MOLECULAR BIOLOGY TECHNIQUES IN CANCER RESEARCH

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But first…

Introduce yourself to the class:

- Name
- 2 truths and 1 lie

But first..

B	I	N	G	O
Has travelled to another country	Plays a musical instrument	Has a pet cat	Speaks more than two language	Is left-handed
Likes to read books	Has met a celebrity	Can do a handstand	Loves to cook or bake	Plays on a sports team
Has been on TV	Can ride a bicycle	Can do a magic trick	Has watched an entire TV series in one weekend	Is in a school club
Likes to draw or paint	Has never broken a bone	Loves playing video games	Knows how to code	Has been to a different continent
Loves to dance	Has been to a concert	Knows how to swim	Collects something interesting	Has a summer birthday

Overview of the course

Date	1st Lecture	2nd Lecture
16th July	Icebreakers, ground rules and maybe and Intro to Molecular Biology and NGS	
17th July	Intro to Molecular Biology and NGS	Data analysis 1
18th July	Experiment: Nucleic acid extraction	Data analysis 2
19th July	Single-cell DNA, RNA and protein technologies	Proteomics, spatial technologies and epigenomics Data analysis 3
20th July	Data analysis 4	Experiment: Staining our own cells
21st July	Experiment: Gel electrophoresis	Data analysis 5 Data analysis 6
22nd July	Preparation for the final presentation	Final presentation and closing ceremony

INTRODUCTION TO MOLECULAR BIOLOGY AND NGS

Objectives

1. Understand the basic concepts of molecular biology, including DNA, RNA, and proteins.
2. Introduce the principles and applications of Next Generation Sequencing (NGS).

What is molecular biology?

Molecular biology is the study of the molecular basis of biological activity

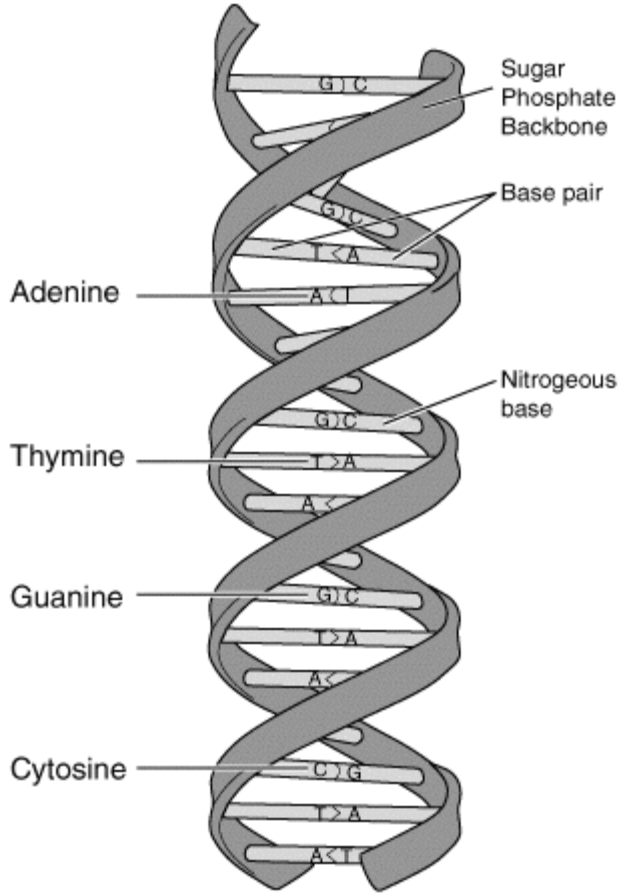
What is molecular biology?

It can also be referred to as multi-omics

- Genomics
- Transcriptomics
- Proteomics
- Epigenomics
- Etc

DNA (Deoxyribonucleic Acid)

- Structure: Double helix composed of nucleotides.
- Nucleotides: Adenine (A), Thymine (T), Cytosine (C), Guanine (G).
- Function: Genetic blueprint for living organisms.
- How can DNA be studied?: **Sequencing, Electrophoresis** and Southern Blotting, Microarrays, etc.
- Terminology: Genomics



RNA (Ribonucleic Acid)

Structure: Single-stranded molecule.

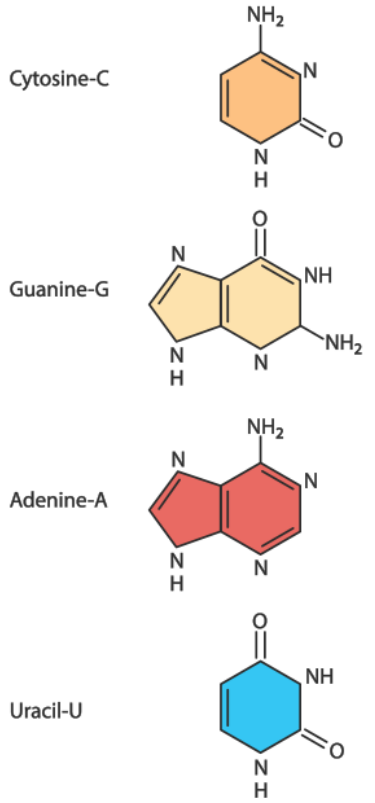
Nucleotides: Adenine (A), Uracil (U), Cytosine (C), Guanine (G).

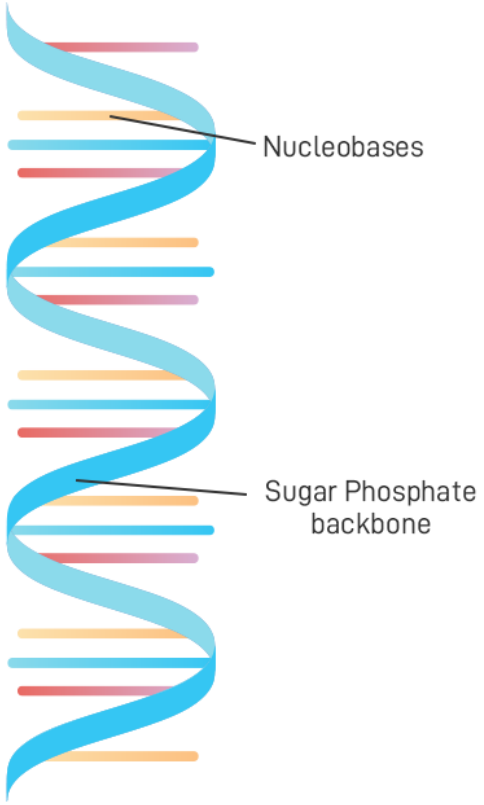
Types: mRNA (messenger), tRNA (transfer), rRNA (ribosomal), etc.

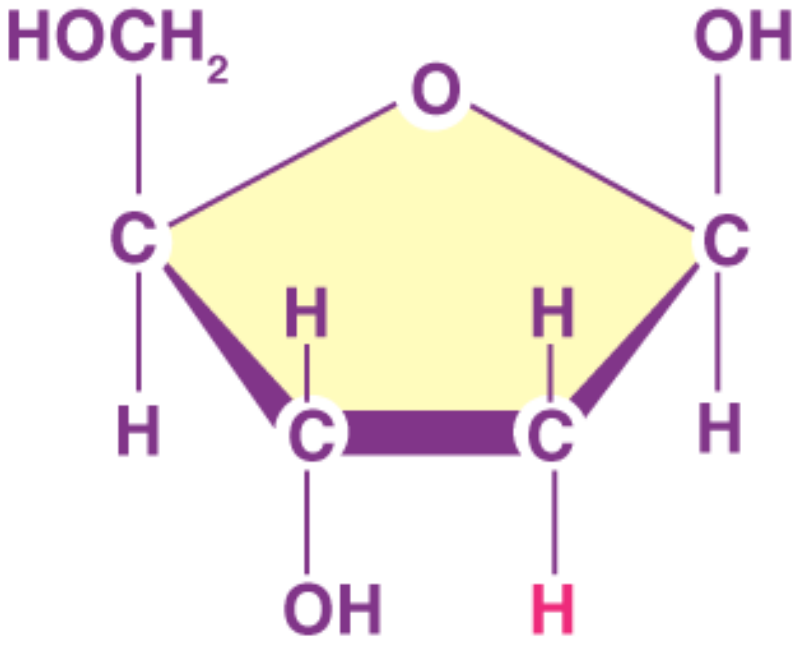
Function: Transfers genetic code from DNA in the nucleus for protein synthesis in the cytoplasm.

How can RNA be studied: **Sequencing, Electrophoresis** and Northern Blotting, in situ Hybridisation

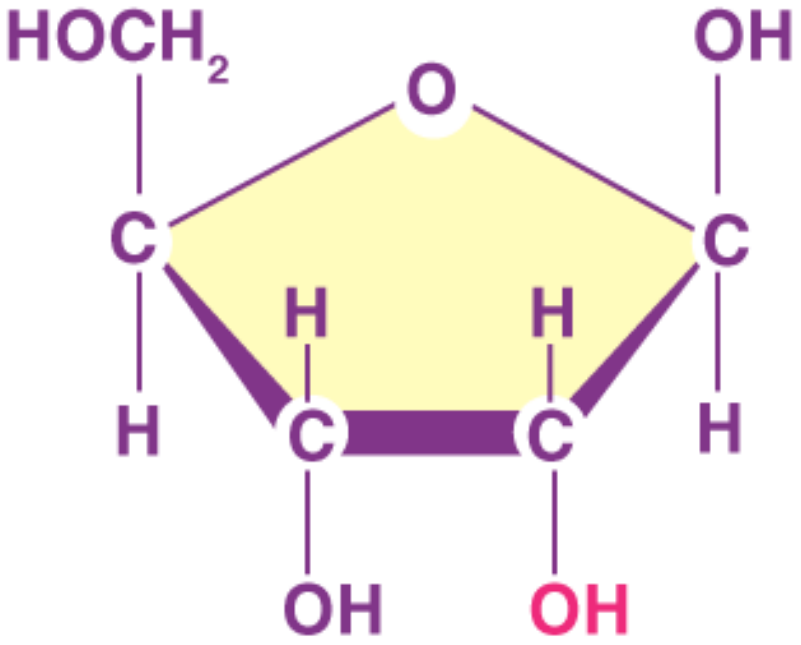
Terminology: Transcriptomics







2- Deoxyribose



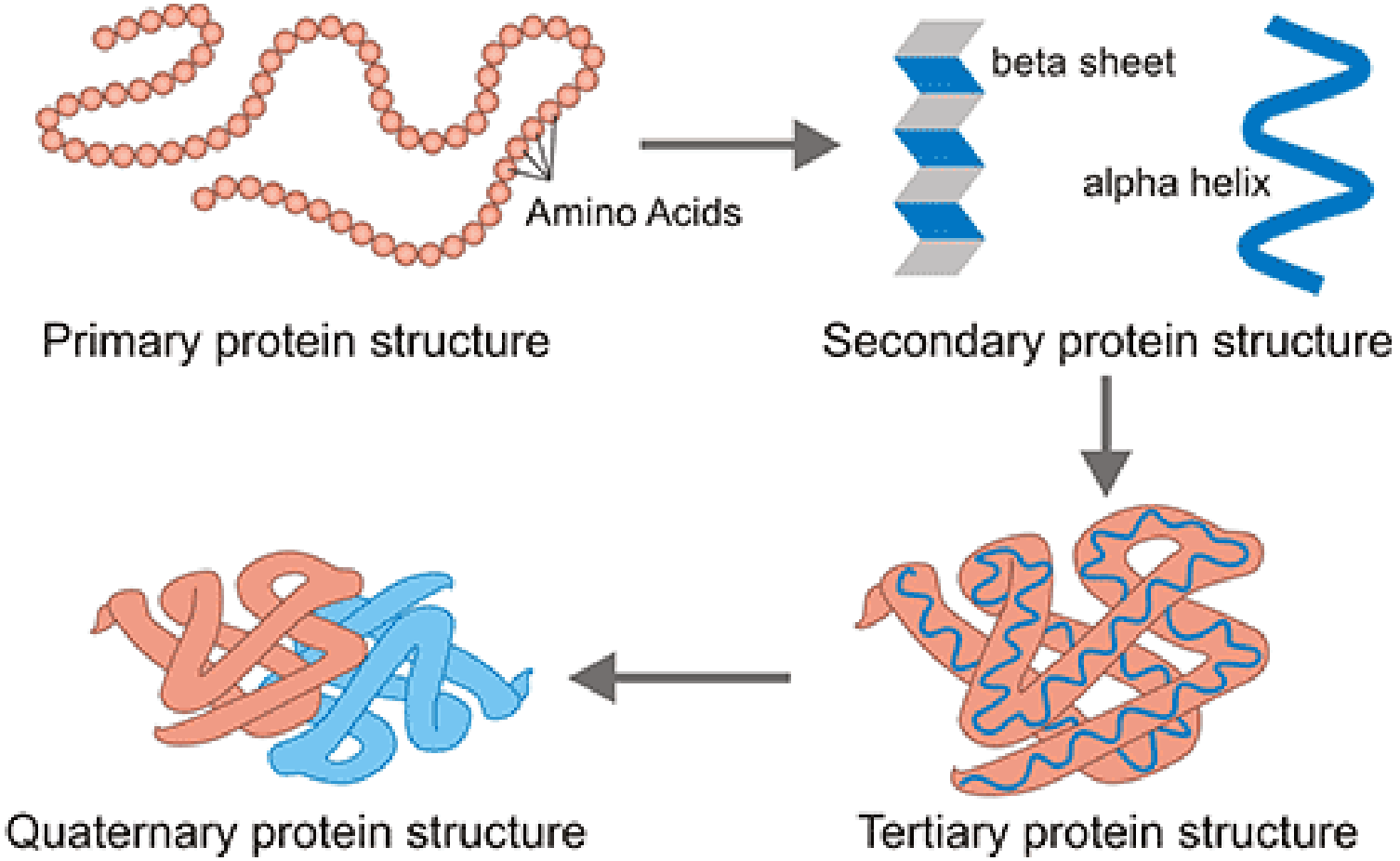
Ribose

Proteins

- Structure:

Structure Level	Primary Structure	Secondary Structure	Tertiary Structure	Quaternary Structure
Description	Linear sequence of amino acids.	Alpha-helices and beta-sheets.	3D folding of a single polypeptide.	Assembly of multiple polypeptides.

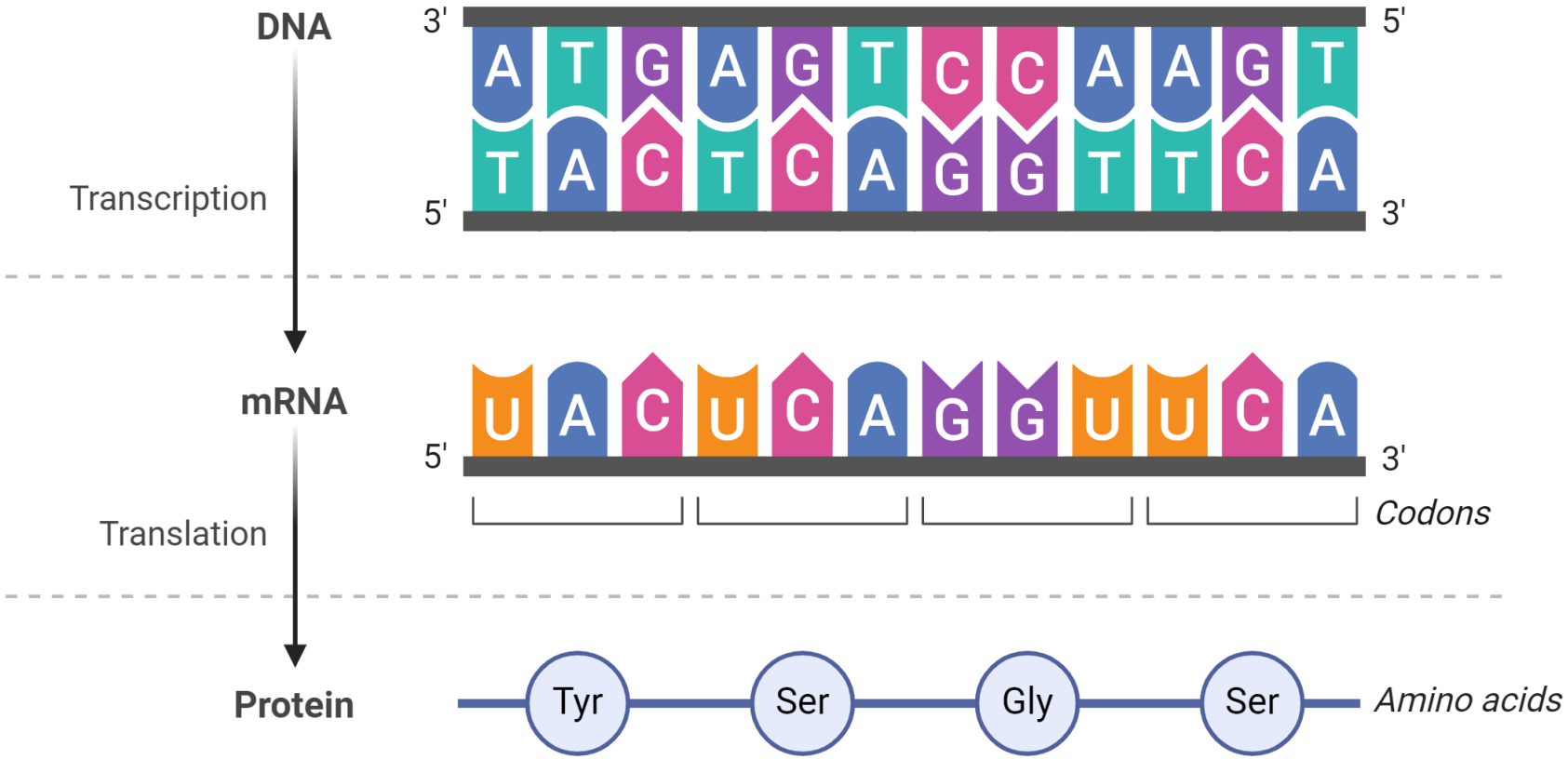
Proteins


Amino Acids
beta sheet
alpha helix
Primary protein structure
Secondary protein structure
Tertiary protein structure
Quaternary protein structure

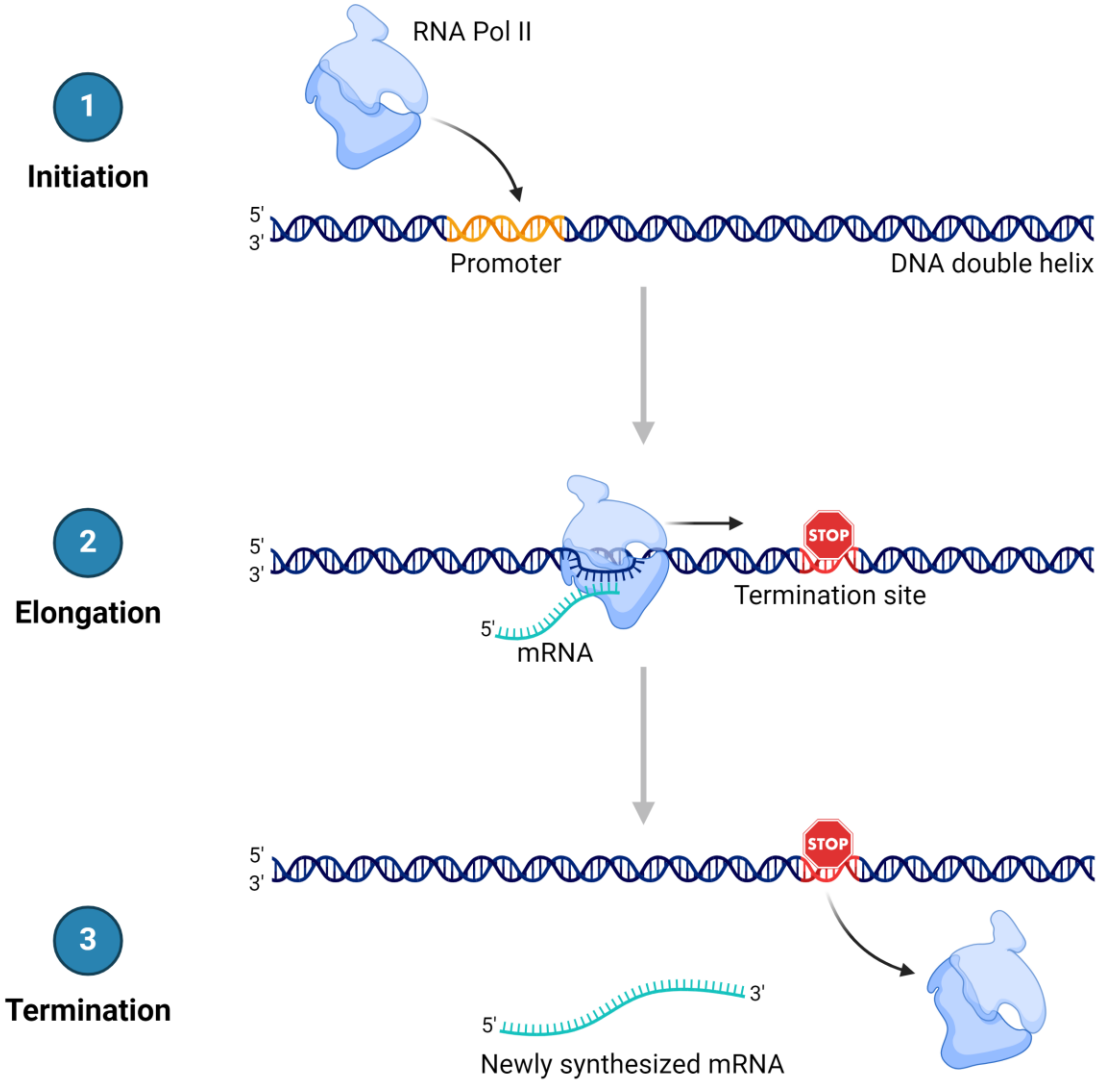
Proteins

- Function:
  - Catalyze metabolic reactions (enzymes).
  - Structural components (collagen, keratin).
  - Transport molecules (hemoglobin).
  - Immune response (antibodies).
- How can proteins be studies?: **Electrophoresis** and Western blotting, ELISA, X-ray Crystallography , **Microscopy**, etc.
- Terminology: Proeteomics

Central Dogma of Molecular Biology: Simplified



Eukaryotic Transcription



Central Dogma: Transcription

1. Initiation

- Promoter Binding: RNA polymerase binds to the promoter region of DNA.
- DNA Unwinding: The DNA double helix unwinds, exposing the template strand.

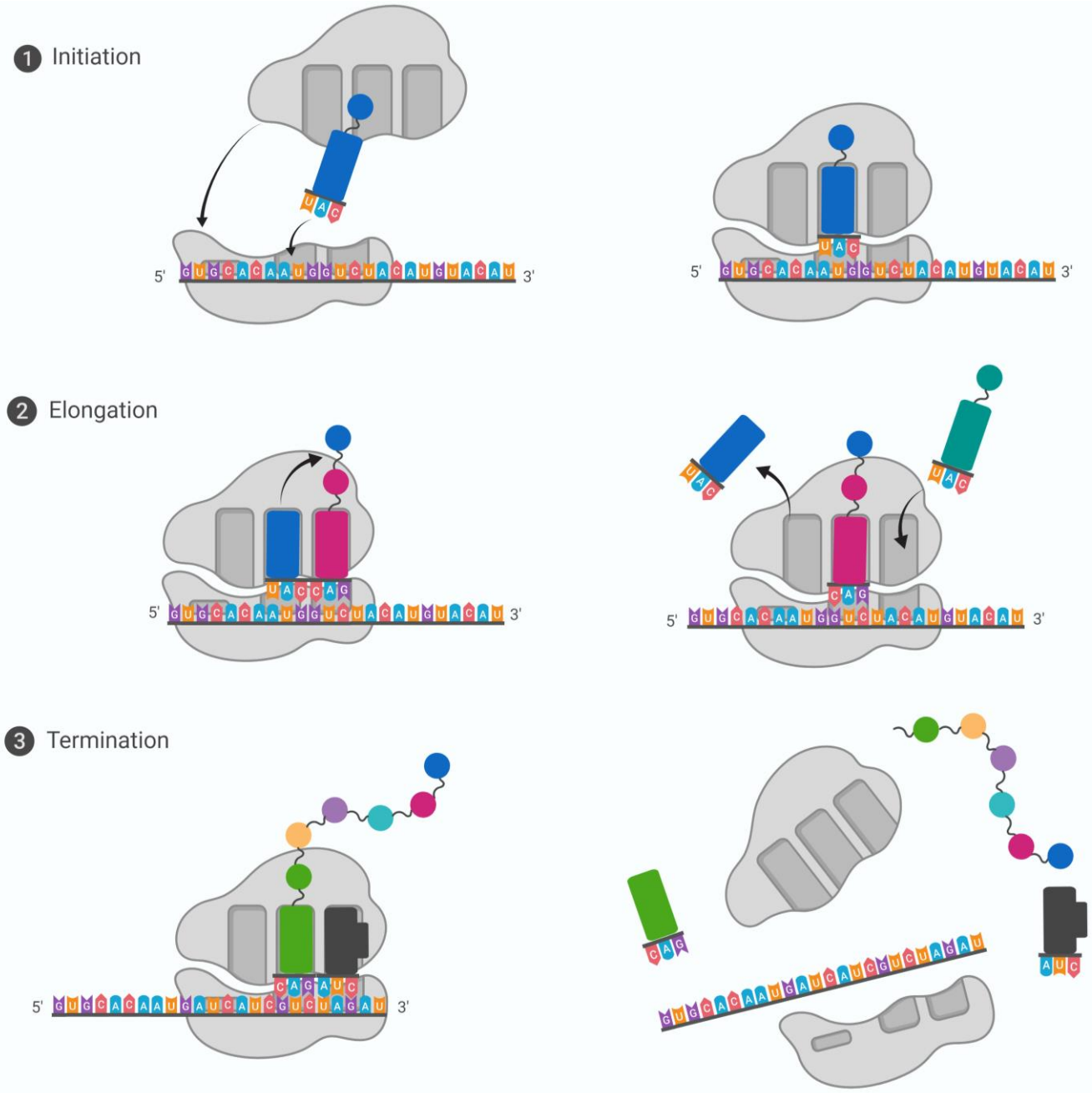
2. Elongation

- RNA Synthesis: RNA polymerase synthesizes RNA by adding ribonucleotides complementary to the DNA template/non-coding strand (read in the 3' to 5' direction)
- Direction: RNA is synthesized in the 5' to 3' direction.

3. Termination

- Termination Signal: RNA polymerase reaches a stop sequence in the DNA template.
- RNA Release: The newly synthesized RNA molecule is released from the DNA template.

Translation



Central Dogma: Translation

1. Initiation

- mRNA Binding: The small ribosomal subunit binds to the mRNA at the start codon (AUG).
- tRNA Binding: The initiator tRNA carrying methionine binds to the start codon.
- Ribosome Assembly: The large ribosomal subunit joins to form the complete initiation complex.

2. Elongation

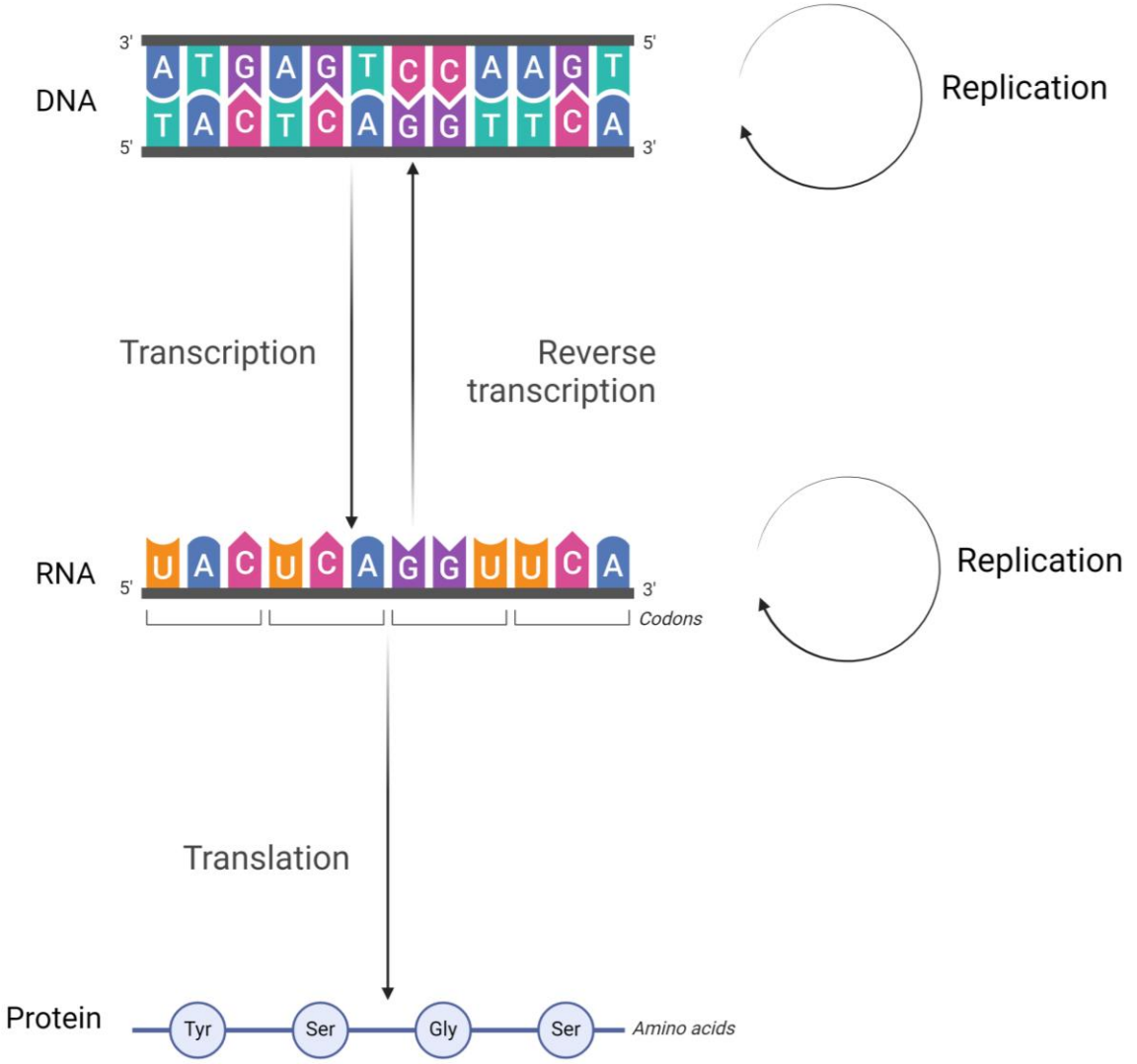
- Codon Recognition: The next tRNA, carrying an amino acid, binds to the mRNA codon in the A site of the ribosome.
- Peptide Bond Formation: The ribosome catalyses the formation of a peptide bond between the amino acid in the P site and the amino acid in the A site.
- Translocation: The ribosome moves along the mRNA, shifting the tRNA from the A site to the P site, and the empty tRNA exits from the E site.

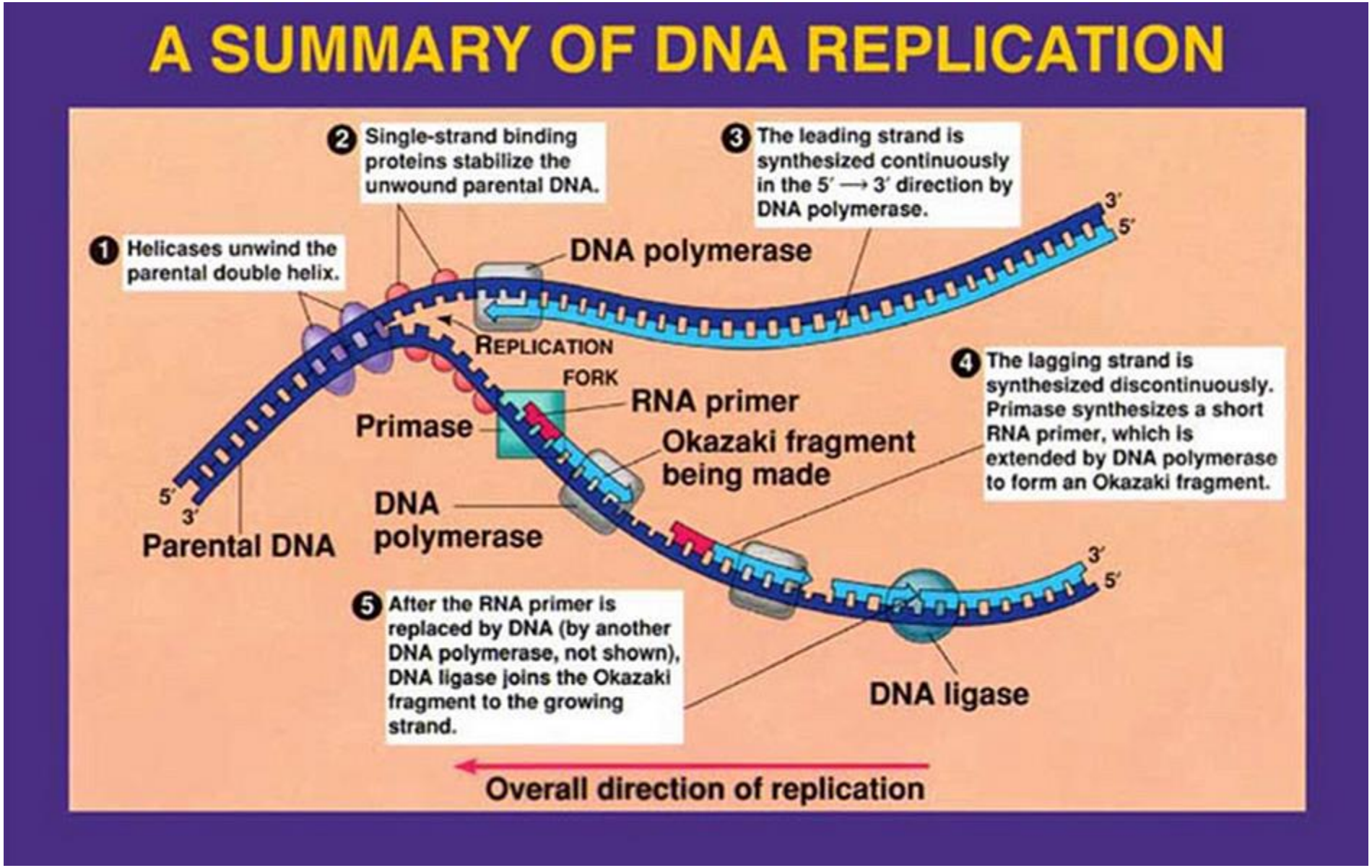
3. Termination

- Stop Codon Recognition: The ribosome reaches a stop codon (UAA, UAG, or UGA) on the mRNA.
- Release of peptide: release factors trigger the release of the synthesised polypeptide
- Ribosome Disassembly: The ribosomal subunits disassemble and are ready to initiate another round of translation.

Transcription and Translation Summary

Central Dogma of Molecular Biology: Complex




A SUMMARY OF DNA REPLICATION
1 Helicases unwind the parental double helix.
2 Single-strand binding proteins stabilize the unwound parental DNA.
3 The leading strand is synthesized continuously in the 5′ → 3′ direction by DNA polymerase.
4 The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5 After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.
DNA polymerase
REPLICATION FORK
RNA primer
Primase
Okazaki fragment being made
DNA polymerase
Parental DNA
DNA ligase
3′
5′
5′
3′
3′
5′
Overall direction of replication

Central Dogma: DNA Replication

1. Initiation

- Replication begins at specific sequences called origins of replication.
- Helicase unwinds the DNA double helix, creating replication forks.
- Primase synthesizes RNA primers complementary to the DNA strand.

2. Elongation

- Leading Strand Synthesis: DNA polymerase continuously synthesizes the leading strand in the 5′ to 3′ direction.
- Lagging Strand Synthesis: The lagging strand is synthesized discontinuously as Okazaki fragments, each initiated by an RNA primer.

-DNA ligase joins Okazaki fragments on the lagging strand by forming phosphodiester bonds.

3. Termination

- Replication continues until the replication forks meet.
- RNA primers are removed and replaced with DNA by DNA polymerase I.
- DNA ligase seals any remaining nicks in the sugar-phosphate backbone, completing the replication process.

Central Dogma: RNA processing (eukaryotes)

- 5′ Capping: A 5′ cap is added to the RNA.
- Polyadenylation: A poly-A tail is added to the 3′ end of the RNA.
- Splicing: Introns are removed, and exons are joined together.

Central Dogma: Reverse transcription

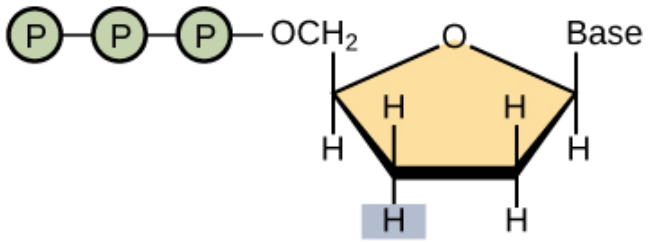
- Reverse transcription is the synthesis of DNA from RNA
- Driven by RNA-dependent DNA polymerases -> reverse transcriptase
- Occurs in retroviruses, prokaryotes and eukaryotes
- Telomerase is an example of a reverse transcriptase in eukaryotes

Objectives

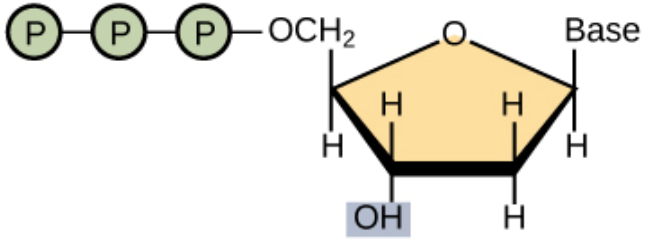
✓Understand the basic concepts of molecular biology, including DNA, RNA, and proteins.

2. Introduce the principles and applications of Next Generation Sequencing (NGS).

Sanger sequencing



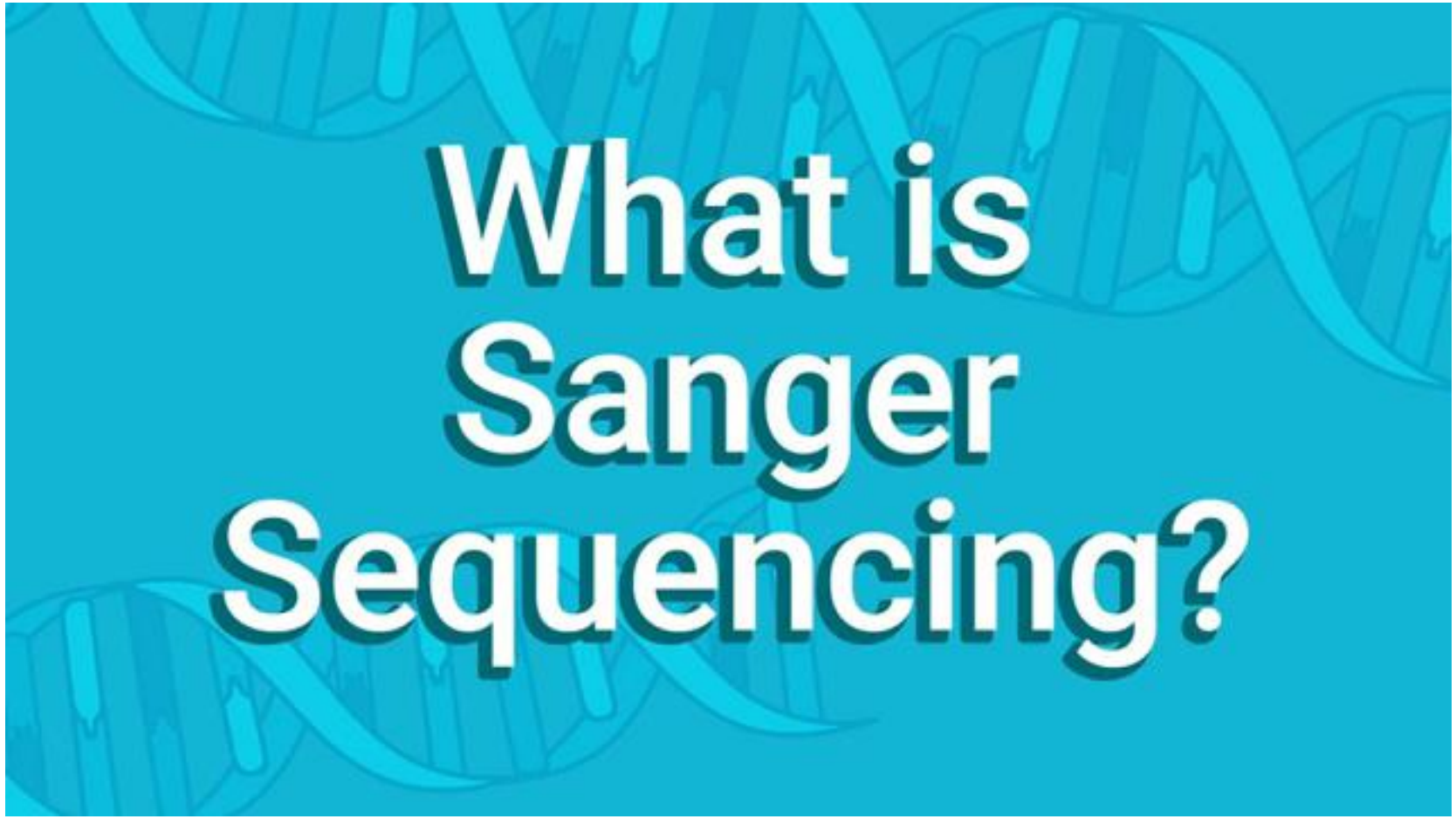
Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

1. The reaction mix contains:
   - DNA template
   - Primer
   - DNA polymerase
   - dNTPs (deoxynucleotides)
   - Fluorescence-labelled ddNTPs (dideoxynucleotides)
2. Chain termination:
   - DNA polymerase adds dNTPs to extend the primer.
   - Incorporation of a ddNTP terminates the chain because ddNTPs lack a 3'-OH group required for forming the next phosphodiester bond.
   - This results in a mixture of DNA fragments of varying lengths, each ending with a ddNTP
3. Fragment separation and analysis:
   - DNA fragments separated by electrophoresis
   - Fluorescent signal is analysed and ordered from shortest to longest

Sanger sequencing



What is Next Generation Sequencing?

- NGS a form of massively parallel sequencing
- Simultaneously sequences multiple DNA fragments

Workflow

- Sample preparation
- Sequencing
- Data analysis

Sample Preparation

1. Extract nuclei acids
2. Fragment nucleic acids
3. Library preparation

1. Bulk nucleic acid extraction

- The nucleic acid extraction we have seen so far
- The most common type of nucleic acid extraction
- Nucleic acids are extracted from a population of cells or tissue
- The cell in which the nucleic acid originally came from cannot be determined
- The greatest identification you can make is which sample the nucleic acids came from

1. Extract nucleic acids

Extract DNA or RNA from cells.

More on this in a later lesson…

1.5. What happens to the nucleic acids next?

What

- Quantify nucleic acids
  - Concentration
  - Yield
- Assess quality of extracted nuclei acids
  - Purity
  - Integrity

How?

- Absorbance methods
- Fluorescence methods
- Electrophoresis

2. Fragmentation

- Most sequencing technologies can only handle short sequences/reads
- Sequences are fragmented during sample preparation and put back together during data analysis

3. Library preparation

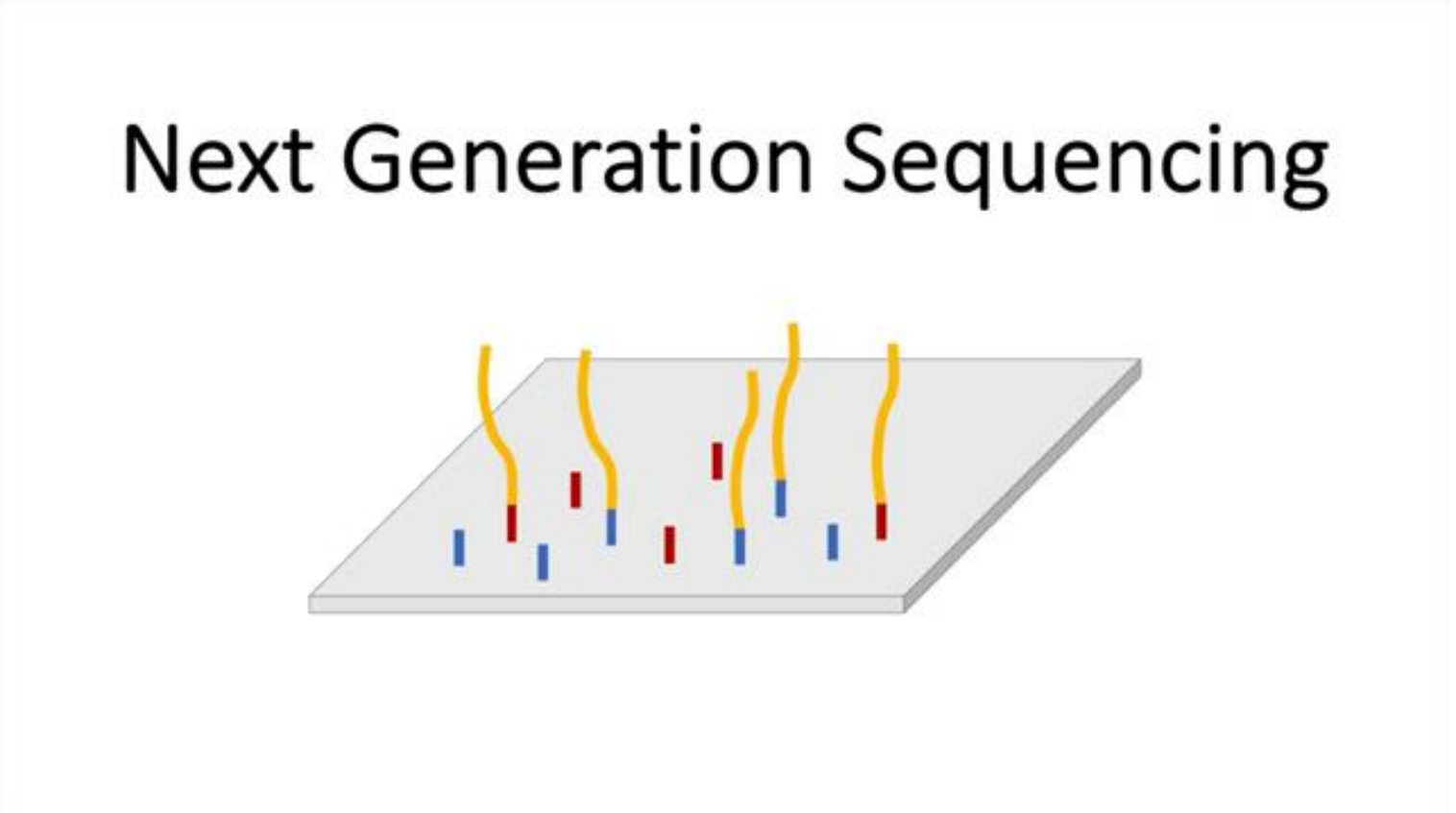
What is a library?

- DNA fragments with adaptor sequences added.
- These adaptors make the DNA compatible with a specific sequencing platform
- PCR is performed to increase DNA

What is the difference between library preparation for DNA vs RNA?

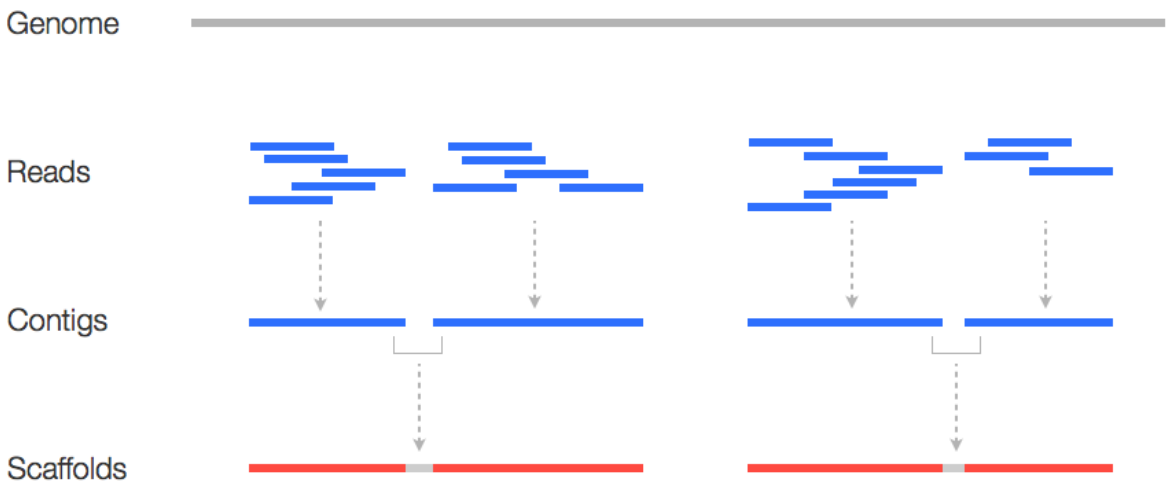
- Reverse transcription of RNA to make cDNA

Sequencing



Data analysis

1. Base calling: Identifying nucleotides from raw data.
2. Alignment: Mapping reads to reference genome.
3. Assembly: Constructing sequences from reads.



Data analysis: Assembly

Applications of NGS in cancer research

Single-cell RNA sequencing reveals differential expression of EGFL7 and VEGF in giant-cell tumor of bone and osteosarcoma

Pan-cancer analysis of whole genomes

A pan-cancer analysis of the microbiome in metastatic cancer

Proteomic analysis of the urothelial cancer landscape

Objectives

- ✓Understand the basic concepts of molecular biology, including DNA, RNA, and proteins.
- ✓ Introduce the principles and applications of Next Generation Sequencing (NGS).

Summary

- DNA -> RNA -> Proteins
- But DNA and RNA can self-replicate and RNA -> DNA
- RNA is less stable than DNA
- A typical sequencing workflow involves sample preparation, sequencing and data analysis
- NGS is like Sanger sequencing but allows multiple DNA fragments to be sequences simultaneously

Any questions?

References

- https://teachmephysiology.com/biochemistry/protein-synthesis/
- Fåhraeus, R. Has translation in the nucleus found its purpose? Nature Reviews. Molecular Cell Biology 25, 1–2 (2023).
- Translation: DNA to mRNA to protein | Learn Science at Scitable. https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393/.
- Reverse Transcription Basics | Thermo Fisher Scientific - IE. https://www.thermofisher.com/uk/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/rt-education/reverse-transcription-basics.html#:~:text=What%20is%20reverse%20transcription%3F,as%20well%20as%20in%20retroviruses.