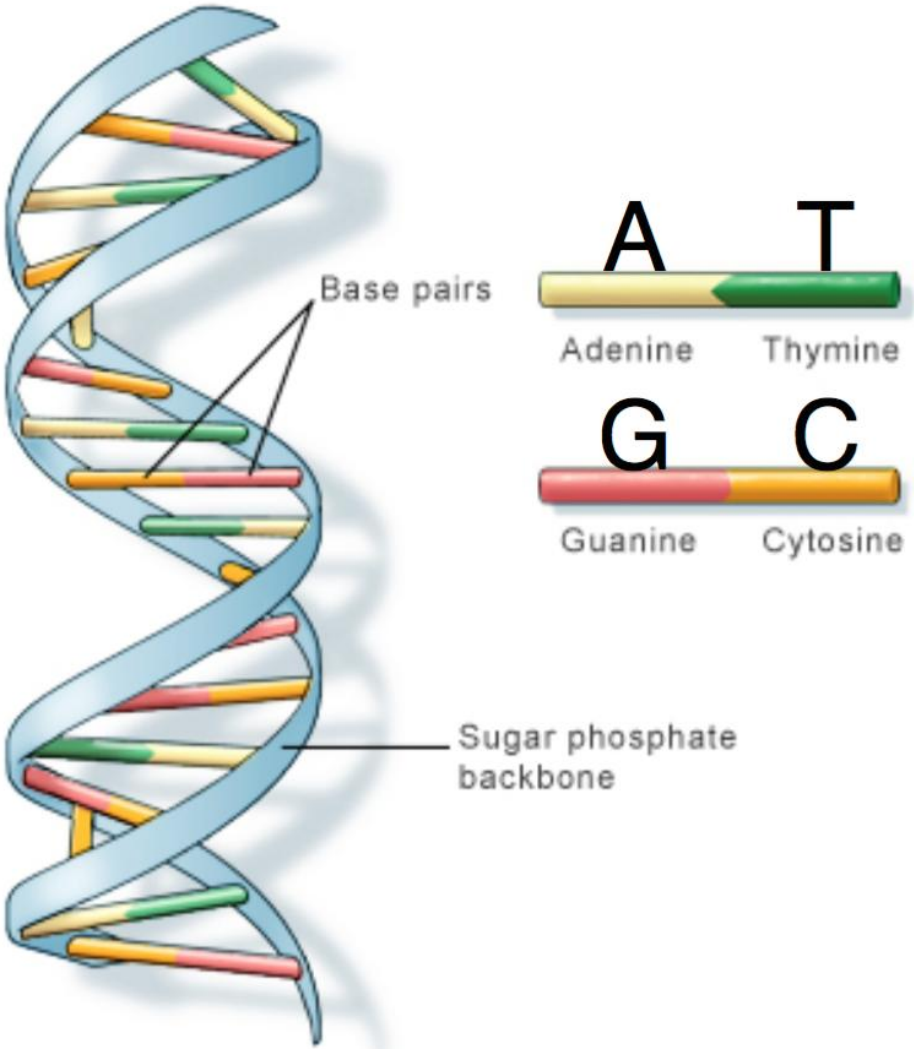
DNA SEQUENCING



Subject: Principle of Genetic Engineering
Sub. Code: MBT-201
M.Sc Biotechnology-II semester

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Professor
Department of Biotech & Microbiology

What is DNA sequencing?

Process of determining the sequence of nucleotides

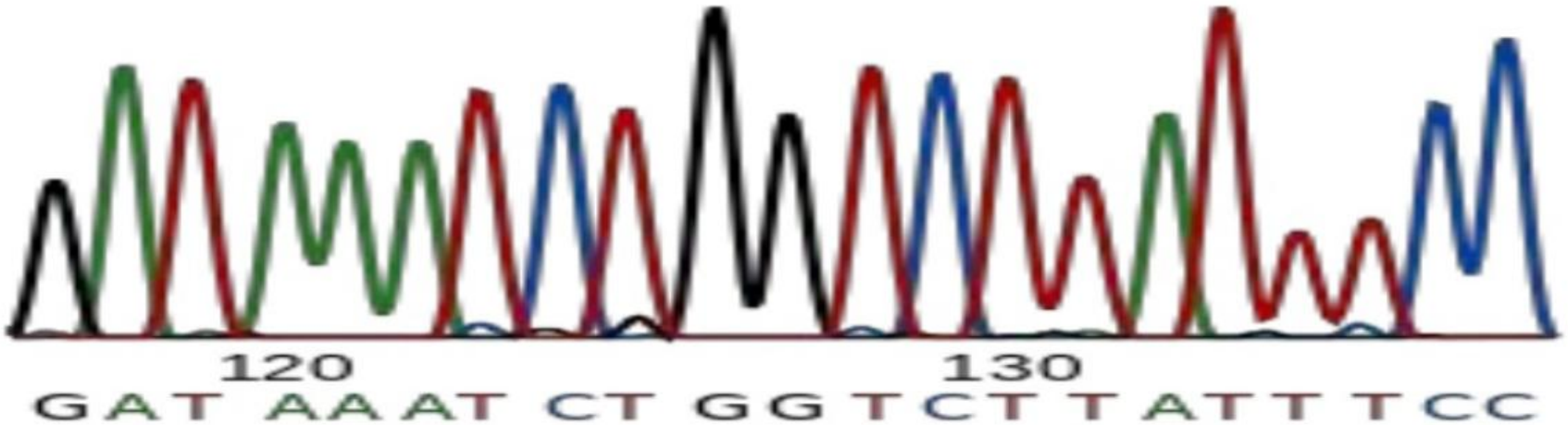
Adenine

Thymine

Cytosine

Guanine

in a piece of DNA.



Sanger sequencing

✓ Fredrick Sanger with his colleagues developed this method of sequencing in the year 1977, for which he was awarded Nobel in 1980

✓It is also known as Chain termination method, Dideoxy sequencing or Enzymatic method

✓It was the most widely used DNA sequencing method for almost 40 years, bringing successful completion of the Human Genome Project (HGP) in 2003

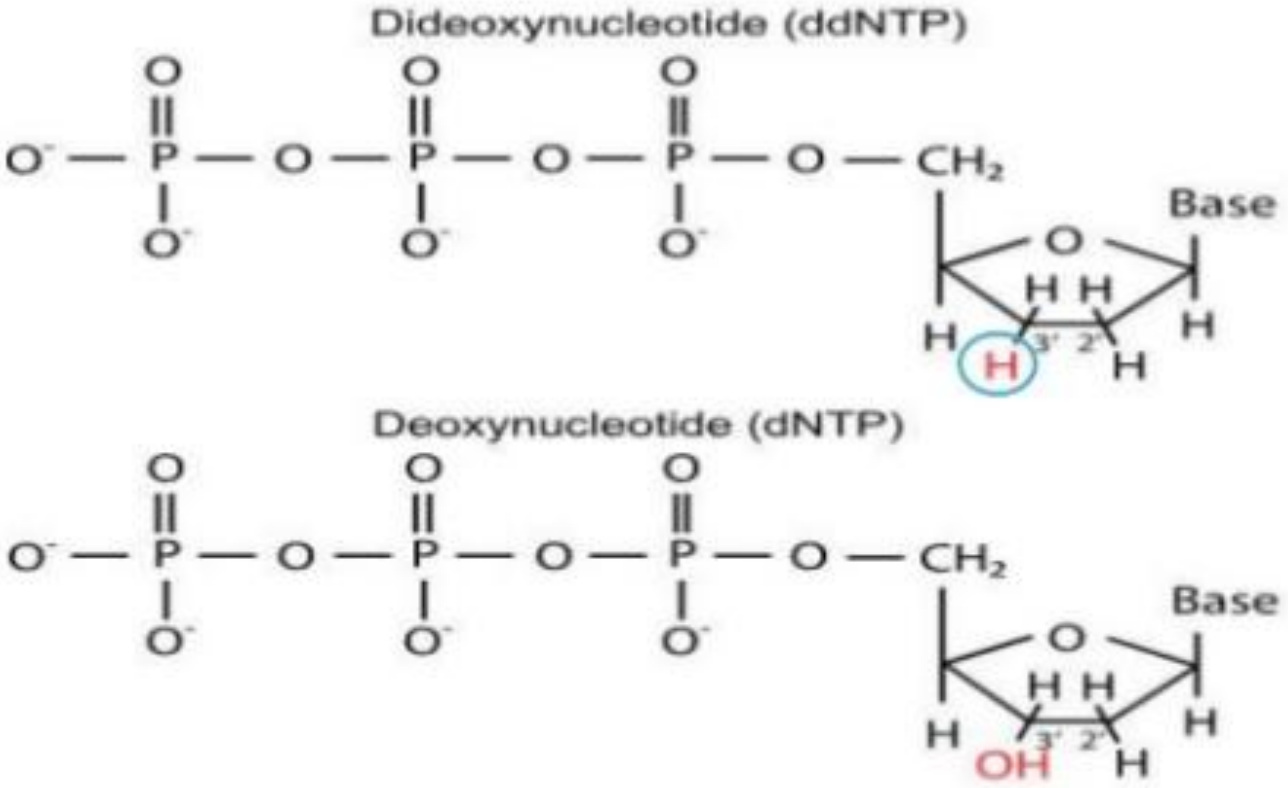
✓Sanger sequencing method is based on the chain termination by the use of Dideoxynucleotides (ddNTPs)

- *Most common approach used to sequencing DNA.*
- *Invented by **Frederick Sanger - 1977***
- *Nobel prize - 1980*
- *Also termed as -*
  - *chain termination*
  - *dideoxy method*
  - *sanger sequencing*

Clip slide

What is Dideoxynucleotide (ddNTPs)

✓ A *dideoxynucleotide* (ddNTP) is an artificial molecule that lacks a hydroxyl group at the 3' carbon of the ribose sugar

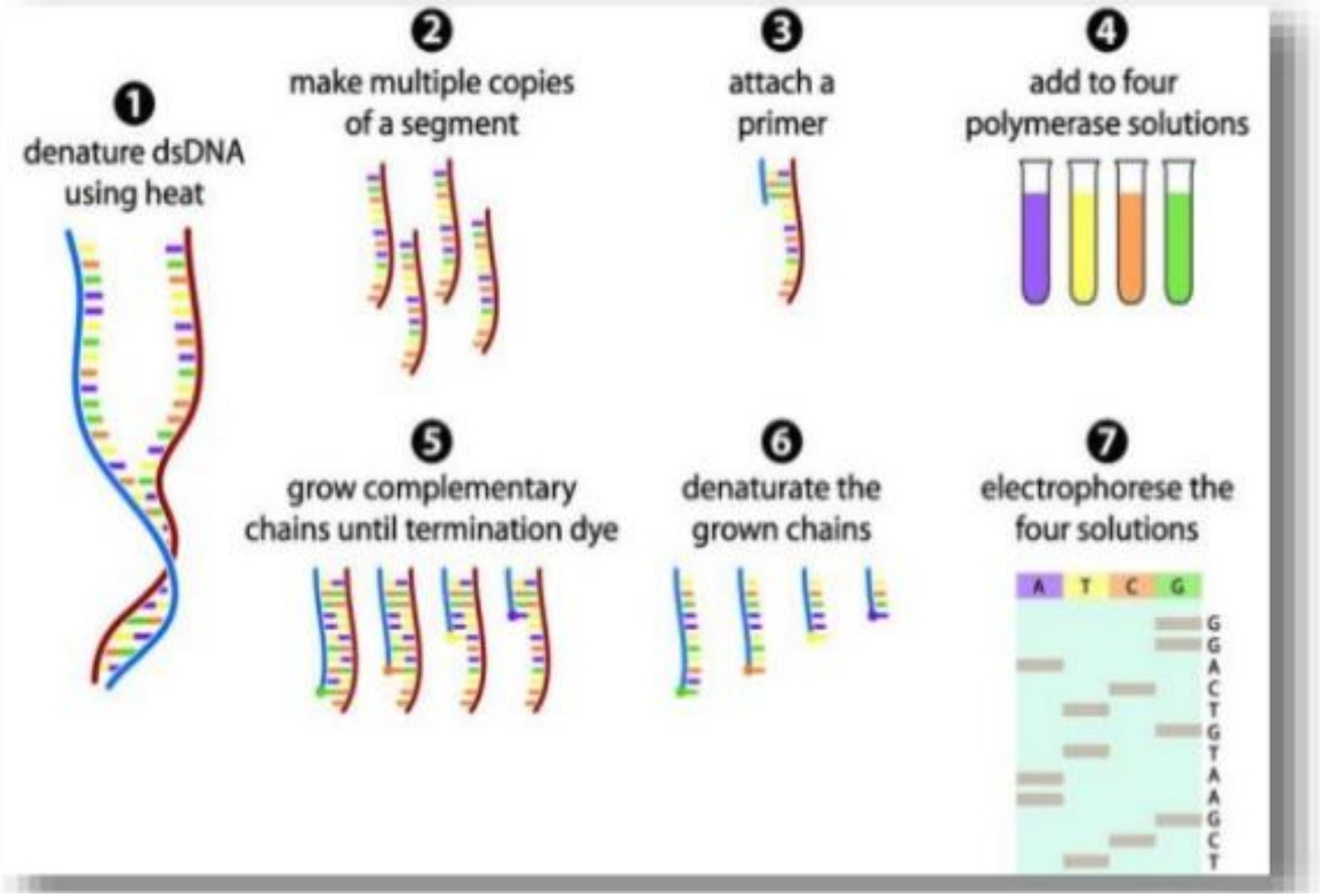


Requirements

DNA sequencing is performed in four separate tubes, each containing

i. Single stranded DNA to be sequenced
ii. DNA polymerase
iii. Primers
iv. The four dNTPs (dATP, dCTP, dTTP and dGTP)
v. Small amount of one of the four ddNTPs (ddATP or ddCTP or ddTTP or ddGTP)

Sanger sequencing process



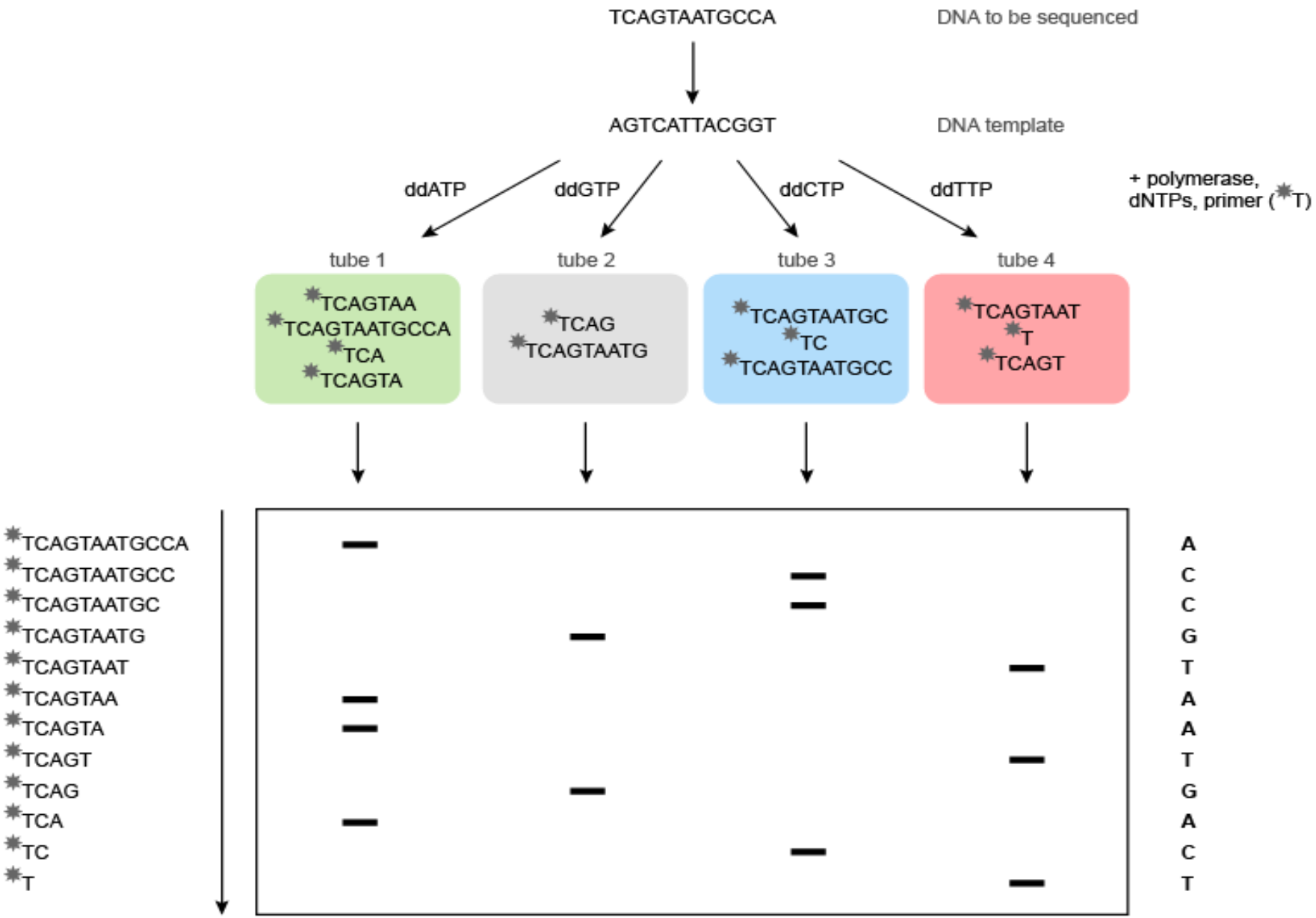


Figure 6 | Sanger (dideoxy) sequencing

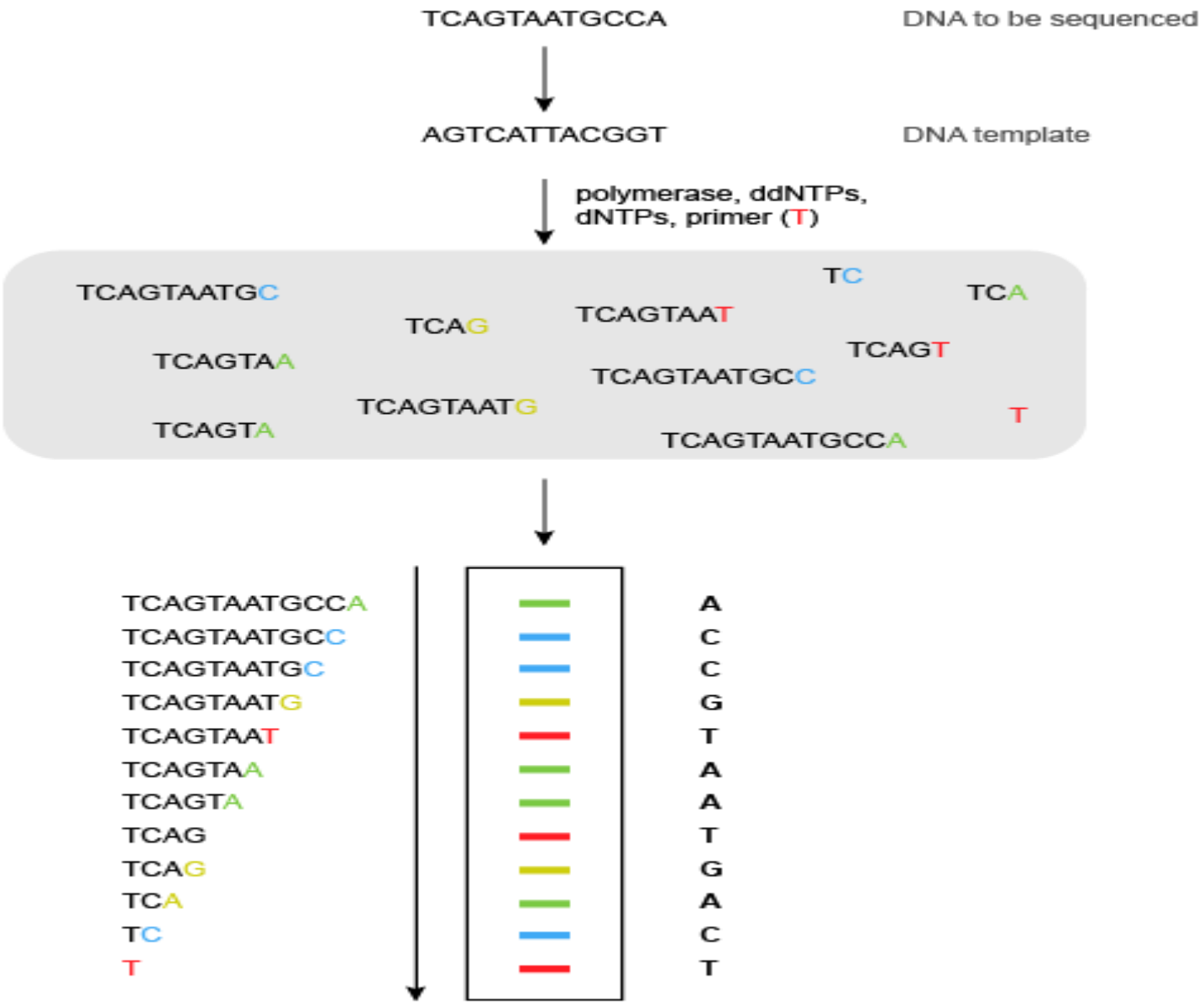
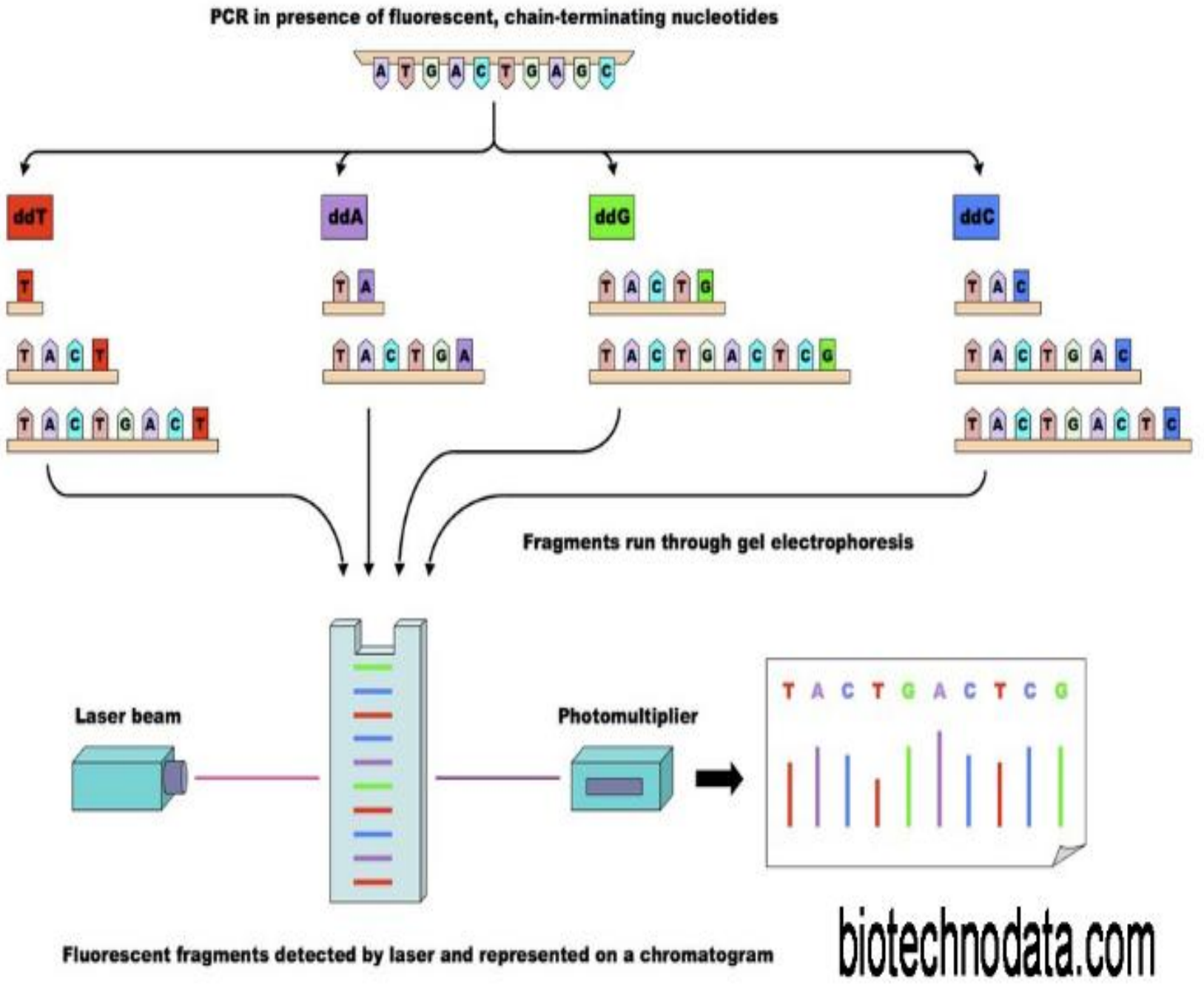


Figure 7 | Fluorescent Sanger (dideoxy) sequencing


PCR in presence of fluorescent, chain-terminating nucleotides
A T G A C T G A G C
ddT
ddA
ddG
ddC
T
T A
T A C
T A C T
T A C T G
T A C T G A
T A C T G A C
T A C T G A C T
T A C T G A C T C
T A C T G A C T C G
Fragments run through gel electrophoresis
Laser beam
Photomultiplier
T A C T G A C T C G
Fluorescent fragments detected by laser and represented on a chromatogram
biotechnodata.com

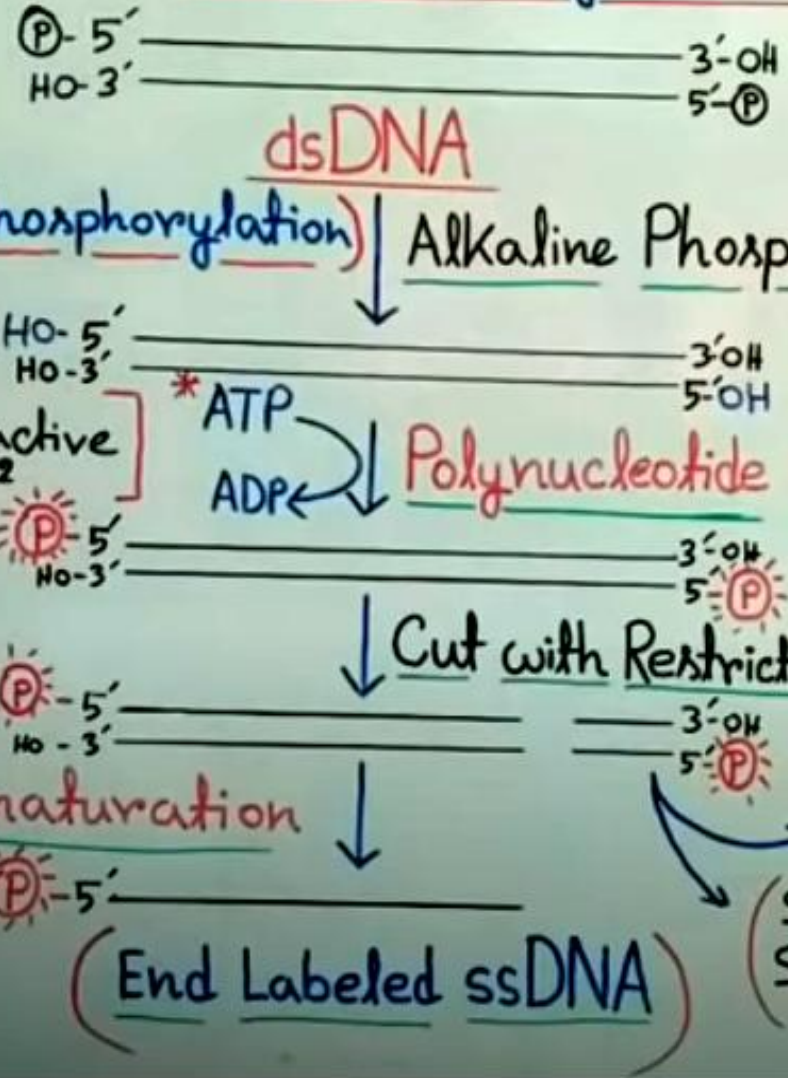
Maxam and Gilbert Method

- In 1976–1977, Allan Maxam and Walter Gilbert developed a DNA sequencing method based on chemical modification of DNA and subsequent cleavage at specific bases

I. Chemical Modification of DNA; radioactive labeling at one 5' end of the DNA (typically by a kinase reaction using gamma-^{32}P ATP)
II. Purification of the DNA fragment to be sequenced
III. Chemical treatment generates breaks in DNA
IV. Run on the gel

MAXAM-GILBERT DNA SEQUENCING

[Chemical Degradation Method]



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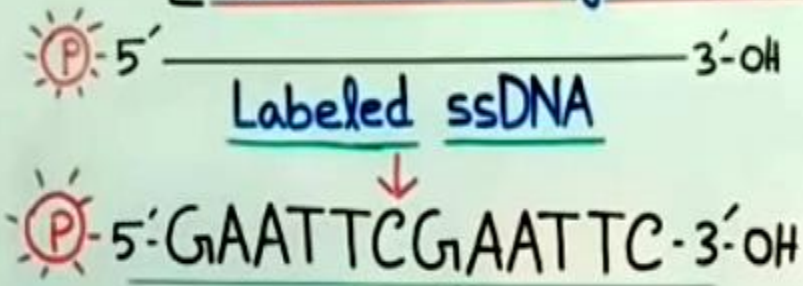
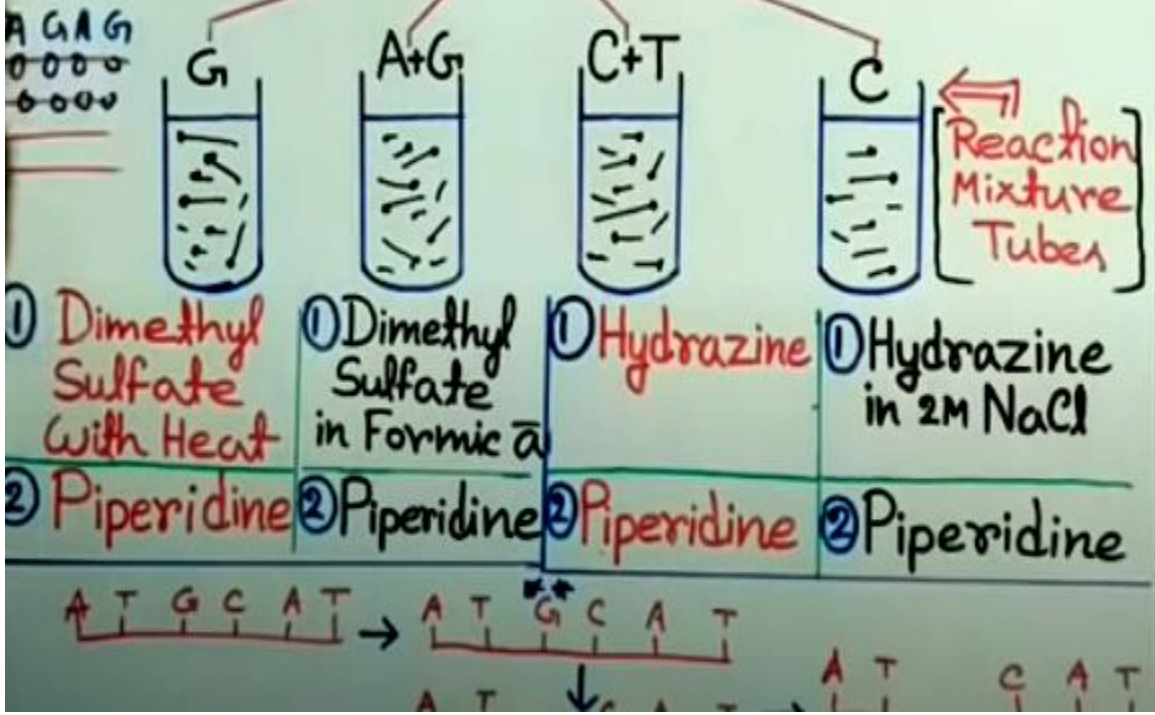
MAXAM-GILBERT DNA SEQUENCING

[Chemical Degradation Method]





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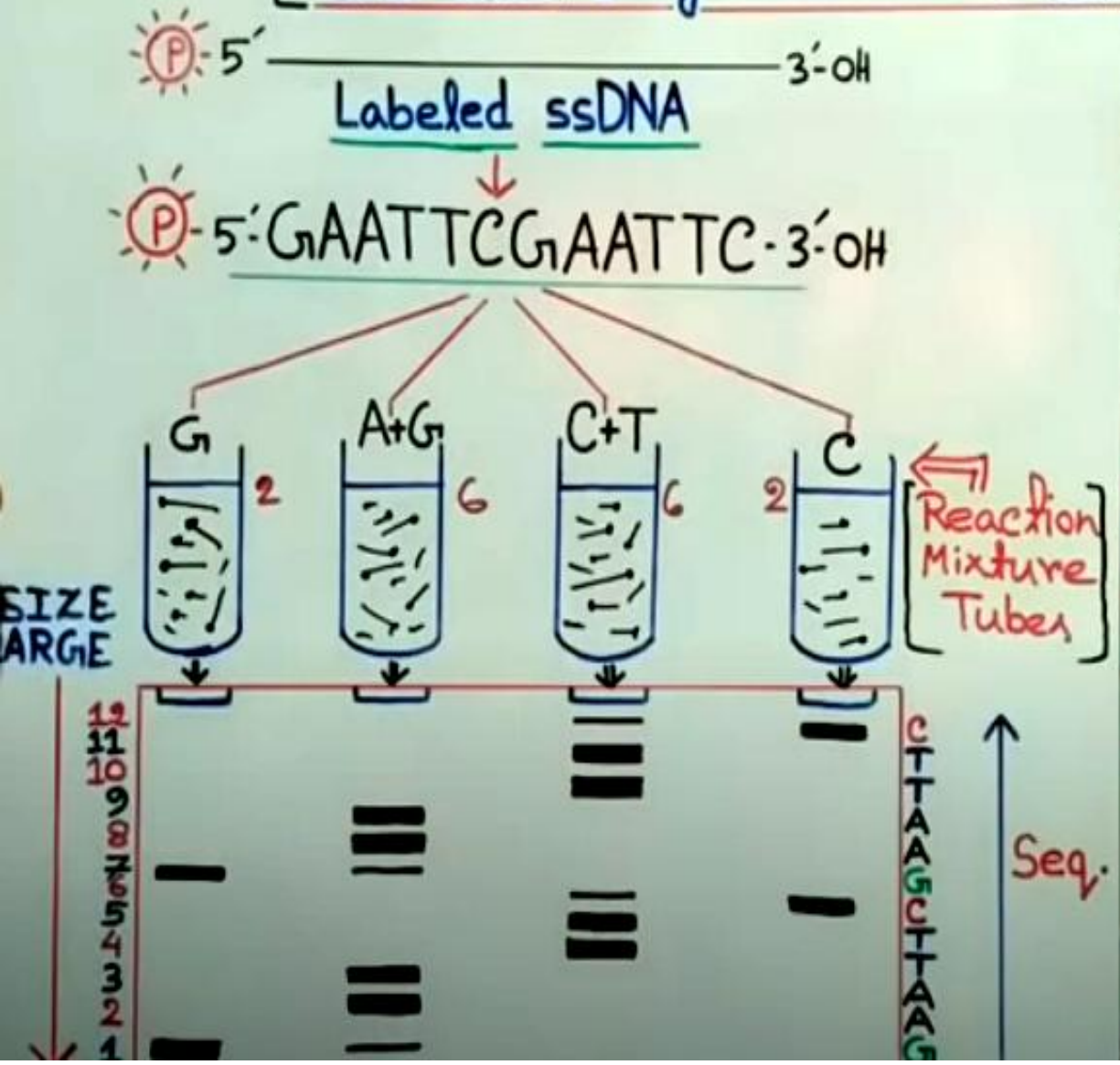
MAXAM-GILBERT DNA SEQUENCING

[Chemical Degradation Method]



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Chemical Modification and Cleavage

- Base Modification using Dimethyl sulphate
  - Purine
    - Adenine
    - Guanine
  - Only DMS------- G
  - DMS+ Formic acid-------G+A

- Cleavage of Sugar Phosphate backbone using Piperidine

Chemical Modification and Cleavage

- Base modification using Hydrazine
  - Pyrimidine
    - Cytocine
    - Thymidine
  - Hydrazine----- C+T
  - Hydrazine + NaCl--------C

- Cleavage of Sugar Phosphate backbone using Piperidine