

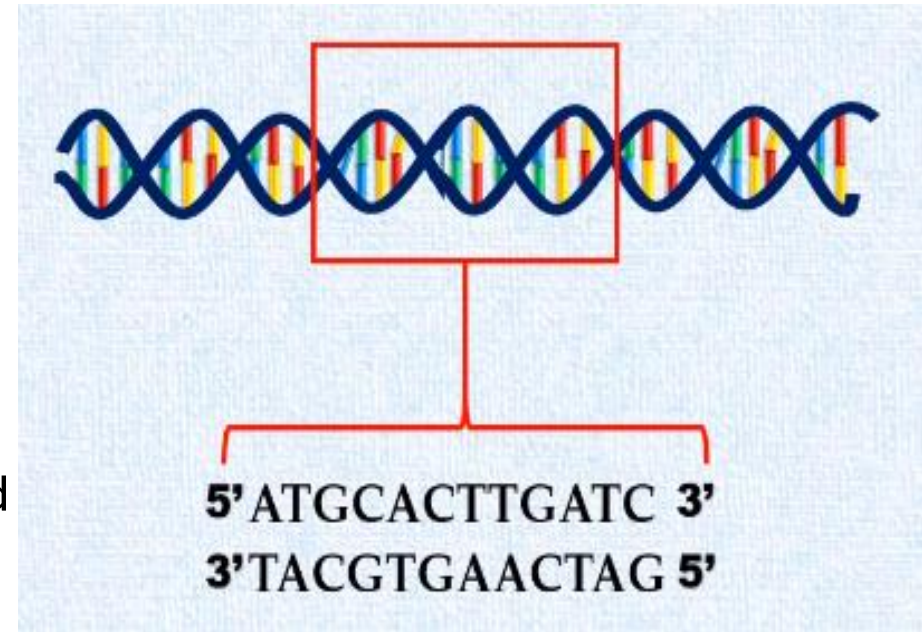
DNA sequencing

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Most slides from Dr Walaa

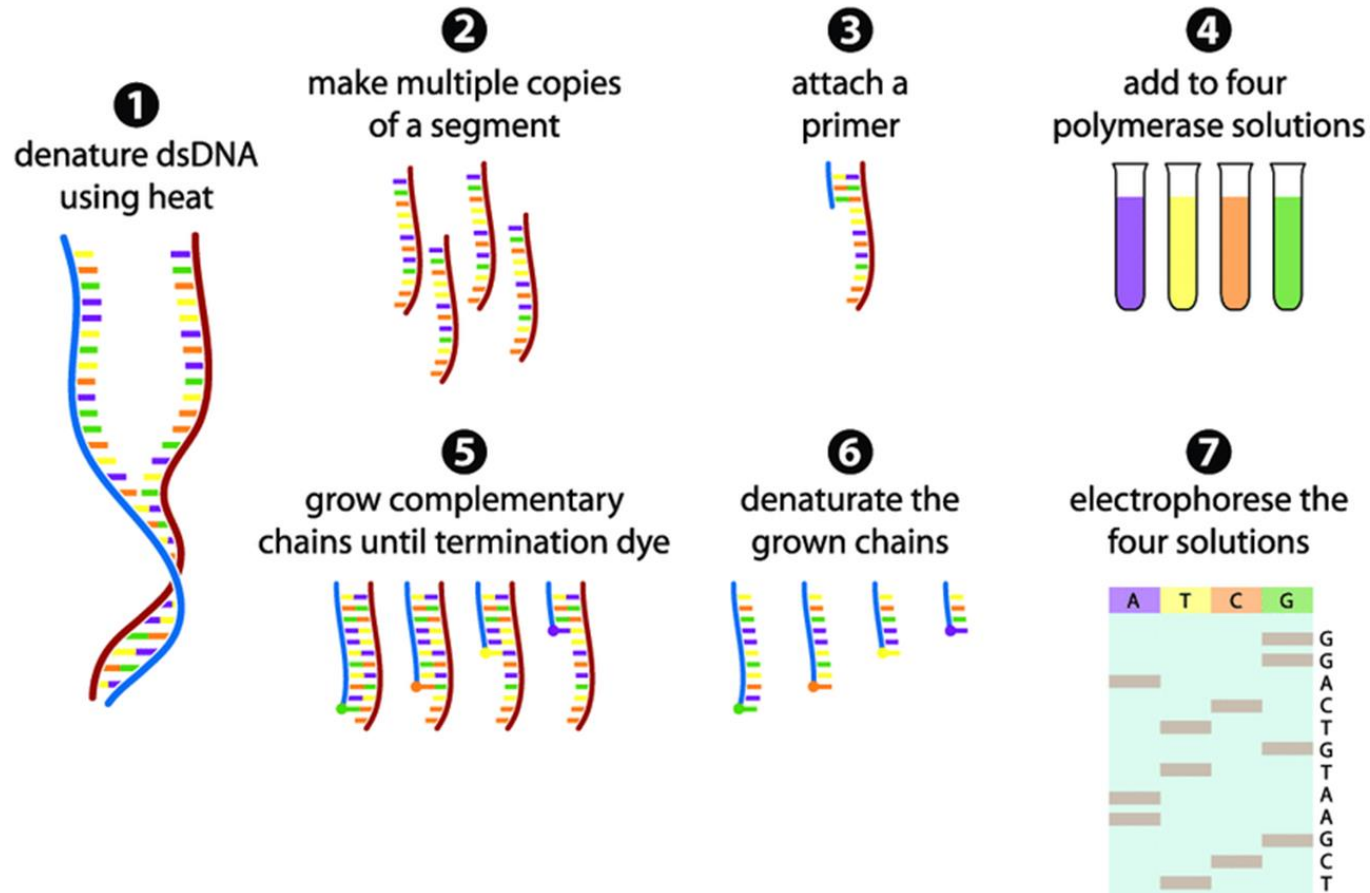
Definition

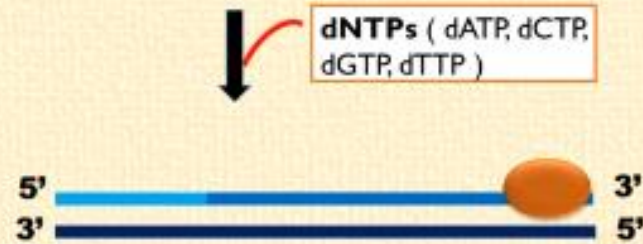
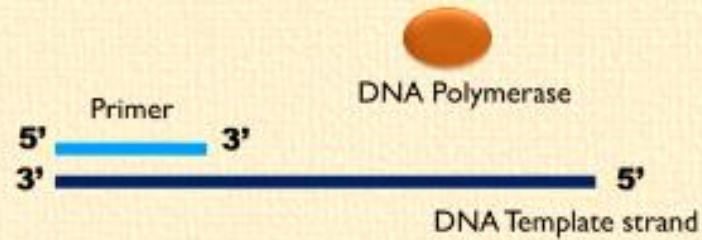
- Technique for determining the exact sequence of nucleotides in a DNA molecule
- Can look at entire genome or part of it
- Applications
 - **Molecular biology:** associations with diseases and phenotypes, and identify potential drug targets
 - **Evolutionary biology**
 - **Virology:** sequencing is one of the main tools in virology to identify and study the virus



Sanger (dideoxy method) sequencing

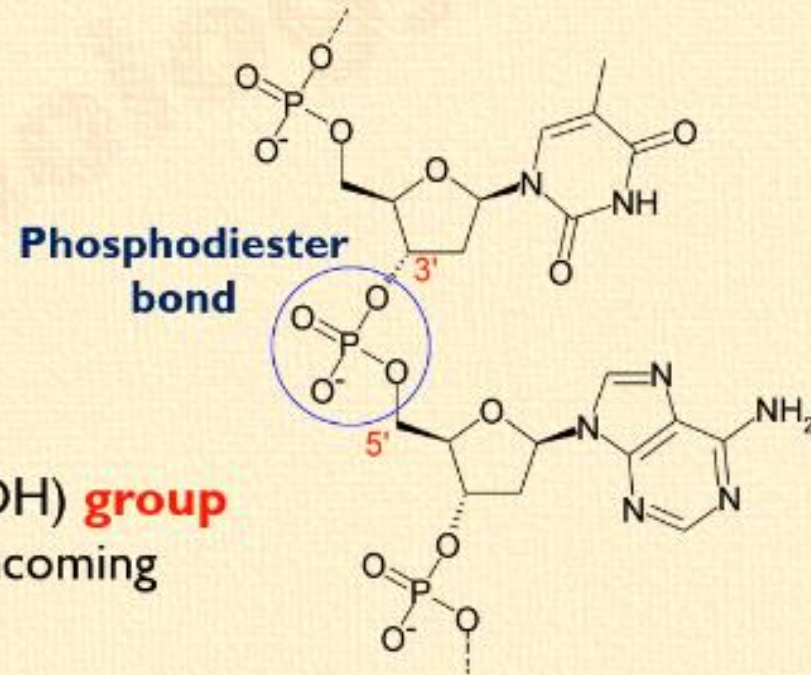
- Developed by Frederick Sanger in 1977 (was later awarded Nobel)



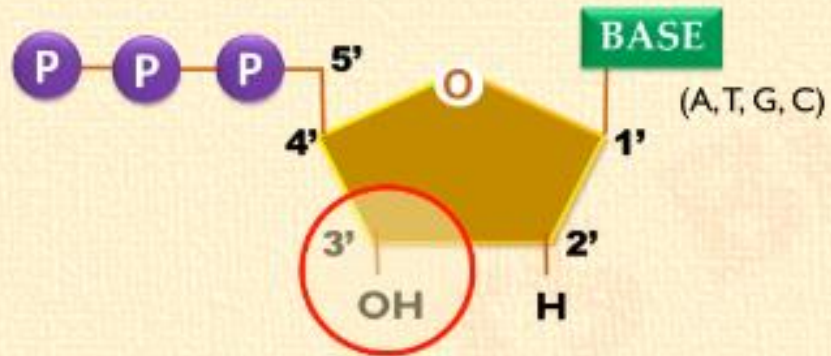


Primer provides **initial 3' hydroxyl (3'OH) group** to form phosphodiester bond with the incoming dNTP.

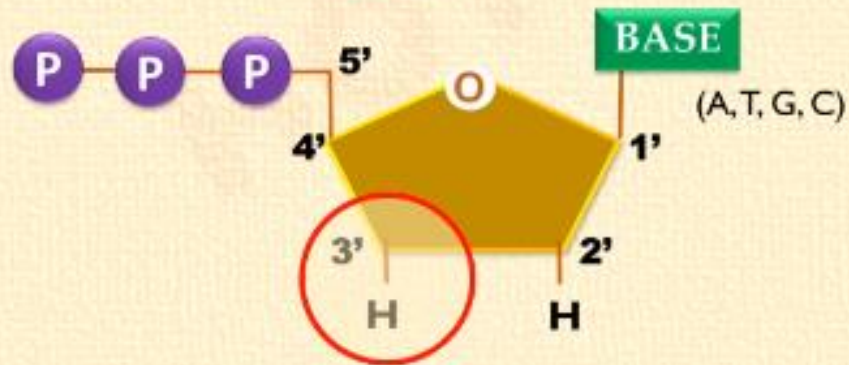
Primer is essential for
DNA synthesis



Sanger DNA sequencing technique makes use of **modified**
deoxynucleotides known as **DIDEOXYNUCLEOTIDES**.



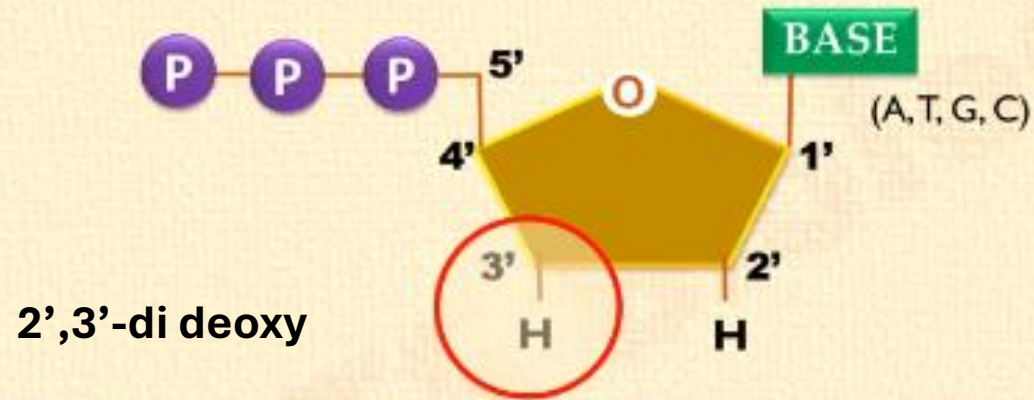
Deoxynucleotide (dNTP)



Dideoxynucleotide (ddNTP)

When a **ddNTP** is added in a DNA synthesis reaction.....

DNA synthesis will **TERMINATE** with the incorporation of ddNTP.



ddNTPs are also known as **chain terminating nucleotides**.

Sanger Sequencing is also known as **chain termination method** or **dideoxy DNA sequencing**.

“controlled termination of synthesis”

This DNA sample is taken in 4 different test tubes

Sanger Sequencing

ddNTP (small amounts)

ddATP

1

ddCTP

2

ddGTP

3

ddTTP

4

Common reaction components

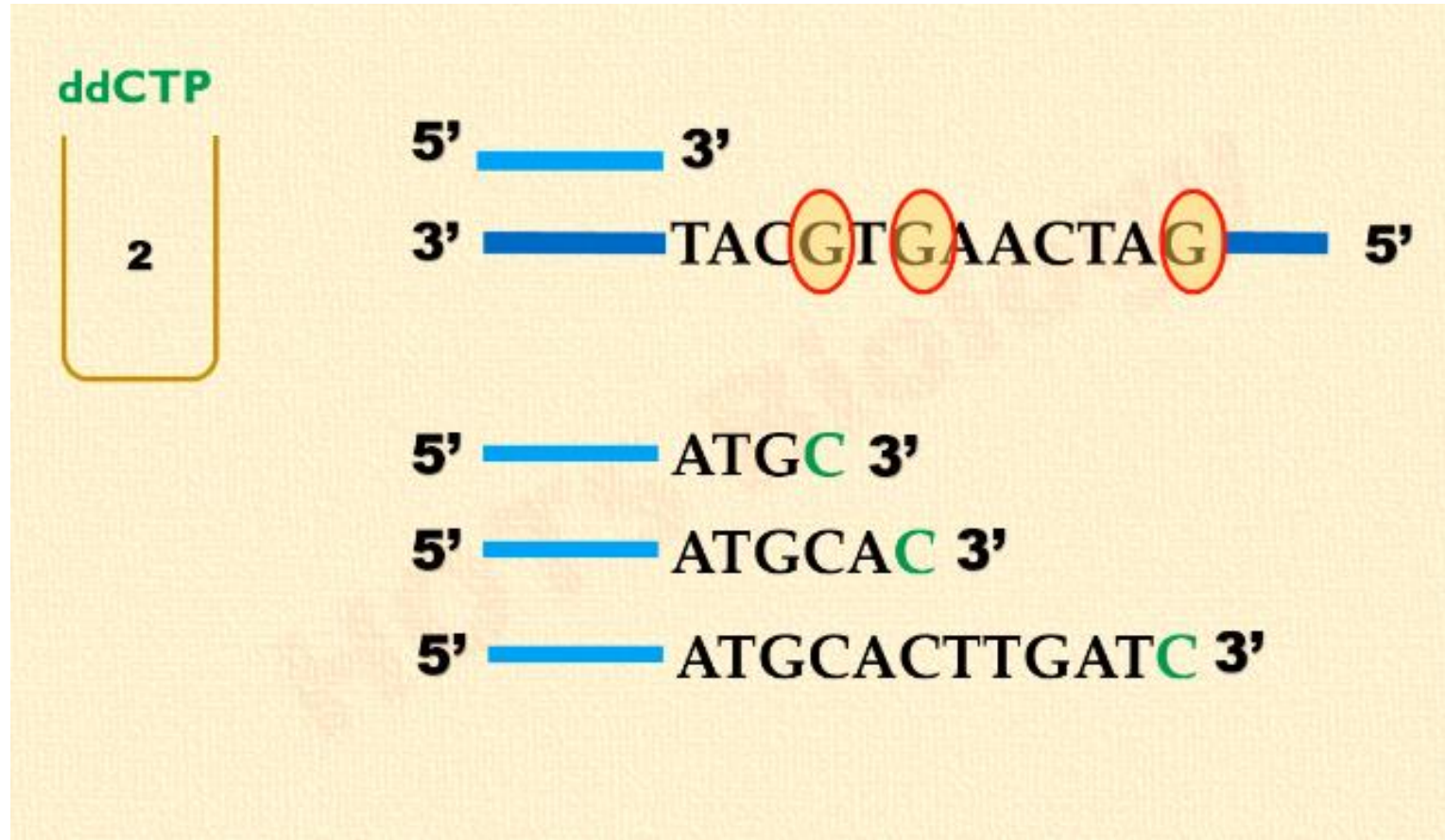
DNA template strand (many copies) 3' ——— TACGTGAACTAG ——— 5'

DNA primer (many copies, radiolabeled) 5' ——— 3'

DNA polymerase

dNTPs – dATP, dCTP, dGTP, dTTP (large amounts)

The ddCTP will add C, but it cannot form the next phosphodiester link, and so further chain lengthening is stopped



3' — TACGTGAACTAG — 5'

ddATP

1

ddCTP

2

ddGTP

3

ddTTP

4

5' — A 3'
5' — ATGCA 3'
5' — ATGCACTTGA 3'

5' — ATGC 3'
5' — ATGCAC 3'
5' — ATGCACTTGATC 3'

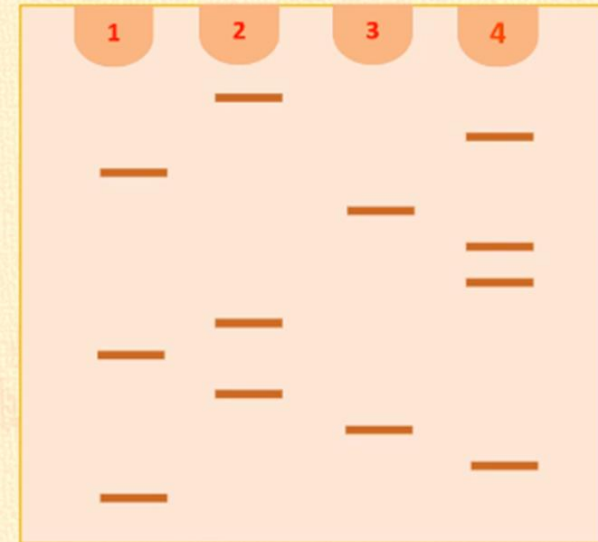
5' — ATG 3'
5' — ATGCACTTG 3'

5' — AT 3'
5' — ATGCACT 3'
5' — ATGCACTT 3'
5' — ATGCACTTGAT 3'



Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis



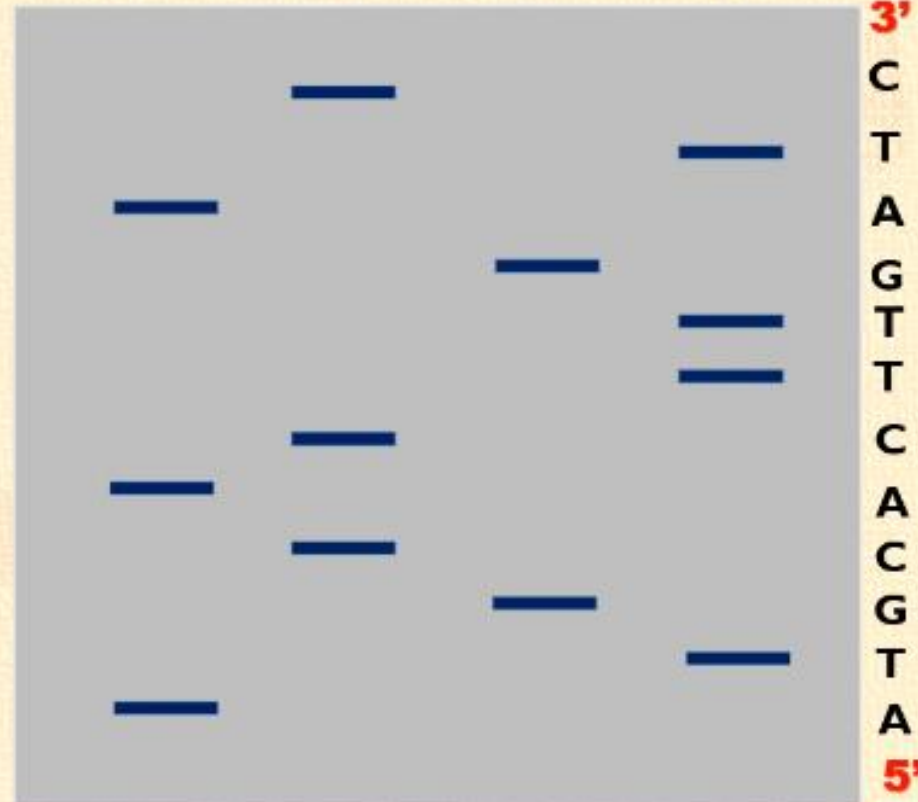
Autoradiography

Largest Fragment



Smallest Fragment

A **C** **G** **T**



3'
C
T
A
G
T
T
C
A
C
G
T
A
5'

Top



Direction
of reading
the
sequence

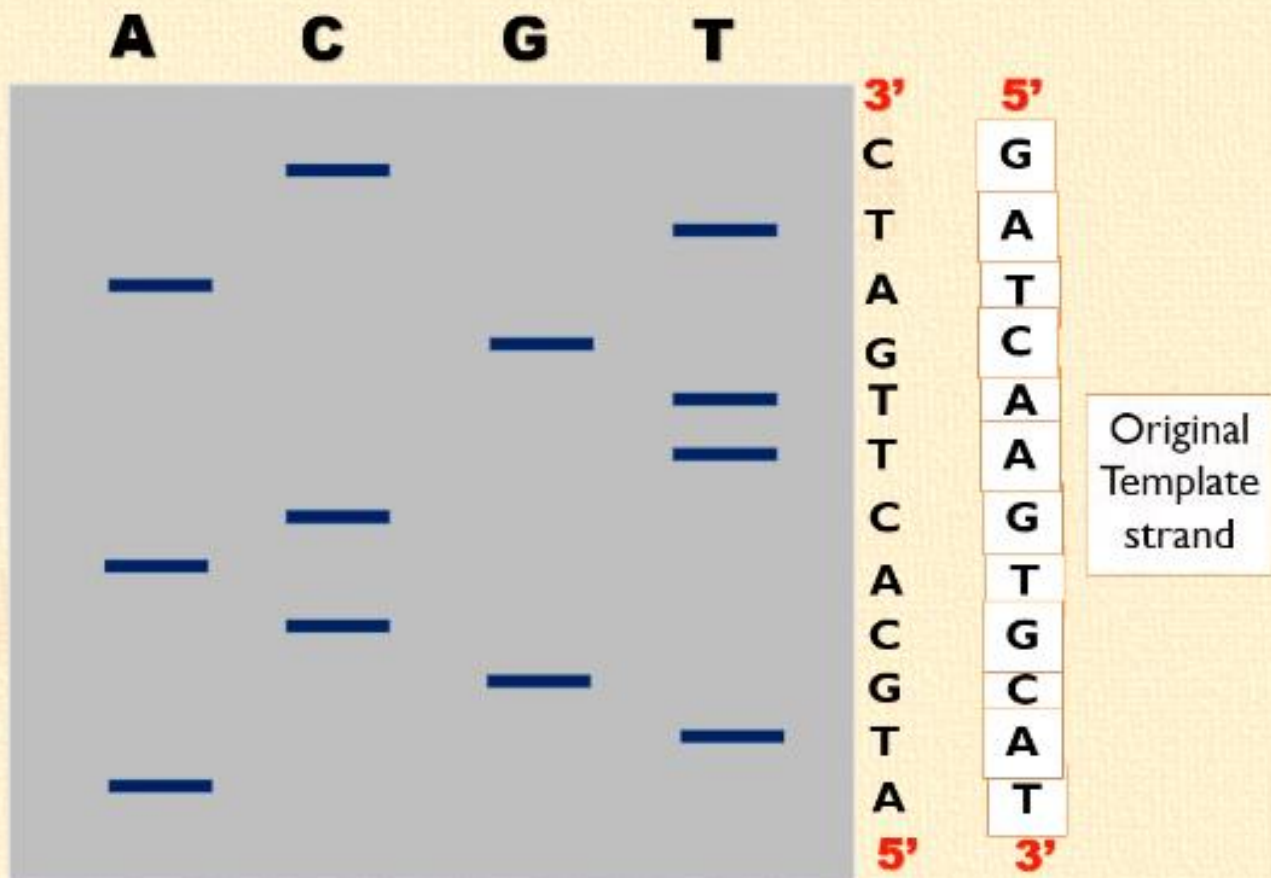
Bottom

Autoradiography

Largest Fragment



Smallest Fragment



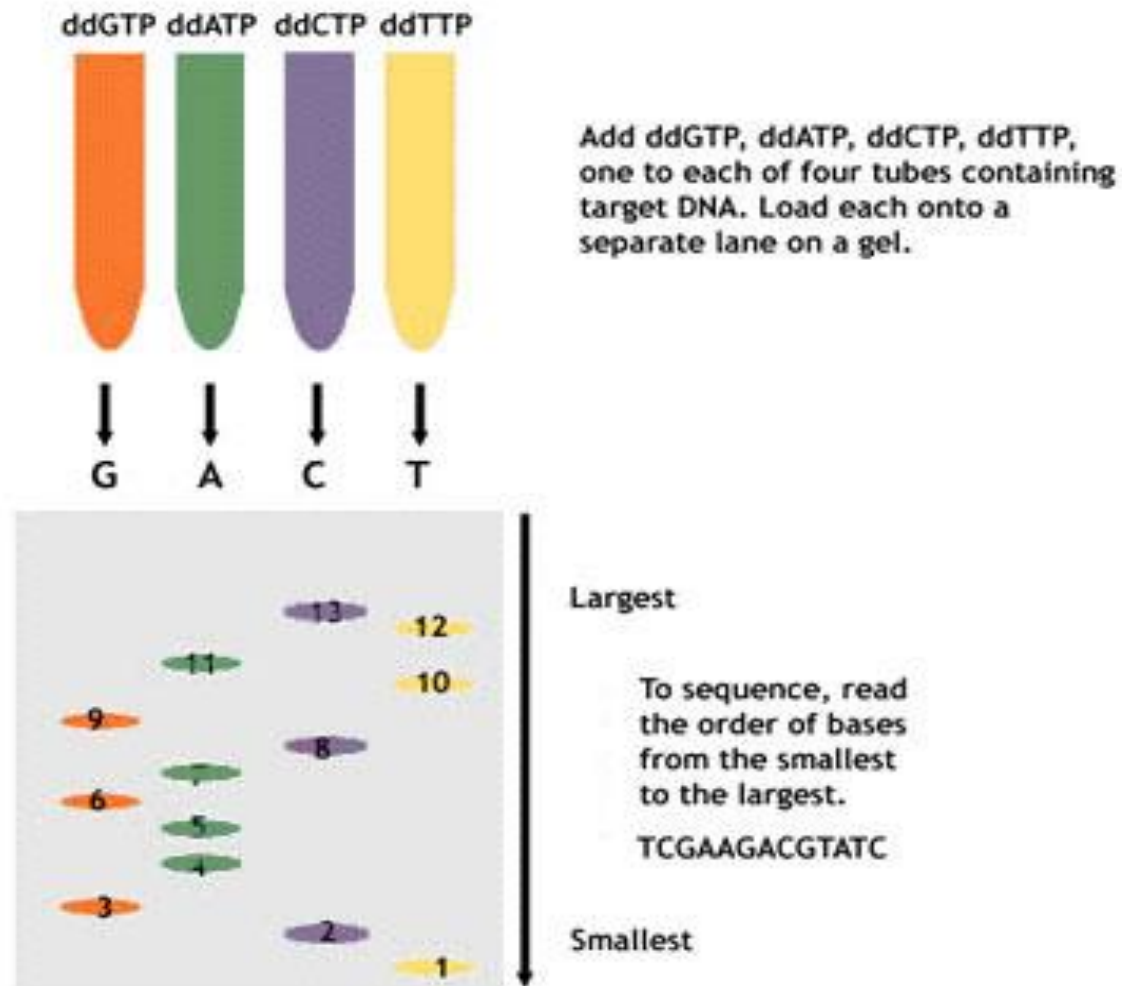
Chain termination method (Sanger dideoxy method)

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 **dideoxynucleoside 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 3'-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

Reading at home

- Read or see below videos about next generation sequencing (NGS)
- <https://www.youtube.com/watch?v=CZeN-IgjYCo>
- <https://youtu.be/WKAUtJQ69n8?si=hrRprb1hhnlYxhCF>

Understand:

How fast it is to sequence entire genome vs HGP

Read depth and coverage

Sanger vs NGS

Problem based learning (PBL)

	II-Critical thinking: 1-Observe, identify and predict health problems based on previous experience and make decisions based on evidence rather than opinion 2- Draw conclusions about the collected data (inference).	
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	3- Maintain good communication habits, such as active listening and respect. 4-Improve problem-solving skills. 5-Demonstrate knowledge of resources and tools available to support lifelong learning.	
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Example PBL: Molecular Diagnostics for Inherited Cancer Syndromes

- Sarah a 28-year-old woman, visits her primary care physician with concerns about her family history of cancer
- Her mother and maternal aunt were both diagnosed with breast cancer in their 30s, and her maternal grandfather had pancreatic cancer
- **Sarah is wondering if she should undergo genetic testing to assess her own cancer risk**

Intended Learning Outcomes

- Understand the concept of inherited cancer syndromes and their genetic basis
- Discuss the indications and implications of genetic testing for cancer risk assessment

Questions to answer

- 1. What are some examples of inherited cancer syndromes, and what are the underlying genetic mechanisms?
- 2. What factors should be considered when determining if a patient is a candidate for genetic testing for cancer risk assessment?
- 3. What do you advise in this case?

Examples of inherited cancer syndromes

- **Hereditary Breast and Ovarian Cancer Syndrome (HBOC):**
 - Caused by mutations in BRCA1 and BRCA2 genes, which are involved in DNA repair
- **Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer):**
 - Caused by mutations in DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2)
 - These mutations lead to an increased risk of colorectal, endometrial, and other cancers.
- **Familial Adenomatous Polyposis (FAP):**
 - Caused by mutations in the APC gene, which regulates cell growth and division
 - These mutations result in the development of numerous colorectal polyps and a high risk of colorectal cancer.

Factors to consider when determining if a patient is a candidate for genetic testing

- Personal and family history of cancer, including types of cancer and ages at diagnosis
- Number and degree of affected relatives
- Presence of rare or bilateral cancers
- Ancestry
- Patient preferences and psychosocial readiness
- Availability of validated genetic tests and their implications for patient management

What do you advise?

- Given strong family history of breast and pancreatic cancers at young ages raises suspicion for an inherited cancer syndrome, possibly HBOC
- Genetic testing for BRCA1 and BRCA2 mutations would be appropriate
- Implications for testing:
 - More intensive screening
 - Prophylactic mastectomy/ oophorectomy
- Pre- and post-test genetic counseling would be essential

PBL tips and tricks

- Read the clinical stem carefully
- Review the intended learning outcomes
- Analyze each question systematically
- Apply your knowledge
- Consider the implications
- Use evidence-based reasoning
- Collaborate with your peers
- Reflect on your learning
- **Embrace uncertainty**

PBL1: Liquid Biopsy for Cancer Monitoring

- **Clinical Stem:** Mahmoud is a 62-year-old man with a history of colorectal cancer, completed treatment with surgery and adjuvant chemotherapy 6 months ago
- His oncologist recommended a liquid biopsy to monitor for minimal residual disease (MRD) and potential recurrence
- **Intended Learning Outcomes**
 - Understand the concept of liquid biopsy and its applications in cancer monitoring
 - Explain the advantages of liquid biopsy over traditional tissue biopsy
 - Discuss the potential implications of detecting MRD and its impact on patient management
- **Questions**
 - 1. What is a liquid biopsy, and what types of biomarkers can be detected using this technique?
 - 2. How does liquid biopsy differ from traditional tissue biopsy, and what are the advantages of using liquid biopsy for cancer monitoring?
 - 3. What is minimal residual disease (MRD), and how can detecting MRD through liquid biopsy impact patient management and outcomes?

PBL2: Next-Generation Sequencing for Precision Oncology

- **Clinical Stem:** Dr. Osama, an oncologist, is treating Yasser, a 55-year-old patient with metastatic pancreatic cancer. Despite initial treatment with chemotherapy, Yasser's disease has progressed
- Dr. Osama is considering using next-generation sequencing (NGS) to identify potential targeted therapies or clinical trial opportunities for Yasser
- **Intended Learning Outcomes**
 - Understand the principles and applications of NGS in precision oncology
 - Discuss the potential benefits and limitations of using NGS for guiding personalized cancer treatment
 - Explain the process of variant interpretation and its implications for clinical decision-making
- **Questions**
 - 1. How can NGS be used to identify potential targeted therapies for cancer patients?
 - 2. What are the advantages and limitations of using NGS in precision oncology compared to traditional single-gene testing?
 - 3. How are variants identified through NGS interpreted and classified, and what are the challenges and implications of variant interpretation for clinical decision-making?

PBL3: Organoids for Personalized Cancer Treatment

- Sarah, a 42-year-old woman with advanced gastric cancer, has not responded well to standard chemotherapy regimens
- Her oncologist Dr. Reem, is considering enrolling her in a trial of patient-derived organoids to test the efficacy of different chemotherapy drugs and targeted agents to guide Sarah's treatment
- **Intended Learning Outcomes**
 - Understand the concept of patient-derived organoids and their applications in cancer research and personalized medicine
 - Discuss the advantages and limitations of using organoids for drug screening and treatment selection
 - Explain the process of generating and characterizing patient-derived organoids
- **Questions**
 - 1. What are patient-derived organoids, and how are they generated from cancer patients?
 - 2. How can organoids be used for drug screening and personalized treatment selection in cancer?
 - 3. What are the advantages and limitations of using organoids for predicting patient response to therapy compared to traditional 2D cell culture or animal models?

PBL4: Single-Cell Sequencing for Cancer Heterogeneity

- **Clinical Stem:** Dr. Nadia is a translational researcher is studying intratumoral heterogeneity in prostate cancer using single-cell RNA sequencing (scRNA-seq)
- She hopes to identify distinct subpopulations of cells within the tumor that may contribute to treatment resistance and metastasis
- **Intended Learning Outcomes**
 - Understand the concept of intratumoral heterogeneity and its clinical implications
 - Explain the principles and advantages of single-cell RNA sequencing
 - Discuss the potential applications of single-cell sequencing in cancer research and precision medicine
- **Questions**
 - 1. What is intratumoral heterogeneity, and how does it contribute to cancer progression and treatment resistance?
 - 2. How does single-cell RNA sequencing work, and what are its advantages over bulk sequencing methods?
 - 3. What are the potential applications of single-cell sequencing in cancer research, and how can it inform precision medicine approaches?