هي الطريقة يلي أنا بعلها بالمختبر عشان أعرف
ايش هو ال sequenc of the
DNA (coding neuclotide in DNA)
سواء whole DNA أو fragment
معين

WHAT IS DNA SEQUENCING ?

- The term DNA Sequencing refers to is the process of determining the precise order of nucleotides (A/T/C/G) in a DNA molecule.

WHAT IS DNA SEQUENCING ?

- This method reveals the sequence of the genetic code which provides the instructions for building and maintaining an organism.
- DNA Sequencing is fundamental in fields like genetic biology, biotechnology, medical research
- Also, DNA Sequencing allows scientists to study genetic variation, mutations, and evolutionary relationship.

HISTORICAL MILESTONES

الطريقة يلي ظهرت فيها هاي التقنية

The Past

Sanger Sequencing
- 1977

Introduction of chain-termination method

يتطلب جهد ووقت كبير

Manual Sequencing
- 1980s

Labor-intensive yet groundbreaking → analysis قد رو يحللو for whole human genom

The Present

Next-Generation Sequencing (NGS)
- 2000s

High-throughput capabilities

أخذ منهم ١٠-٢٠ سنة تقريبا

The Future

Third-Generation Sequencing (TGS)
- Ongoing

Long-read and real-time sequencing advancements

يستخدم نفس Concept لل sanger لكن صاروا يقدرو يحللو automated ملايين fragments في خلال يوم أو يومين

شغالين هسا بالNGS وعم بطوّرو بالTGS

HISTORICAL MILESTONES ...

- At the **end of the 70s** the first two rapid nucleic acid sequencing techniques were published almost simultaneously:
- 1. The Chemical Method of Allan **Maxam** and Walter **Gilbert**, and
- 2. The Enzymatic Chain Termination Method of Frederick **Sanger**, S. Nicklen, and A. R. Coulson.
  - In 1980, Sanger and Gilbert received the ***Nobel Prize*** in Chemistry for the development of DNA sequencing technology.
    - more popular
- Without changing the underlying concept of both methods, some improvements have been done over the years by applying different strategies, by developing various modifications and by automation.

DNA fragment هل راح تكسّر DNS هل راح يكون فيها aliquot على كل G؟ هلأ المفروض ان chemical يلي بحضّرو reaction تكون مزروعة بحيث انها تعمل Breaking في كل molecule وكل molecule (millions) بكون عندي كتير من one molecule (من كتير مفهوم) Breaking at one (G) بصير عندي

MAXAM-GILBERT CHEMICAL METHOD

- The Maxam-Gilbert chemical method starts by obtaining a set of double-stranded DNA molecules labelled at the 5' end with dNTPs having a radioactive isotope (usually ^{32}P), by the action of polynucleotide kinase.
- Four aliquots of the same sample are then treated under different conditions. The chemical treatment generates breaks in a small proportion of one or two of the four nucleotides in each of the four reactions (G, A+G, C, C+T). The chemical agents used in each case are:

هاد الدرتيم هو يضيف

Break the DNA fragment at G

1) DMS (G)
2) Formic acid (A+G)
3) Hydrazine (C+T)
4) Hydrazine plus salts (C)

5 end يلي بدي اشتغل عليها بعمل إلها من ناحية labelling ① fragment ② يلي بدي اكملها sample 4 aliquots بحضرمنها sequncing (identical in the DNA sequnce) وبضيف كلهم الـ chemical reagent

- This generates a series of fragments, ranging in size from the radiolabelled end to the first "cut" site on each molecule.
- As the treatment is gentle, only one nucleotide per DNA molecule is cleaved, resulting in molecules of different sizes. The products of the four reactions are separated on a polyacrylamide gel. A radiographic plate is attached to observe the radioactive banding pattern formed.

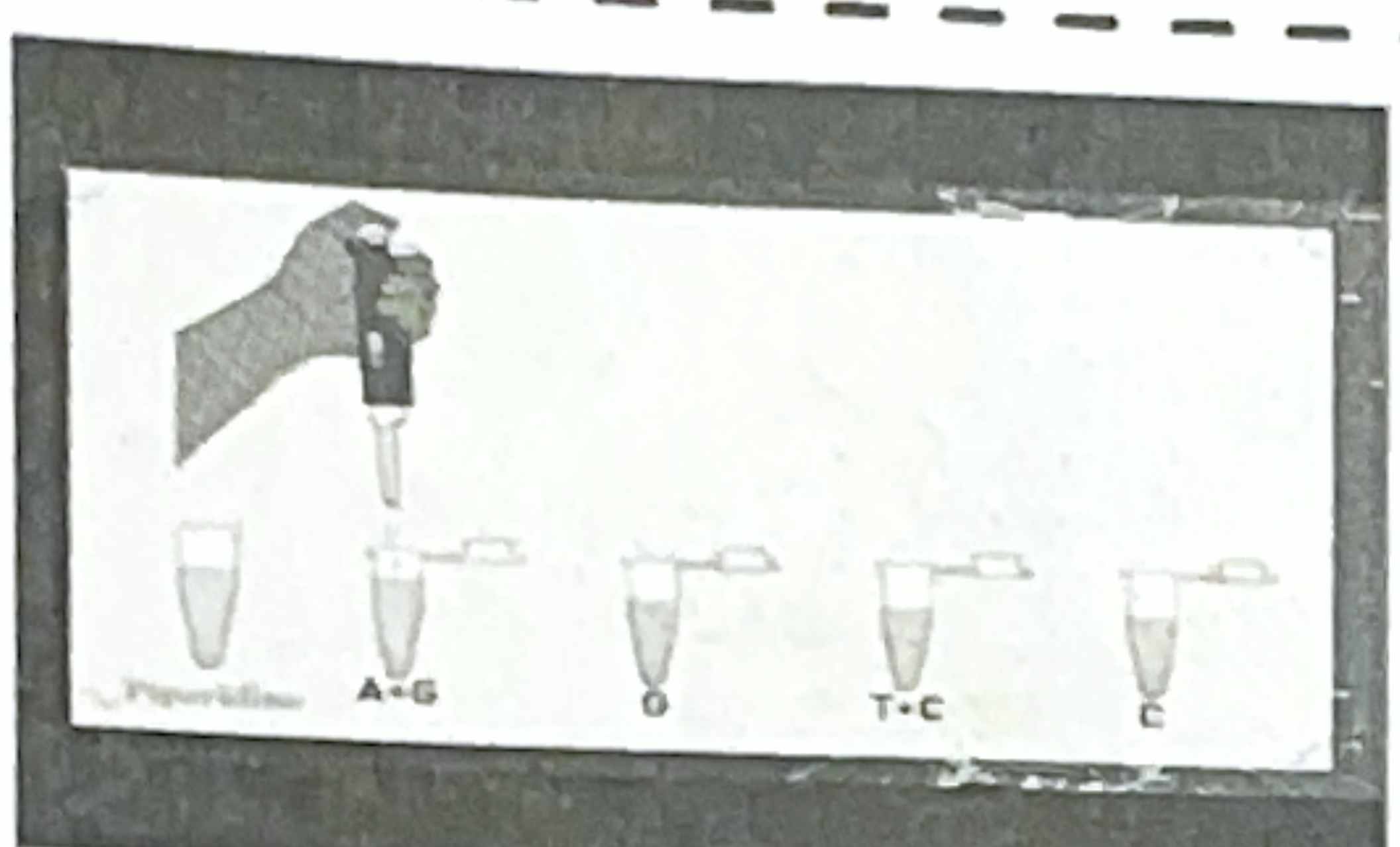
they will result in Breaking of the fragment at a selective point : chemical agents وطبيعة

It can read ← software ففي automatic لو كان
these bands, so they can gusse the DNA sequncing
based on patterns and sizes of the fragments
under each column

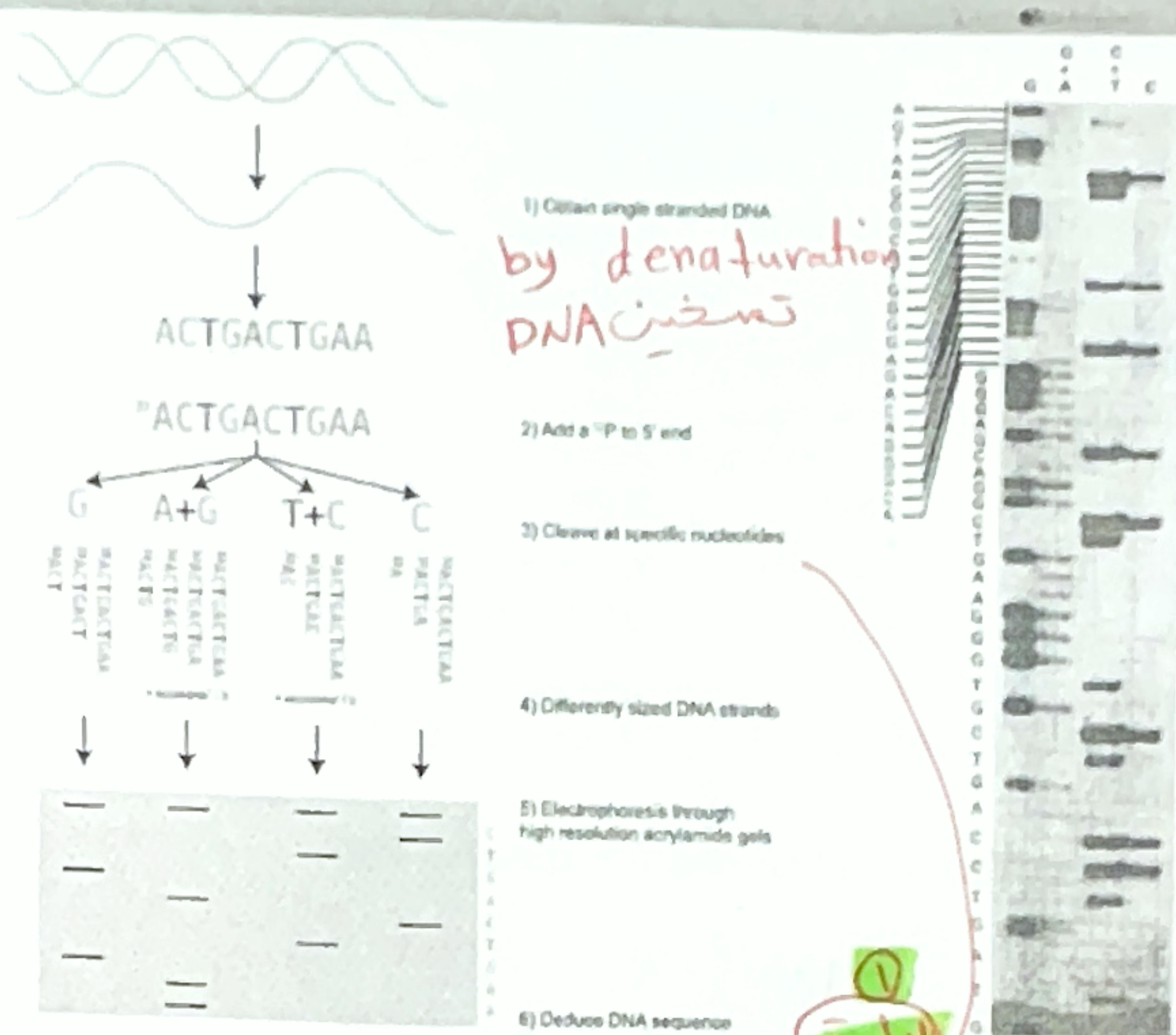
MAXAM-GILBERT CHEMICAL METHOD

الجامعة الهاشمية
The Hashemite University

- An example of Maxam-Gilbert Sequencing Technique, showing specific cleavage of DNA backbone yielding different sized labelled DNA fragments.



https://www.youtube.com/watch?v=B5Dj8PL4E0



ما فهمت الشرح يلي على الفيديو لانه كان مش واضح

وهاد راح ينتج كذى using (4) reagents
diffirent fragments (broken at certain size) tube من كل
↓
بعملهم running على gel electroph... وراح يصير separation لهم
according to the size of these radiolabeled fragments

MAXAM-GILBERT CHEMICAL METHOD

الجامعة الهاشمية
The Hashemite University

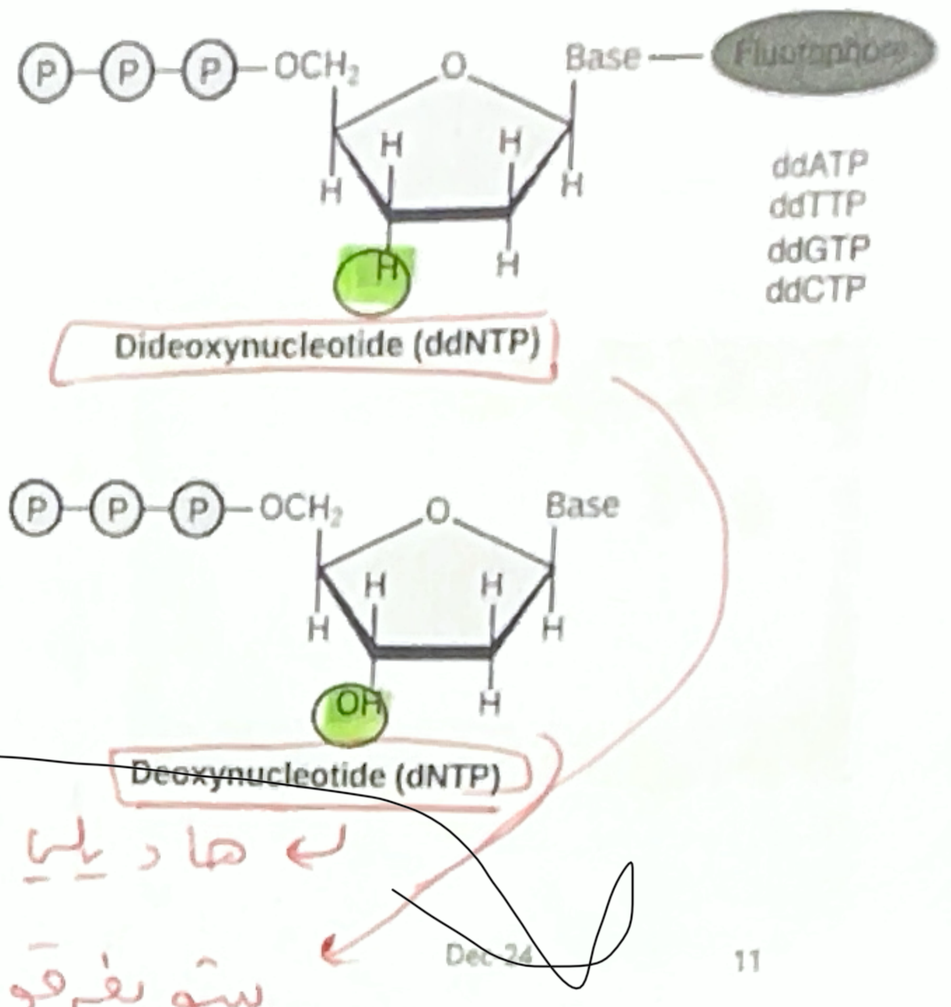
- Maxime Gilbert was a significant breakthrough
- The 1st widely adopted method for DNA sequencing. (لانها أول شي طلعت)
- It represents the 1st generation of DNA sequencing methods.
- This method allows the reading of about 100 sequence bases. (مش أكثر)
- however, it is less commonly used today, due to its complexity and the availability of more advanced methods.
- It has been largely replaced by faster and easier methods, like single sequencing or modern next generation sequencing (NGS) techniques.

① ممكن احزر هدول ال fragment, they correspond to what
الشخص يلي بشتغل بهاي التجربة بكون زي بحل ال buzzle, بقدر يحزر
من ال patterns الموجودة كذى بعدم يشوف هدول ال bands وين
مكانهم، وين موجودين تحت اي column, ممكن يحزر ايش
هم هدول ال fragments، فبتاخذ الموضوع وقت لو كان manually

DNA polymerase فكرتها باختصار انه بتعتمد على ← فكرتها
↳ to build a complementary DNA strand using the
4 nucleotides + modefied ← mixture) بتضيف على
deoxynecleotide

Sanger Enzymatic Method

- The **enzymatic Sanger chain termination method** is based on the use of ddNTPs. As with the chemical method, four different reaction mixtures are made. Each reaction mixture contains:
  - **DNA** to be sequenced: plasmid with the "problem" DNA fragment or PCR product.
  - The four **dNTPs**: dATP, dCTP, dTTP and dGTP
  - **DNA polymerase I** لانه زي ما بعرف polymerase طابعة ر
  - A radiolabelled or fluorescently labelled **primer**: a 17-20 base oligonucleotide complementary to the DNA fragment to be sequenced. يبين منا الصغر لازم primer يعهد له الطريق
  - A **ddNTP** (at a low concentration): a dideoxynucleotide, which lacks a free 3' OH end, so that when incorporated into a DNA strand stops DNA elongation.
    - For Sanger sequencing ratio of dNTP : ddNTP ≈ 100:1



لا هاد يلي احنا بغرفه موجود بالDNA

H ، OH ستو بفرقو عنا بعض ؟
(ddNTP) (dNTP)

هي ال neocleophile يلي بسببها بصير عندي OH ، يعني اذا ماكان في another DNTP جنبه ، وبسبب ال DNA ، بسبب ال phosphodiester bond مارح يقدر ال polymerase يبني

لاني بدي اسمح لل polym.. PNA يبني بشكل طبيعي بس بدي إياه يوقف عند مكان معين، وين بوقف؟ عند G أو C، T، A مب أنا شو وظيفة على mix

Sanger Enzymatic Method

لو وظيفة مثلاً ddCTP يوقف عنا Cytosin

[redacted]

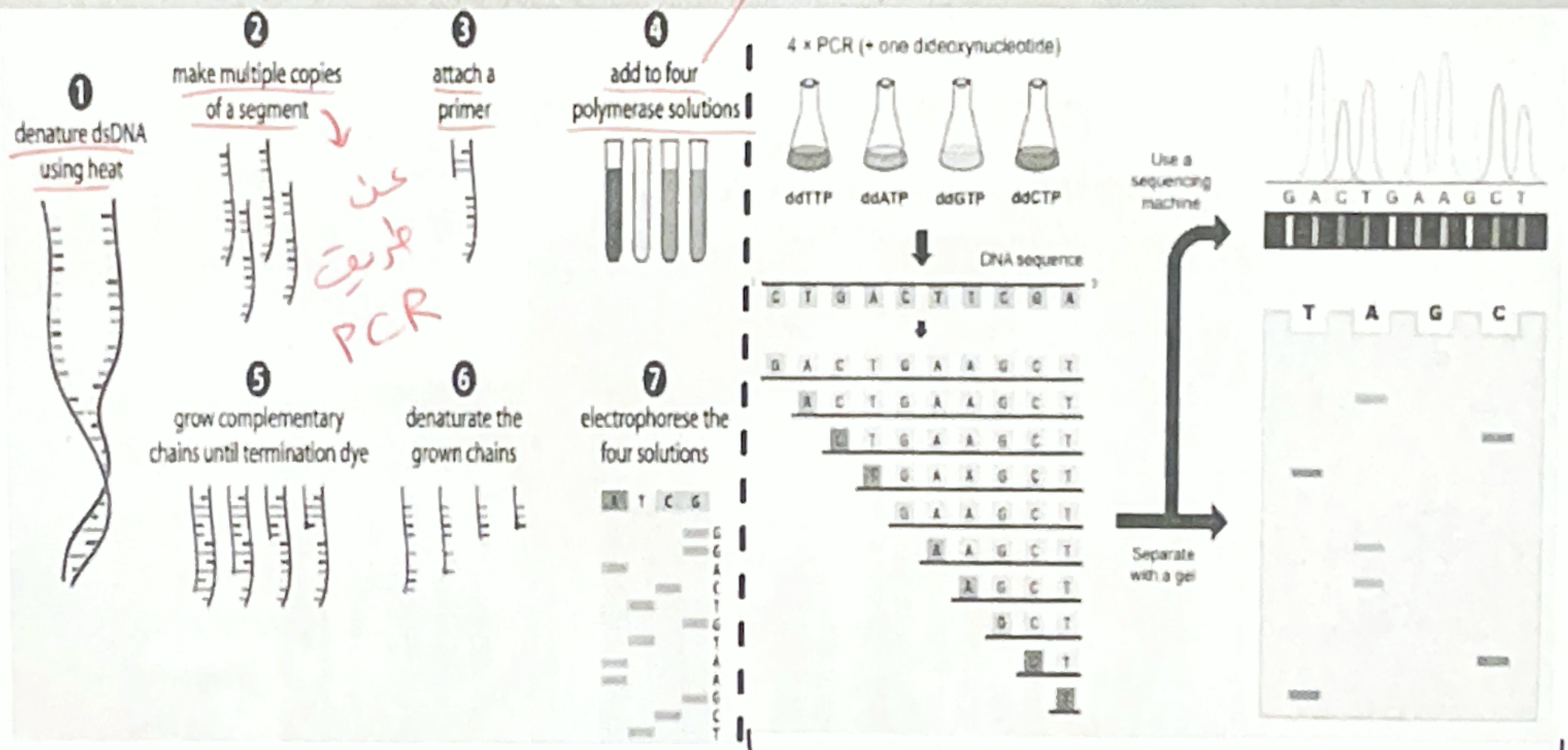
- Sanger sequencing, also known as dideoxy sequencing or chain termination method, identifies the order of nucleotide bases in DNA. This method relies on chain termination by modified nucleotides called dideoxynucleotide triphosphates (ddNTPs).
- it was the first DNA sequencing method and remains highly regarded for its accuracy and the length of the reads it produces (~500-1000 bp).
- The technique involves the incorporation of ddNTPs into a growing DNA chain during replication. These ddNTPs lack a 3' hydroxyl group, which prevents the addition of further nucleotides, thus terminating the chain. By incorporating fluorescent or radioactive labels, the terminated fragments can be detected and analyzed to determine the DNA sequence.
- Sanger sequencing was crucial for the Human Genome Project, completed in 2003, marking a significant milestone in genomics. Today, it remains a fast and cost-effective method for reading small, targeted regions of the genome. Its applications include testing for known familial variants, validating NGS results, and single gene sequencing.

فاراهو جابو ddNTP صنعا H بدل OH وانت هيك بتوقف DNA polymerase ، ببطل يقدر يضيف another neoclotid تفاعل الـ enzymatic chain termination عشان هيك اسمها

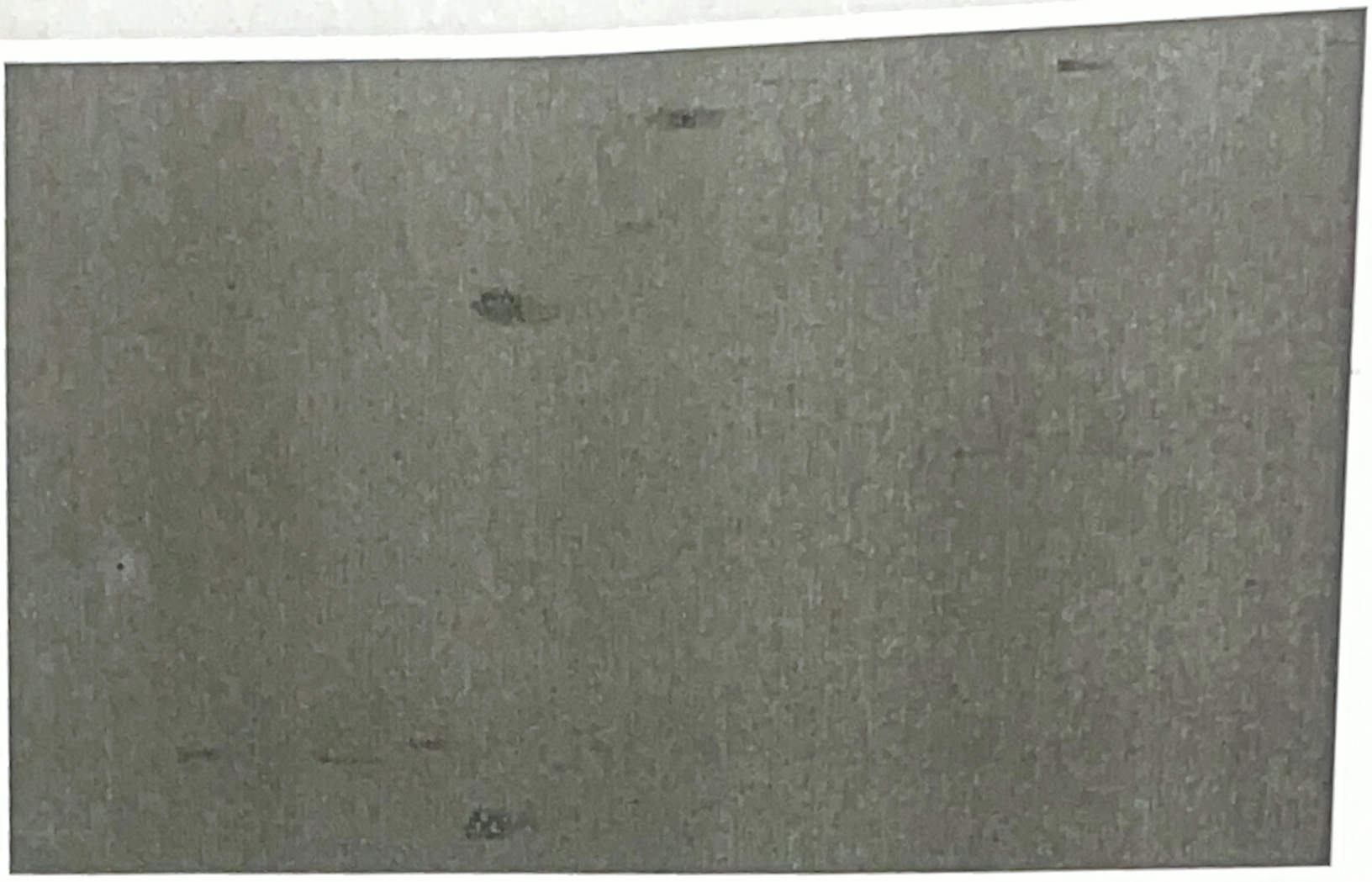
Chain Termination By ddNTP Incorporation

كل tube راح يكون خاص لمعرفة sequnce of certain neocleutide (T, or C, or A, G)

Sanger Enzymatic Method



Sanger Enzymatic Method



https://youtu.be/dVRB4CaLizc?feature=shared

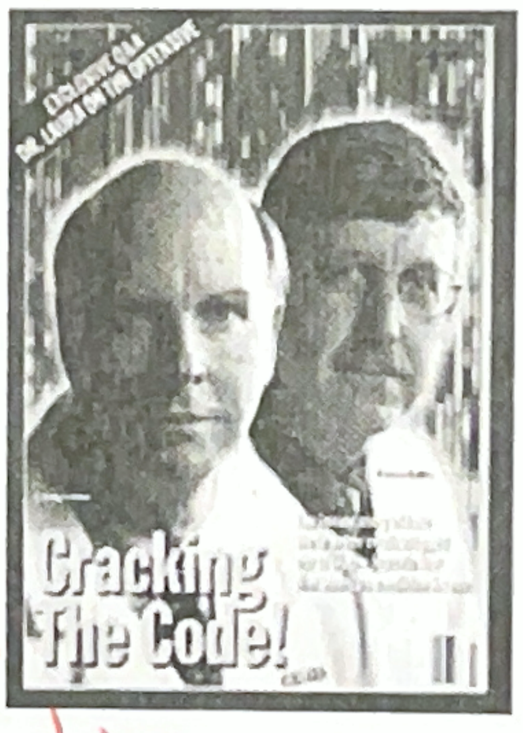
Sanger Sequencing Enabled Genome Sequencing!

انتشرت

1995 First bacterial genome (*Haemophilus influenzae*) sequenced

2001 First draft of the human genome published

كثر وكانت الطريقة الرئيسية يلي عن طريقها قدرو انهم يجلو drafting for the human genom for the first time



بدل ما أنت تحضّر (4) mixture تحضّر 1 mix بس
بتضيف عليه 4 ddNTP بس لازم يكون كل واحد بلون
fluoresecently labelled

AUTOMATIC SEQUENCING

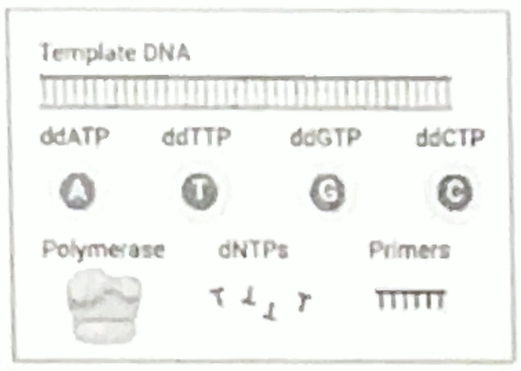
1. Automatic sanger-based sequencing

Aspect	Manual Sanger Sequencing	Automated Sanger Sequencing
Reading Method	User reads all four gel lanes from bottom to top.	Computer reads the bands in capillary gel.
Detection of ddNTP	Identified by the band's position in a specific lane (corresponding to ddNTP).	Laser excites fluorescent tags, and a computer detects the emitted light.
Fluorescence	No fluorescence; bands are detected using radioactive or UV light.	Fluorescent tags attached to ddNTPs emit distinct light signals.
Data Output	Manual interpretation of gel bands.	Automated chromatogram with fluorescent peaks for each nucleotide.
Efficiency	Time-consuming and requires manual effort.	Faster, with less manual input and higher throughput.

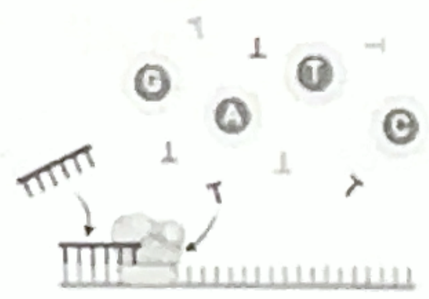
← يعني بقدر أحلل عدد كبير من ال samples في نفس الوقت (آلاف أو أكثر)
← اي automation بكون نسبة الخطأ فيه مقارنة بال manual أقل

AUTOMATIC SANGER-BASED SEQUENCING

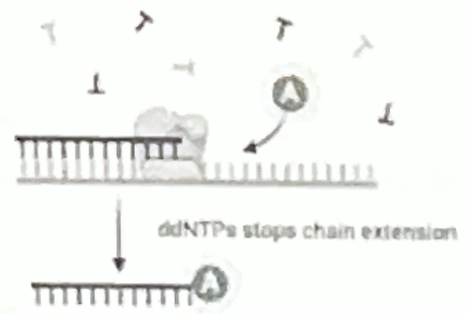
Reagents



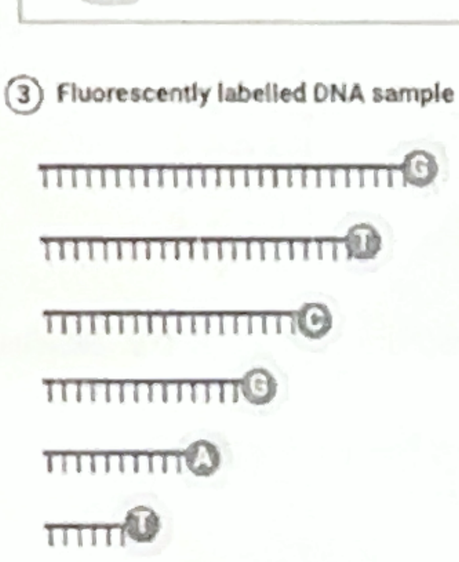
① Primer annealing and chain extension



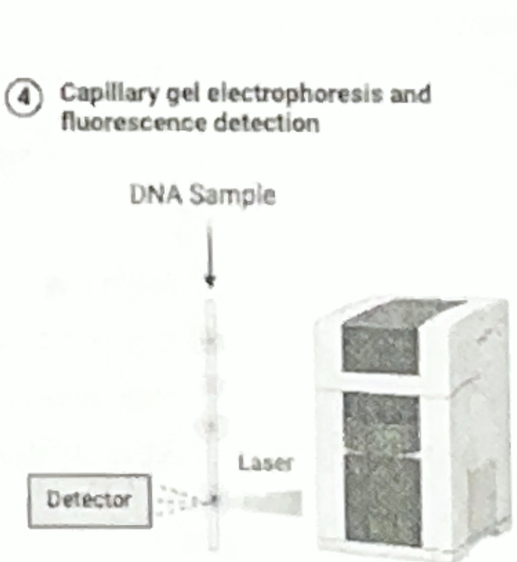
② ddNTP binding and chain termination



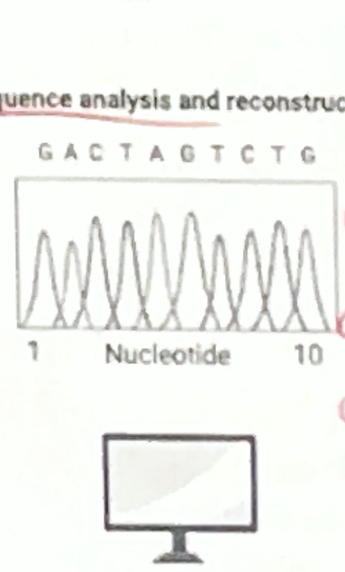
③ Fluorescently labelled DNA sample



④ Capillary gel electrophoresis and fluorescence detection



⑤ Sequence analysis and reconstruction



→ بكون output على شكل chromatogram
اذا كنا بحكي عن fluoresecently labeled nucleotide

نفس خطوات ال mannual بس آخر شي بختلف بالمودل detector

2. Next-Generation Sequencing (NGS)

- Techniques based on the Sanger method are suitable for sequencing relatively small DNA fragments (up to 1000 bp), using cloning vectors such as plasmids or directly PCR products. However, for mass sequencing of larger fragments, such as the entire genome of an organism, ***Next generation sequencing*** (NGS) is used. These are a group of mass sequencing techniques.
  - Library Preparation: DNA is fragmented, and adaptors are attached to the fragments.
  - Amplification: The fragments are amplified using PCR.
  - Sequencing: The amplified fragments are read in parallel using platforms like Illumina or Ion Torrent.
  - Data Analysis: Bioinformatics tools interpret the sequences.

- Watch: https://www.youtube.com/watch?v=CZeN-IgjYCo

① زي ما قلنا بالبداية انه concept s NGS بعتمد على الفكرة الرئيسية للـ Sanger بس الـ steps بتكون more automated + high throughput capabilities

② amplification نفس فكرة PCR بس it's localized PCR لانها بتجبر انه يكون على buliding (fixed chip يعني الـ amplification عم بيصير للـ DNA وهي fixed on a surface)، اما الـ Conventional بكون الـ amplification in a solution

← ماشرحته (شكلها معلومات مكررة)

Next-generation Sequencing (NGS)

- NGS refers to advanced DNA sequencing technologies that allow for the rapid sequencing of large amounts of DNA. Unlike traditional Sanger sequencing, NGS is high-throughput, enabling the simultaneous sequencing of millions of fragments.

- Next-generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies.
- Read lengths typically range, depending on the technology used, between 100 – 600 bp.

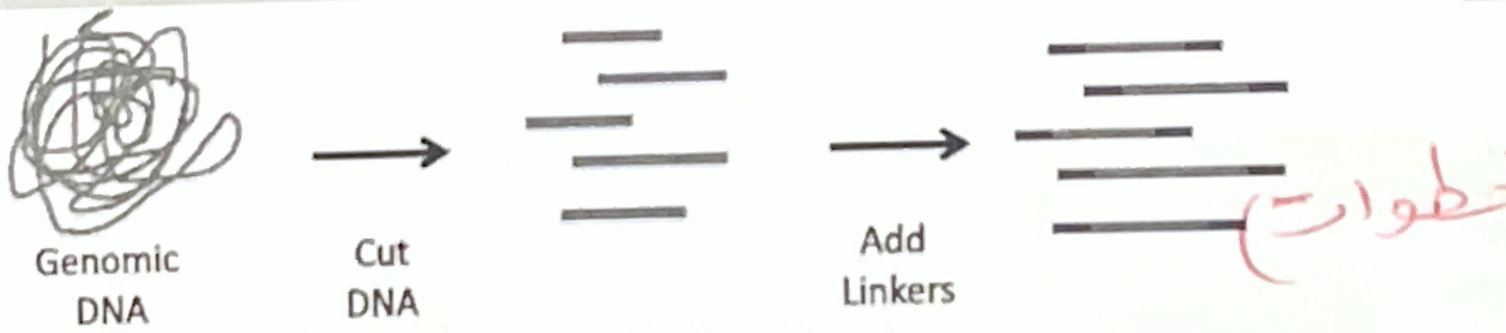
Advantages
- High throughput and scalability
- Cost-effective for large-scale projects
- Capable of sequencing entire genomes, exomes, or transcriptomes

Limitations
- Requires advanced computational resources for data analysis
- High (initial) costs for instruments

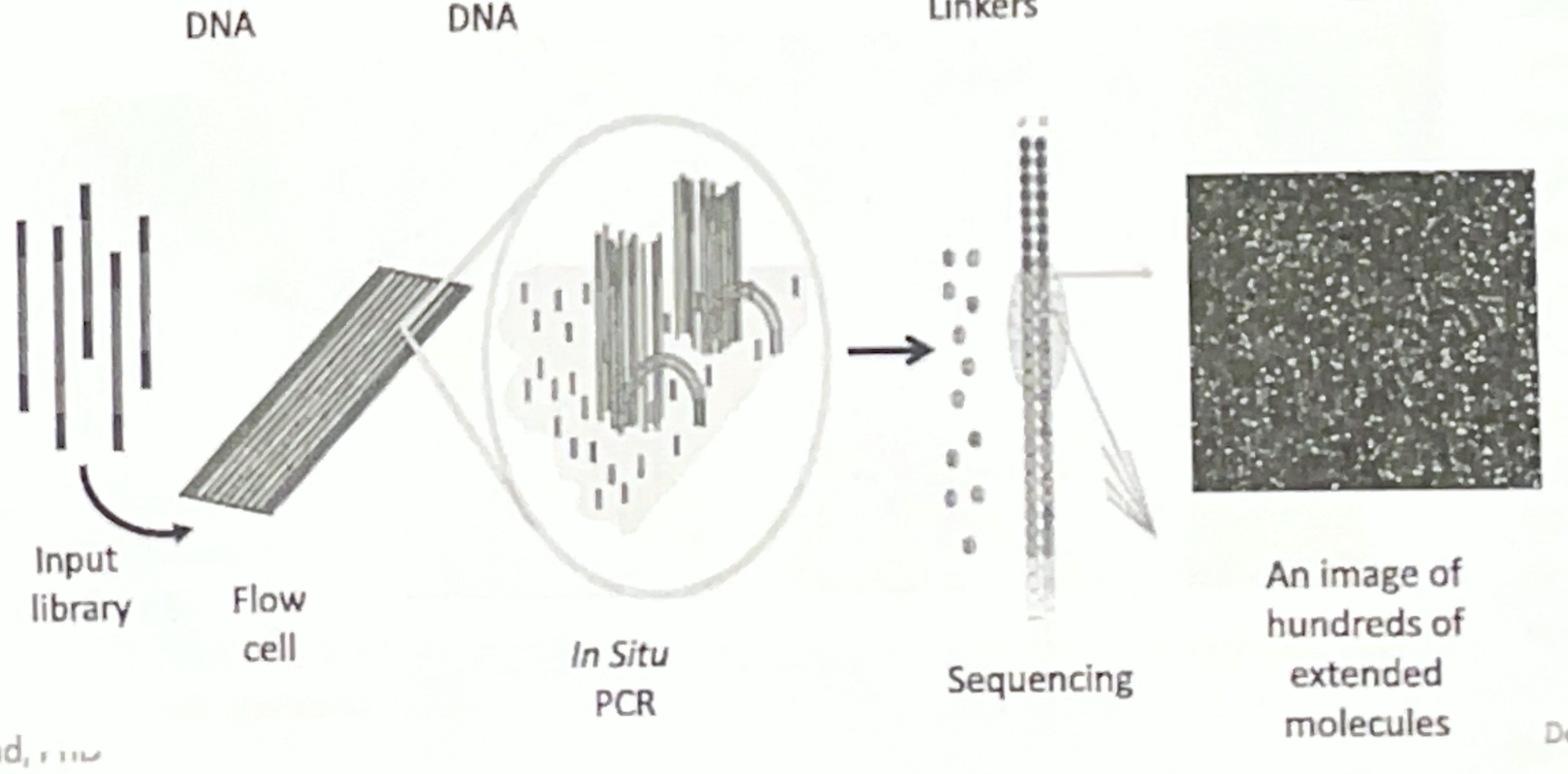
- Key Steps in NGS

1. Sample Preparation
   - DNA is fragmented, and adapters are added to the ends for amplification and sequencing.
2. Library Preparation
   - Fragments are amplified on a solid surface (flow cell) using techniques like bridge amplification.
3. Sequencing by Synthesis (SBS)
   - DNA is synthesized base-by-base with fluorescently labeled nucleotides, and the sequence is recorded.
4. Data Analysis
   - The resulting short reads are aligned to a reference genome or assembled de novo.

← احفظو الفيديوهات المحطوطة بالملاحظات المكتوبة ملونة وصفحة



(تلخيص للخطوات)



Next-generation Sequencing (NGS)

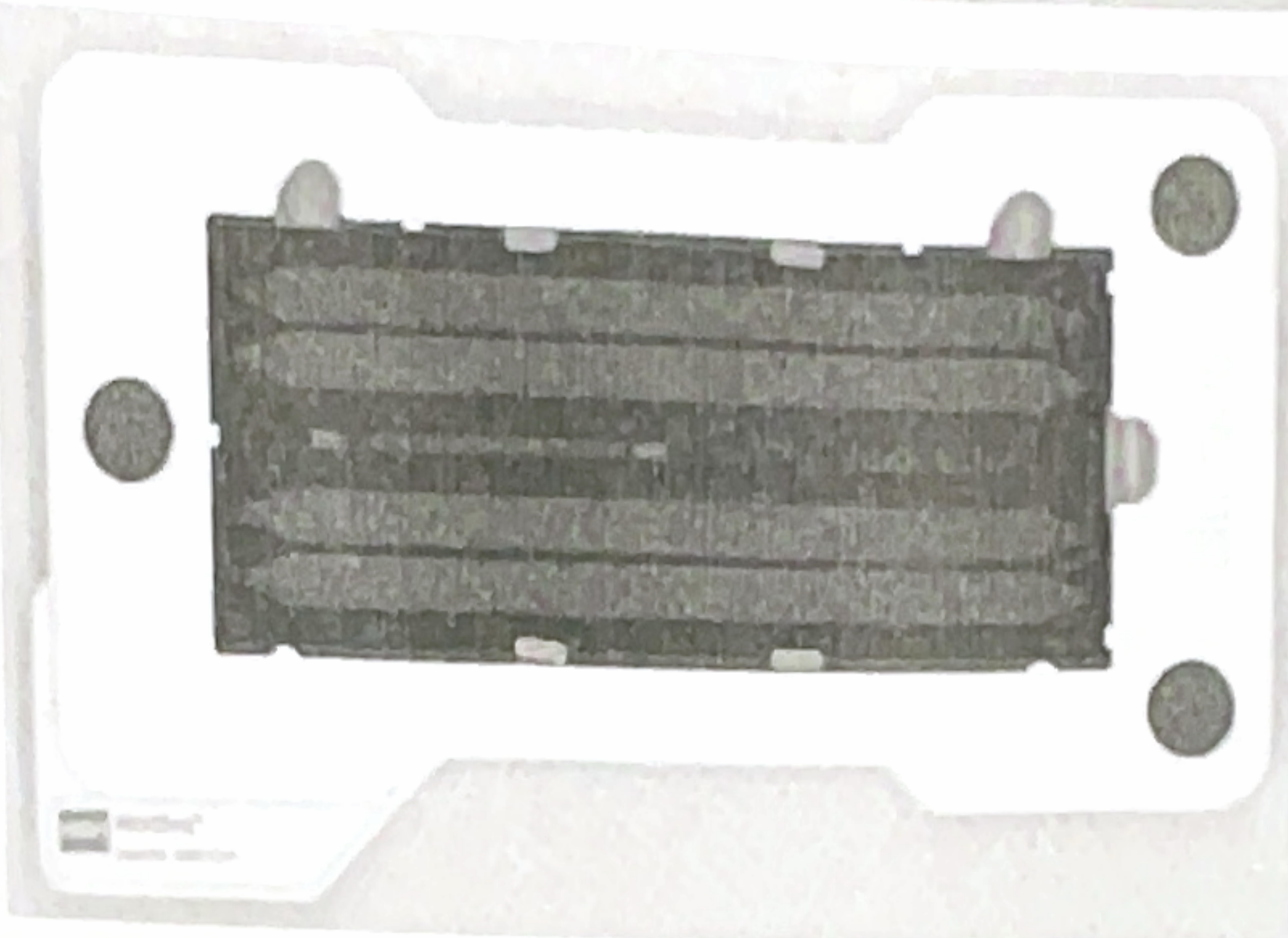
- In Next-Generation Sequencing (NGS), **DNA adaptors** are short, synthetic double-stranded DNA sequences that are attached to the ends of DNA fragments during library preparation. These adaptors serve multiple essential roles in the sequencing process:
- **Key Functions of DNA Adaptors in NGS:**
- **Facilitating Attachment to Sequencing Platforms:**
  - Adaptors contain sequences that enable the DNA fragments to bind to the surface of a sequencing flow cell (e.g., in Illumina sequencing) or to beads (e.g., in Ion Torrent sequencing).
- **Priming for Amplification and Sequencing:**
  - They include sequences recognized by primers for PCR amplification, which generates sufficient copies of each fragment for sequencing.
  - During sequencing, the adaptors provide binding sites for sequencing primers.
- **Indexing/Barcoding:**
  - Adaptors often include **index sequences (barcodes)** that allow the multiplexing of multiple samples in a single sequencing run. These indices enable bioinformatics tools to differentiate reads originating from different samples.
- **Compatibility with Sequencing Chemistry:**
  - Adaptors are designed to be compatible with the sequencing platform's specific chemistry and workflow. For example, Illumina adaptors have regions that enable cluster formation on the flow cell.

Next-generation Sequencing (NGS)

- **Illumina Sequencing** is a widely-used next-generation sequencing (NGS) technology that allows for the high-throughput sequencing of DNA. It uses a method called **sequencing by synthesis (SBS)**, which involves capturing DNA fragments on a solid surface and incorporating labeled nucleotides one base at a time.

https://www.youtube.com/watch?v=fCd6B5HRaZ8

Illumina NextSeq Flow Cell



400,000,000 DNA fragments X 300 nucleotides

=

120,000,000,000 nucleotides in 29 hours

← هاي chip الصغيرة بصير عليها تقريبًا 400 billion DNA fragment

كل fragment ال length إلها 300 nucleotids , عشان هيك

بنسميها high througpot لانها تحلل عددها كل من ال fragments

في نفس اللحظة وفي مكان صغير وبوقت قليل

Illumina Sequencing Benchmarks

مقارنة

NextSeq 500

1 Technician
29 hours
$4000
120,000,000,000 nucleotides
Human genome X 40

vs.

The Human Genome Project

>1000 Scientists
13 years (1990-2003)
$3,000,000,000
24,000,000,000 nucleotides
= (1) Human genome X 8

The Sanger method remained the primary technique for the majority of sequencing, while newer techniques, such as **pyrosequencing** and **sequencing by synthesis** (as seen in Illumina technology), started to emerge later and contributed to genome sequencing efforts in subsequent projects.

The Human Genome Project (HGP)

(قرأتها قراءة)

- The Human Genome Project (1990–2003) was a landmark international research initiative aimed at mapping and sequencing the entire human genome. The goal was to identify all the genes in human DNA and understand their function, providing a foundation for advances in medicine and biology.
- **Scope**: Sequenced 3 billion DNA base pairs of the human genome.
- **Output**: Identified approximately 20,000–25,000 genes.
- **Collaboration**: Involved scientists from multiple countries and institutions.
- **Impact**: Revolutionized understanding of genetic diseases, drug discovery, and personalized medicine.

Illumina Sequencing Is Scalable

بقدر اشتغل على أكثر من جهاز بنفس الوقت

HiSeq X Ten: Single device has 15X capacity of the NextSeq 500

Cost for sequencing of a human genome (at 40X): ~$1000

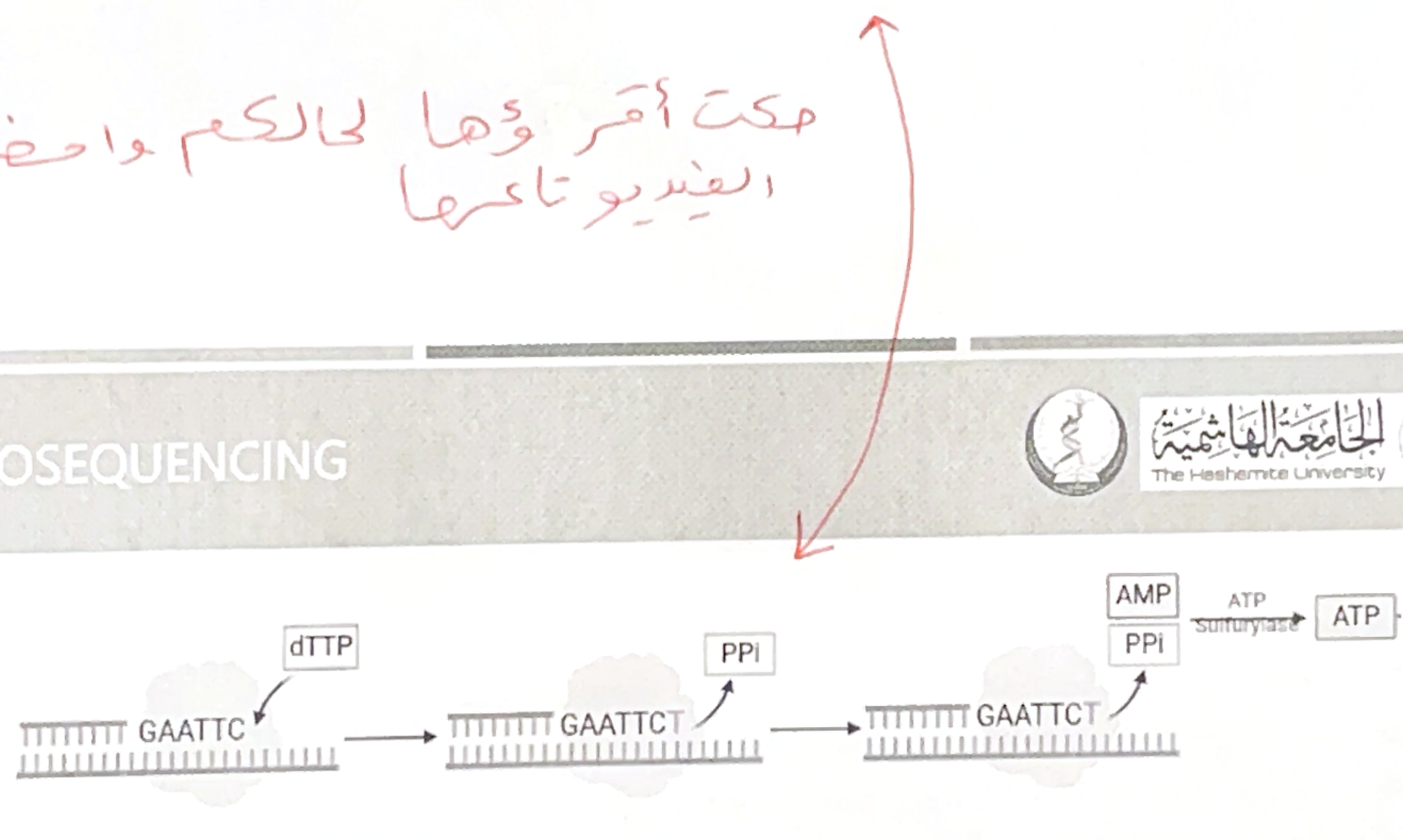
3. Pyrosequencing

- automated sequencing technique is pyrosequencing. It allows the determination of DNA sequences on a large scale, using luminescence. This technique requires:
- eDNA to be sequenced: single-stranded
- Primer
- dNTPs
- DNA polymerase
- 3 enzymes: sulphurylase (and the substrate adenosine 5'-phosphosulphate or APS), luciferase (and the substrate luciferin) and apyrase.

- The process occurs in successive three-step cycles. In the first step, one of the 4 dNTPs is added. If it is complementary to the base of the template strand to be copied, it is incorporated into the strand being extended by DNA polymerase, releasing a PPi. This reacts with APS to give ATP, which is used to oxidise luciferase, a process in which it emits light. If the dNTP that has been added to the reaction medium is not complementary, it is degraded by apyrase before the next dNTP is added. The emitted light is captured by a camera. The results are plotted as a pyrogram.
- This technique allows the sequencing of small and medium-sized DNA fragments (100 – 500 BP).

https://www.youtube.com/watch?v=pollGc5uj7A

ممكن أمرّ وها لحالكم واحضرو الفيديو تاعرها



https://www.youtube.com/watch?v=RYVaKwTSQjQ

THIRD-GENERATION SEQUENCING (TGS)

كتير لا DNA fragmentation ما بحتاج

- Key Features:
  - Real-time sequencing of single molecules.
  - Long-read capabilities.
- Technologies:
  - PacBio (SMRT): Long reads, high accuracy.
  - Oxford Nanopore: Portable and versatile.

amplification يعني ما بحتاج one molecule of DNA بس

← بحكت هاي الـ technology معهم بس لسا في سوء سليبات ي low accuracy ي

APPLICATIONS IN DRUG DISCOVERY

- Key Areas:
  - Target Identification: Detect mutations or gene variations linked to disease.
  - Biomarker Discovery: Identify genetic signatures for drug response.
  - Pharmacogenomics: Tailoring treatments based on genetic profiles.
- **Example:** Using sequencing to study tumor genomes for targeted therapies.

CHALLENGES AND FUTURE DIRECTIONS

- Current Challenges:
  - Cost and data management for large-scale projects.
  - Ethical concerns in genomics.
- Future Innovations:
  - Integrating AI for data analysis.
  - Expanding accessibility to low-resource settings.
  - Improving accuracy for clinical applications.

study hard , You are almost done

THE END ...

Any questions? ☺

Sara Jammain
Artery academi