

Maxam Gilbert Sequencing Method

Types

- Two most popular DNA sequencing method available.
 - Maxam–Gilbert sequencing method
 - Sanger sequencing method

Maxam-Gilbert sequencing

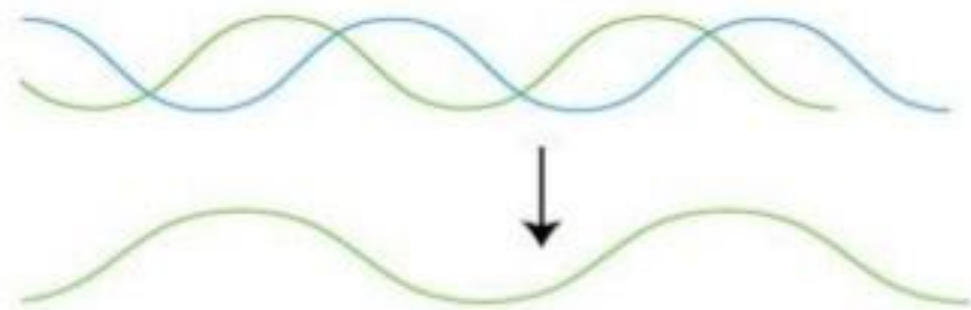


- **Maxam-Gilbert sequencing** is a method of DNA sequencing developed by Allan Maxam and Walter Gilbert in 1976-1977.
- This method is based on nucleobase-specific partial chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides.

Procedure



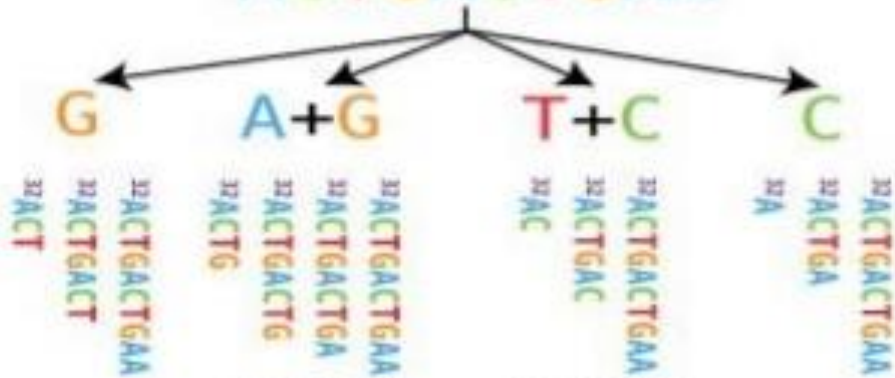
- Denature a double-stranded DNA to single-stranded by increasing temperature.
- Radioactively label one 5' end of the DNA fragment to be sequenced by a kinase reaction using gamma-³²P.
- Cleave DNA strand at specific positions using chemical reactions.
- For example, we can use one of two chemicals followed by piperidine. Dimethyl sulphate selectively attacks purine (A and G), while hydrazine selectively attacks pyrimidines (C and T). The chemical treatments outlined in Maxam-Gilbert's paper cleaved at G, A+G, C and C+T. A+G means that it cleaves at A, but occasionally at G as well.
- Now in four reaction tubes, we will have several differently sized DNA strands.



1) Obtain single stranded DNA

ACTGACTGAA

³²ACTGACTGAA



+ occasional G's

+ occasional T's

2) Add a ³²P to 5' end

3) Cleave at specific nucleotides

4) Differently sized DNA strands

Electrophoresis

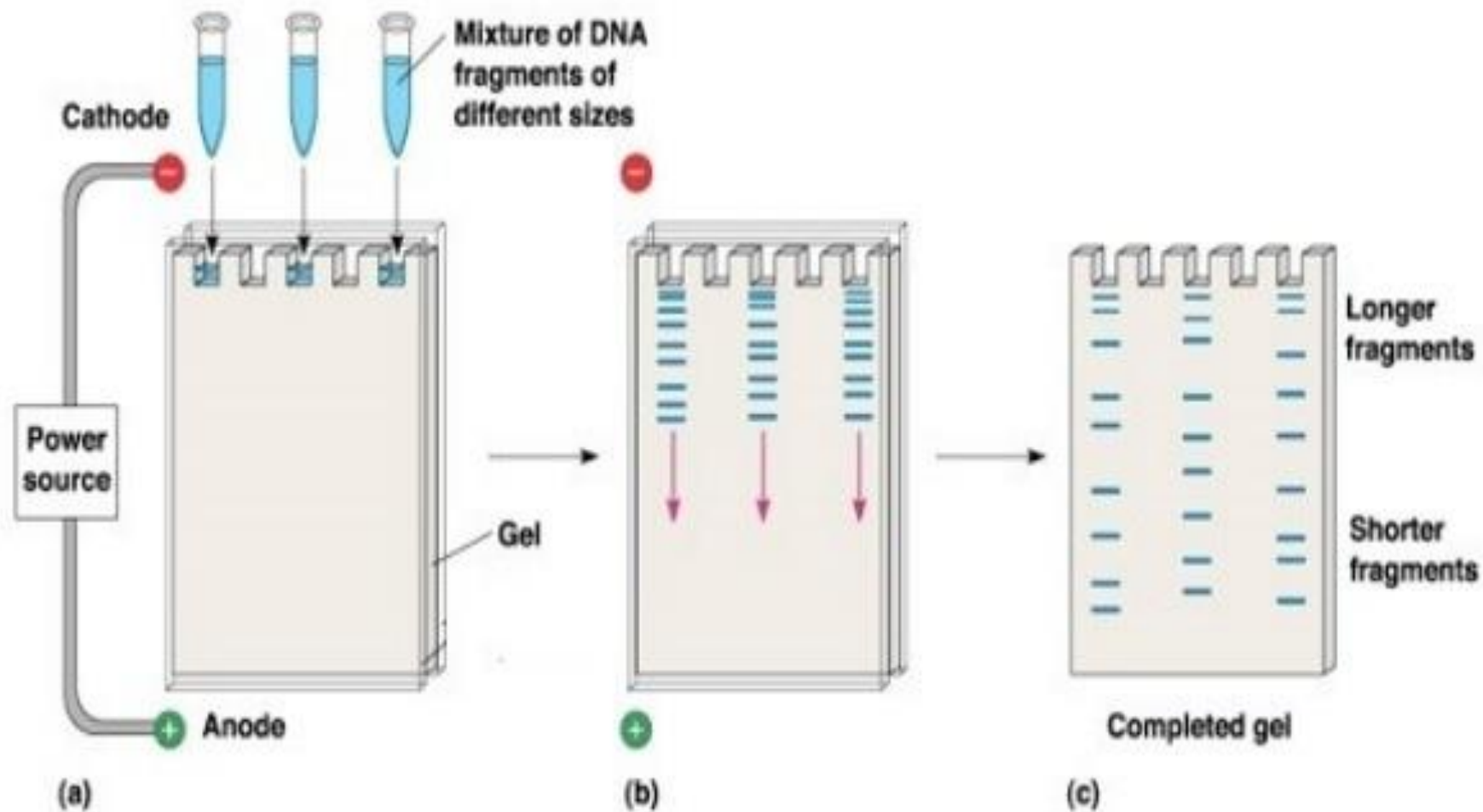


- A method used in research laboratories for separating molecules according to their size and electrical charge.
- An electric current is passed through a medium that contains the mixture of molecules.
- Each kind of molecule travels through the medium at a different rate, depending on its electrical charge and molecular size. Smaller molecule goes faster.
- Separation of the molecules occurs based on these differences.

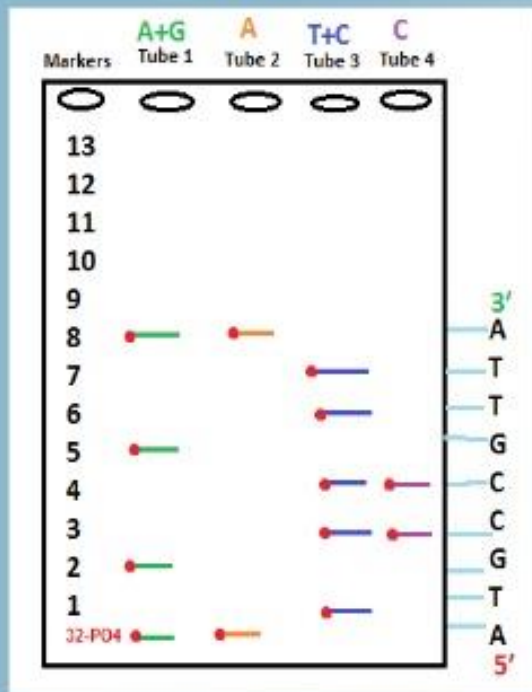
Electrophoresis



- Fragments are electrophoresed (migrate) in acrylamide gels for size separation.
- These gels are placed under X-ray film, which then yields a series of dark bands which show the location of radiolabeled DNA molecules.
- The fragments are ordered by size and so we can deduce the sequence of the DNA molecule



If we see the sequence on gel from 5 prime to 3 prime, and compare it, it is the same sequence that we have at first



:Fragment sequence that was selected



:Fragment sequence identified from the Gel



Advantages



- No premature termination due to DNA sequencing. So, no problem with polymerase to synthesize DNA.
- Stretches of DNA can be sequenced which can not be done with enzymatic method.
- Purified DNA can be read directly,
- Homopolymeric DNA runs are sequenced as efficiently as heterogeneous DNA sequences,
- Can be used to analyze DNA-protein interactions (i.E., Footprinting),
- Can be used to analyze nucleic acid structure and epigenetic modifications to DNA

Disadvantages



- ❑ Not widely used.
- ❑ Use of radioactivity and toxic chemicals
- ❑ It requires extensive use of hazardous chemicals
- ❑ It has a relatively complex set-up/technical complexity
- ❑ It is difficult to "scale-up", and cannot be used to analyze more than 500 base pairs
- ❑ The read-length decreases from incomplete cleavage reactions, and
- ❑ It is difficult to make Maxam-Gilbert sequencing based DNA kits

Limitations



- Gel electrophoresis is limited to 700-900 bp, with 400-500 bp more commonly attained
- The first 15-40 bp are often difficult to interpret
- Sequencing techniques based on slab gel electrophoresis require cumbersome gels, buffers, time spent loading and running the gels, autoradiography and analysis; all lower the amount of DNA that can be sequenced
- To overcome the limitations of slab gel electrophoresis and the manual reading of DNA sequences, other innovations were introduced.

COMPARISON

<u>Sanger Method</u>	<u>Maxam Gilbert Method</u>
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

