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FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Chemical Modification and Cleavage

- Ploy nucleotide Kinase radioactive label at one 5' end of the DNA using gamma-^{32}P

5′ G A C G T G C A A C G A A 3′

^{32}P **5′ G A C G T G C A A C G A A 3′**

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

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Chemical Modification and Cleavage

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

- Cleavage of Sugar Phosphate backbone using Piperidine

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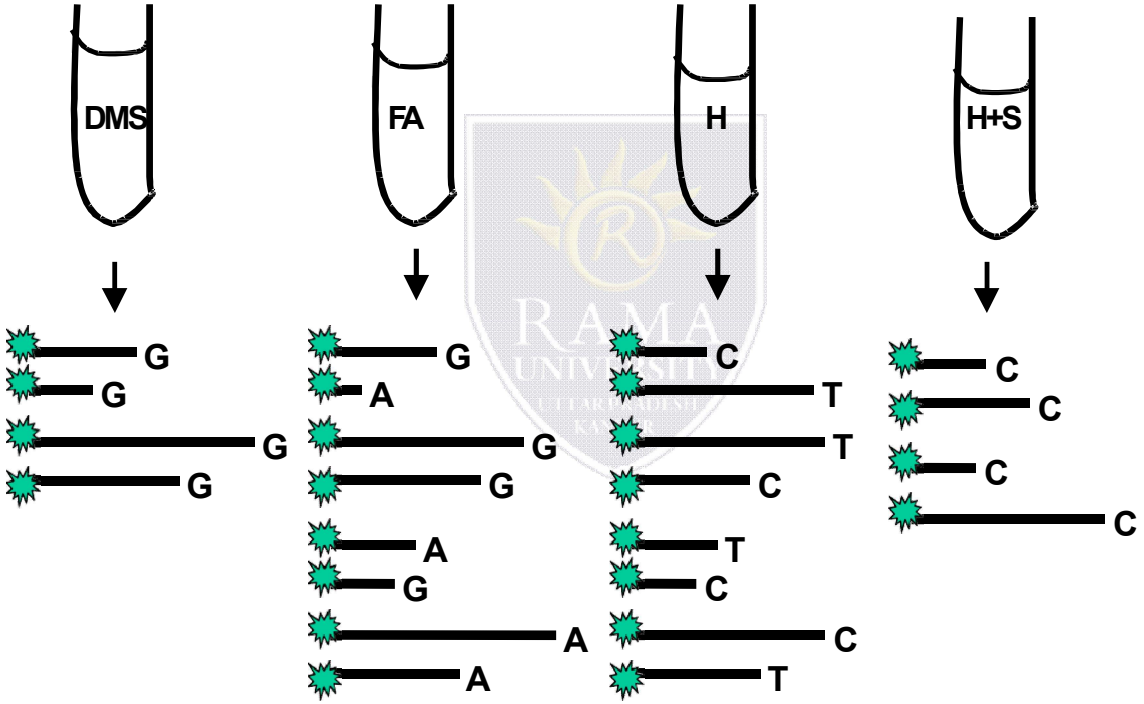
Maxam Gilbert Sequencing

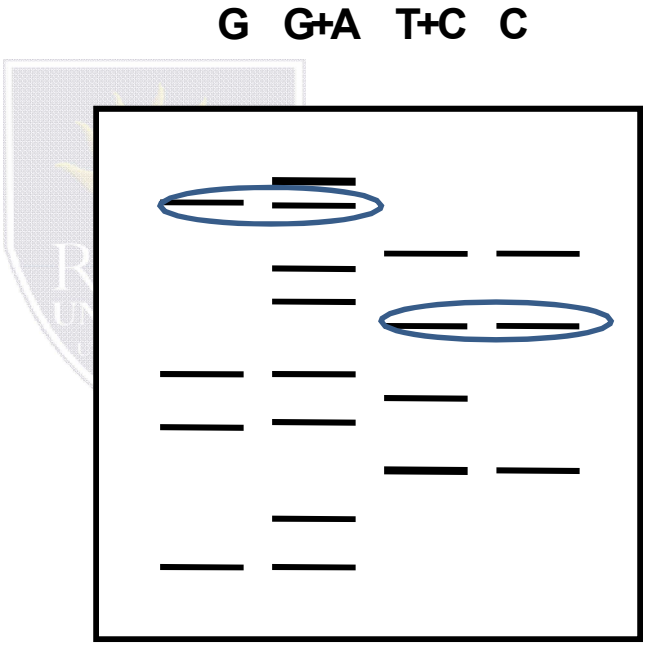


^{32}P **5′ G A C G T G C A A C G A 3′**

Maxam-Gilbert Sequencing

Longer fragments

Shortest fragments



3′ A G C A A C G T G C A G 5′

Sequencing gels are read from bottom to top (5′ to 3′).

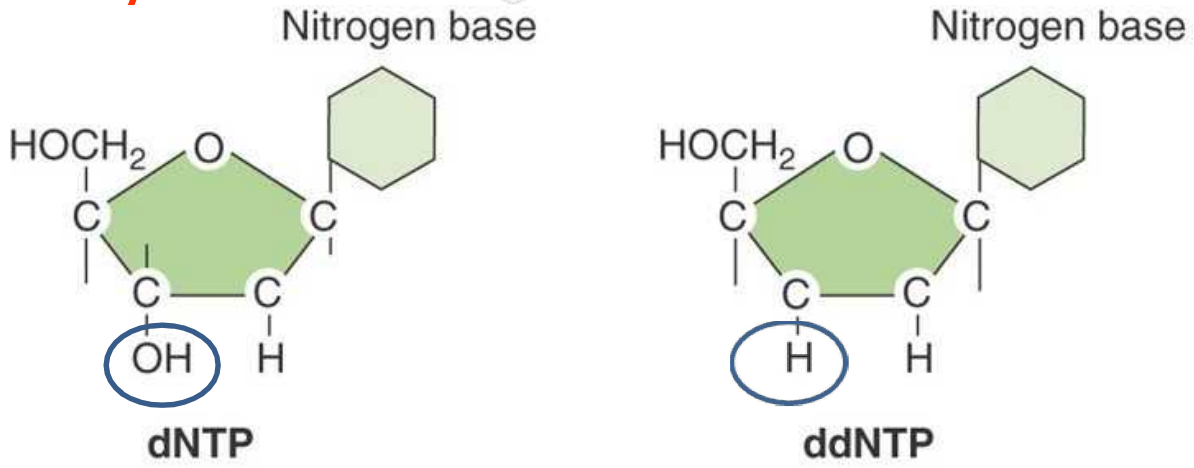
^{32}P 5′ G A C G T G C A A C G A

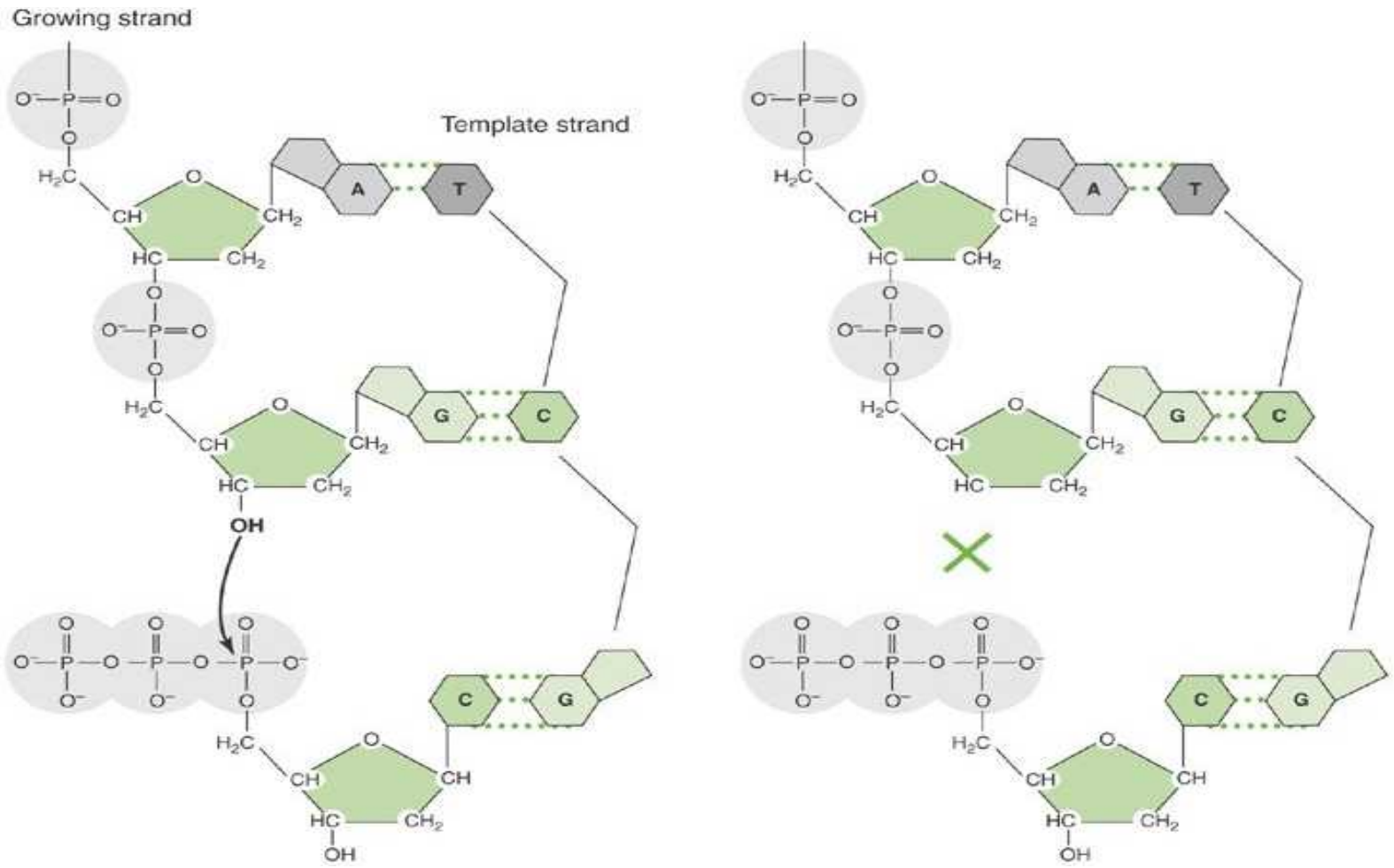
Maxam Gilbert Sequencing: Process Summarized

1. Label 5'- end of DNA
2. Aliqot DNA sample in 4 tubes
3. Perform base modification reaction
4. Perform Cleavage reaction
5. Perform Gel Electrophoresis
6. Perform Autoradiography
7. Interpret results

Sanger, Chain Termination Sequencing

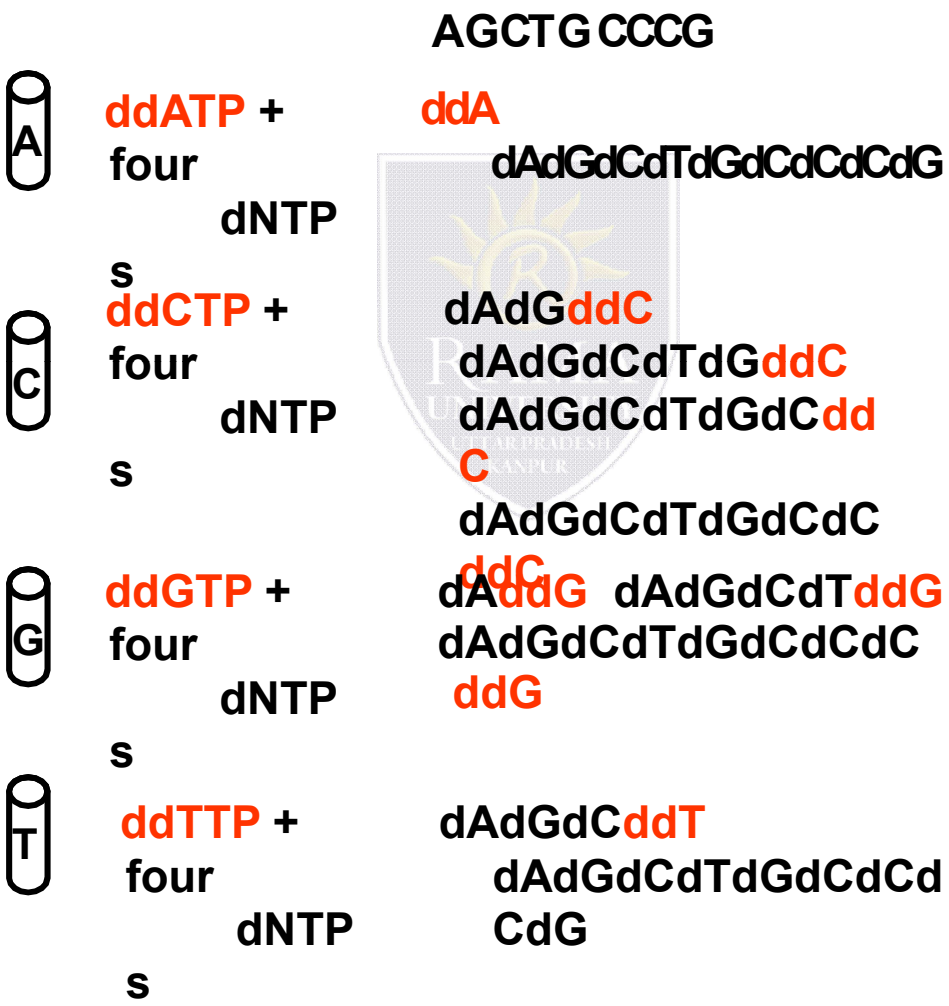
- It is PCR based method
- A modified DNA replication reaction
- Growing chains are terminated by dideoxynucleotides





The 3′-OH group necessary for formation of the phosphodiester bond is missing in ddNTPs

Sanger, Chain Termination Sequencing

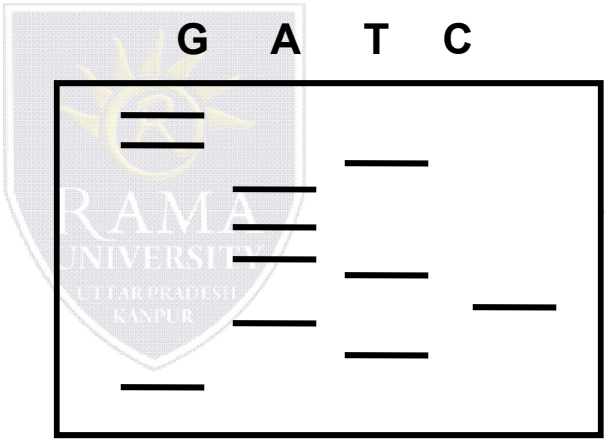


Chain Termination Sequencing

Chain Termination Sequencing

Longer fragments

Shorter fragments



3′ GGTAAATCATG 5′

Sequencing gels are read from bottom to top (5′ to 3′)

Sanger Sequencing: An Example

5'-TACACGATCGA-3'

3'-ATGTGCTAGCT-5'

Denature the sequence

Use only forward primer i.e. using 3'-5

Amplification in ddTTP

3’-ATGTGCTAGCT-5’

5’-T-3’

5’-TACACGAT-3’

Amplification in ddATP

3’-ATGTGCTAGCT-5’

5’-TA-3’

5’-TACA-3’

5’-TACACGA-3’

5’-TACACGATCGA-3’

Amplification in dGTTP

3’-ATGTGCTAGCT-5’

5’-TACACG-3’

5’-TACACGATCG-3’

Amplification in ddCTP

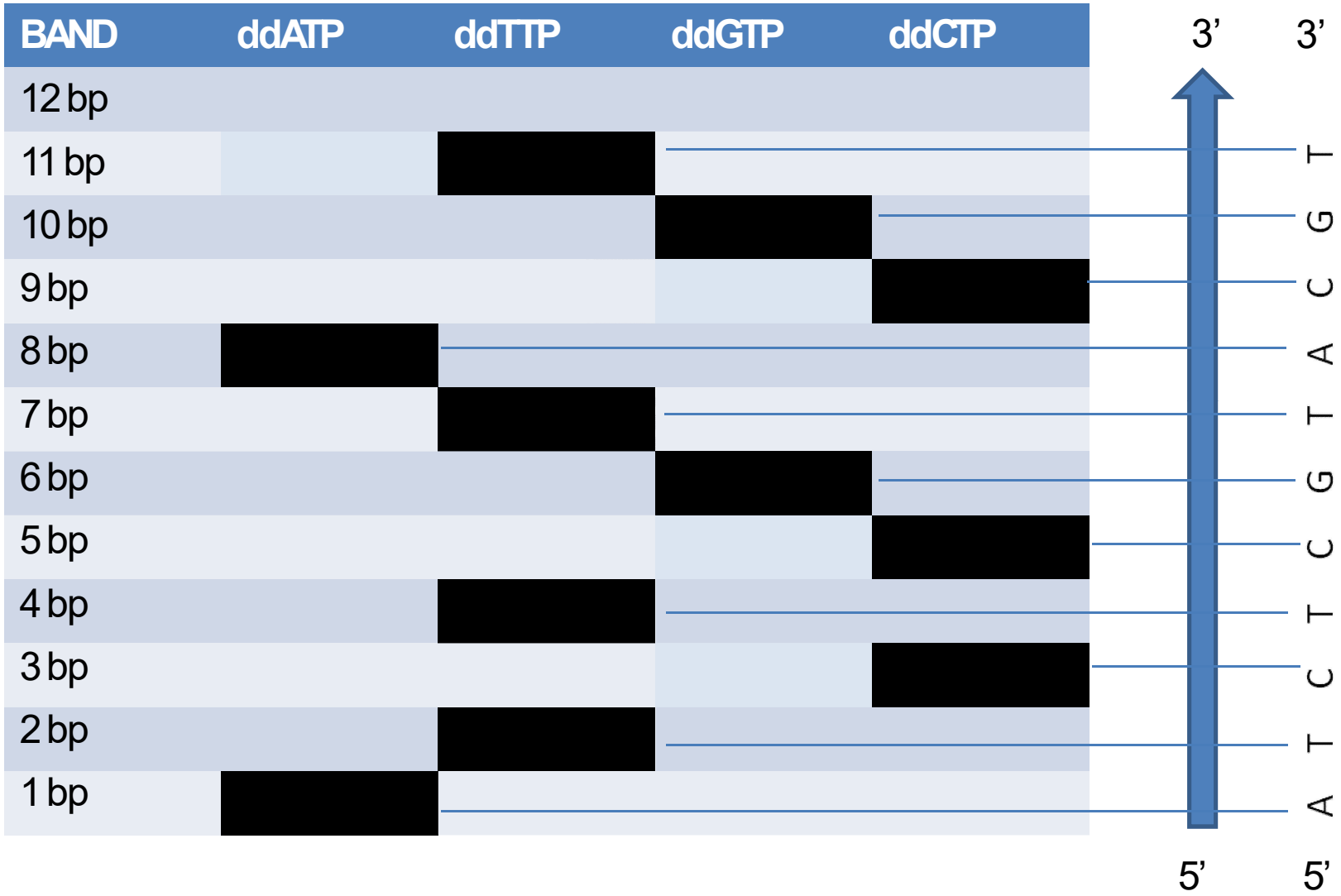
3’-ATGTGCTAGCT-5’

5’-TAC-3’

5’-TACAC-3’

5’-TACACGATC-3’

Reading Sequence



Sanger Sequencing: Process Summarized

1. Get enough quantity of DNA (Run PCR)
2. Aliqot DNA into four different tubes
3. Prepare PCR reaction mix as below:
   - Primer, taq PM, template(ss DNA), dNTPS (All) and ddNTPs(ddATP, ddGTP,ddCTP & ddTTP respectively)
4. Run PCR
5. Perform Gel Electrophoresis
6. Interpret results