
بسم الله الرحمن الرحيم
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DNA SEQUENCING METHOD

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•Summary

Introduction	What is DNA Sequencing method?
History	Who and when it was practiced first?
The Method	How it is performed?
Advantages	How does it revolutionize biological sciences?
Future	How long will it give benefits?

•Deoxyribonucleic Acid

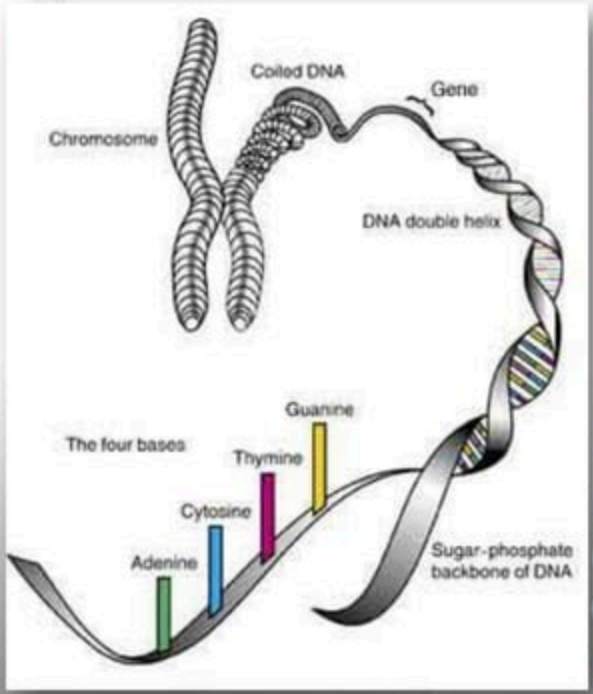
- Deoxyribonucleic acid (DNA) is a nucleic acid that functions include
  - Storage of genetic information
  - Self-duplication & inheritance
  - Expression of the genetic message
- DNA's major function is to code for proteins. Information is encoded in the order of the nitrogenous bases.

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

- **PURPOSE**
  - Deciphering “code of life”
  - Detecting mutations
  - Typing microorganisms
  - Identifying human halotypes
  - Designating polymorphisms

•DNA SEQUENCING METHODS

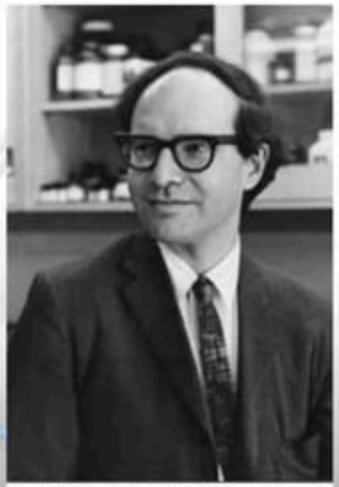
•Historically there are two main methods of DNA sequencing

1. Maxam and Gilbert method
2. Sanger method

Modern sequencing equipment uses the principles of the Sanger technique.

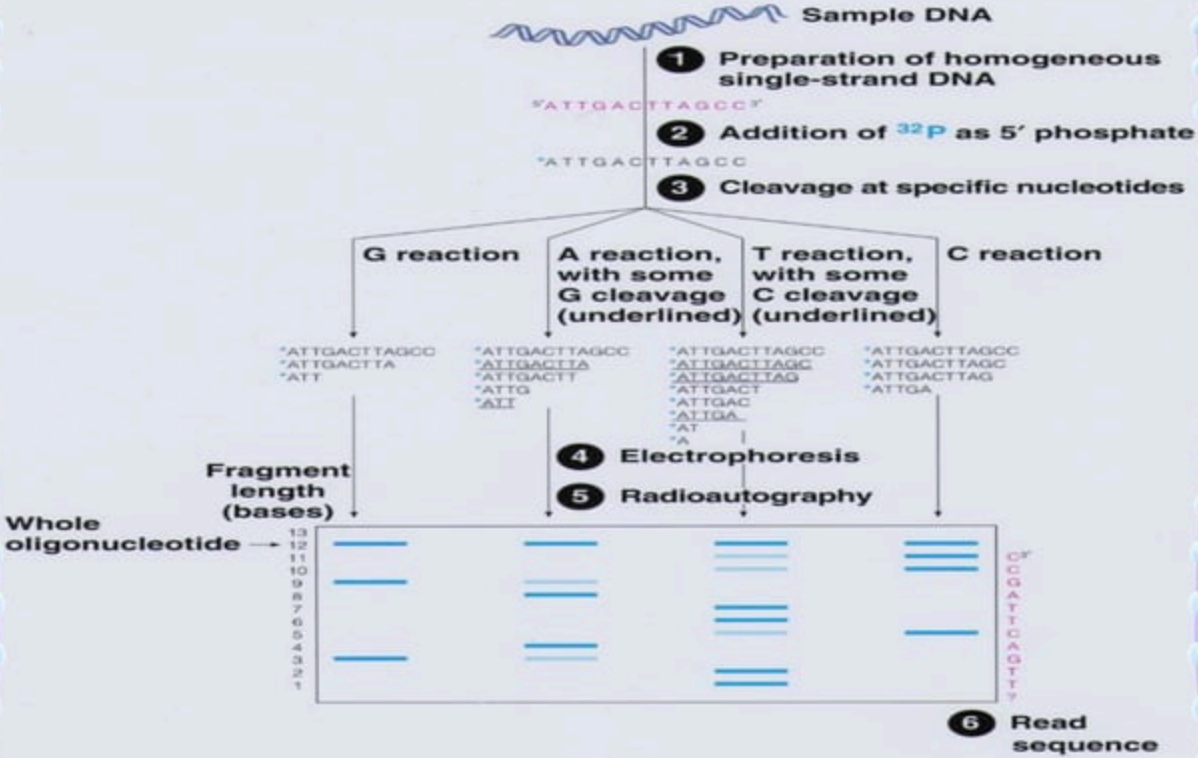
•MAXAM & GILBERT METHOD

- A. M. Maxam and W.Gilbert-1977
- Chemical Sequencing
- Treatment of DNA with certain Chemicals ➔ DNA cuts into Fragments ➔ Monitoring of sequences



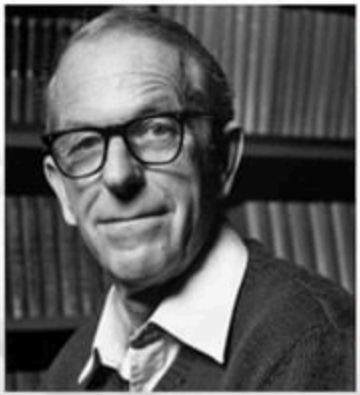
Walter Gilbert (1932 -)

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



•SANGER METHOD

- Most common approach used for DNA sequencing .
- Invented by Frederick Sanger - 1977
- Nobel prize - 1980
- Also termed as Chain Termination or Dideoxy method



•SANGER METHOD

- **The chain termination reaction**

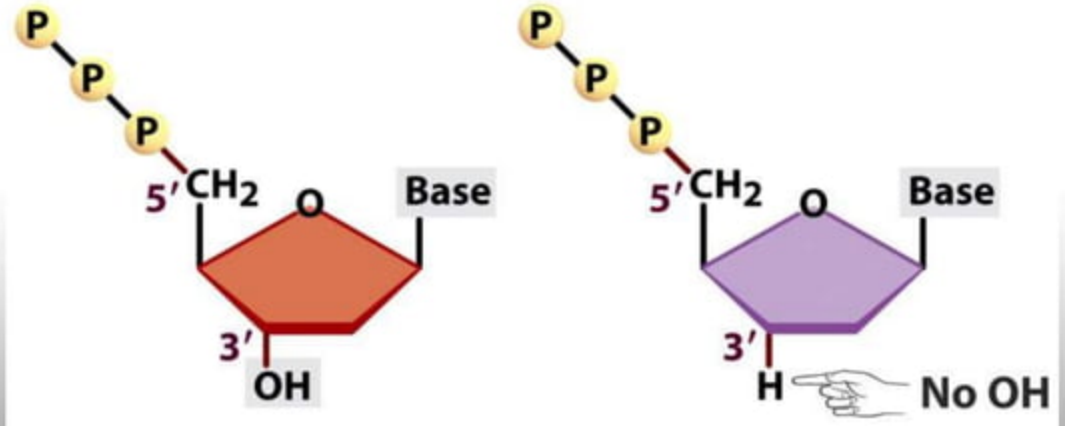
- Dideoxynucleotide triphosphates (ddNTPs) chain terminators
    - havig an H on the 3'C of the ribose sugar (normally OH found in dNTPs)

- ssDNA ➔ addition of dNTPs ➔ elongation

- ssDNA ➔ addition of ddNTPs ➔ elongation stops

•DEOXY VERSUS DIDEOXY

ddNTPs terminate DNA synthesis.



Normal dNTP
(extends DNA strand)

ddNTP
(terminates synthesis)

Figure 19-6a Biological Science, 2/e

•PRINCIPLE

ssDNA

↓

Enzymatic synthesis of complementary polynucleotide chains

↓

Termination at specific nucleotide positions

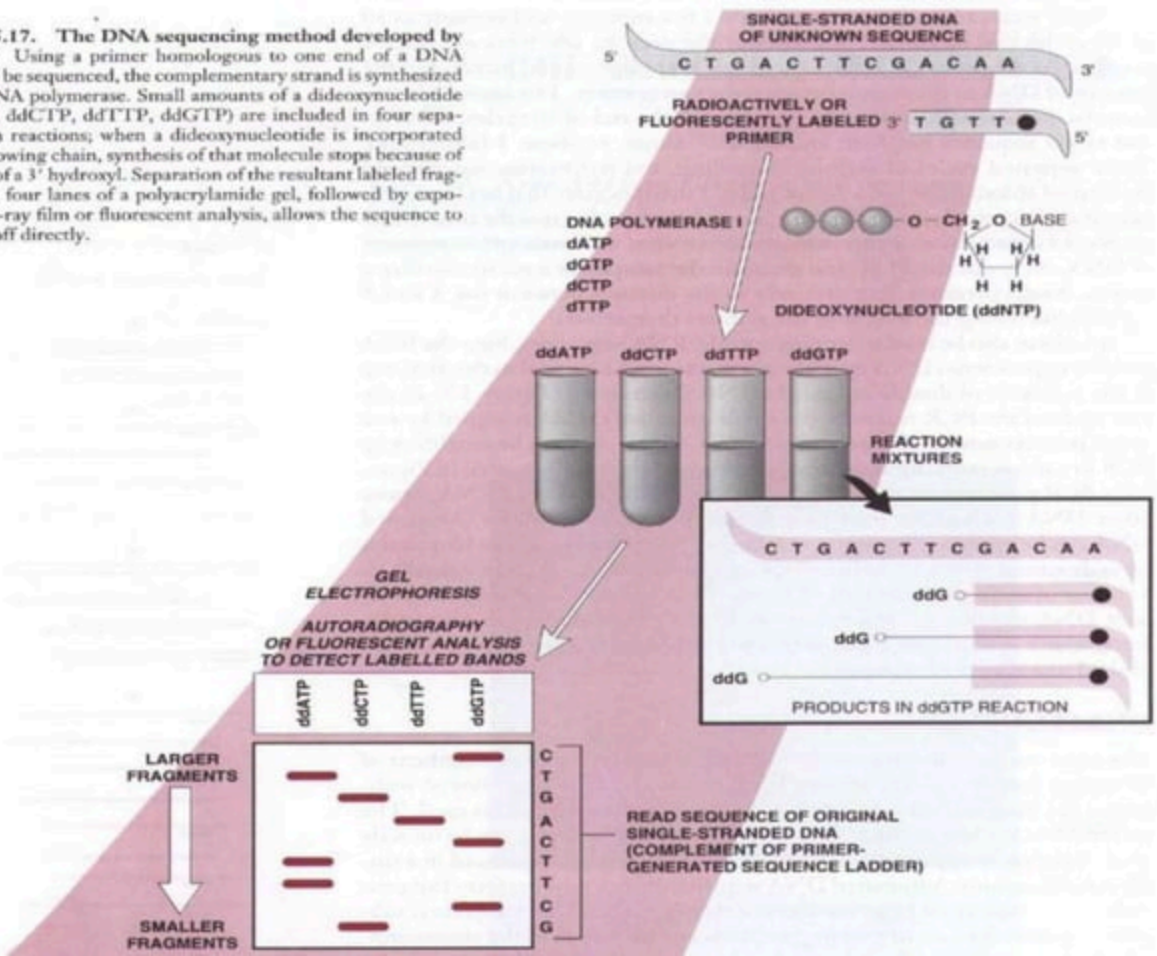
↓

Separate by Gel Electrophoresis

↓

Read DNA Sequence

Figure 5.17. The DNA sequencing method developed by Sanger. Using a primer homologous to one end of a DNA strand to be sequenced, the complementary strand is synthesized using DNA polymerase. Small amounts of a dideoxynucleotide (ddATP, ddCTP, ddTTP, ddGTP) are included in four separate such reactions; when a dideoxynucleotide is incorporated into a growing chain, synthesis of that molecule stops because of the lack of a 3′ hydroxyl. Separation of the resultant labeled fragments in four lanes of a polyacrylamide gel, followed by exposure to x-ray film or fluorescent analysis, allows the sequence to be read off directly.



•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

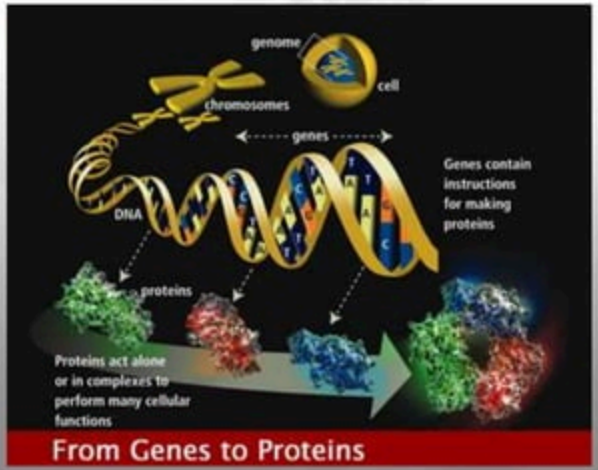
• Medicine: can be used to help detect the genes which are linked to various genetic disorders such as muscular dystrophy.

• 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

•The Human Genome Project

- The biggest challenge for the life sciences
- 15 years project (NIH, DOE of USA)
- Primary goal ➔ Sequence base pairs of human beings that form DNA
- Identifying & mapping approx. 20K-25K genes
- Significance ➔ Physical & functional •standpoint



Animation Page

http://www.youtube.com/watch?v=oYpIlbl0aF8

Thank You / Good Luck