
# 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

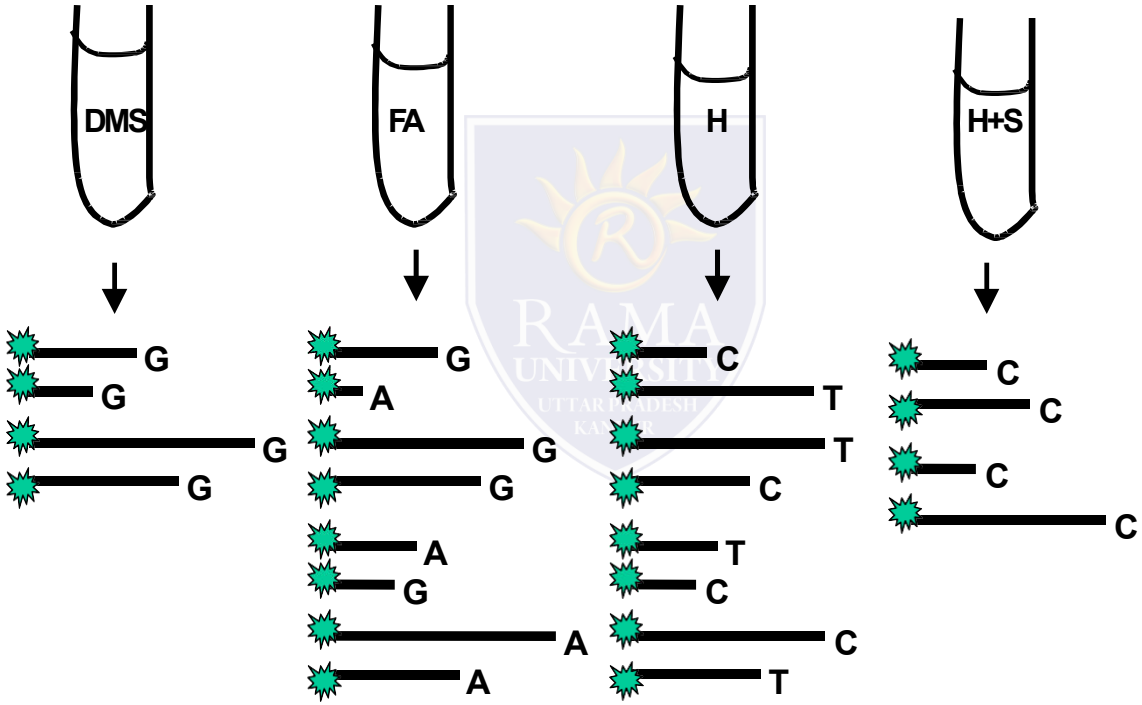
- Cleavage of Sugar Phosphate backbone using Piperidine

# Chemical Modification and Cleavage

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

- Cleavage of Sugar Phosphate backbone using Piperidine

# Maxam Gilbert Sequencing



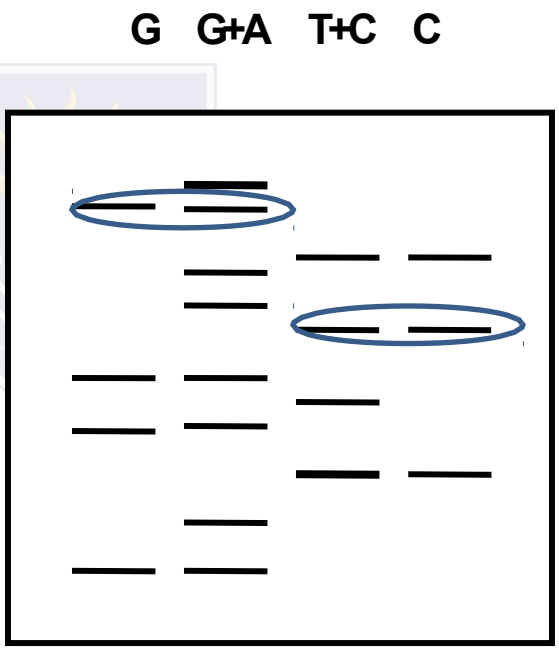

$^{32}P$ **5′ G A C G T G C A A**

**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′

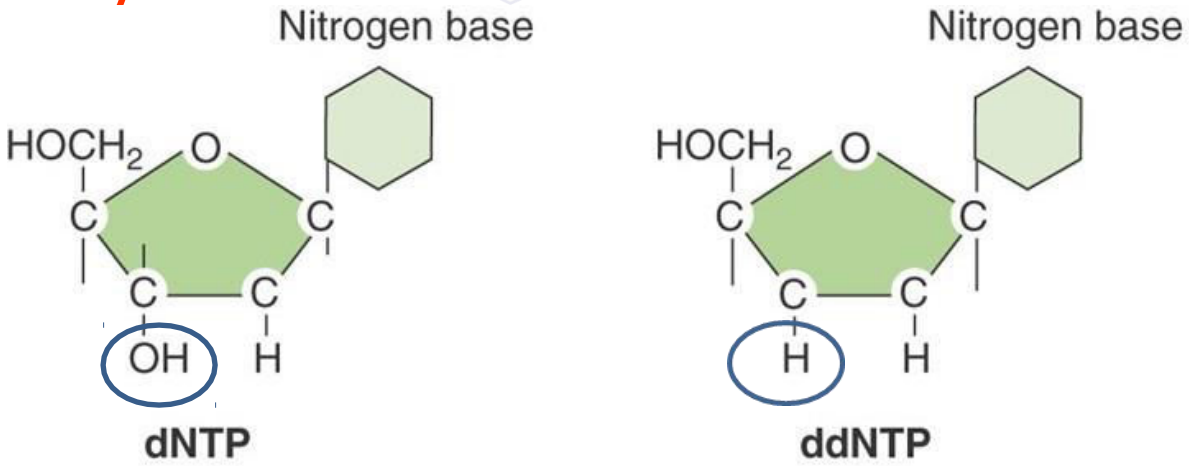
Sequencing gels are read from bottom to top (5′ to 3′).

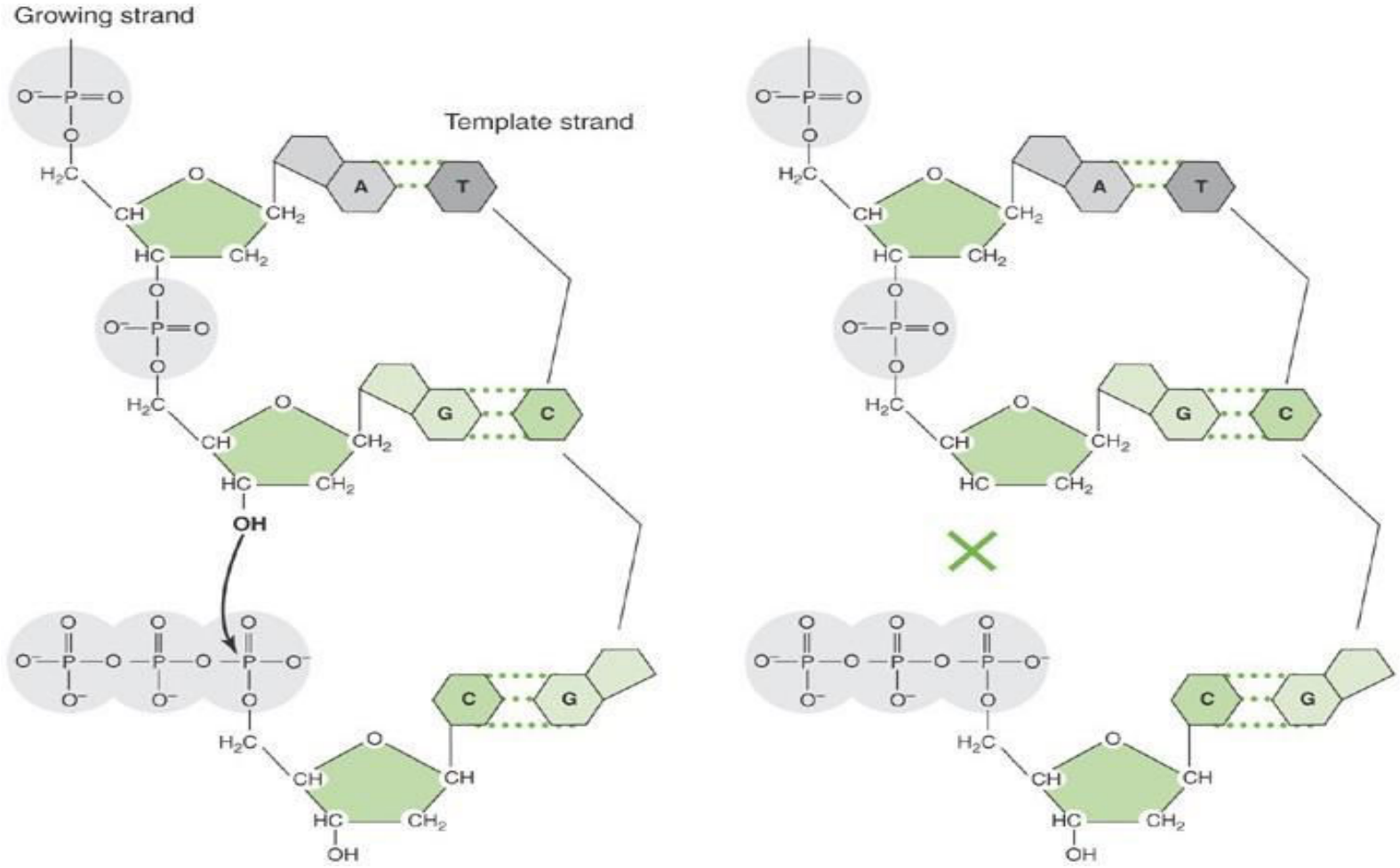
$^{32}$P 5′ G A C G T G C A A C G A

# 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

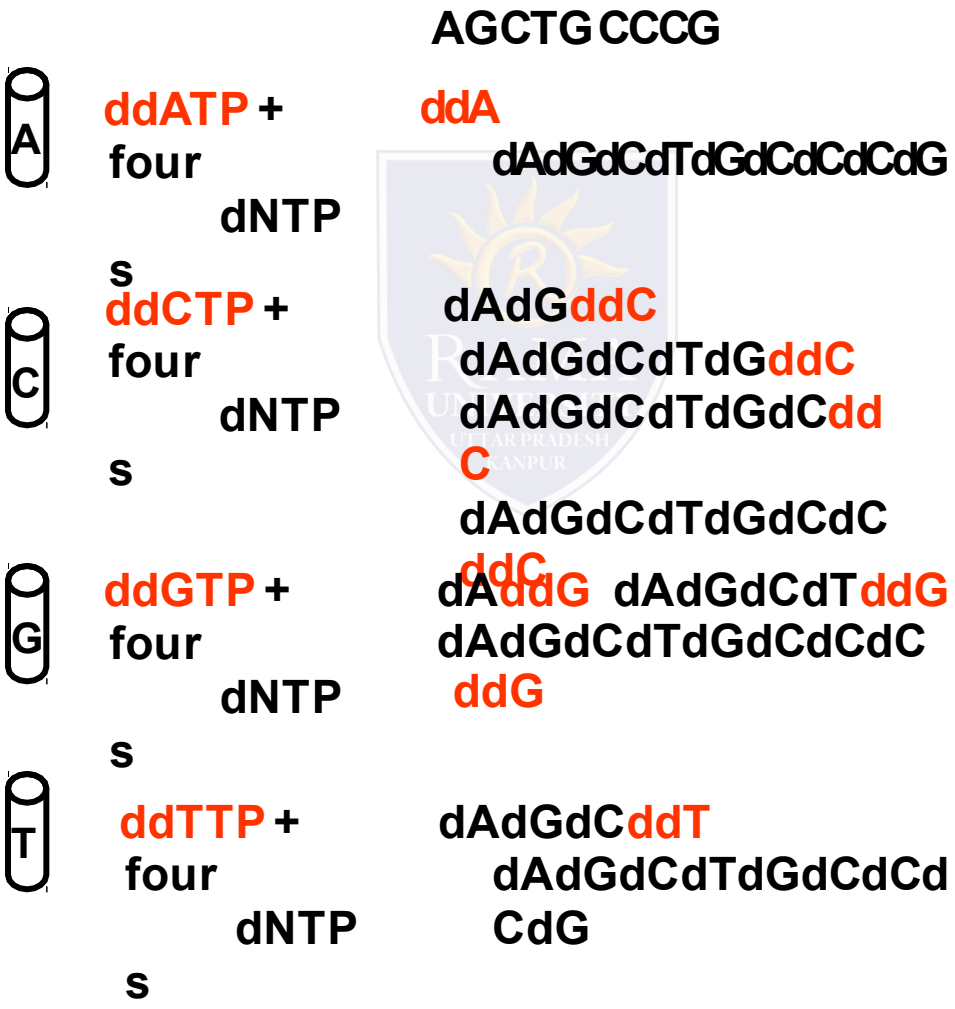
- It is PCR based method
- A modified DNA replication reaction
- Growing chains are terminated by dideoxynucleotides






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

# Sanger, Chain Termination Sequencing

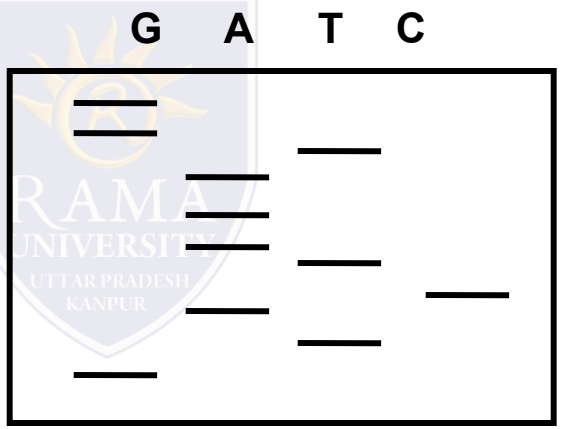


# Chain Termination Sequencing

Chain Termination Sequencing

Longer fragments


Shorter fragments


3′ G G T A A A T C A T G 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