Elective Paper :

Unit 2 .

Q1] History of Sequencing

→ Foundations of DNA Sequencing

1871 - 1952

① The history of DNA sequencing can be tracked back to the late 19th century when scientist like Friedrich Miescher laid the groundwork in cell chemistry & nucleic substances. By 1929, the chemical composition of nucleic acids & the structure of DNA's backbone were understood, revealing the existence of nitrogen containing bases (A, C, T, G) & alternating sugar phosphate backbone.

The Revolutionary Discovery 1944 - 1952

In 1944, Oswald Avery, Maclyn McCarty's groundbreaking research demonstrated that DNA, not protein carries genetic information & can transform cellular properties. This discovery led to significant shift in scientific focus towards DNA research.

In 1952, Erwin Chargaff & Linus Pauling made important contributions by revealing crucial chemical properties of DNA, including the base pairing rules (A=T & C=G)

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## The Sanger Method (1905–1982)

The first sequencing methods were introduced in 1960s with a heavy focus on RNA sequencing due to its relative simplicity. It was not until 1977 that Frederick Sanger developed a method for DNA sequencing that became the gold standard for over a decade. Sanger's method enabled a major milestone, the ~~sequing~~ sequencing of the first whole genome of a bacteriophage in 1982.

## Second generation sequencing (1996–2006)

The emergence of second generation sequencing technologies began with the introduction of pyrosequencing in 1996. These methods including Roche/454, SOLiD & Illumina allowed for high throughput sequencing & significantly reduced the associated costs. Roche/454 life sciences released the first high throughput sequencer in 2005, followed by Illumina's Genome Analyzer in 2006, propelling the field of genomics into new era.

## Third Generation Sequencing (2008)

Third generation sequencing technologies revolutionized DNA sequencing by providing long read capabilities. Pacific Biosciences SMRT sequencing uses fluorescent nucleotides to generate long reads. & Oxford Nanopore Technologies nanopore technology measures disruptions in electrical current as individual bases pass through a nanopore. These technologies have facilitated denovo genome assembly & structural analysis.

## Maxam Gilbert

DNA sequencing refers to methods for determining the order of the nucleotide bases adenine, guanine, cytosine & thymine in a molecule of DNA

Two main methods are widely known to be used to sequence DNA.

1. The chemical Method (also called Maxam-Gilbert method after its inventors)
2. The chain Termination Method (also known as Sanger dideoxy method)

Maxam gilbert technique depends on the relative chemical liability of different nucleotide bonds, whereas the Sanger

... elongation of DNA sequences by incorporating dideoxynucleotides into the sequences.

The chain termination method is the method more usually used because of its speed & simplicity.

① In 1976–1977, Allan Maxam & Walter Gilbert developed a DNA sequencing method based on chemical modification of DNA & subsequent cleavage at specific bases.

② The method requires radioactive labelling at one end & purification of the DNA fragment to be sequenced.

③ Chemical treatment generates breaks at a small proportions of one or two of the four nucleotide based in each of four reactions (G, A+G, C, C+T)

④ Thus a series of labelled fragments is generated from the radiolabelled end to the first 'cut' site in each molecule.

⑤ The fragments in the four reactions are arranged side by side in gel electrophoresis for size separation.

⑥ To visualize the fragments the gel is exposed to xray film for autoradiography yielding a series of dark bands each corresponding to a radiolabelled DNA fragment from which the sequence may be inferred.

## II Sanger Sequencing

1. The first major breakthrough in sequencing technology was made by Fredrick Sanger in 1977, when he & his colleagues introduced the "dideoxy chain termination method for sequencing DNA molecules also known as 'Sanger sequencing'. It earned him his second Nobel Prize

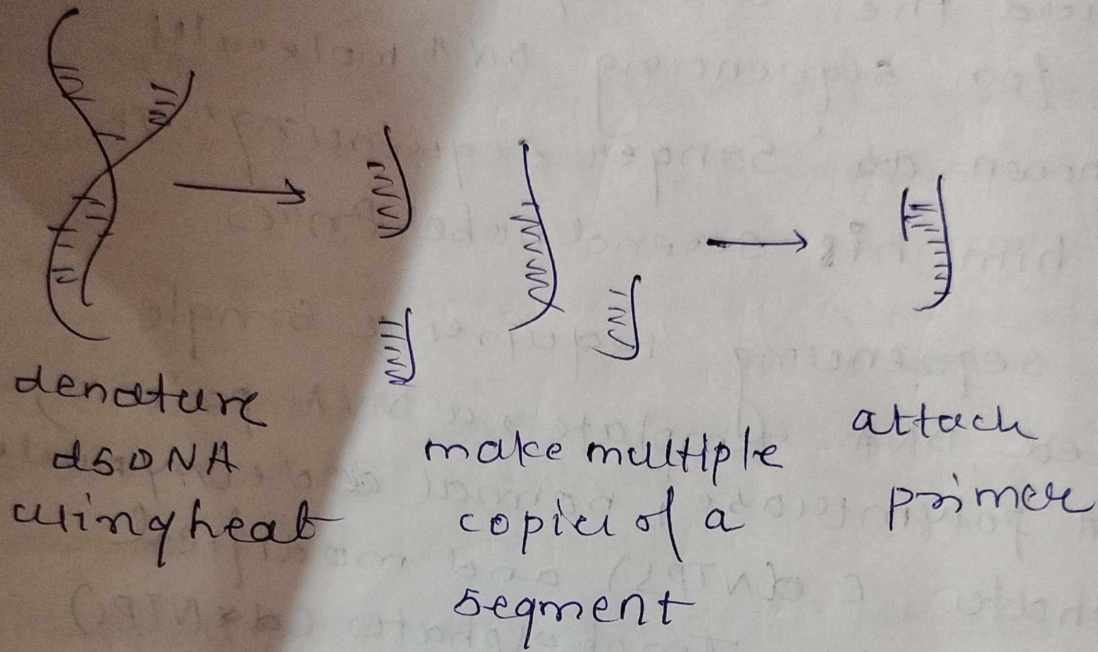
2. Sanger sequencing requires a single stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleotide triphosphates (dNTPs) and modified dideoxynucleotide triphosphates (ddNTPs)

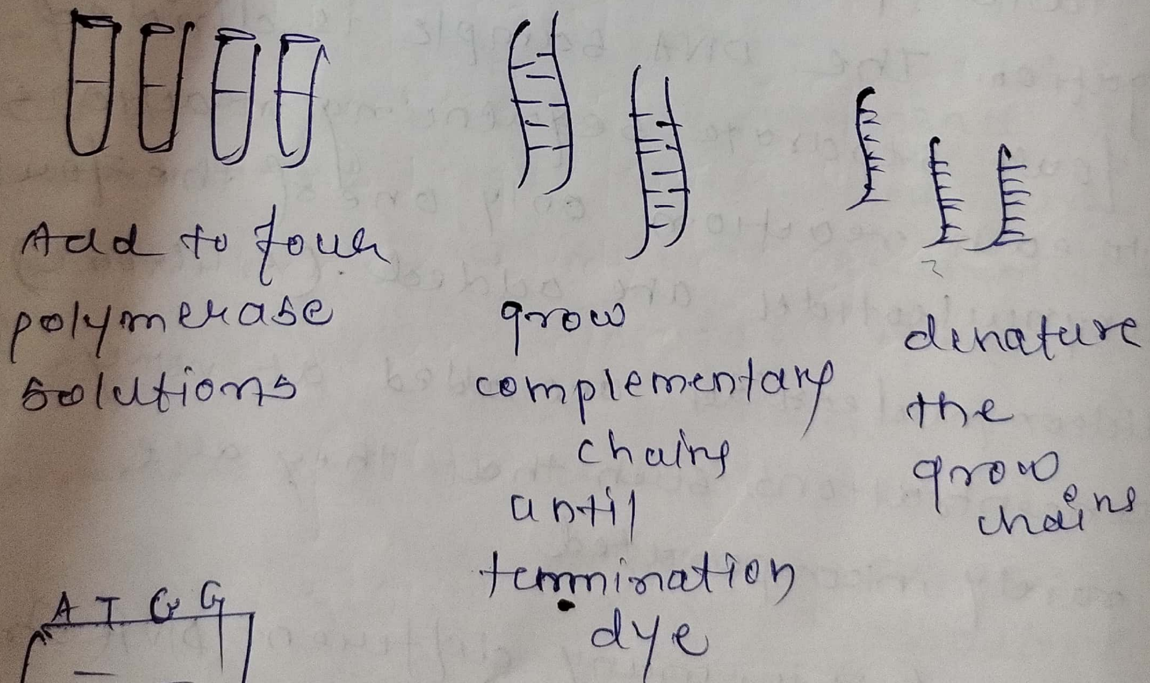
3. The latter of which terminate DNA strand elongation. The DNA sample is divided into four separate sequencing reactions & to each reaction only one of the four dideoxynucleotides are added. (A, T, G or C)

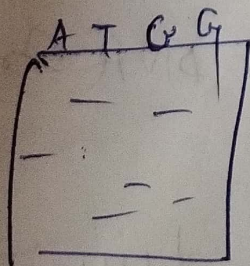
3. The dideoxynucleotides are added at very low concentrations, such that they are very rarely incorporated

4. Because of this, many different DNA strand lengths are formed, each with radioactive nucleotide at their terminus.

By 'lining up' all the varied lengths strands from all four reactions, one is able to see where each nucleotide occurs.




Electrophoresis this four soln

However Sanger sequencing lacked Automation & was extremely time consuming.

Q5)-

| First Generation | Second Generation |
| --- | --- |
| ① A low throughput method used to determine a portion of nucleotide seq of an individual genome | ① A high throughput method used to determine a portion of the nucleotide seq of an individual genome |
| ② Dideoxy chain termination method | ② Massive parallel sequencing. |
| ③ First commercialized by Applied Biosystem | ③ Dominant platform is Illumina |
| ④ Can only process a single DNA fragment at a time | ④ Processes millions of fragments simultaneously at a time |
| ⑤ A less sensitive method | ⑤ Highly sensitive |
| ⑥ limit detection is 5-20 | ⑥ limit detection is less than 1%. |

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(7) cost effective
Fast
up to 20
sample

(7) Time consuming
& cost effective
upto 20 sample

(8) Gold standard
for clinical
research sequencing

(8) Becoming
common in
clinical
labs

4 a) NGS (Next Generation Sequencing)

NGS is massively parallel sequencing technology that offers ultra high throughput, scalability & speed. The technology is used to determine the order of nucleotide in entire genome or targeted regions of DNA or RNA. NGS has revolutionized the biological science allowing labs to perform a wide variety of applications & study biological system at a level never before possible.

Applications of NGS

1. Rapidly sequence whole genomes
2. Deeply sequence target regions
3. utilize RNA sequencing (RNA seq) to discover novel RNA variants & splice sites, or quantify mRNAs for