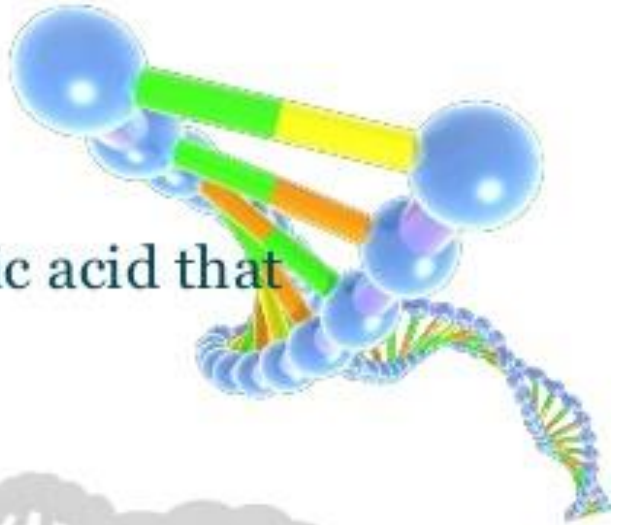
# DNA SEQUENCERS

Course code: **M.Sc./Zoo-408**

Course title: **Advanced Instrumental Techniques**

**Lahore College for Women University, Lahore**

# •Deoxyribonucleic Acid



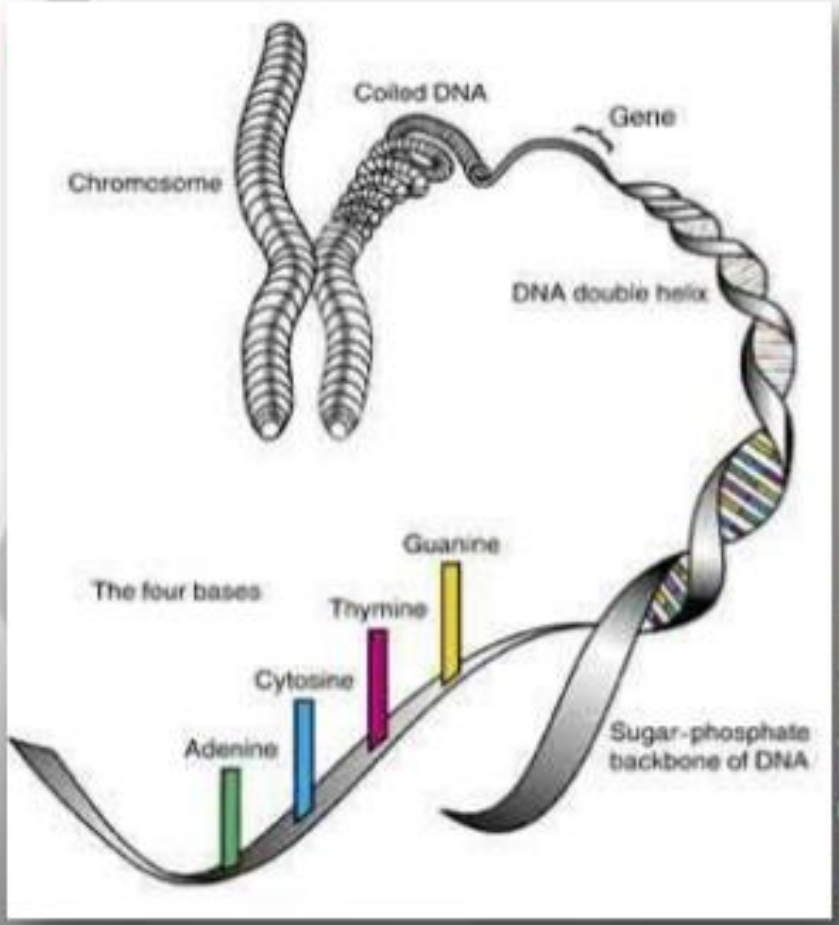
- Deoxyribonucleic acid (DNA) is a nucleic acid that functions include
  - Storage of genetic information
  - Self-duplication & inheritance
  - Expression of the genetic message
- DNA's major function is to code for proteins. Information is encoded in the order of the nitrogenous bases.

Adenosine | Cytosine | Guanine | Thymine

# •Watson & Crick Model of DNA

**KEY FEATURES OF A DNA**

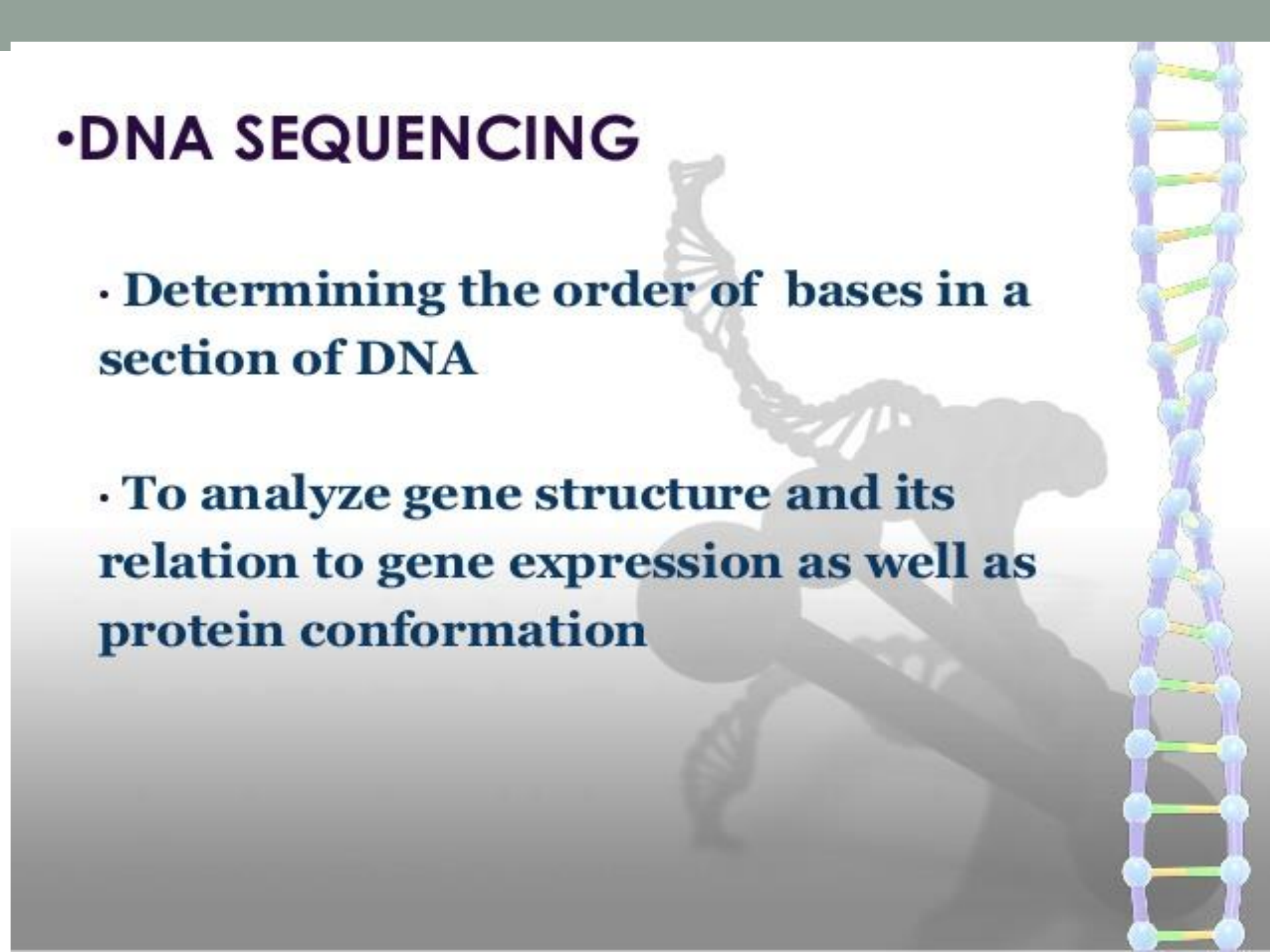
- DNA is composed of 2 chains of nucleotides that form a double helix shape.
- The two strands are antiparallel.
- The backbone of the DNA molecule is composed of alternating phosphate groups and sugars.
- The complimentary nitrogenous bases form hydrogen bonds between the strands.
- A is complimentary to T and G is complimentary to C.



# •DNA SEQUENCING

- Determining the order of bases in a section of DNA

- To analyze gene structure and its relation to gene expression as well as protein conformation



# •DNA SEQUENCING METHODS

•Historically there are two main methods of DNA sequencing

*1. Maxam and Gilbert method*
*2. Sanger method*

Modern sequencing equipment uses the principles of the Sanger technique.

# SANGER SEQUENCING OR CHAIN TERMINATION METHOD

# HISTORY

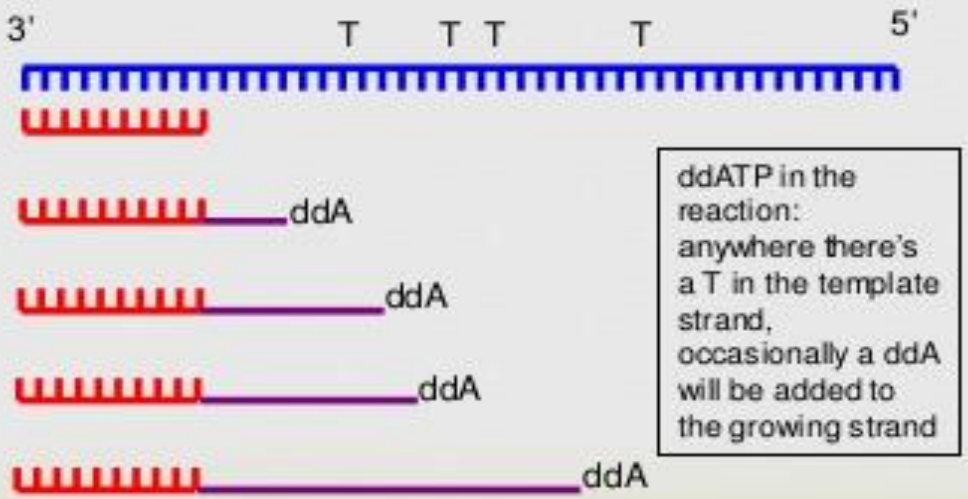
- Developed by Frederick Sanger and colleagues in 1977,
- it was the most widely used sequencing method for approximately 25 years after its discovery.
- He got NOBEL PRIZE in 1980.

# INTRODUCTION

- It is method to find out the nucleotides Sequence of unknown DNA strand.
- More recently, Sanger sequencing has been upgraded as "Next-Generation" sequencing methods, especially for large scale genome analyses and for obtaining especially long DNA sequence reads (>500 nucleotides).

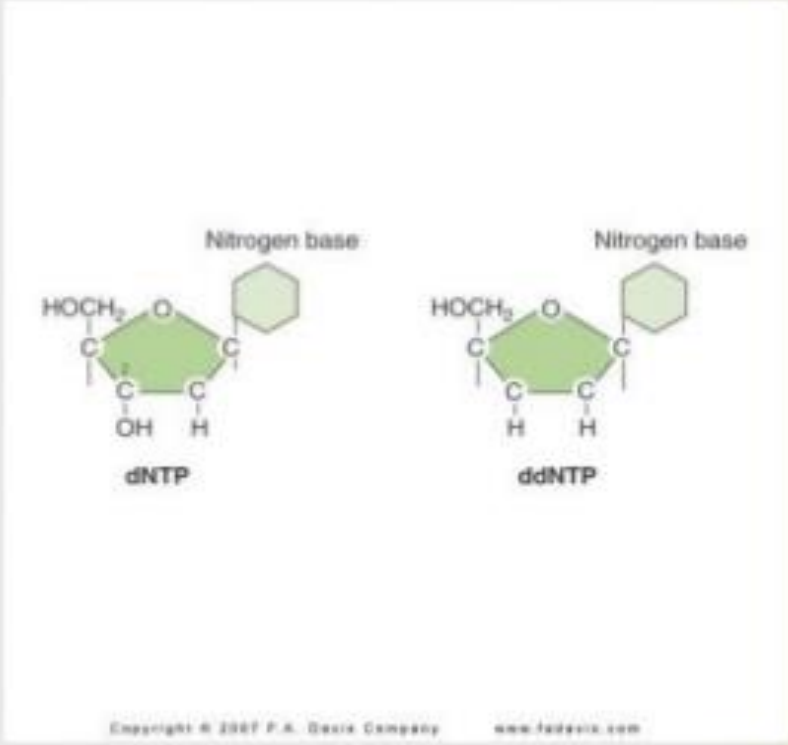
# BASIC PRINCIPLE

- This method generally is an In-Vitro synthesis of DNA strand and by using terminators (di-deoxynucleotide) the growing strand terminates at specific site.
- Upon termination the strands are overlap to got original sequence of unknown DNA Strand.



# REQUIREMENTS

- Single Stranded template
- Primer
- DNA polymerase
- Di-Deoxynucleotide
- ✓ The 3'-OH group necessary for formation of the phosphodiester bond is missing in ddNTPs)
- ✓ Every nucleotide have its specific ddNTP form i.e., ddATP, ddGTP etc

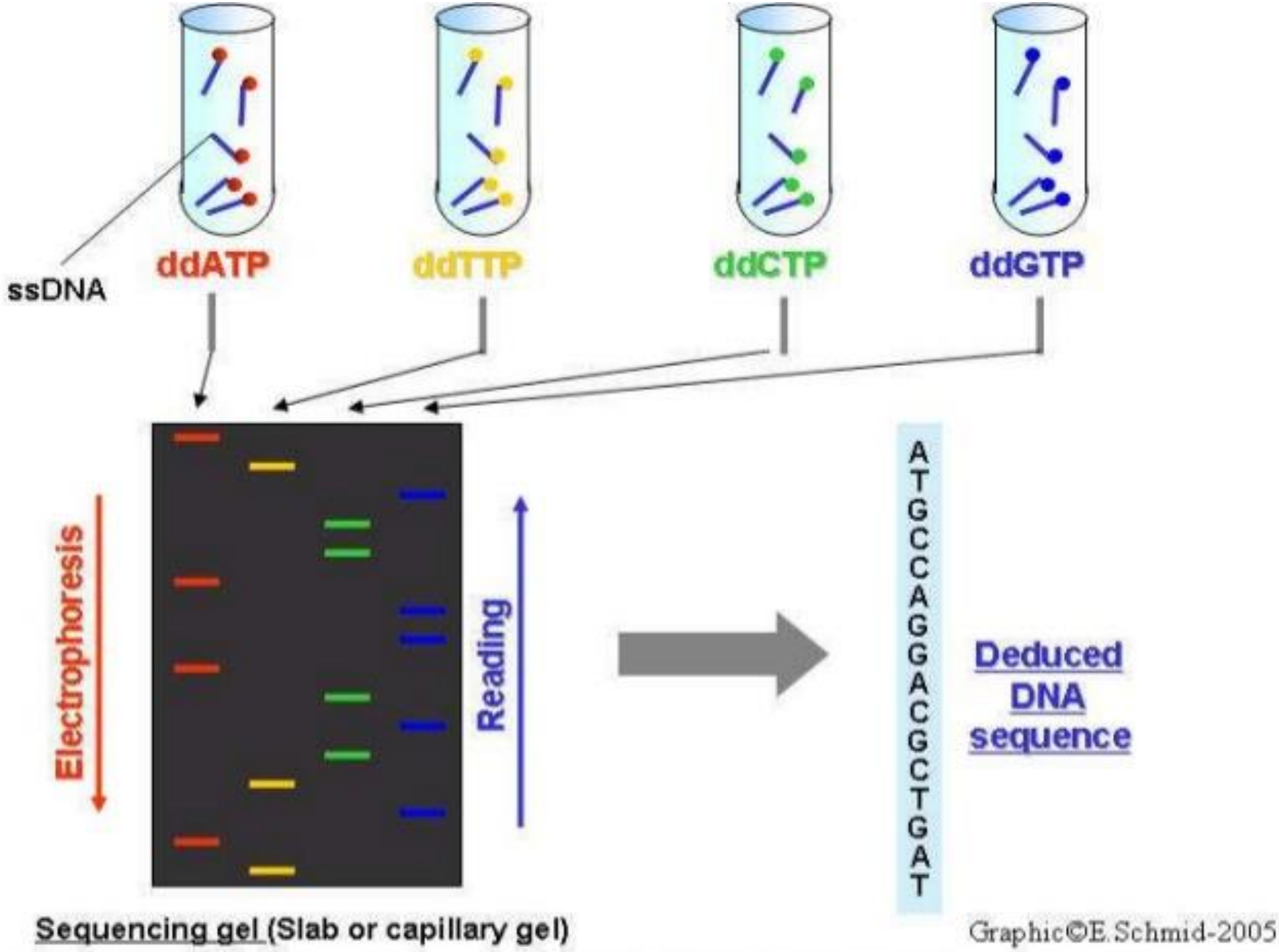


# PROCEDURE

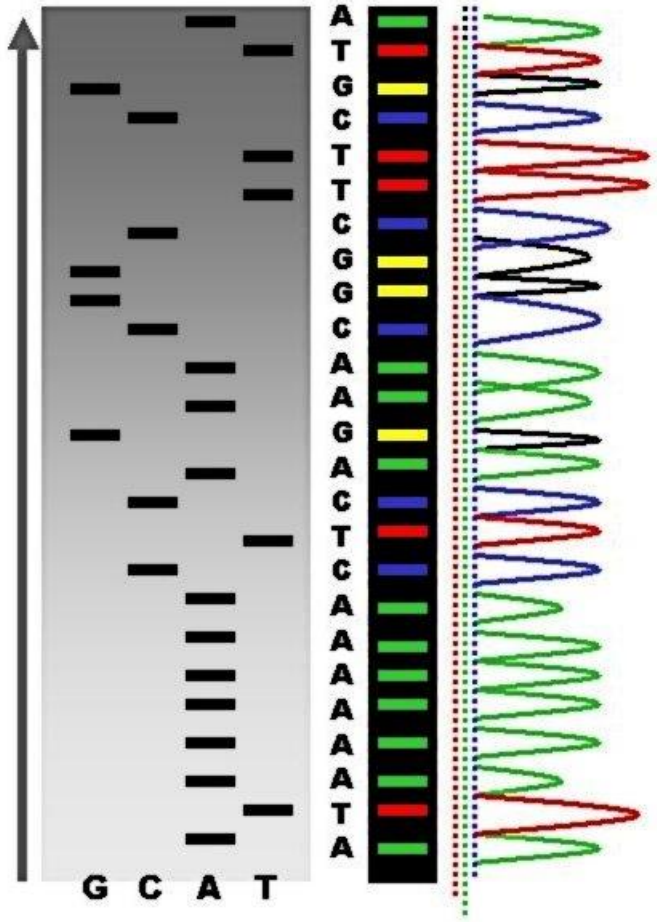
- Steps:
  1. Denaturation
  2. Primer attachment and extension of bases
  3. Termination
  4. Gel electrophoresis

# Cont….

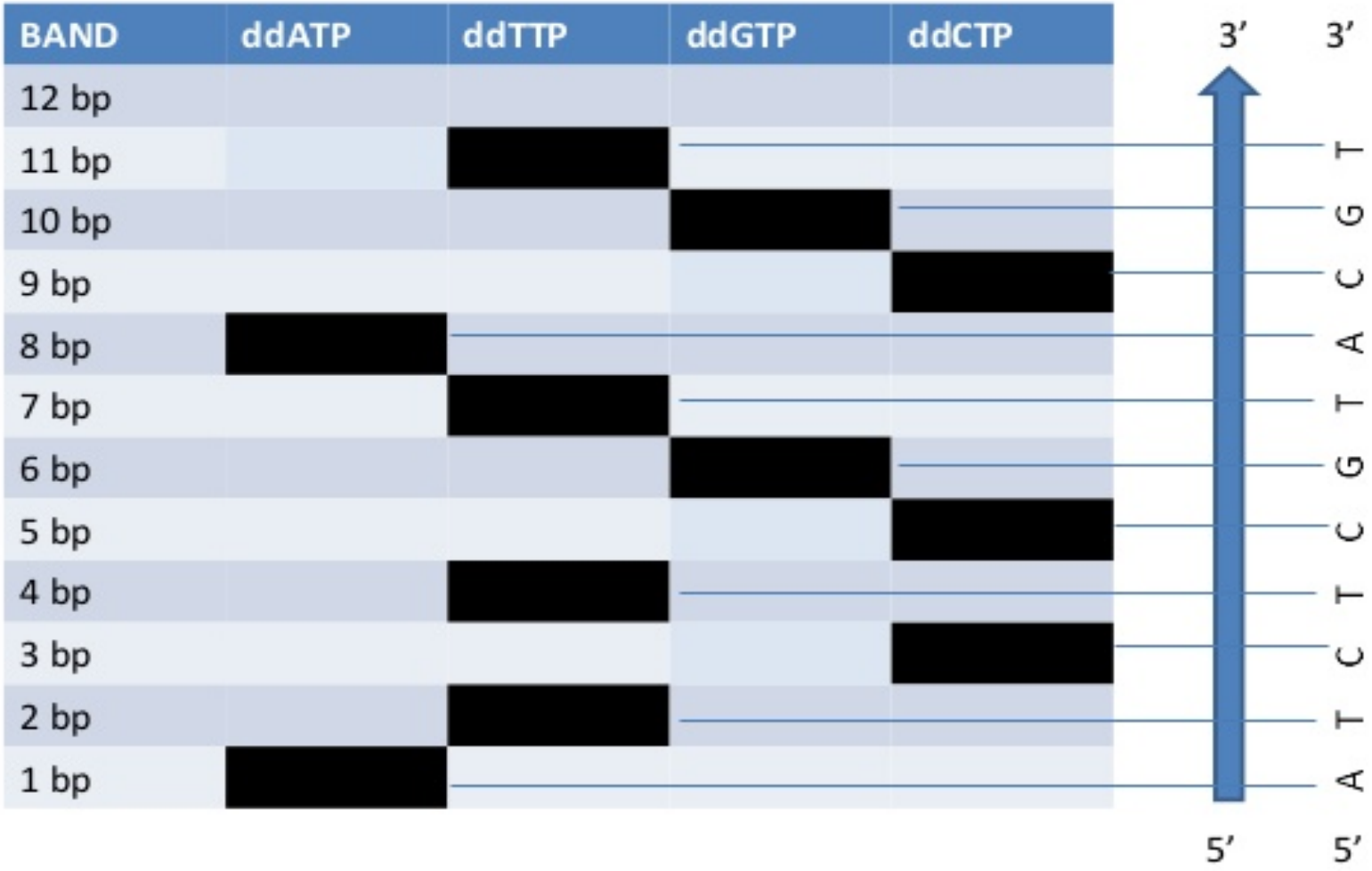
- The DNA template is treated with heat so that it becomes single stranded
- A short, single-stranded primer which is radioactively labelled is added to the end of the DNA template
- Add template DNA and primer in 4 Tubes.
- Now add ddNTPs In tubes in the way that single tube contain one type of ddNTP.
- Extension is start and band formed of various sizes.
- The fragments of DNA are separated by electrophoresis
- Overlap these sequences to find out sequence of Target DNA.


ssDNA
ddATP
ddTTP
ddCTP
ddGTP
Electrophoresis
Reading
ATGCCAGGACGCTGAT
Deduced DNA sequence
Sequencing gel (Slab or capillary gel)
Graphic©E.Schmid-2005

# Sample: Dye Sequencing Output



# 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)
1. Run PCR
2. Perform Gel Electrophoresis
3. Interpret results

# MAXAM-GILBERT SEQUENCING METHOD

# INTRODUCTION

- Maxam–Gilbert sequencing is a method of DNA sequencing developed by Allan Maxam and Walter Gilbert in 1976–1977.
- Maxam–Gilbert sequencing was the first widely adopted method for DNA sequencing, and, along with the Sanger dideoxy method.
- method based on chemical modification of DNA and subsequent cleavage at specific nitrogenous bases.

# PRINCIPLE

- purification of the DNA fragment that to be sequenced and labeled with radioactive material.
- Chemical treatment generates breaks at a specific nitrogenous bases and thus a series of labelled fragments is generated. The fragments in the four reactions are arranged side by side in gel electrophoresis for size separation.
- The fragments visualize in X-ray for autoradiography.
- To visualize the fragments,the gel is exposed to X-ray film for autoradiography,yielding a series of dark bands each corresponding to a radiolabelled DNA fragment,from which the sequence may be inferred.

# 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

# 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

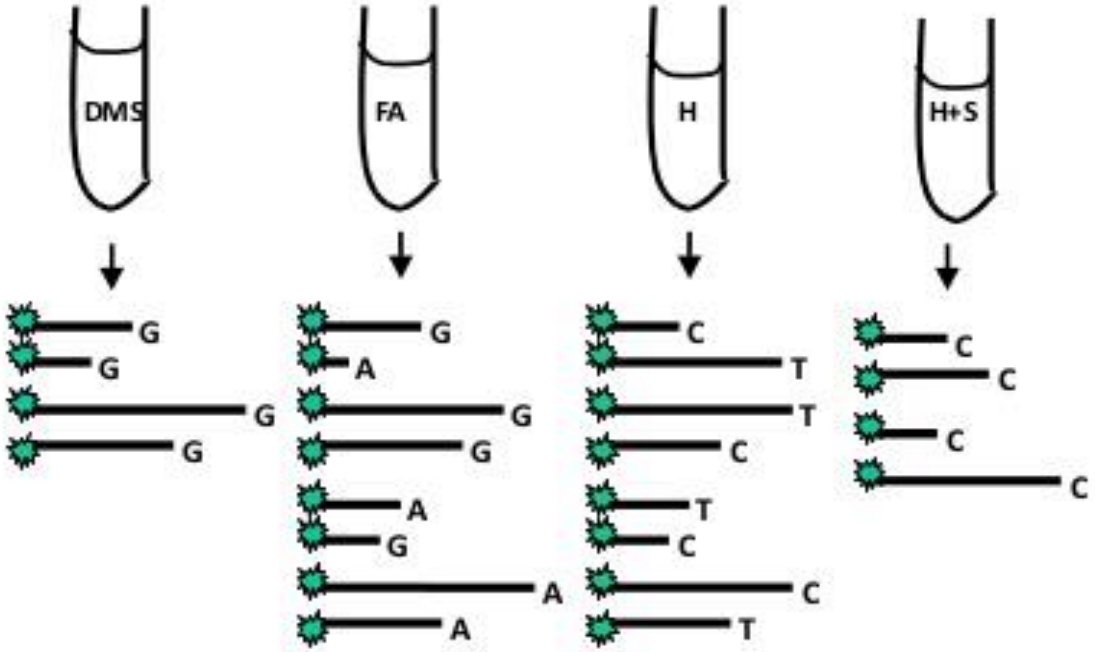
- Cleavage of Sugar Phosphate backbone using Piperidine

# Chemical Modification and Cleavage

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

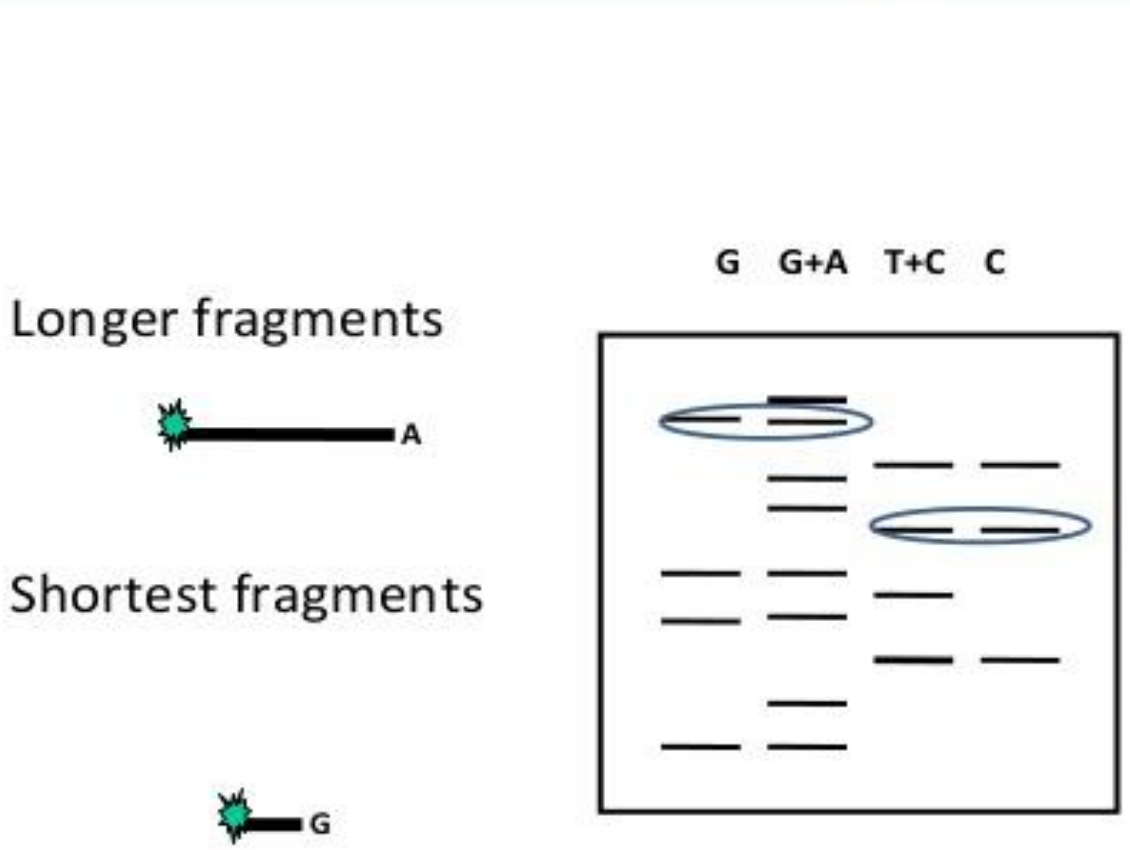
- Cleavage of Sugar Phosphate backbone using Piperidine

# Maxam Gilbert Sequencing




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

# Maxam-Gilbert Sequencing



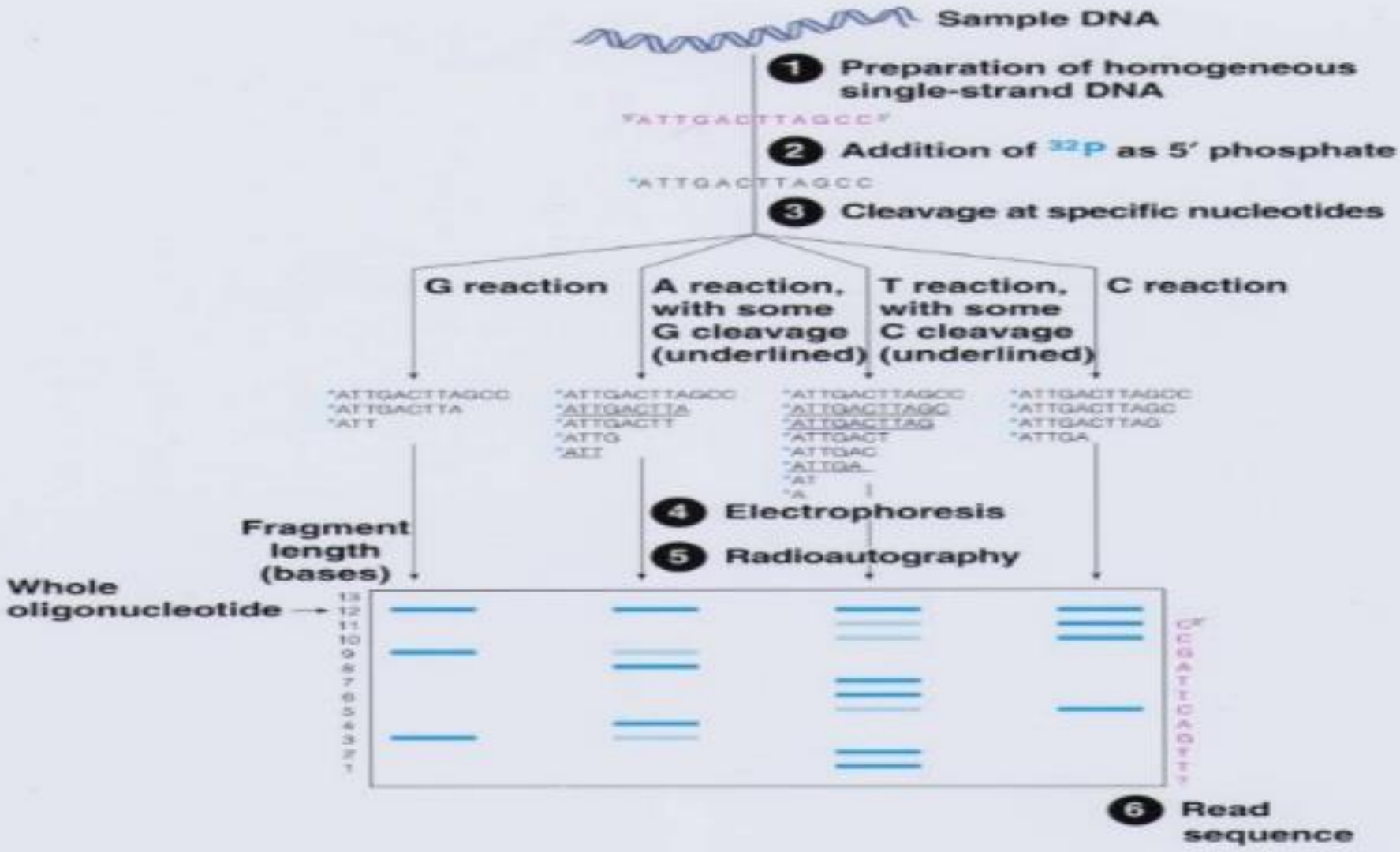

Sequencing gels are read from bottom to top (5′ to 3′).

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

# 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

**Figure 4A.4 Sequencing an oligonucleotide by the Maxam-Gilbert method**



# •COMPARISON

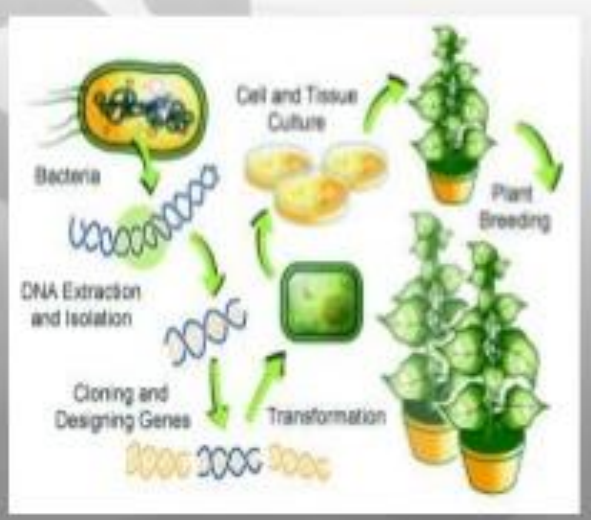
| Sanger Method | Maxam Gilbert Method |
|---|---|
| Enzymatic | Chemical |
| Requires DNA synthesis | Requires DNA |
| Termination of chain elongation | Breaks DNA at different nucleotides |
| Automation | Automation is not available |
| Single-stranded DNA | Double-stranded or single-stranded DNA |

# •Applications of DNA Sequencing

• Forensics: to help identify individuals because each individual has a different genetic sequence


• Medicine: can be used to help detect the genes which are linked to various genetic disorders such as muscular dystrophy.

• Agriculture: The mapping and sequencing of a genome of microorganisms has helped to make them useful for crops and food plants.



- **Advantages**
  - Improved diagnosis of disease
  - Bio pesticides
  - Identifying crime suspects
- **Disadvantages**
  - Whole genome cannot be sequenced at once
  - Very slow and time consuming