DNA REPLICATION

Dr. Sami Mamand

Molecular Biology

Semester I

Week 4

10-28-2025

Outline

- DNA Replication
- Mechanism of DNA replication
- Difference between prokaryote and eukaryote in DNA replication

Objectives

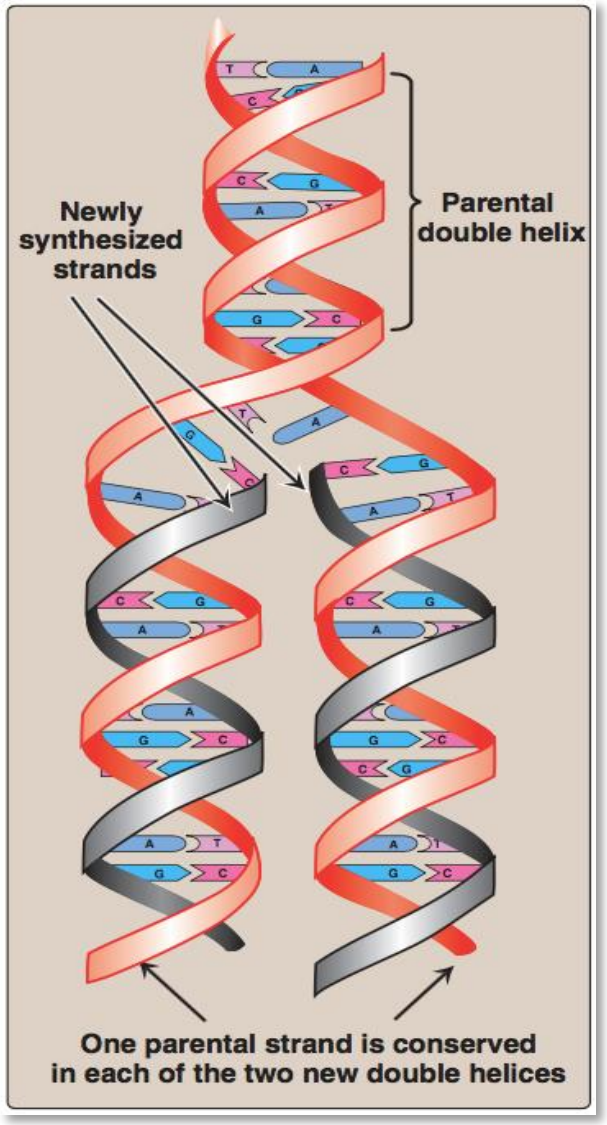
- **Define** DNA replication
- **Describe** the **basic requirements** for DNA replication, including substrate, template, primer, enzymes, and proteins.
- **Explain** the **mechanism** of DNA replication and distinguish among the stages: **initiation, elongation, and termination.**
- **Differentiate** between **prokaryotic and eukaryotic DNA replication** in terms of enzymes, origin, and process.
- **Identify** the **enzymes and proteins** involved in DNA replication and describe their specific functions.
- **Discuss** the **applications** of DNA replication in biotechnology, including **PCR, gene cloning, genome sequencing, and CRISPR technology.**

Introduction to Molecular Biology

- DNA is made up of millions of **nucleotides**, which are composed of deoxyribose sugar, with phosphate and a base.
- **DNA Replication:** is the process by which a DNA makes a copy of itself. DNA replication is **semi-conservative**, meaning that each strand in the DNA acts as a **template** for the synthesis of a new complementary strand.

- Any mistake in copying - **Mutation**

- **DNA mutation** - chromosomal mutation

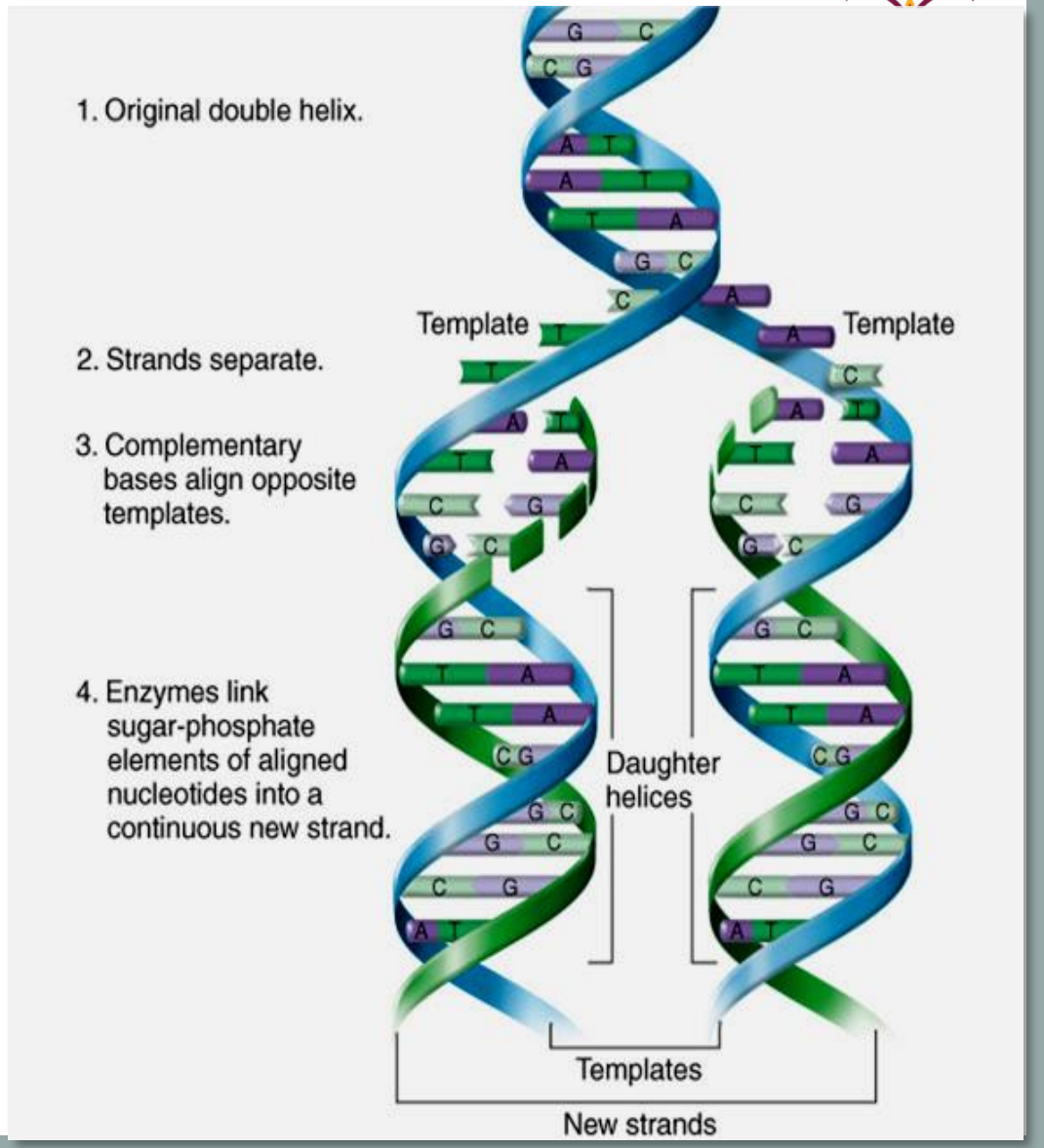


Basic rules of Replication

A. Semi-conservative
B. Starts at the ‘origin’
C. Synthesis always in the 5-3’ direction
D. Can be uni or bidirectional
E. Semi-discontinuous
F. RNA primers required
G. During S-phase of cell cycle

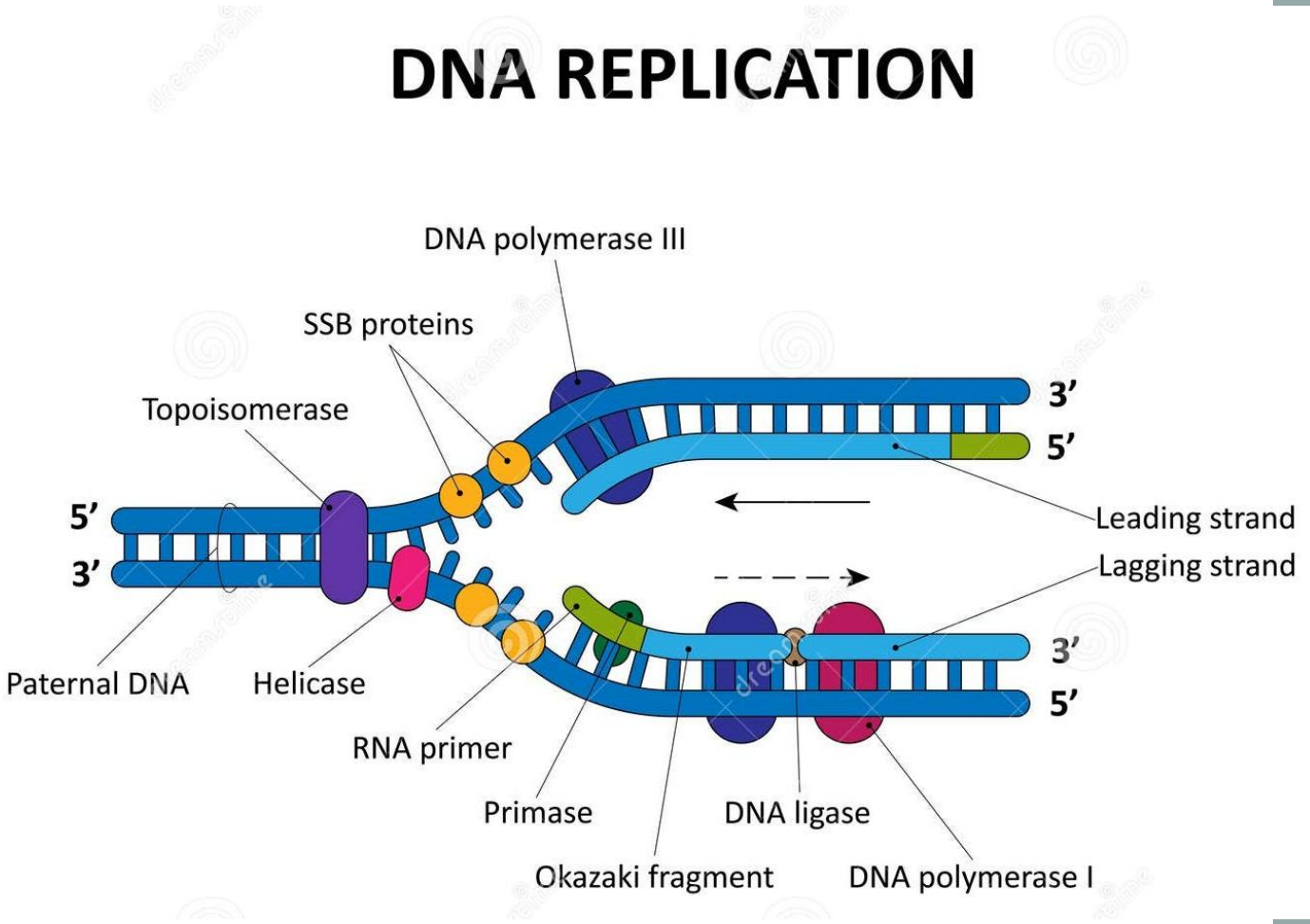
Semi-conservative replication

- One strand of duplex passed on unchanged to each of the daughter cells.
- This 'conserved' strand acts as a **template** for the synthesis of a new, complementary strand by the enzyme DNA polymerase



Basic requirement of DNA Replication

Requirement	Definition	Shown in Image As
1. Template	The **existing parental DNA strands** used to make new strands.	**Paternal DNA** (labeled on the left) and the **leading/lagging strand templates.**
2. Substrate	The **free nucleotides (dNTPs)** that DNA polymerase adds to the new strand.	Not labeled, but implied at the site of **DNA polymerase III and I activity.**
3. Primer	Short **RNA segment** that starts DNA synthesis by providing a 3′-OH group.	**RNA primer** (green section) made by **Primase.**
4. Enzymes	Catalysts that perform the steps of replication.	**Helicase**, **Primase**, **DNA polymerase III**, **DNA polymerase I**, **DNA ligase**, and **Topoisomerase.**
5. Proteins	Accessory proteins that stabilize or assist enzymes.	**SSB proteins** (yellow/orange circles) that keep strands apart during replication.

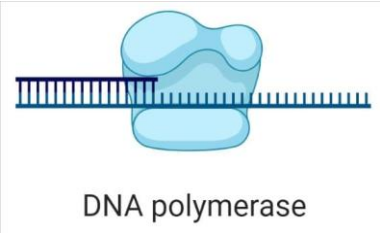


Basic Enzymes and Proteins Required for DNA Replication

Enzyme / Protein	Function / Role	Key Feature / Notes
DNA Helicase	Unwinds and separates the double-stranded DNA by breaking hydrogen bonds.	Acts like a “zipper” that opens the DNA helix ahead of the replication fork.
DNA Topoisomerase (Gyrase)	Relieves tension and supercoiling ahead of the replication fork.	- **Type I:** Cuts one strand and rejoins it. - **Type II:** Cuts both strands and passes another segment through.
Single-Strand Binding Proteins (SSBs)	Bind to separated DNA strands to prevent them from reannealing.	Keep the strands open and stable for replication.
DNA Primase	Synthesizes short RNA primers on the template strand.	Provides the **3′–OH** starting point for DNA polymerase.
DNA Polymerase III	Adds nucleotides to the 3′ end of the primer to synthesize the new strand.	Main replication enzyme; synthesizes both leading and lagging strands.
DNA Polymerase I	Removes RNA primers and replaces them with DNA.	Has **5′→3′** polymerase and **3′→5′** proofreading activities.
DNA Ligase	Joins Okazaki fragments by sealing gaps between DNA fragments.	Forms **phosphodiester bonds** between adjacent fragments.
Nucleases	Cleave phosphodiester bonds within DNA or RNA.	Important for **repair and proofreading**.
Primers (RNA Primers)	Short RNA sequences that initiate DNA synthesis.	Made by **primase**; necessary for polymerase to begin replication.

DNA Polymerase

DNA polymerases are the enzyme that is responsible for adding new nucleotides and synthesizing a new strand of DNA by taking the old fragmented strand as a template.



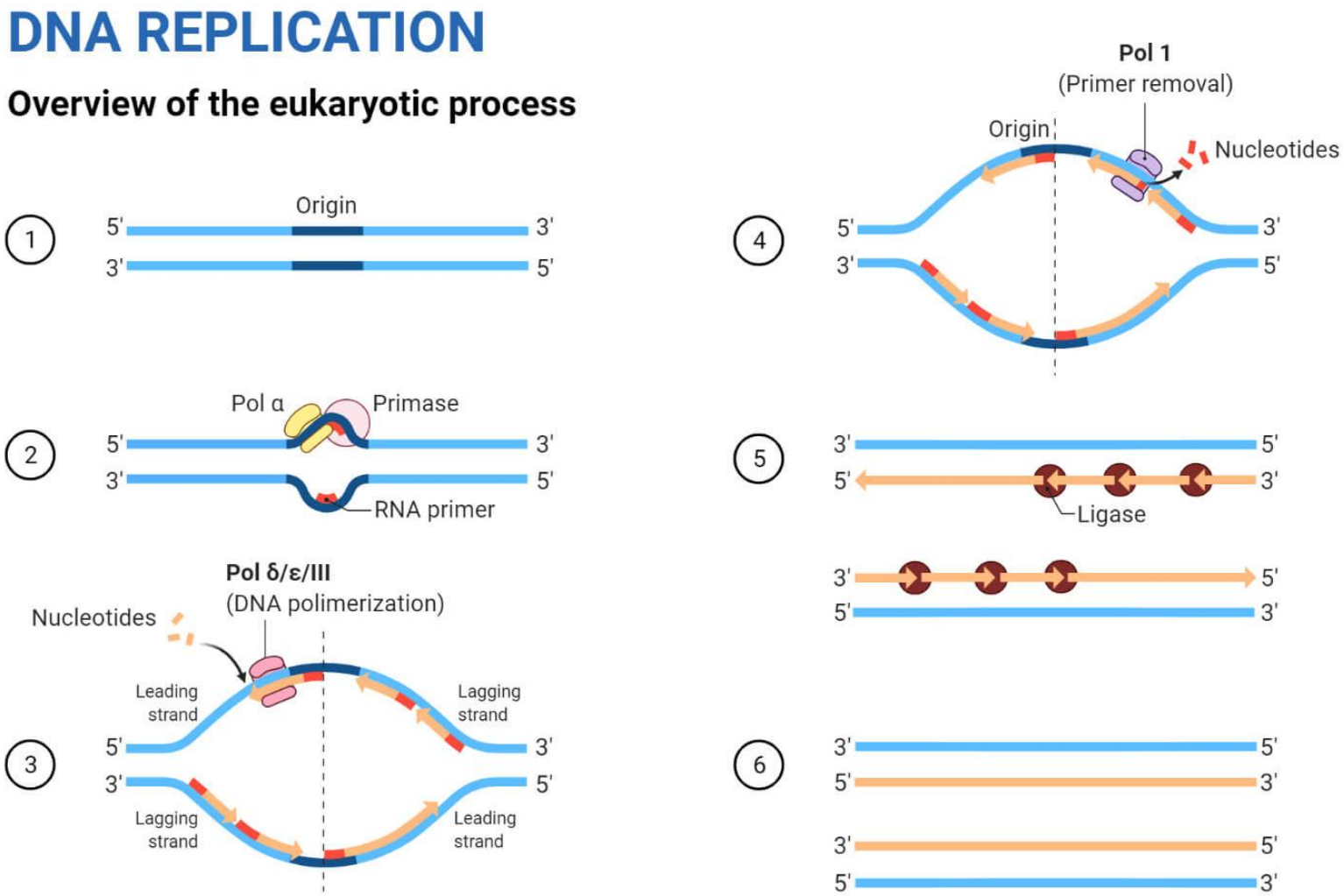
DNA polymerase

Cell Type	DNA Polymerase	Main Function
Prokaryotic	Pol I	Repair; removes RNA primers, fills gaps
	Pol II	DNA repair enzyme
	Pol III	Main replication enzyme
Eukaryotic	Pol α	Primer synthesis (with primase); short DNA extension
	Pol β	DNA repair
	Pol γ	Mitochondrial DNA replication
	Pol δ	Lagging strand synthesis
	Pol ε	Leading strand synthesis & repair

Mechanism of DNA replication

- ***Initiation:***
  - Proteins bind to DNA and open up double helix.
  - Prepare DNA for complementary base pairing.
- ***Elongation:***
  - Proteins connect the correct sequences of nucleotides into a continuous new strand of DNA.
- ***Termination:***
  - Proteins release the replication complex.

Mechanism of DNA replication



Mechanism of DNA replication

Stage	Steps in the Diagram	Process Description	Key Enzymes / Proteins
Initiation	**Steps 1 & 2**	Replication starts at **origins of replication**.- **DNA unwinds** (helicase).- **Primase + DNA Pol α** synthesize short RNA primers to begin synthesis.	**ORC complex, Helicase (MCM), Primase, DNA Polymerase α, SSBs, Topoisomerase**
Elongation	**Steps 3, 4 & 5**	**DNA Polymerases δ & ε** extend new strands.- **Leading strand** synthesized continuously.- **Lagging strand** forms Okazaki fragments.- **Primers removed**, and fragments joined.	**DNA Polymerase δ & ε, RNase H, DNA Polymerase I, DNA Ligase I**
Termination	**Step 6**	-Replication forks meet.- **New DNA molecules** are completed.- The process ends with two identical double helices.	**DNA Ligase I, Topoisomerase II** (resolves supercoils)

Leading Strand and Lagging Strand

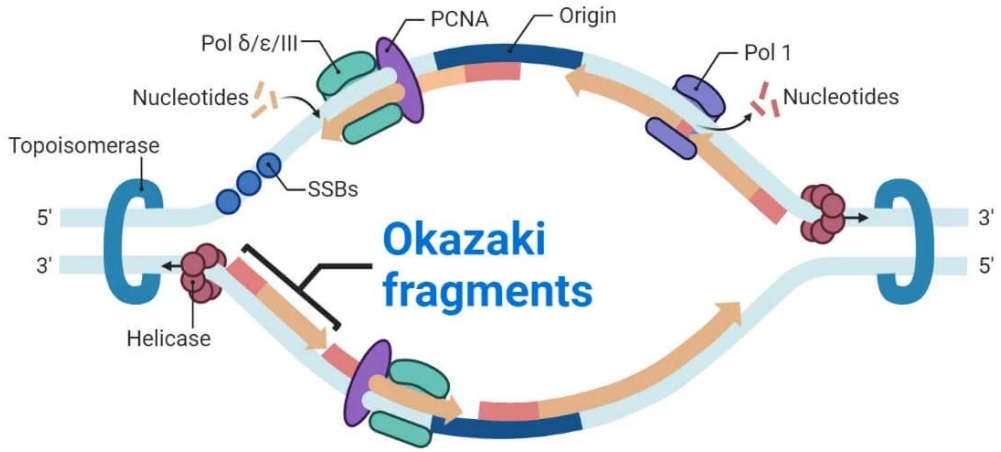
Leading Strand – The DNA strand which runs in 3’ to 5’ direction is synthesized in continuous manner.

Lagging Strand The DNA strand which runs in 5’ to 3’ direction is synthesized in discontinuous manner.

This strand is made in MANY short segments (OKAZAKI FRAGMENTS).

Okazaki Fragments

The two DNA strands run in opposite or antiparallel directions, and therefore to continuously synthesize the two new strands at the replication fork requires that one strand is synthesized in the 5’to3’ direction while the other is synthesized in the opposite direction, 3’to 5’.



Applications of DNA Replication

Genetic Continuity

- DNA replication ensures transfer of genetic information from one generation to the next.

Cellular Function

- Maintains cellular homeostatsis and genetic integrity in all living cells.

Genome Sequencing

- Understanding replication led to human genome sequencing and identification of genetic diseases

Gene Cloning

- Replication princsiples allow gene cloning and recombinant DNA research

PCR (Polymerase Chain Reaction)

- Uses DNA polymerase to replicate DNA in *vitro* for diagnosis, sequencing, and biotechnology

CRISPR–Cas9 Technology

- Based on replication and DNA repair mechanisms–enables precise genome editing

cDNA Synthesis

- Reverse transcription (making cDNA from mRNA) relies on replicative enzymes for molecular biology applications

Reference

- Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2022). *Molecular biology of the cell* (7th ed.). W. W. Norton & Company.
- Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Stryer, L. (2019). *Biochemistry* (9th ed.). W. H. Freeman and Company.
- Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Bretscher, A., Ploegh, H., & Amon, A. (2021). *Molecular cell biology* (9th ed.). W. H. Freeman and Company.
- Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., & Losick, R. (2014). *Molecular biology of the gene* (7th ed.). Pearson Education.