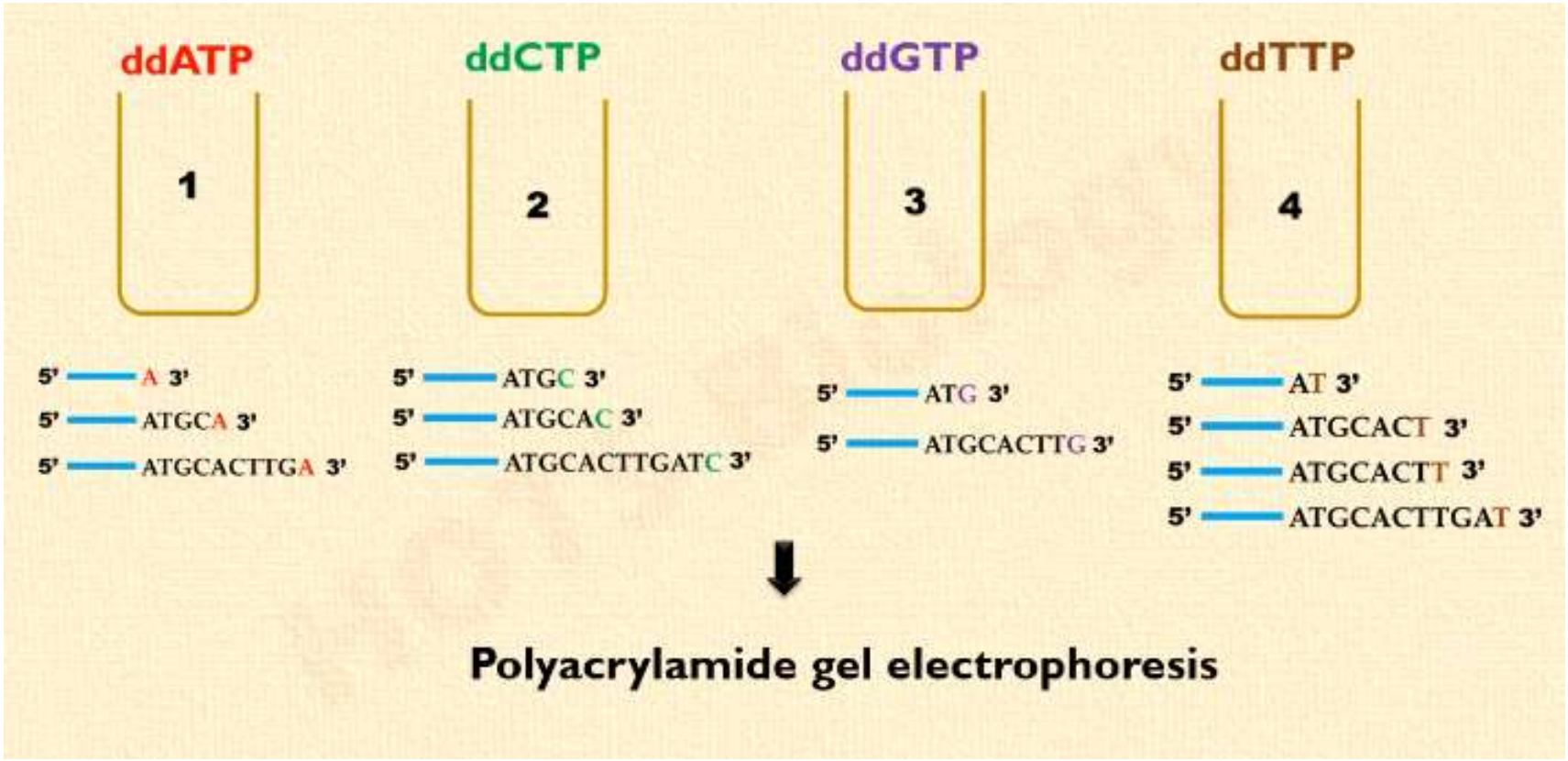
DNA template strand (many copies) 3' TACGTGAACTAG 5'

DNA primer (many copies, radiolabeled) 5' 3'

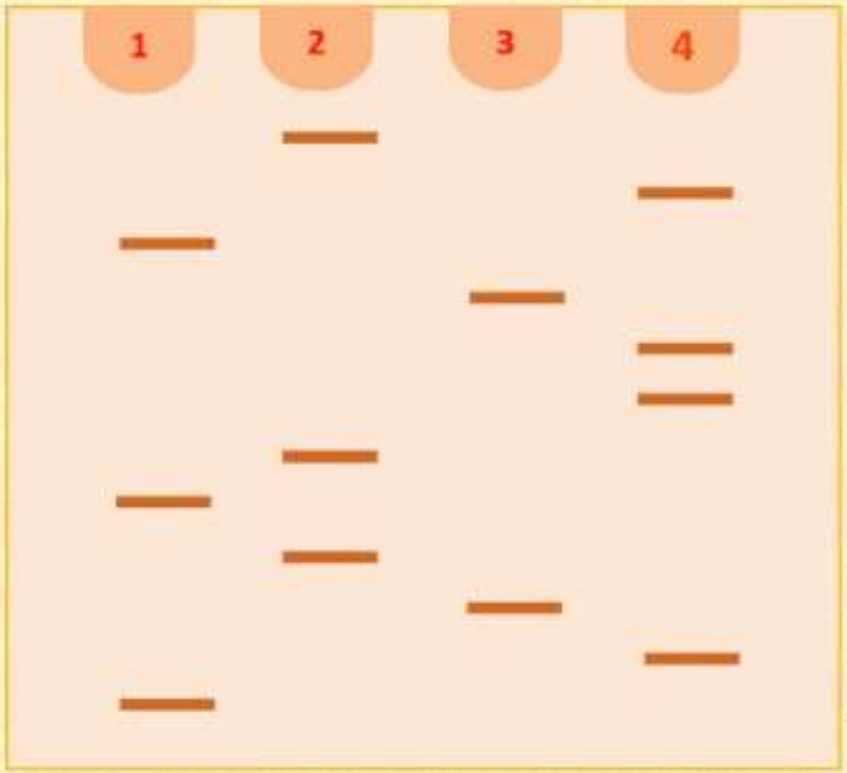
DNA polymerase

dNTPs – dATP, dCTP, dGTP, dTTP (large amounts)


ddCTP
2
5’ 3’
3’ TACGTGAACTAG 5’
5’ ATGC 3’
5’ ATGCAC 3’
5’ ATGCACTTGATC 3’

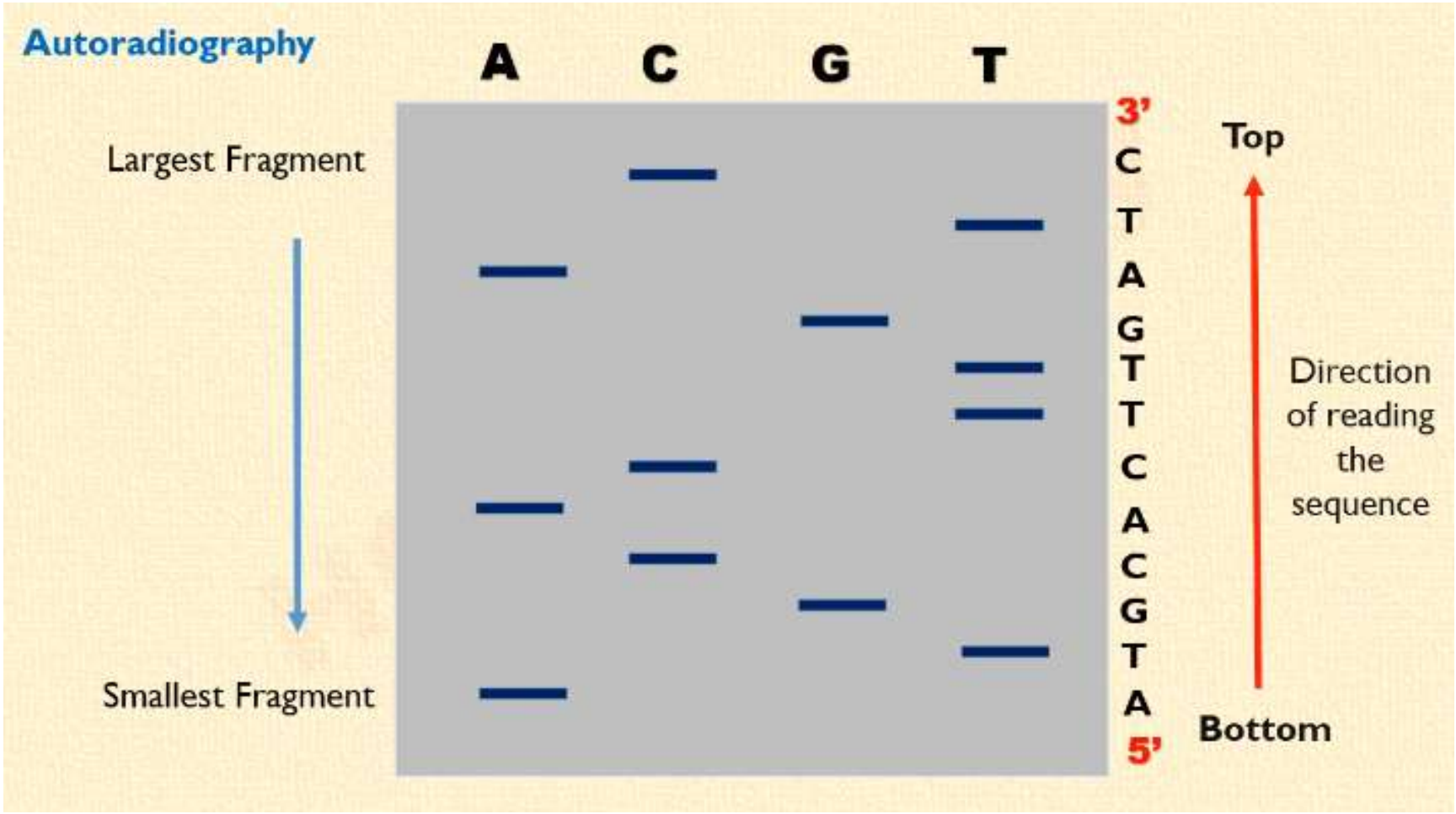

ddATP
ddCTP
ddGTP
ddTTP
1
2
3
4
5' A 3'
5' ATGCA 3'
5' ATGCACTTGA 3'
5' ATGC 3'
5' ATGCAC 3'
5' ATGCACTTGATC 3'
5' ATG 3'
5' ATGCACTTG 3'
5' AT 3'
5' ATGCACT 3'
5' ATGCACTT 3'
5' ATGCACTTGAT 3'
Polyacrylamide gel electrophoresis

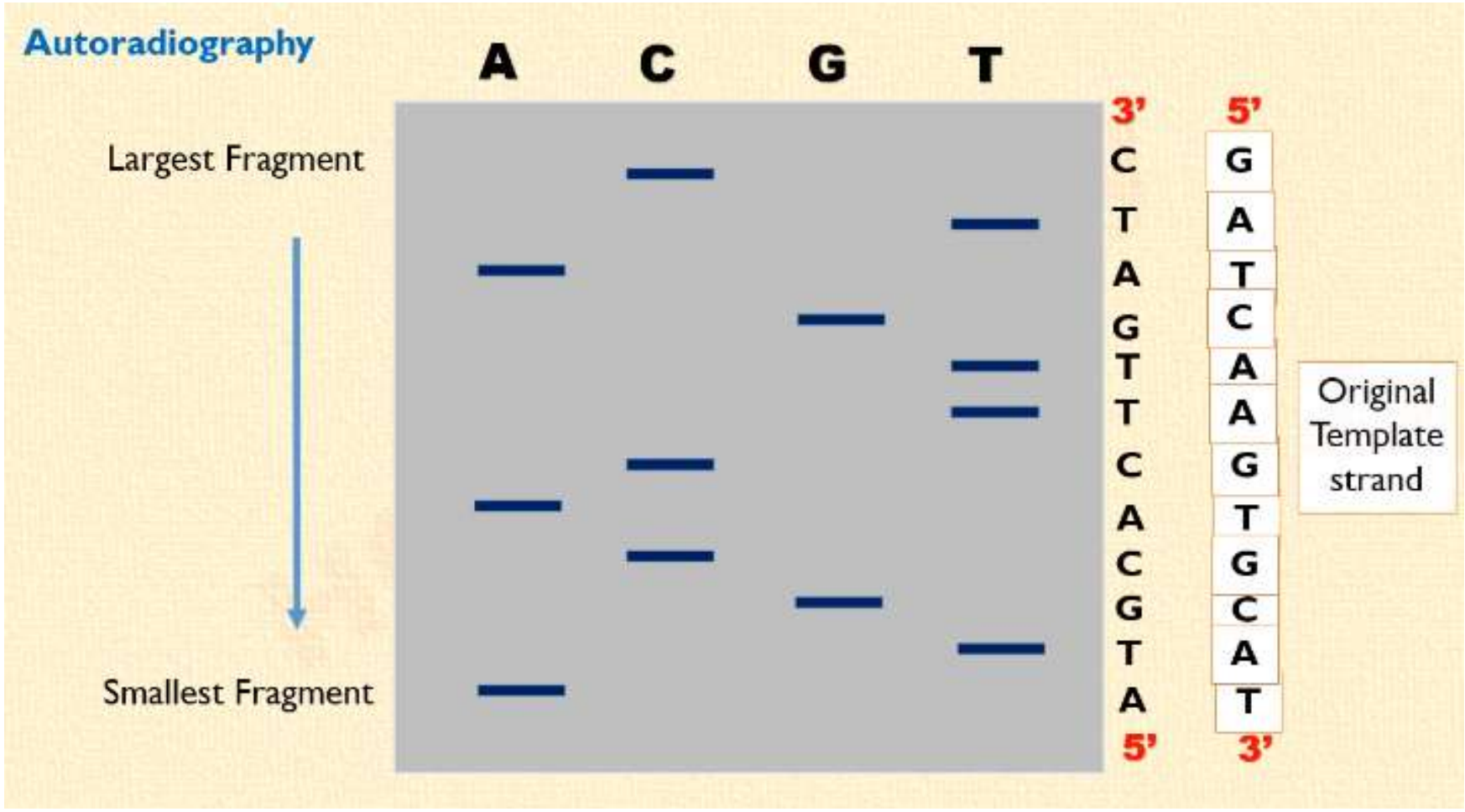
Polyacrylamide gel electrophoresis



Largest Fragment

Smallest Fragment


Autoradiography
A
C
G
T
Largest Fragment
Smallest Fragment
3'
C
T
A
G
T
T
C
A
C
G
T
A
5'
Top
Direction of reading the sequence
Bottom


Autoradiography
A
C
G
T
Largest Fragment
Smallest Fragment
3'
5'
C
T
A
G
T
T
C
A
C
G
T
A
5'
G
A
T
C
A
A
G
T
G
C
A
T
3'
Original Template strand

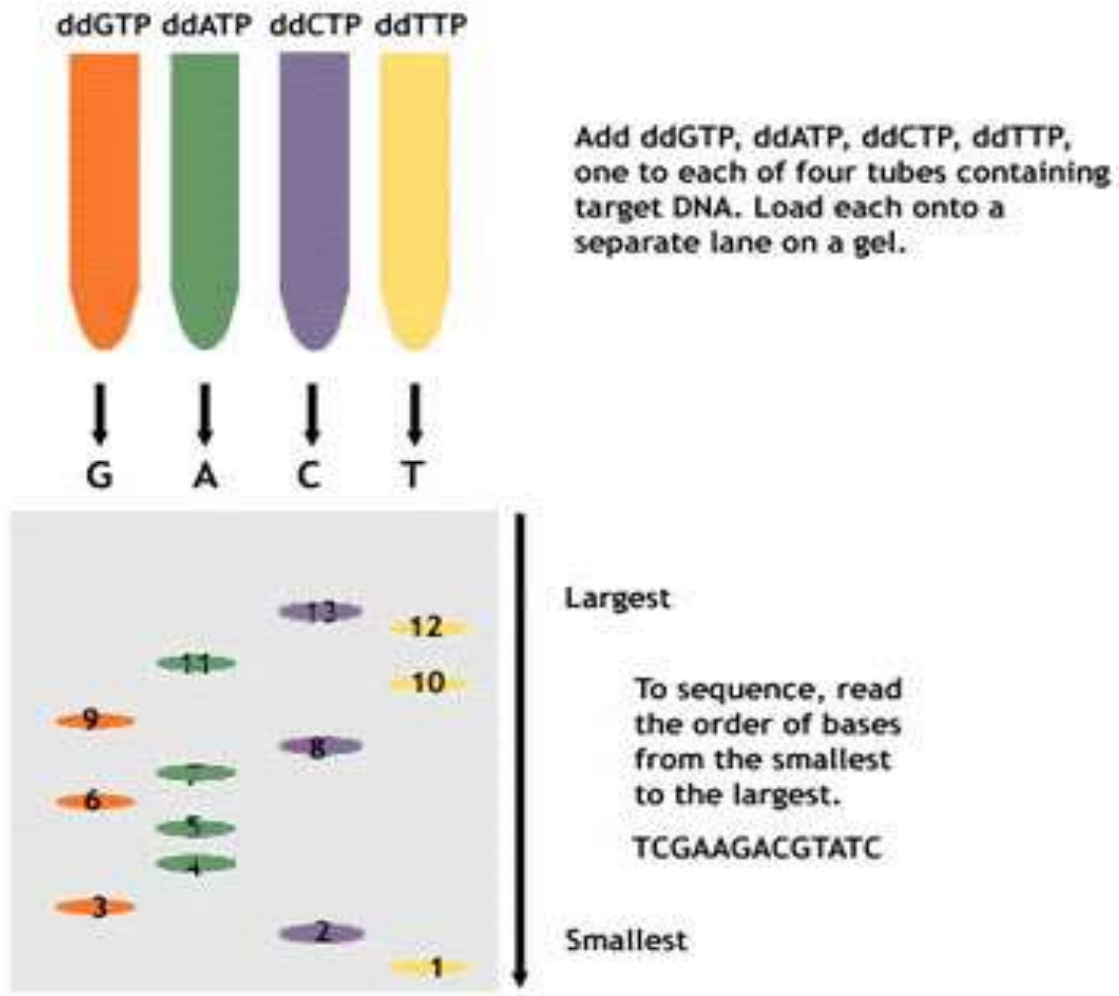
Chain termination method (sanger dideoxy method)

1- The DNA to be sequenced is prepared as single stranded molecule .

2- An incubation mixture is set up containing the following :

- The single stranded DNA template
- DNA polymerase
- **Radioactive primer** complementary to the 3`end of the target DNA.
- All four deoxynucleoside triphosphates (dATP-dGTP-dCTP-dTTP).

The sample is divided into four reaction tubes and a small amount of one of the four **dideoxyribonucleoside triphosphate (ddNTP)** is added to each tube.



3- During incubation, the DNA begins to copy the template molecule by extending the bound primer.

4- As a new DNA strand is synthesized, **every time when dGTP , for example, is incorporated there is a chance to incorporate ddGTP instead.** If this happens, no further chain elongation can occur because ddGTP lacks the 3̀-OH group needed to make the next phosphodiester bond. Thus this particular chain stops at this point.

5-Four sets of chain-terminated fragments are formed corresponding to the positions of A,G,C and T in the sequence.

6-After incubation, all four reaction mixtures are electrophoresed in parallel lanes of a **polyacrylamide gel** and then subjected to autoradiography.

7-The DNA sequence is determined simply by reading the band pattern on the autoradiogram from the bottom of the gel toward the top.

8-We know that each reaction mixture has the same primer therefore all the strands begin with the same sequence