Al-Mustaqbal University is ranked first among Iraqi private universities.

Department of Biochemistry

Third Year – Molecular Biochemistry Laboratory

Experiment-2Title: Simulation of DNA Replication Steps

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1. Aim of the Experiment

To simulate the sequential enzymatic events of DNA replication in order to understand strand synthesis directionality, enzyme coordination at the replication fork, and the structural basis for leading and lagging strand formation.

2. Background

DNA replication is a **semi-conservative process**, meaning that each daughter DNA molecule contains one parental (template) strand and one newly synthesized strand.

Replication proceeds exclusively in the **5′ → 3′ direction**, requiring coordinated activity of several essential enzymes:

- **Helicase** – unwinds the DNA double helix
- **Single-strand binding proteins (SSBs)** – stabilize separated strands
- **Primase** – synthesizes short RNA primers
- **DNA polymerase III (prokaryotes) / DNA polymerase δ & ε (eukaryotes)** – elongate DNA
- **DNA polymerase I (prokaryotes) / RNase H & DNA polymerase (eukaryotes)** – remove RNA primers
- **DNA ligase** – seals phosphodiester backbone nicks

Due to the antiparallel structure of DNA strands, replication occurs differently on the two templates:

- The **leading strand** is synthesized continuously.
- The **lagging strand** is synthesized discontinuously as short **Okazaki fragments**.

This experiment provides a structural and conceptual simulation of replication fork dynamics using physical modeling tools.

3. Principle

DNA replication is based on:

- Complementary base pairing (A–T, G–C)
- Phosphodiester bond formation
- Primer-dependent DNA polymerization
- Antiparallel strand organization

Because DNA strands run in opposite orientations:

- Continuous synthesis occurs toward the replication fork (leading strand).
- Discontinuous synthesis occurs away from the fork (lagging strand), forming Okazaki fragments that must later be ligated.

The simulation models enzymatic activities through structured physical steps.

4. Materials

Material	Purpose
Printed double-stranded DNA template (labeled 5′ and 3′ ends)	Represents parental DNA
Colored nucleotide cards (A, T, G, C)	Simulate complementary base pairing
Short RNA-labeled strips	Represent RNA primers
Adhesive tape or connectors	Simulate DNA ligase activity
Marker pens	Label strand polarity
Worksheet	Record replication steps

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5. Equipment

Equipment	Purpose
Laboratory bench	Experimental workspace
Whiteboard or magnetic board	Visualization of replication fork
Molecular model kit (optional)	3D structural representation
Computer with molecular animation software (optional)	Interactive simulation

6. Procedure

Step 1: Identification of Template Strands

- Label parental strands 5′ and 3′.
- Confirm antiparallel orientation.

Step 2: Initiation

- Manually separate strands to simulate helicase activity.
- Indicate replication fork formation.
- (Instructor note: Emphasize that SSB proteins prevent re-annealing in vivo.)

Step 3: Primer Placement

- Place one RNA primer on the leading strand template.
- Place multiple RNA primers on the lagging strand template.

Step 4: Elongation

- Add complementary nucleotides according to base pairing rules.
- Ensure synthesis proceeds strictly in the 5′ → 3′ direction.
- Construct:
    - A continuous leading strand
    - Discontinuous lagging strand (Okazaki fragments)

Step 5: Primer Removal

- Remove RNA primer strips.
- Replace them with DNA nucleotides to simulate primer replacement.

Step 6: Ligation

- Join Okazaki fragments using adhesive material.
- Confirm formation of two identical semi-conservative daughter DNA molecules.

7. Expected Results

Students should obtain:

- Two semi-conservative DNA molecules
- One continuous leading strand
- One ligated lagging strand
- Correct 5′ → 3′ synthesis orientation
- Proper complementary base pairing

8. Discussion

The simulation demonstrates:

- The necessity of primer-dependent DNA synthesis (DNA polymerases cannot initiate de novo).
- The structural reason for discontinuous lagging strand synthesis.
- The requirement for coordinated enzyme activity at the replication fork (replisome complex).
- The molecular basis of high replication fidelity, including proofreading activity (3′ → 5′ exonuclease function).
- The importance of antiparallel strand orientation in determining replication dynamics.

9. Conclusion

This simulation allows students to visualize and reconstruct DNA replication in a structured laboratory model.

It reinforces understanding of:

- Strand polarity
- Enzyme function
- Replication fork dynamics
- Semi-conservative genetic duplication
- Molecular coordination within the replisome

10. Short Questions

1. Why is an RNA primer required for DNA synthesis?
2. Why does lagging strand synthesis occur in fragments?
3. What is the biochemical function of DNA ligase?
4. Why is DNA replication described as semi-conservative?
5. In which direction does DNA polymerase synthesize DNA?
6. What would occur if helicase failed to function?
7. How does the antiparallel structure of DNA influence replication strategy?

11.References

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