Athar Batch

Genetics

Lecture: 27

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LECTURE 27

HYBRIDIZATION AND BLOT TECHNIQUES

PROBES

- **How can the DNA sequence of interest be picked out of a mixture of thousands or even millions of irrelevant DNA fragments?**

- The answer lies in the use of a probe (a single-stranded sequence of DNA or RNA of variable length used to search for its complementary sequence and can be radioactively or fluorescently labeled to allows its binding to be visualized).

- For example, if the sequence of interest is CCC, the probe will be GGG.
- We can use radioactive or fluorescent dye to mark the probes.

Hybridization of a probe to DNA fragments

- The utility of probes hinges on the phenomenon of hybridization (or annealing) in which a probe containing a complementary sequence binds a single-stranded sequence of a target DNA.
- ssDNA, produced by alkaline denaturation of dsDNA, is first bound to a solid support, such as a nitrocellulose membrane. The immobilized DNA strands are prevented from self-annealing, but are available for hybridization to an exogenous, radiolabeled, ssDNA probe.

- Several routes are used to separate the two DNA strands from each other such as the heat or alkaline denaturation.
- Alkaline denaturation: we use an alkaline solution as a medium for DNA.

Blotting techniques

- blot: بقعة او لطخة
- this technique is used to detect any mutation in the sequence of nucleotides in specific gene.

Southern Blot Technique:

- It is based on the specific base pairing properties of complementary nucleic acid strands. This technique is therefore based on **DNA hybridization.**
- The blot technique was developed by EM Southern in 1975. This is used to detect a **specific segment of DNA in the whole genome.**

- DNA is isolated from the tissue.
- It is then fragmented by **restriction endonucleases.**
- The cut pieces are **electrophoresed on agarose gel.** It is then treated with NaOH to denature the DNA, so that the pieces become single-stranded.
- This is then **blotted (adsorbed)** over to a nitrocellulose membrane. The single-stranded DNA is adsorbed in the nitrocellulose membrane.

- DNA segments run from the negative pole to the positive pole according to the molecular weight of the segment.
- NaOH: alkaline material
- Nitrocellulose membrane: used to be placed over the gel to absorb the DNA.

- The DNA is then fixed on the membrane by baking at 80°C. There will be many DNA fragments on the membrane, but only one or two pieces contain the target DNA.

- The radio active **DNA probe is placed over the membrane.** If the target genes are present in the host DNA, the probe will detect the complementary nucleotide sequence in the host DNA. So the probe is hybridized to the particular pieces of host DNA.

•The membrane is then thoroughly **washed to remove excess probes.**

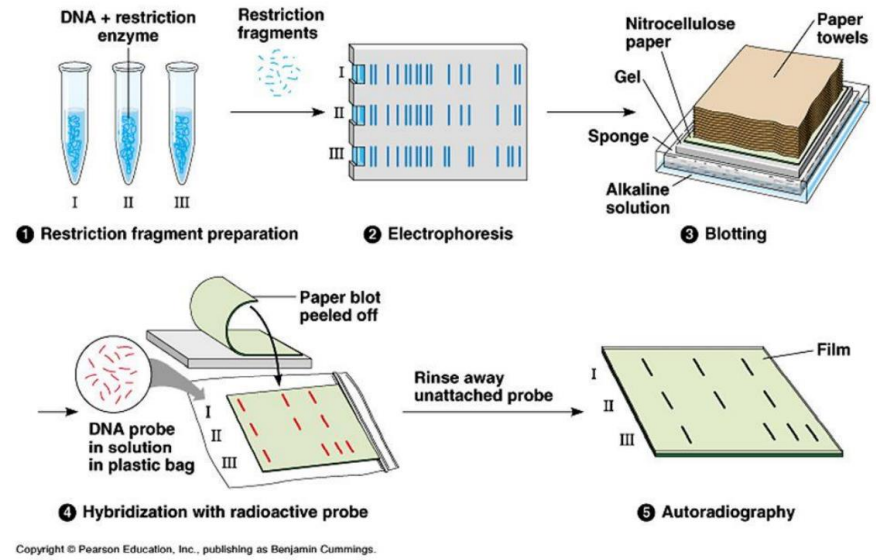
- since the probe is radioactive, it will make marks on the X-ray plate after placing of X-ray plate over the membrane for several days.

• **An** X-ray plate is placed over the membrane in the dark for a few days. The radiation from the fixed probe will produce its mark on the X-ray plate. This is called **autoradiography.**

- If the probe used in the technique is for mutant gene, we can detect if there is a mutation or not.

• **Mutant genes** such as HbS, cystic fibrosis, DMD, PKU as well as presence of viral DNA (hepatitis virus B and C) can be identified by this method.

- DMD: Duchenne Muscular Dystrophy.



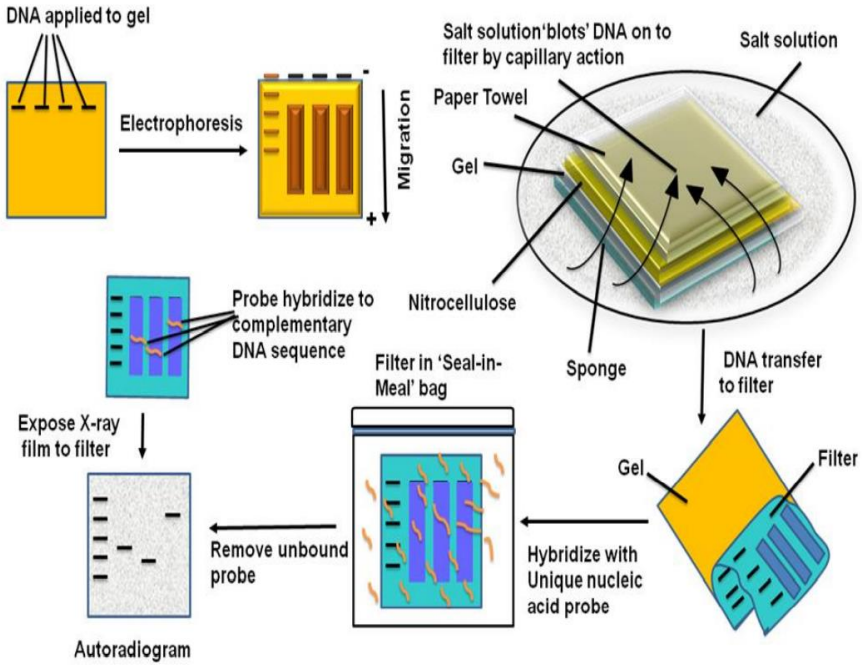
1. DNA extraction
2. Fragmentation by restriction enzymes.
3. Electrophoresis → fragments run in the gel according to their molecular weight.
4. Blotting on nitrocellulose membrane.

مشان تمتص المحلول و توصله للجل

- Place the gel over the sponge
- Place the nitrocellulose membrane over the gel
- Place the paper towels on the nitrocellulose membrane تعمل ثقل عليه مشان تضغطه على الجل

5. Hybridization with radioactive probe
6. Rinse the nitrocellulose membrane from unattached probes.

7. Autoradiography → the DNA fragments appear on the X-ray plate.



تقريبا نفس الصورة اللي فوقها

- Autoradiogram:

نفس فكرة ال Ladder

Northern Blotting for Identifying RNA:

- The Northern blot is used to demonstrate **specific RNA.** The total RNA is isolated from the cell, electrophoresed and then blotted on to a membrane. **This is then probed with radioactive cDNA (RNA-DNA hybridization) or RNA.**
- This is used to detect the gene expression in a tissue

- Southern → detection of specific DNA sequence.
- Northern → detection of specific RNA sequence.
- RNA extraction → electrophoresis → plotting on the membrane → using radioactive probe (DNA or RNA probe).

Western Blot Analysis for Proteins

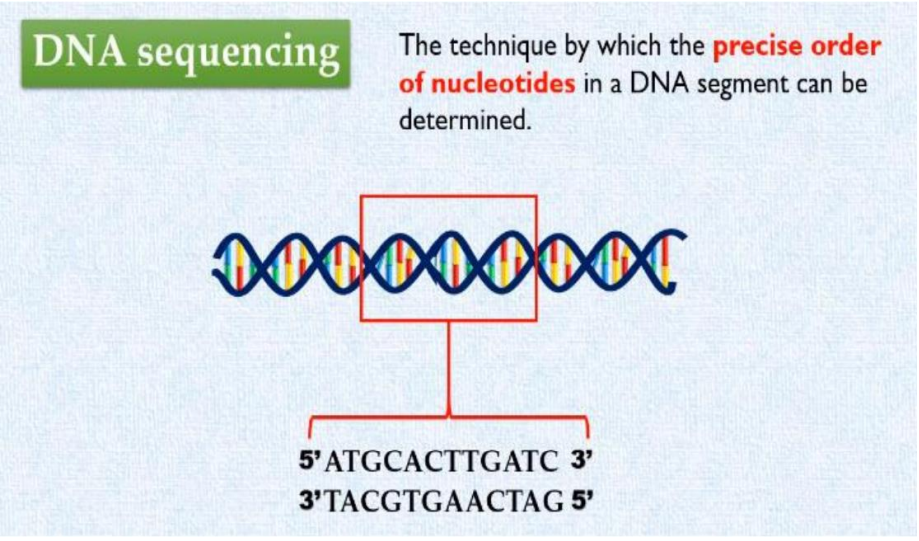
- In this technique, proteins (not nucleic acids) are identified.
- The proteins are isolated from the tissue and electrophoresis is done. The separated proteins are then transferred on to a nitrocellulose membrane.
- After fixation, it is probed with radioactive **antibody and autoradiographed.**
- This technique is very useful to identify the specific protein in a tissue, thereby showing the expression of a particular gene.

➢ protein extraction → electrophoresis → blotting on nitrocellulose membrane → binding with antibodies (like probe in southern and northern blot)

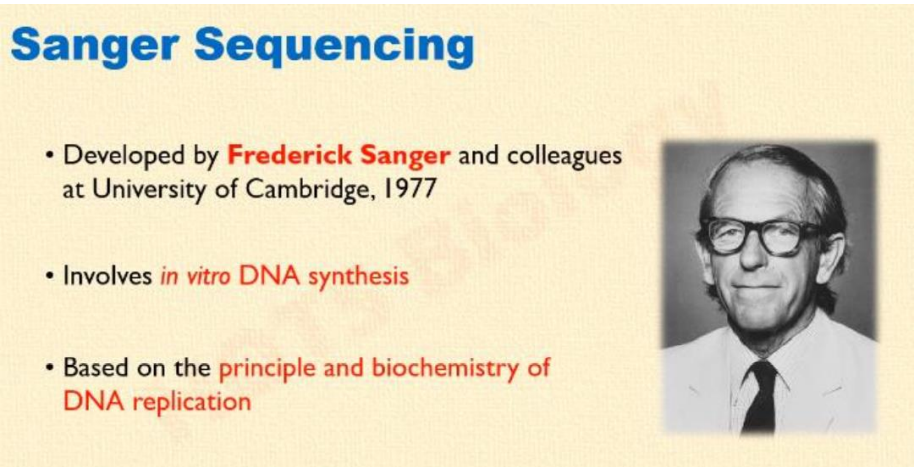
DNA sequencing

By

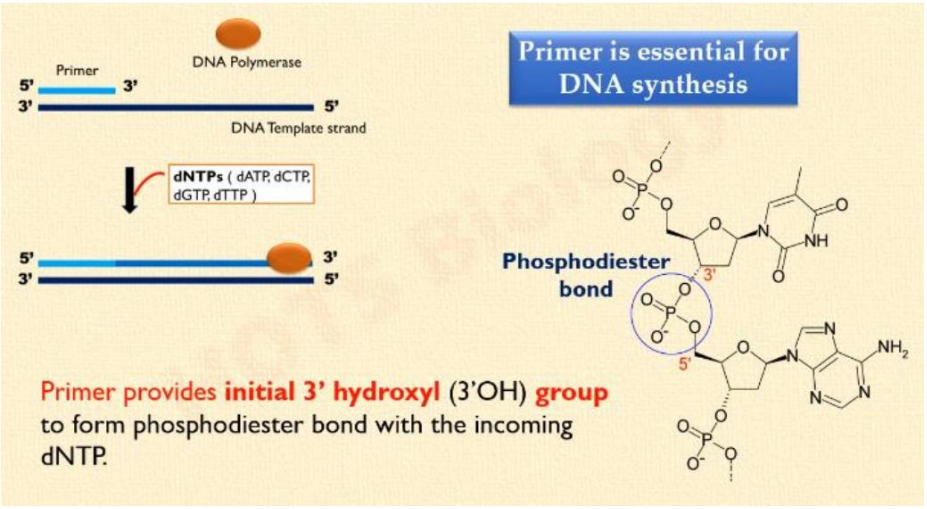
Dr. Walaa Bayoumie El-Gazzar



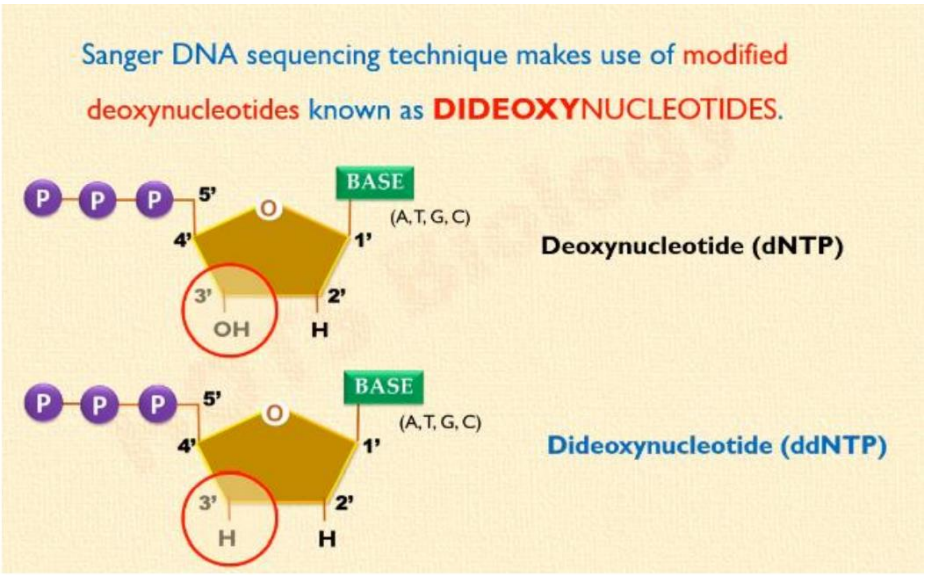
➢ We can determine if the gene is normal or mutated.



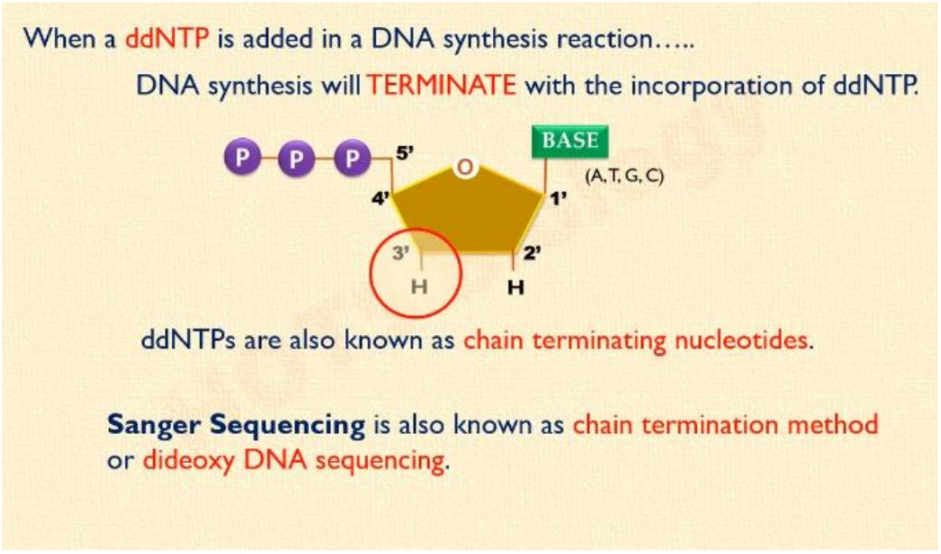
➢ In vitro DNA synthesis with some modification



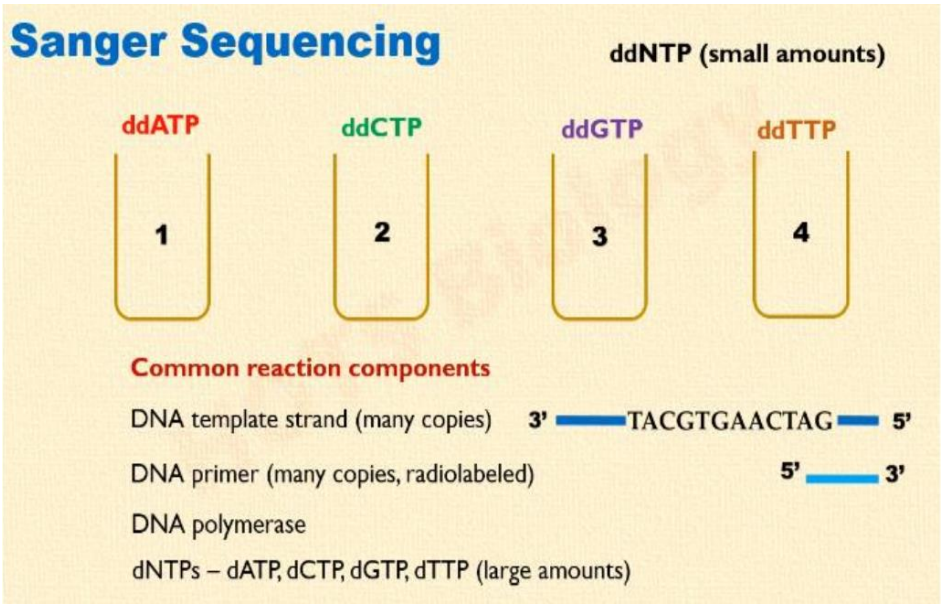
- after denaturation of the DNA, a single strand DNA is available to be used as a template to build its complementary strand.
- This requires the presence of a primer that contain an OH group at the 3` end.



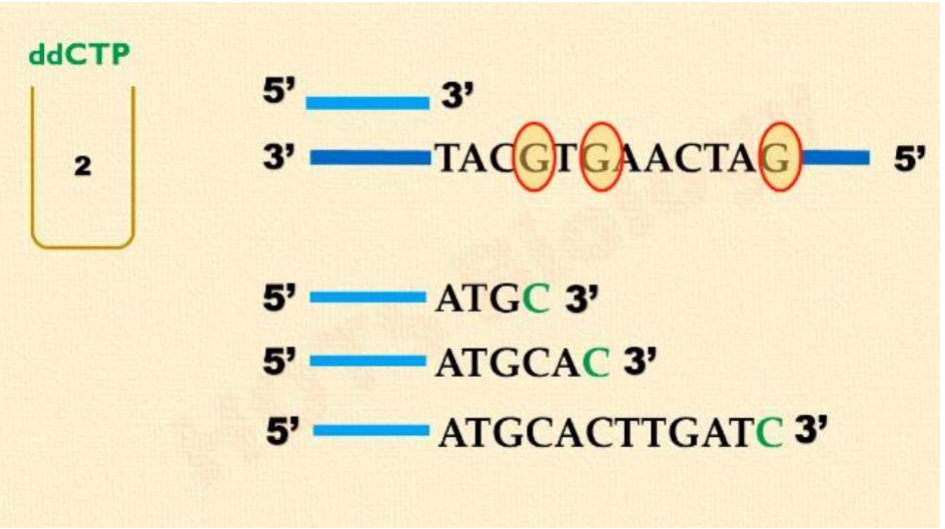
- Deoxyribonucleotide: carbon number 2 binds with H not OH.
- Dideoxyribonucleotide: carbon number 2 and carbon number 3 bind with H but not OH.
- Deoxyribonucleotides can bind to other nucleotides in 3` directions because the carbon number three is bound to OH group.
- Dideoxyribonucleotides cannot bind to other nucleotides in 3` direction because there is no OH group bound to the carbon number 3.



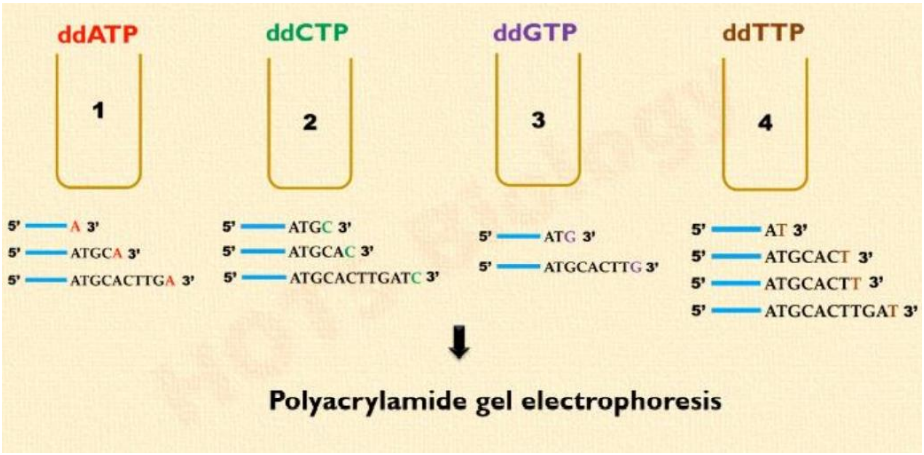
- a tube is filled with a template DNA strand, deoxyribonucleotide triphosphate, primer, required enzymes and dideoxynucleotides triphosphate.



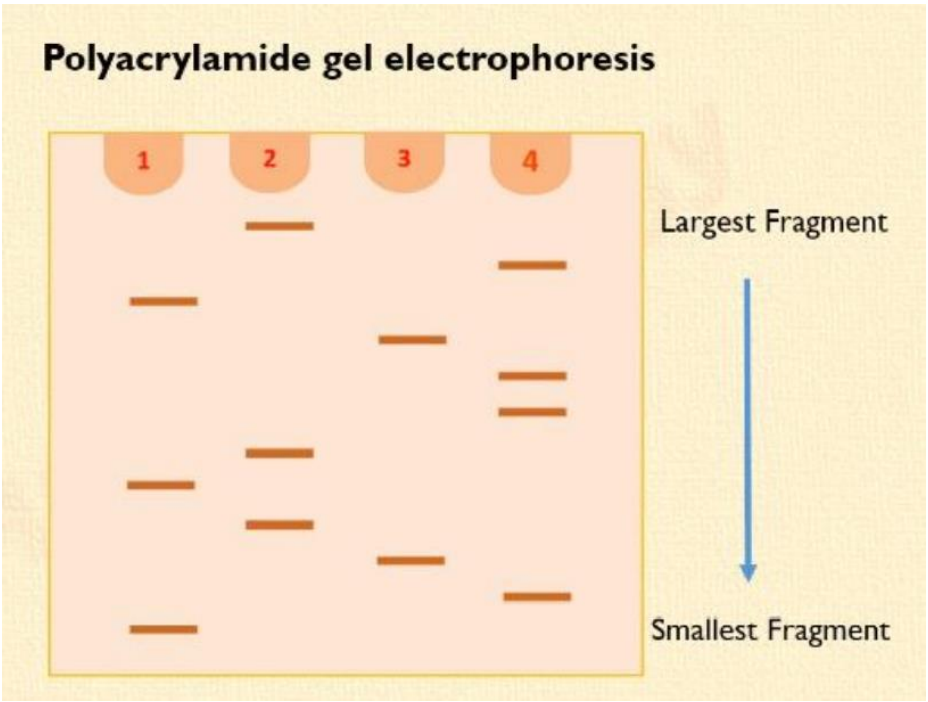
- In each reaction, four tubes are filled with all the materials mentioned previously, but each tube has one type of dideoxyribonucleotide triphosphate (ddATP, ddGTP, ddCTP or ddTTP).



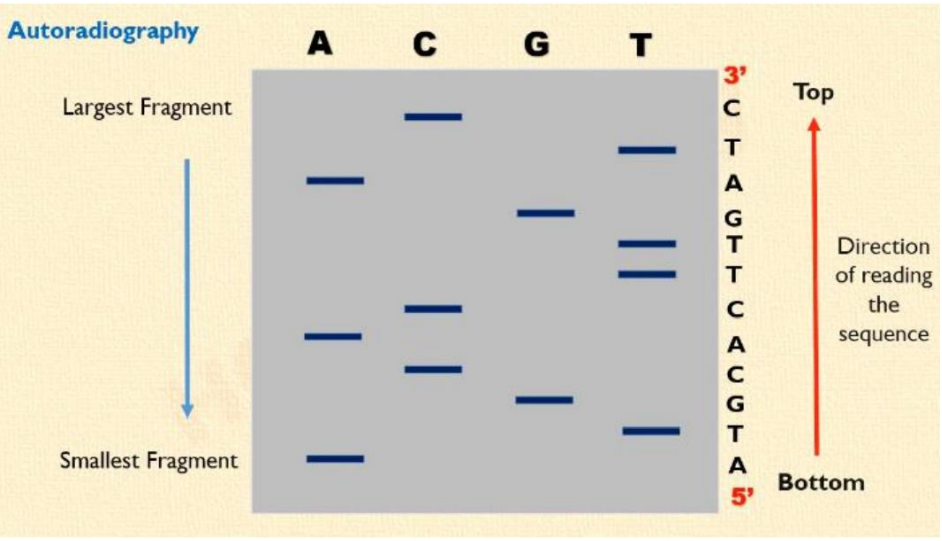
- There is a chance for ddCTP to be paired with one of G nucleotides in the template strand.
- The complementary strand will be terminated after binding of ddCTP to the G nucleotide.
- This results in complementary DNA sequences different in length.



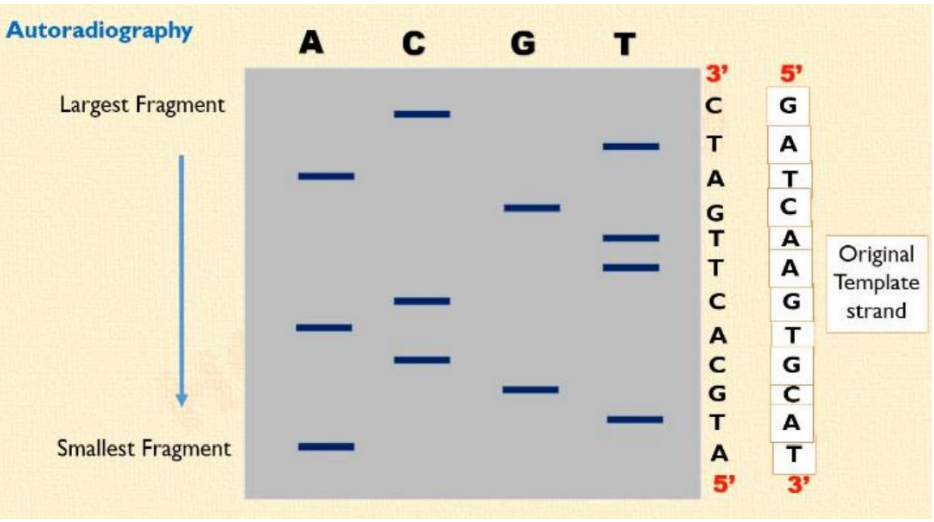
- the result: 4 tubes contain different complementary DNA sequences differs in length.
- The last nucleotide in all DNA chains in one tube is the dideoxyribonucleotide that is added to the tube.



- Placing the DNA segments from the previous step in gel material (DNA segments in the same tube are filled in the same well in the gel, e.g: DNA segments that end with A nucleotide are placed together in one well).
- Then we start electrophoresis to make every DNA segment run in the gel according to the molecular weight of the segment.



- The smallest fragment= primer+ the first nucleotide after the primer.
- In this figure, the first nucleotide after the primer is A.
- The second nucleotide after the primer is T... and so on.



- *the sequence in the last figure was for the complementary strand for the template strand.*
- *We can identify the sequence of the template strand via base-pairing rule.*

- *DNA sequencing is PCR reaction but in four tubes rather than one tube.*

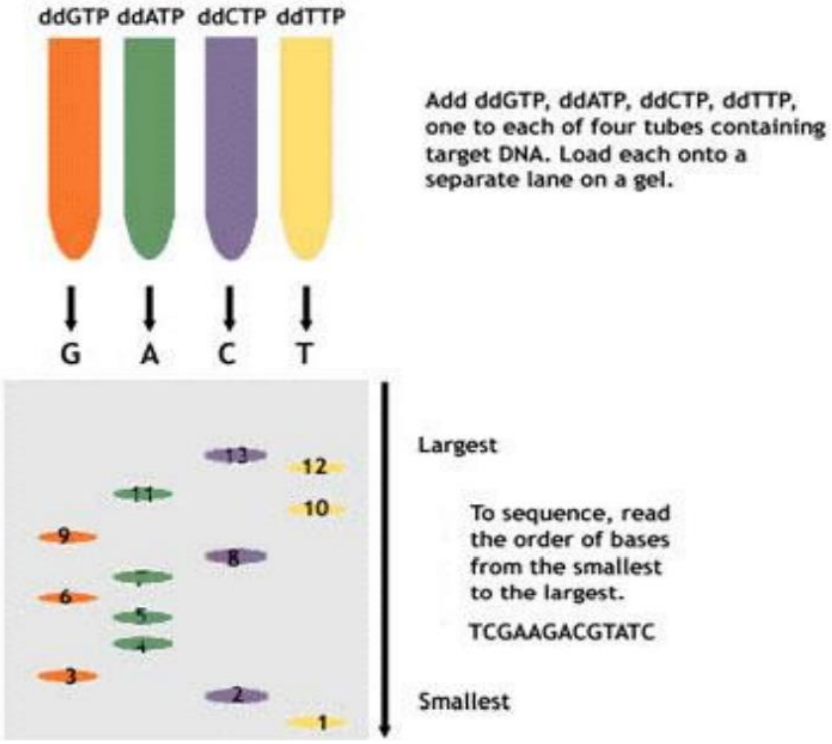
Chain termination method (sanger dideoxy method)

1- The DNA to be sequenced is prepared as single stranded molecule .

2- An incubation mixture is set up containing the following :

- The single stranded DNA template
- DNA polymerase
- **Radioactive primer** complementary to the 3`end of the target DNA.
- All four deoxynucleoside triphosphates (dATP-dGTP-dCTP-dTTP).

The sample is divided into four reaction tubes and a small amount of one of the four **dideoxyribonucleoside triphosphate (ddNTP)** is added to each tube.



3- During incubation, the DNA begins to copy the template molecule by extending the bound primer.

4- As a new DNA strand is synthesized, **every time when dGTP , for example, is incorporated there is a chance to incorporate ddGTP instead.** If this happens, no further chain elongation can occur because ddGTP lacks the 3̀-OH group needed to make the next phosphodiester bond. Thus this particular chain stops at this point.

5-Four sets of chain-terminated fragments are formed corresponding to the positions of A,G,C and T in the sequence.

6-After incubation, all four reaction mixtures are electrophoresed in parallel lanes of a **polyacrylamide gel** and then subjected to autoradiography.

7-The DNA sequence is determined simply by reading the band pattern on the autoradiogram from the bottom of the gel toward the top.

8-We know that each reaction mixture has the same primer therefore all the strands begin with the same sequence

- *Automated sequencers:* نستخدمهم لما بدنا نعرف ال *sequence* على طول بوقت جدا قصير و هي اجهزة متطورة و استخدامها اسهل و تعطي نتائج اكثر دقة