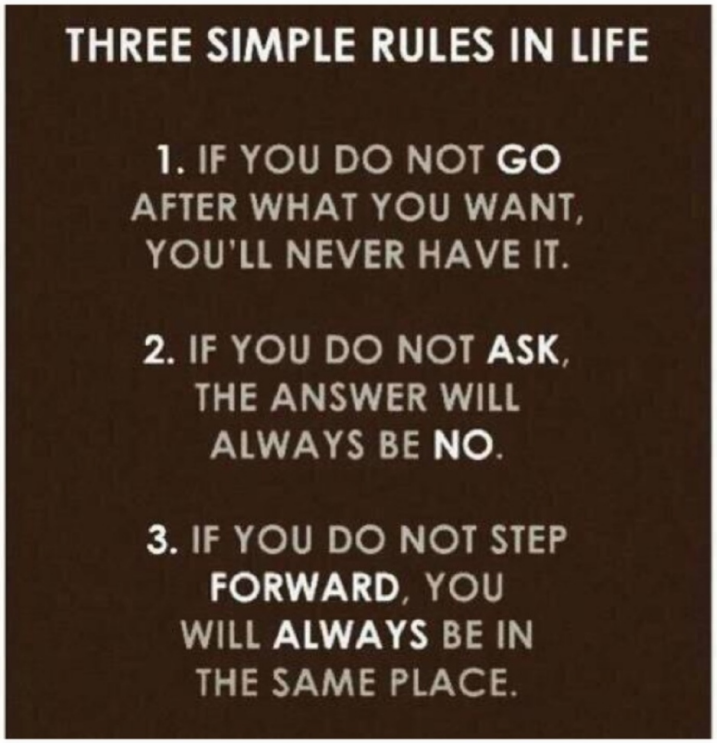
Lecture 14:

DNA replication in prokaryotes

The process of replication

Course 371

Lessons for life



AIMS

- Introduce the DNA replication process and the roles of the enzymes/proteins involved.
- Introduce the terminology given to the DNA replication fork. This includes:
    - The strands names and polarity.
    - The fragments names.

A bit of review

What do we need to replicate DNA?

1. DNA template.
2. DNA building blocks (dNTPs).
3. DNA copier enzyme (DNA Polymerase).
4. Primer (hook so that the copier know and can start working).
5. Mg^{2+} so the DNA copier can work.
6. A number of other helpers (proteins and enzymes).

A bit of review

DNA Polymerase Exonuclease activity

- **Nuclease:** an enzyme that cuts (digest) nucleotide(s).
- **Exo:** at the ends of a DNA molecule.
- **Endo:** in the middle of a DNA molecule.
- **Exonuclease:** ability to remove nucleotide(s) at the end of a molecule.
-

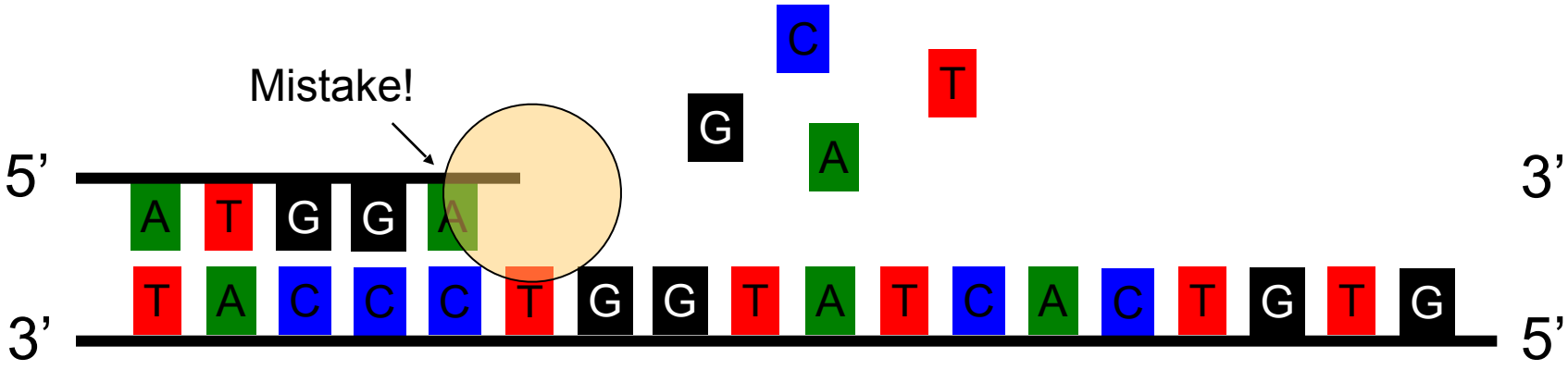
A bit of review

DNA Polymerase Exonuclease activity

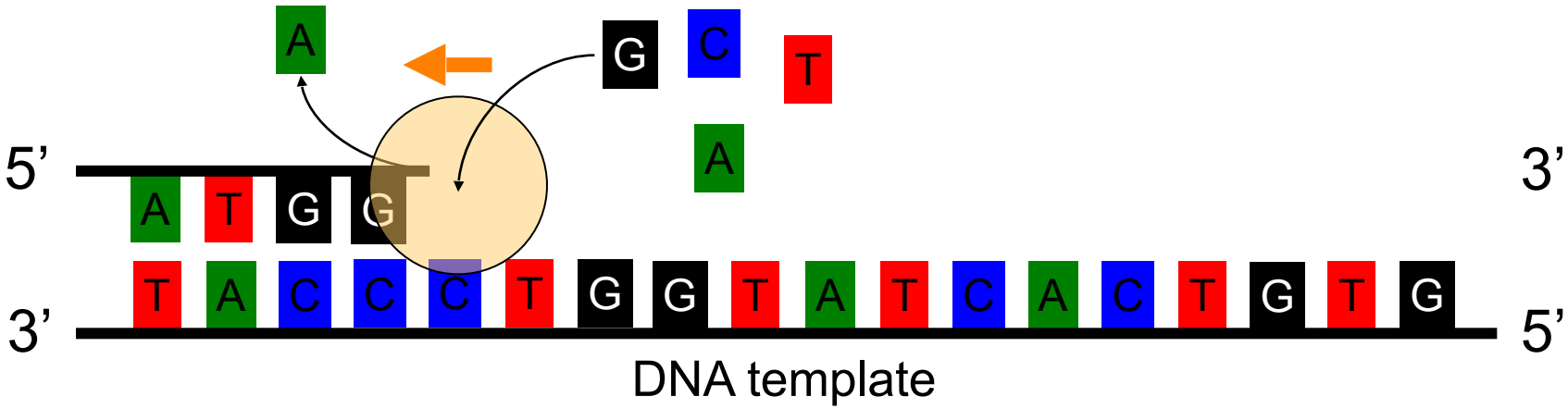
- **DNA Pol I:**
  - 3'→5' exonuclease activity
  - 5'→3' exonuclease activity
- **DNA Pol III:**
  - 3'→5' exonuclease activity

A bit of review

DNA Polymerase 3'→5' Exonuclease activity

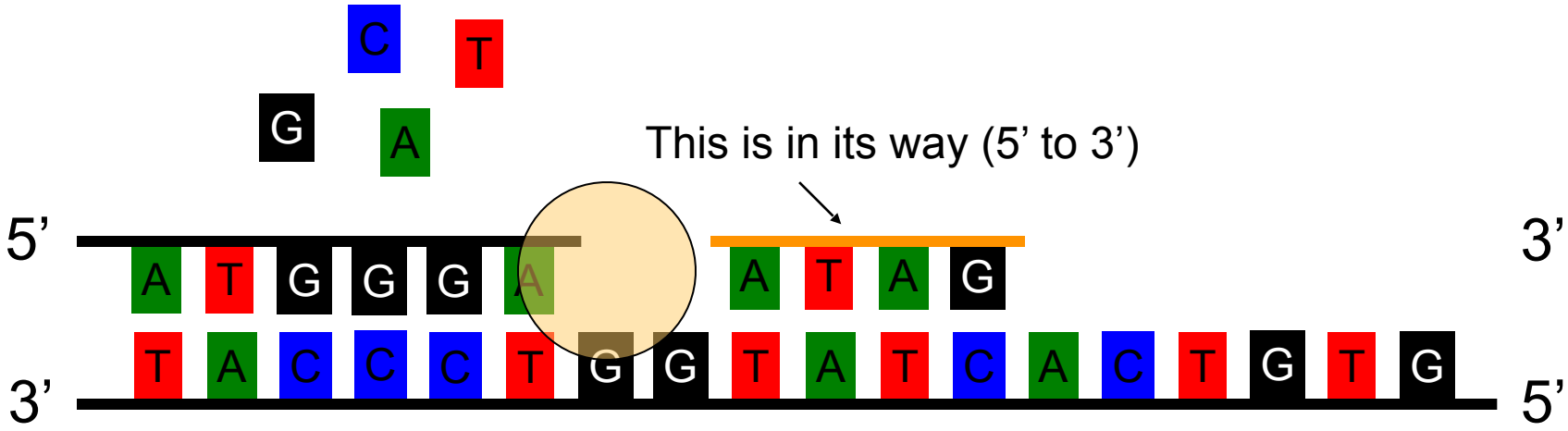


DNA polymerase goes back (**3' to 5'**) and removes wrong nucleotide then adds the correct one (**proofreading**)

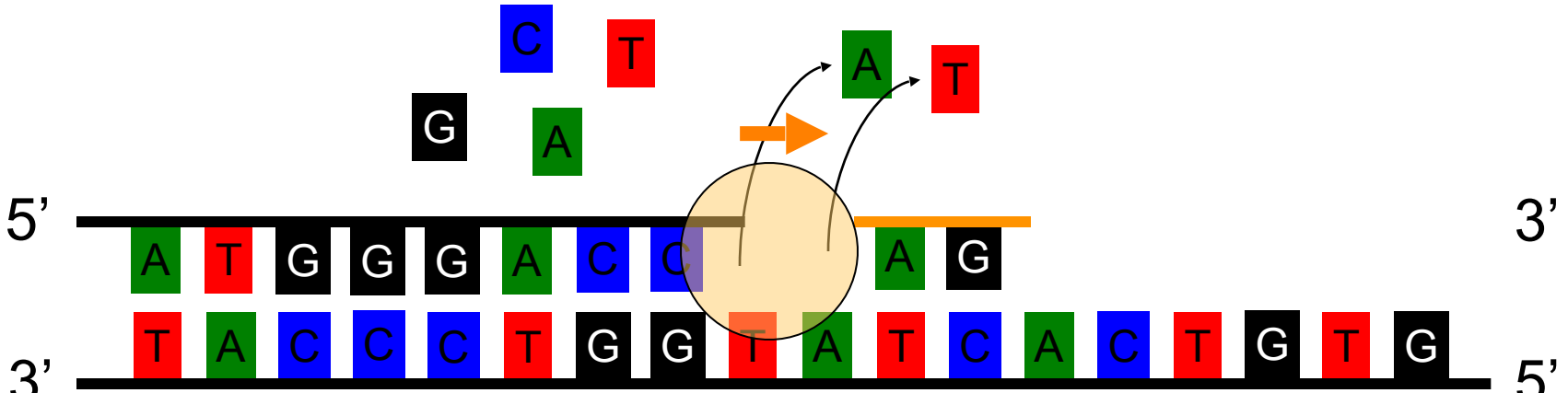


A bit of review

DNA Polymerase I 5'→3' Exonuclease activity

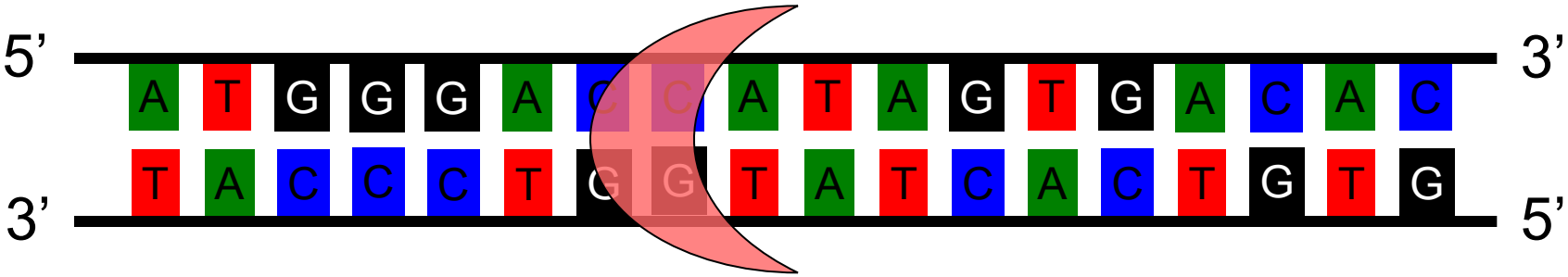


DNA polymerase I removes forward (**5' to 3'**) the nucleotides in its way and adds new ones

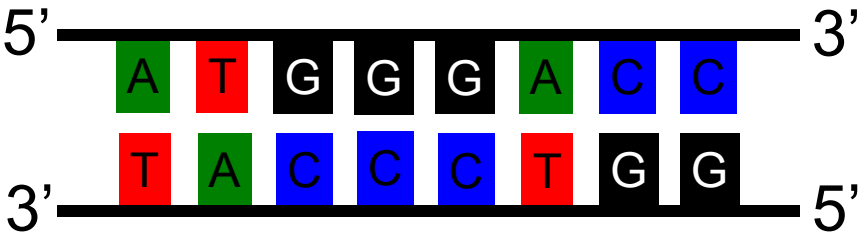


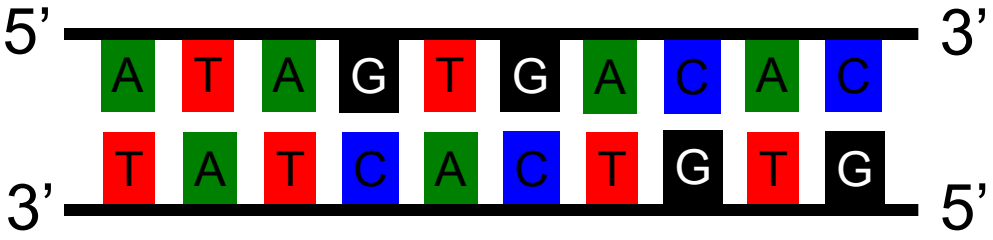
A bit of review

For later in the course (Endonuclease)



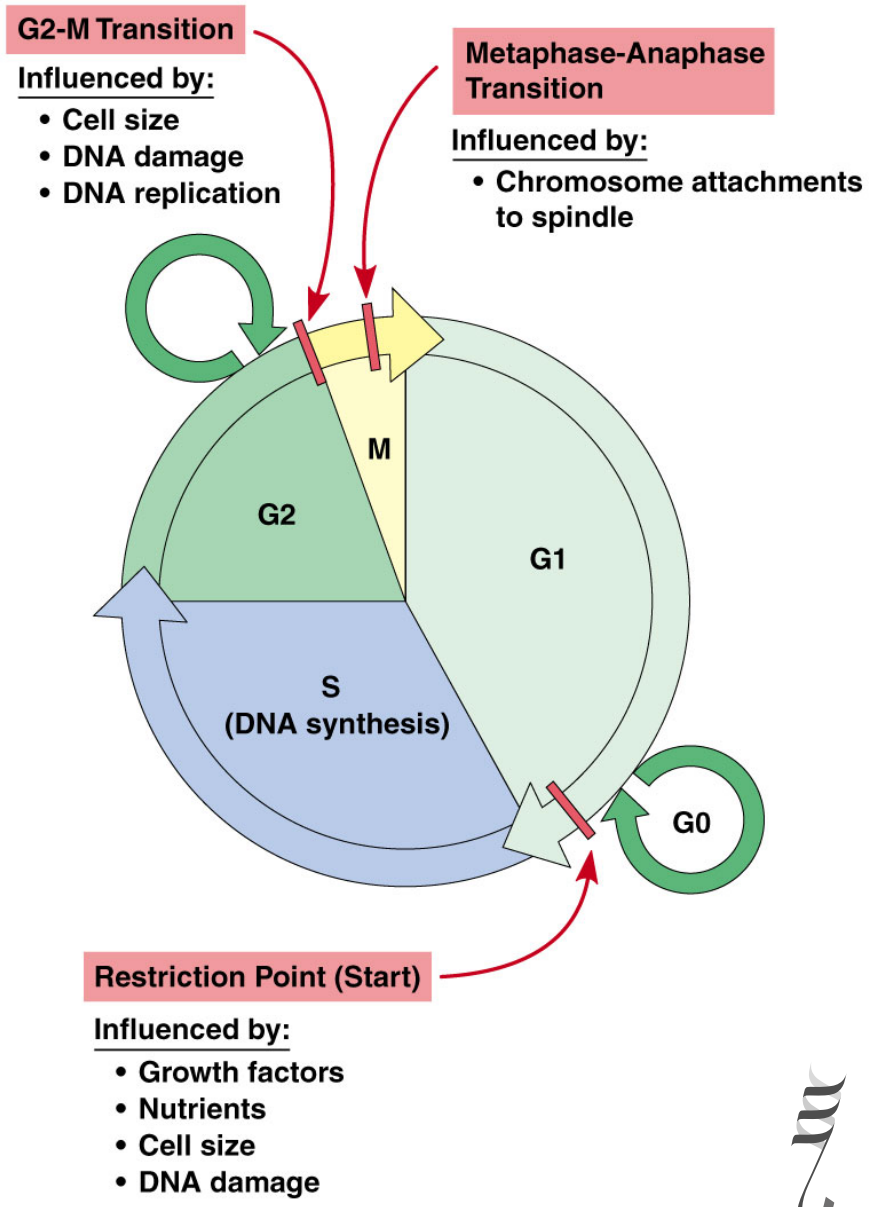
Endonuclease cuts the molecule in the middle





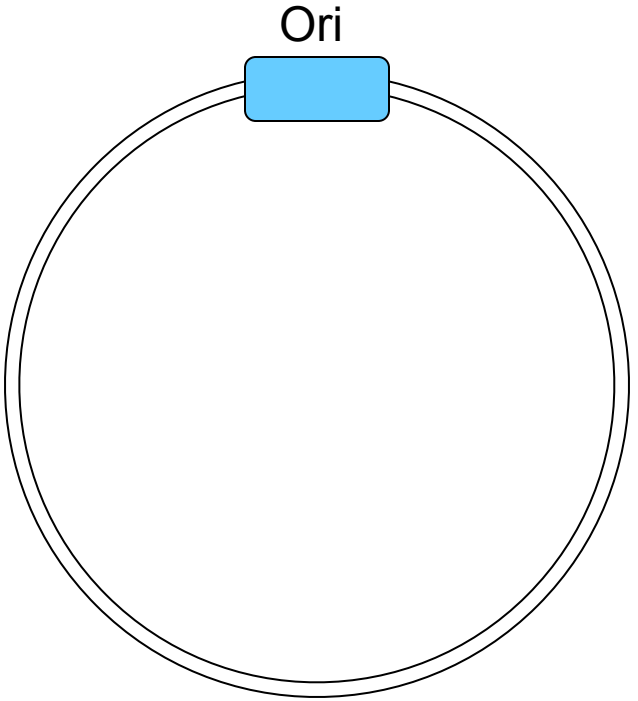
DNA synthesis in the cell cycle

- The cell cycle involves the replication and synthesis of DNA.
- At a specific time in the cell cycle and in the presence of specific conditions, the DNA is replicated.
- The DNA is replicated during the S phase of the cell cycle.



Where replication starts?

- **Initiation of replication start at a specific location:**
  - Replicator.
  - Origin of replication (Ori).

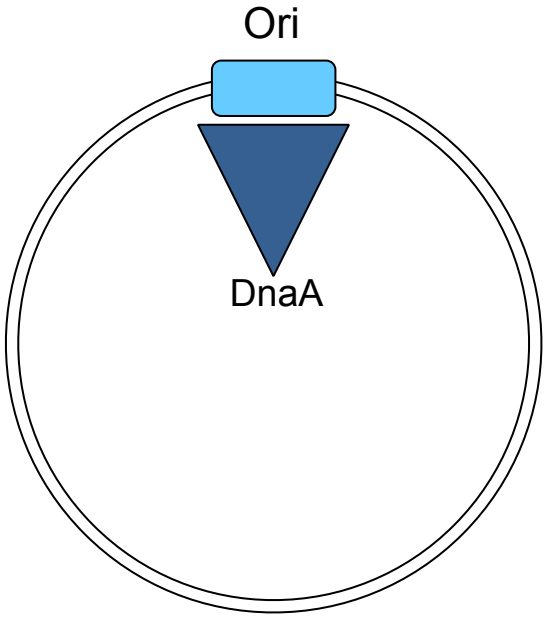


- **Origin of replication has specific characteristics:**
  1. About 245 bp.
  2. A-T rich region.

Remember: A-T hydrogen bonds!

DNA replication process

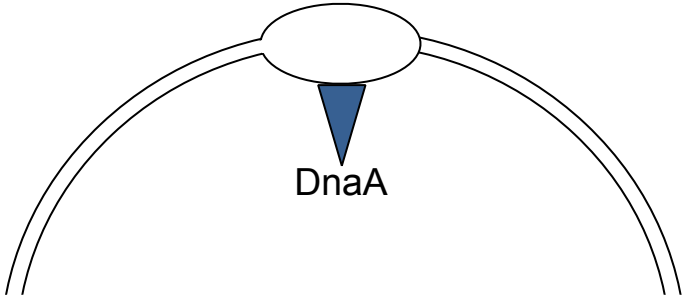
1- **DNA denaturation**



- Initiation protein (**DnaA**) binds to the origin of replication and **denatures** the double strands forming two single strands of DNA (two templates).

DNA replication process

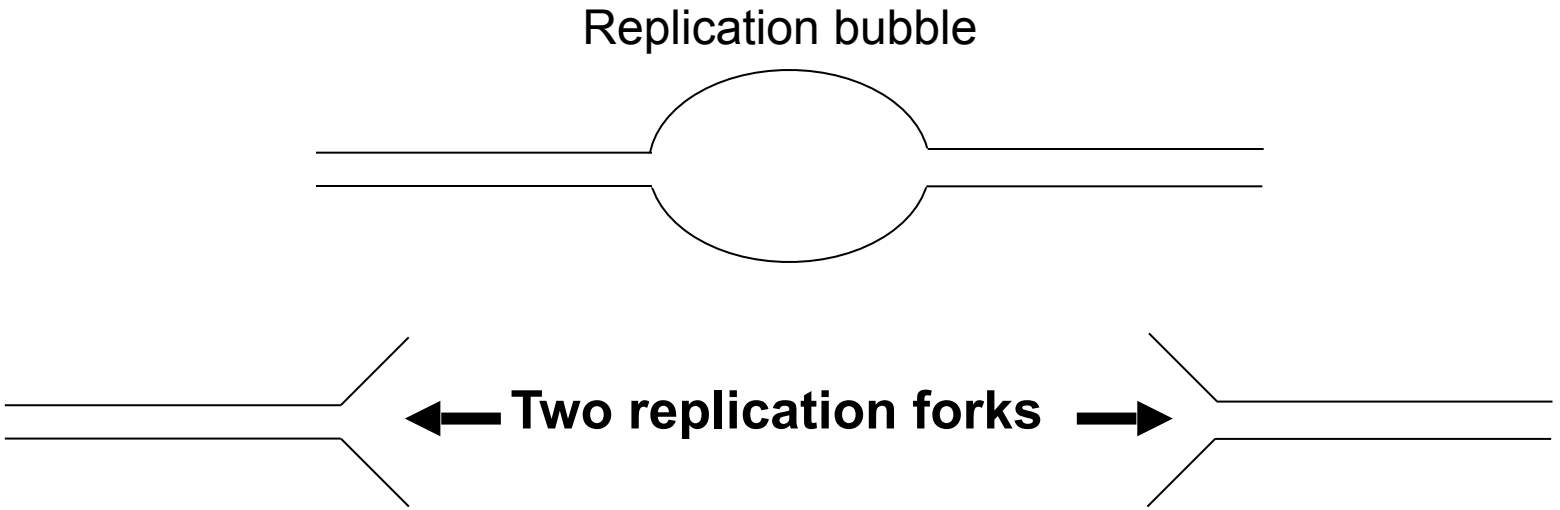
1- **DNA denaturation**



- The region after the separation of the two strands is referred to as (**the replication bubble**).
- The strands represent the two template strands needed for replication.
- Each side of the replication bubble represent a **replication fork**.

DNA replication process

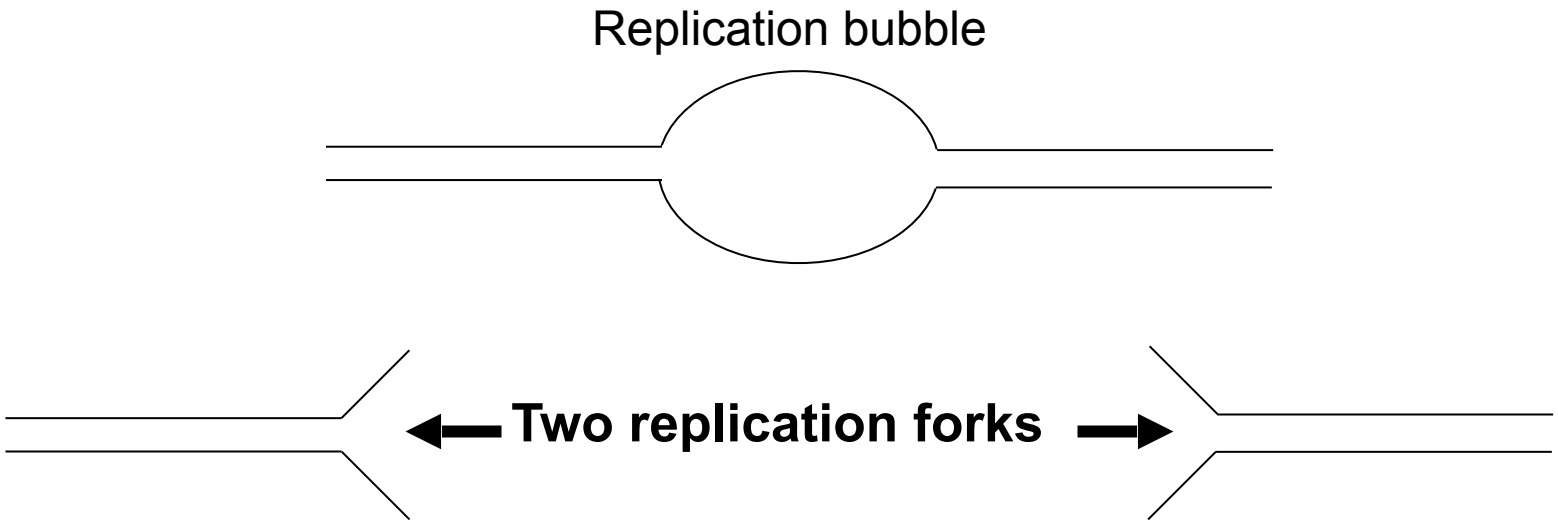
1- DNA denaturation/DNA melting



- The replication bubble creates two replication forks going to opposite directions.
- This is referred to as a **bidirectional replication.**

DNA replication process

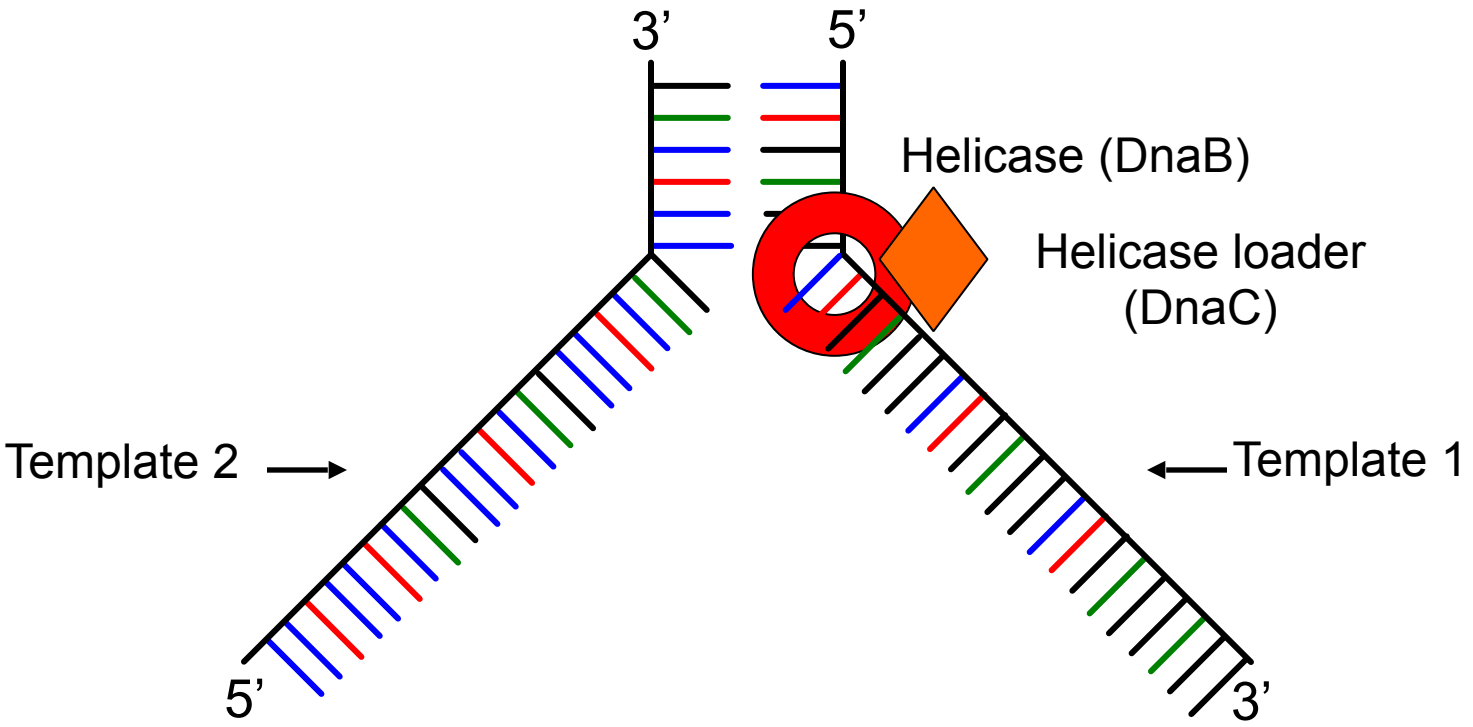
1- DNA denaturation/DNA melting



The two forks are identical in the process of replication and for the sake of ease of understanding, we will consider the process on one replication fork.

DNA replication process

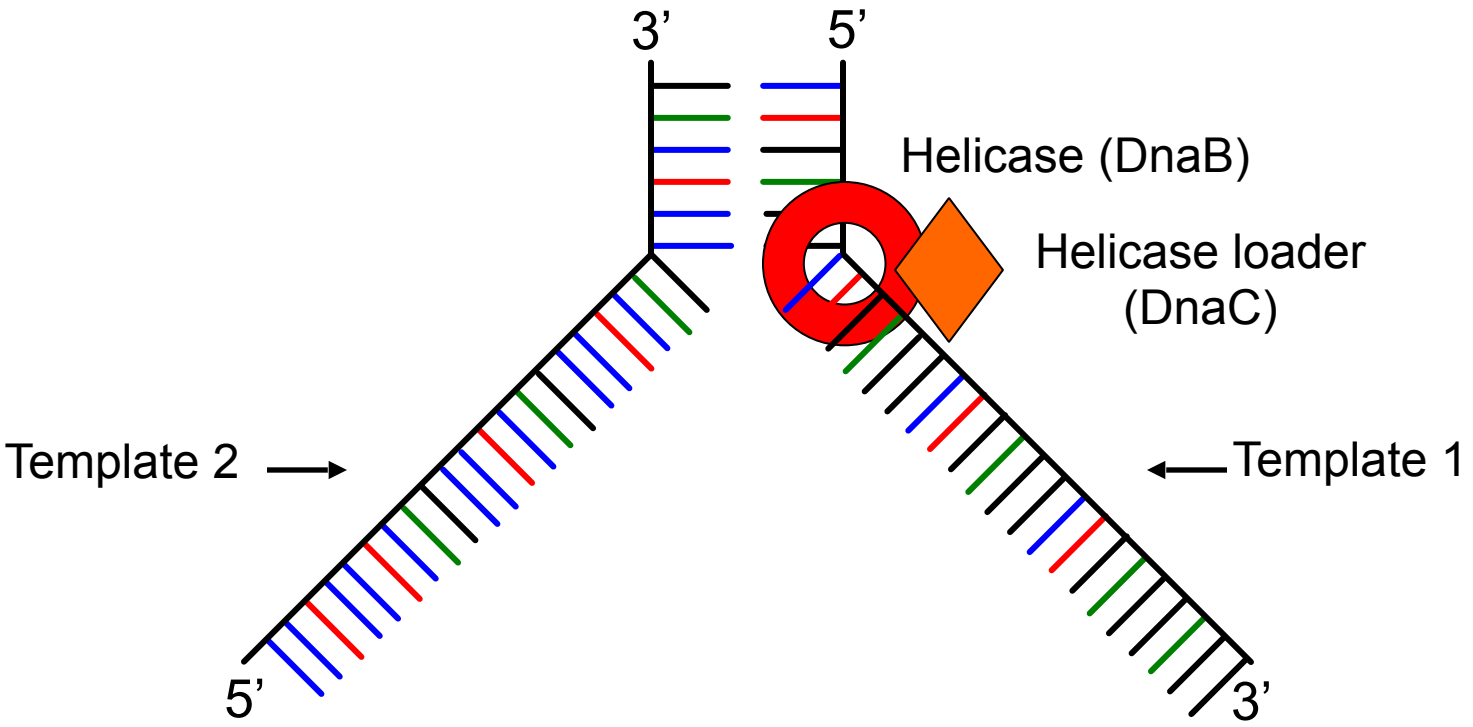
2- **Helicase loading**



DNA helicase (DnaB) is loaded to the fork by the helicase loader (DnaC).

DNA replication process

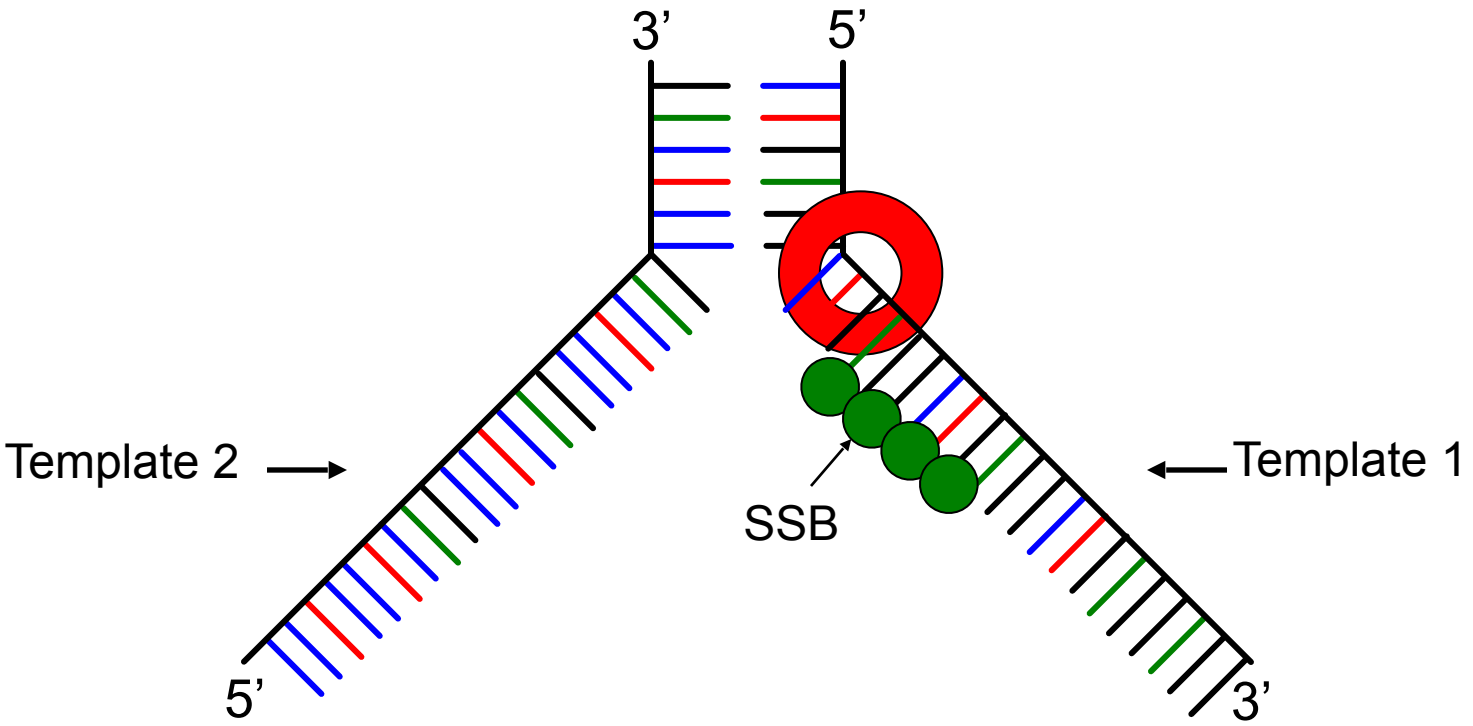
2- **Helicase loading**



DNA helicase breaks hydrogen bonds between bases and untwist the DNA.

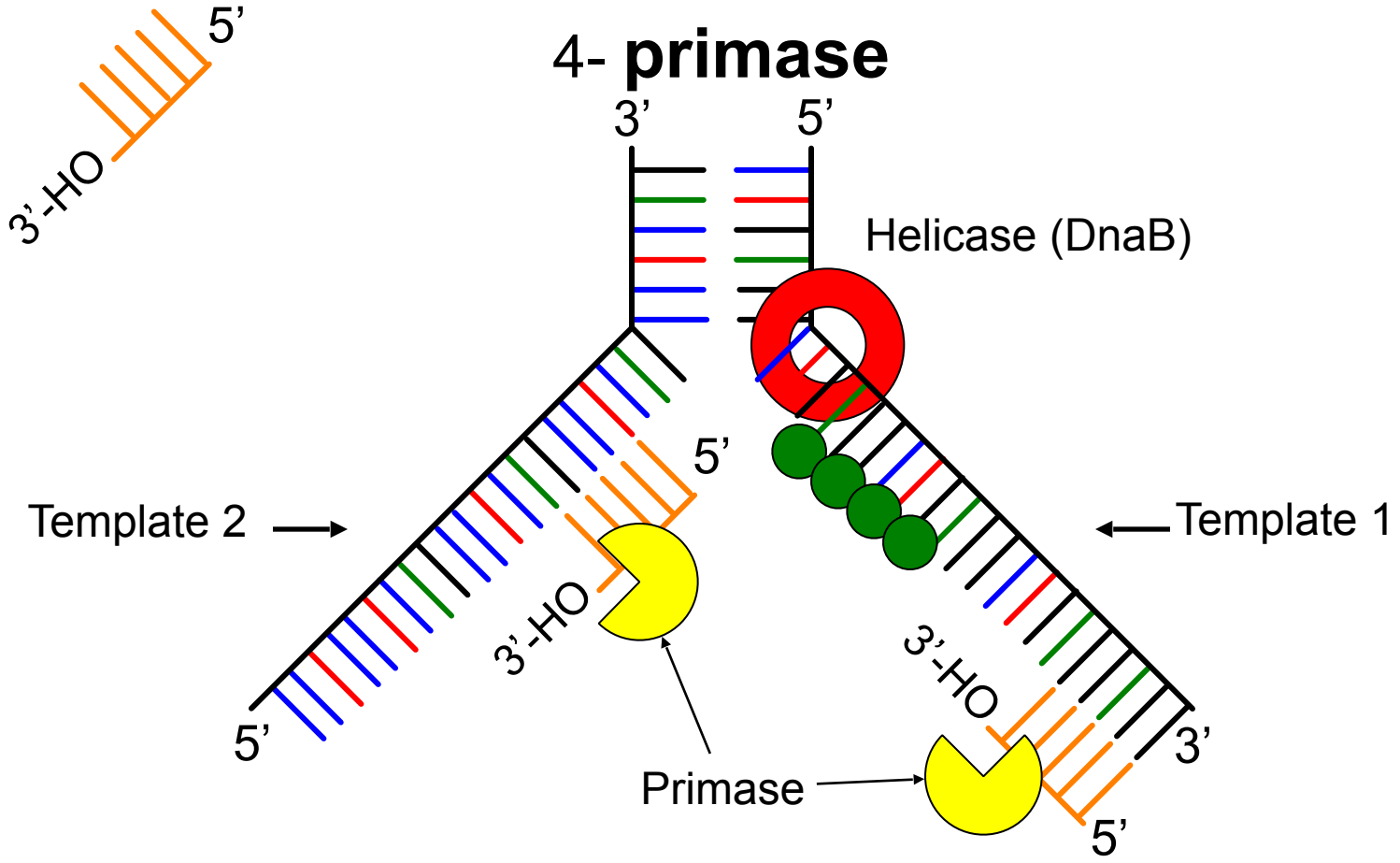
DNA replication process

3- Single strand DNA binding proteins(SSB)



Single strand DNA binding protein binds to ssDNA and prevent the strands from re-annealing (coming back together).

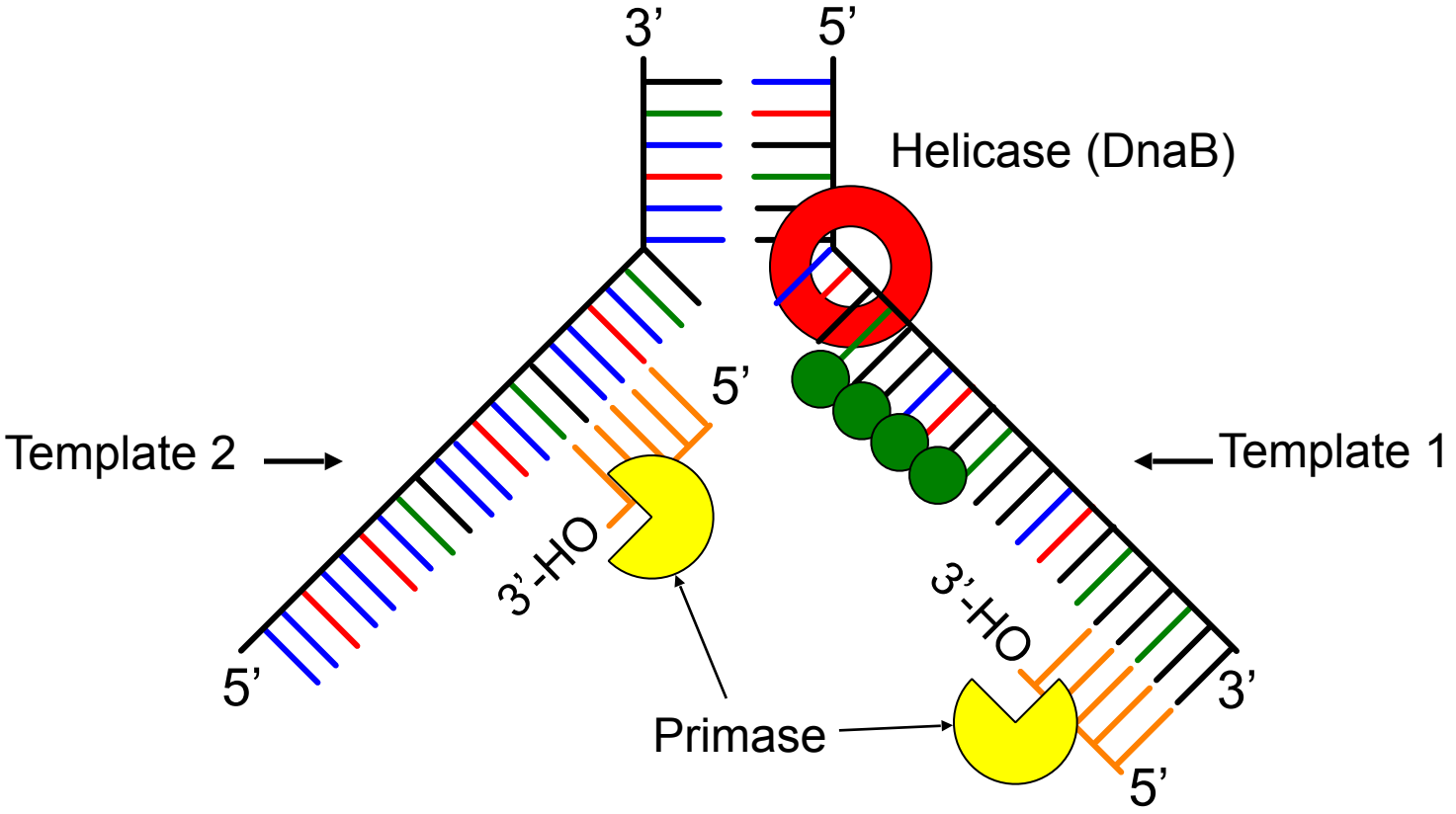
DNA replication process



Primase adds (an RNA primer) at the 5’ providing a 3’-OH for new strand synthesis on both templates.

DNA replication process

4- DNA primase



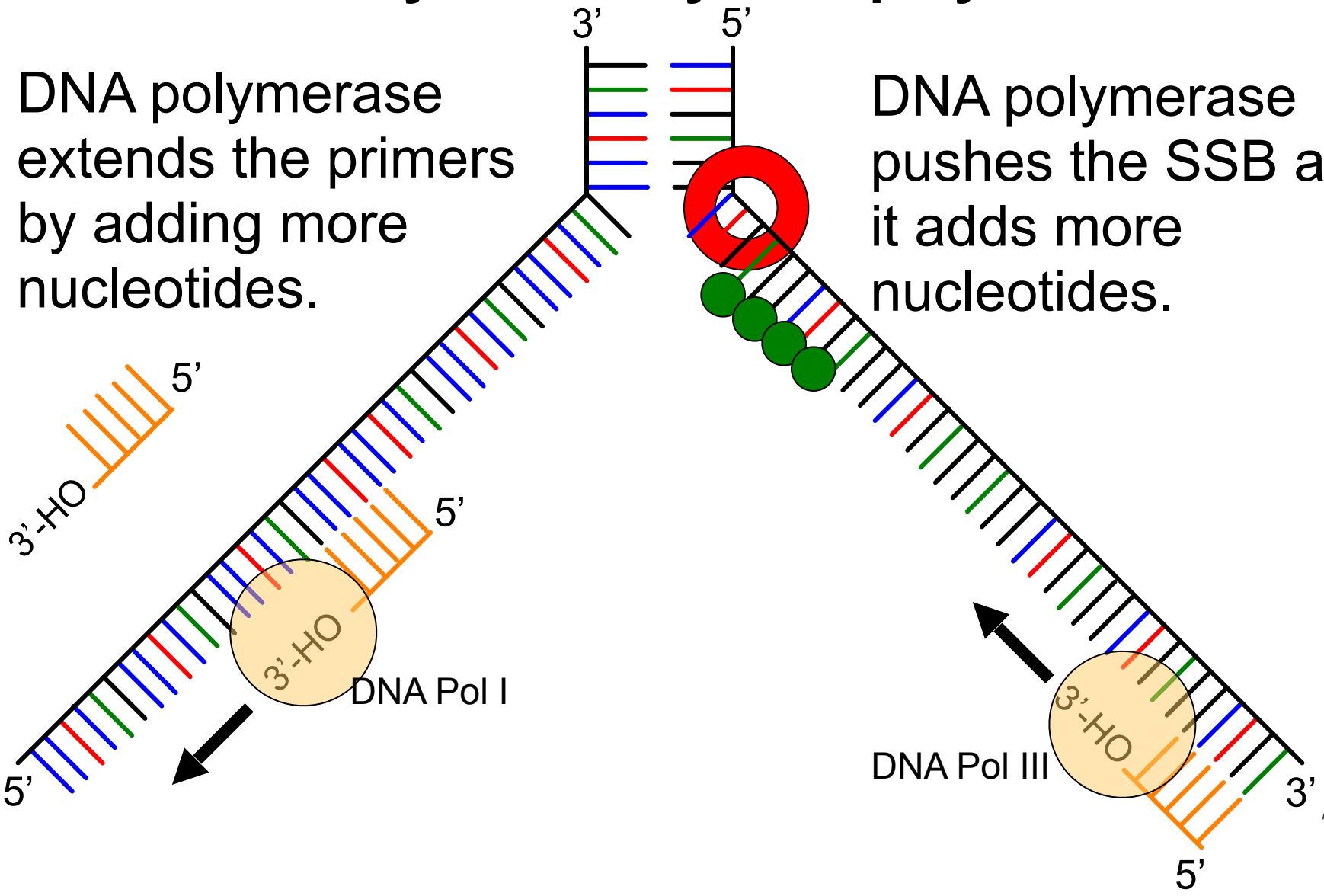
Primase add a single primer on the leading strand and multiple primers on the lagging strand.

DNA replication process

5- DNA synthesis by DNA polymerase

DNA polymerase extends the primers by adding more nucleotides.

DNA polymerase pushes the SSB as it adds more nucleotides.



DNA replication process

5- **DNA synthesis by DNA polymerase**

Remember!

DNA has two strands (two templates) that have opposite polarity

Template 1: 5’ ➔ 3’
Template 2: 3’ ➔ 5’

DNA polymerase synthesis DNA only 5’➔3’

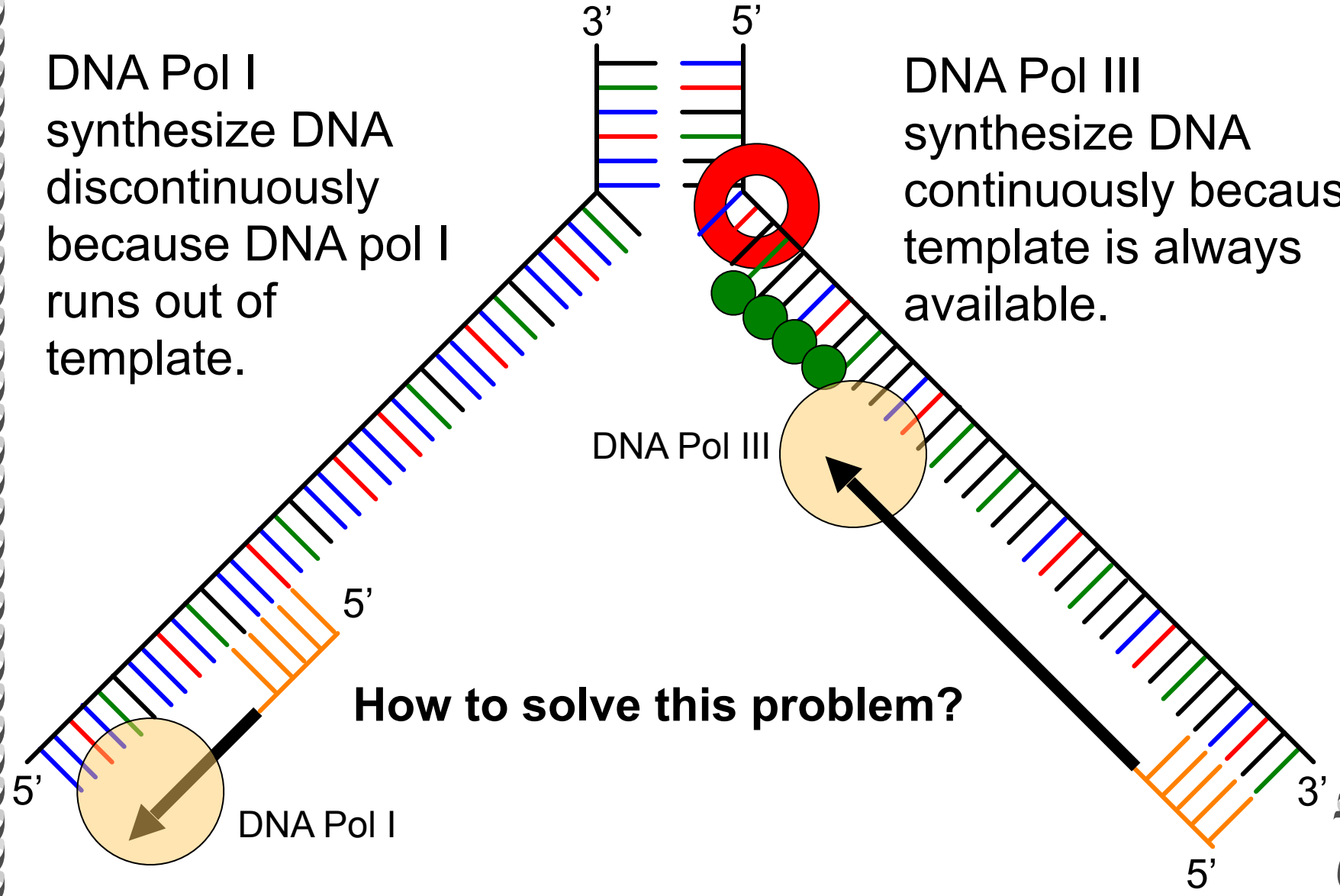
This makes DNA synthesis continuous on one strand and one discontinuous the other strand

DNA replication process

5- DNA synthesis by DNA polymerase

DNA Pol I synthesize DNA discontinuously because DNA pol I runs out of template.

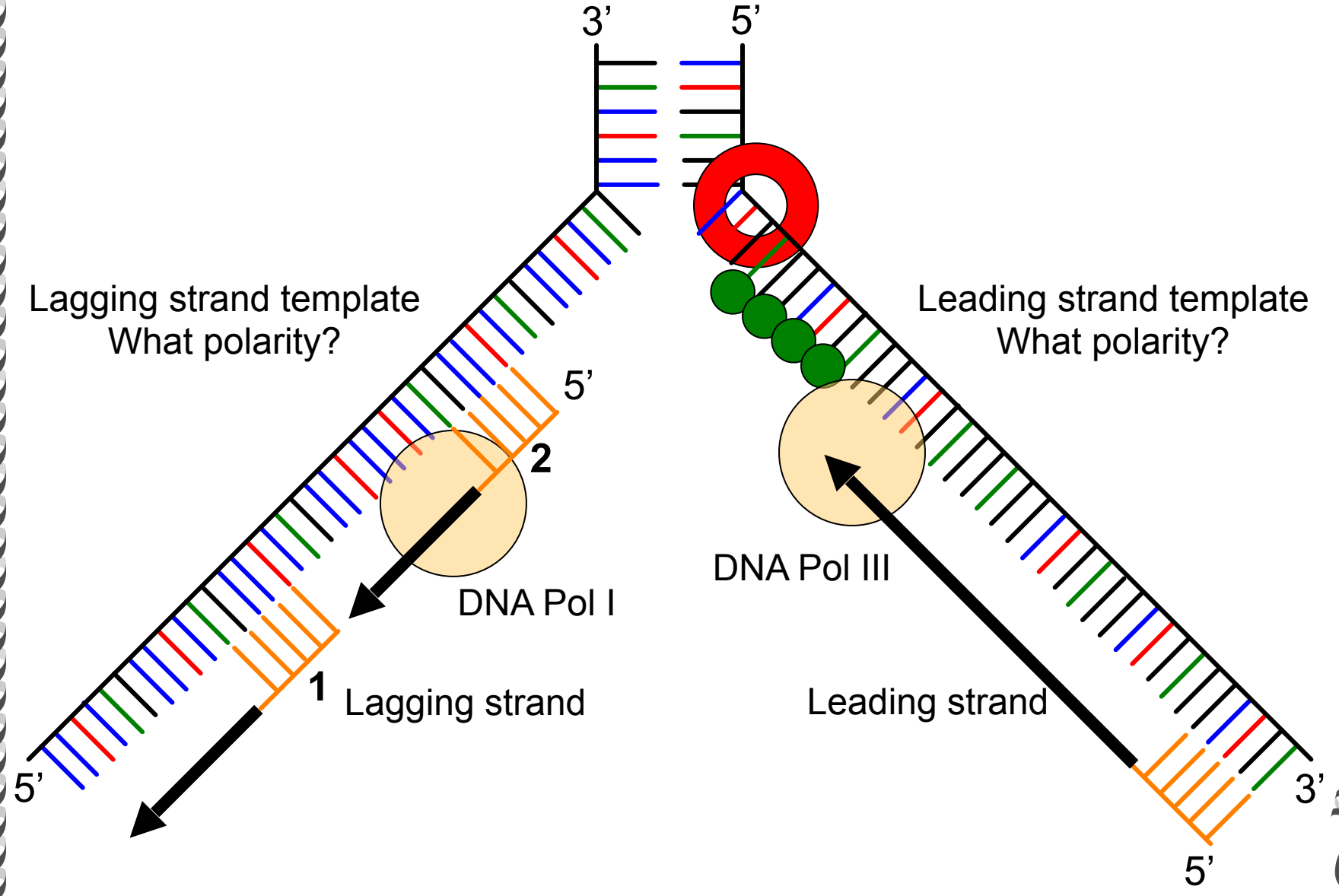
DNA Pol III synthesize DNA continuously because template is always available.



How to solve this problem?

DNA replication process

5- DNA synthesis by DNA polymerase



DNA replication process

5- **DNA synthesis by DNA polymerase**

More primers synthesized as the replication fork moves forward and more template is provided for DNA Pol I

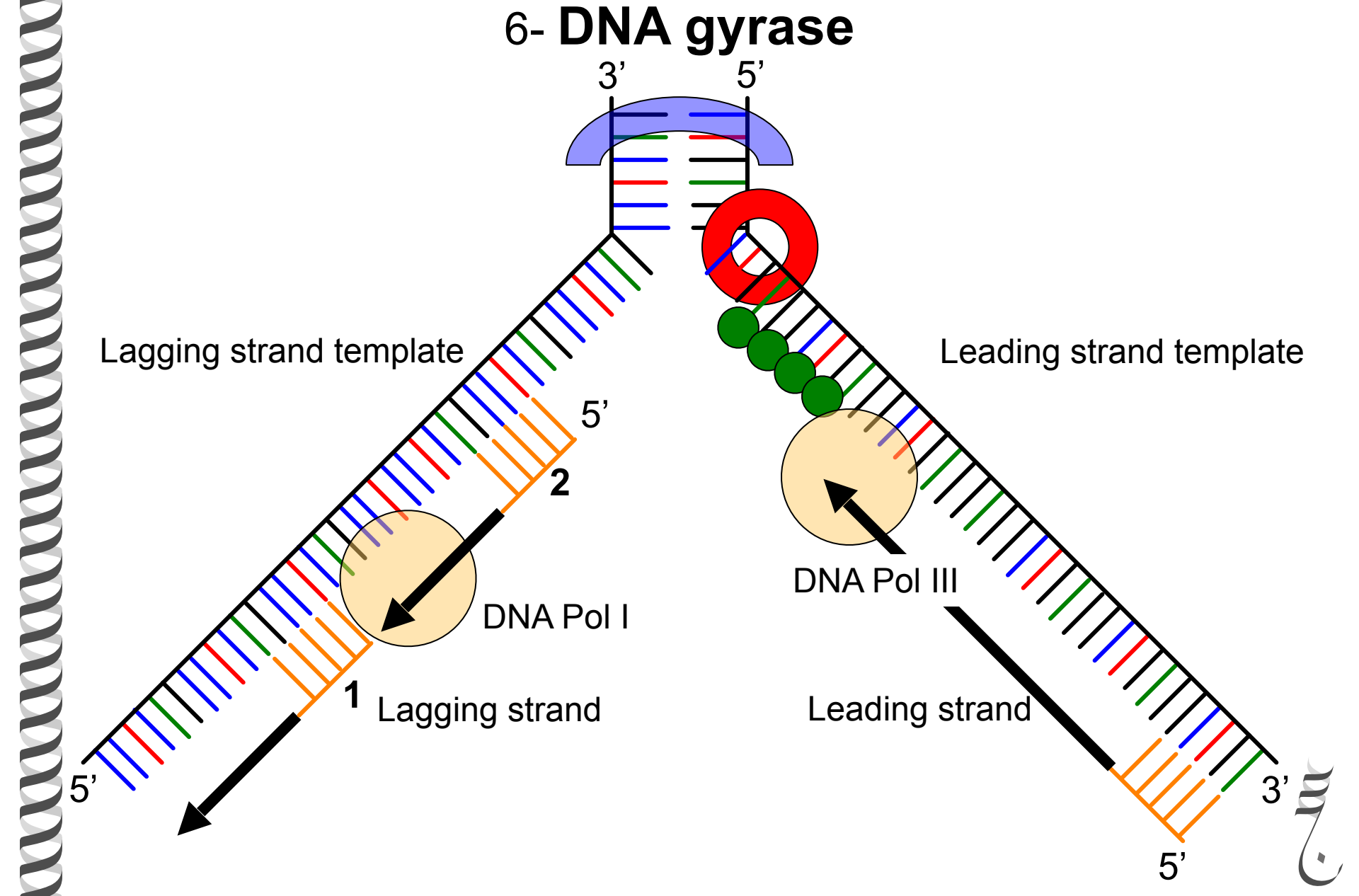
DNA replication process

5- DNA synthesis by DNA polymerase

So DNA is replicated in

<u>Semi-dis-continuous</u> way

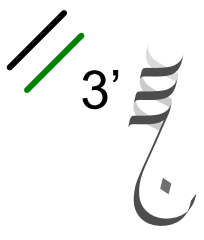
DNA replication process



DNA replication process

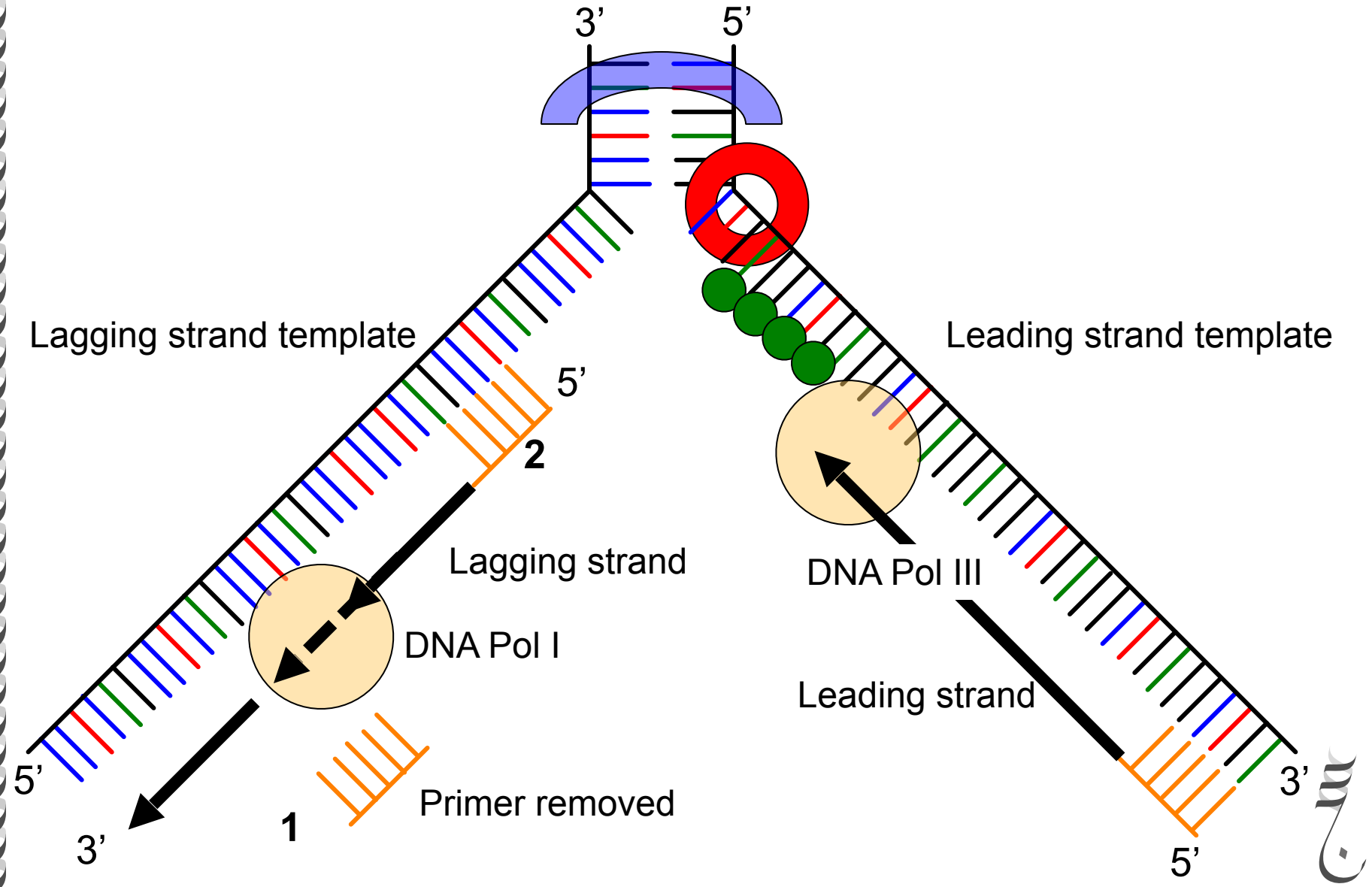
6- **DNA gyrase**

As the replication fork, the tension generated by the untwisting DNA is relaxed by DNA gyrase



DNA replication process

7- Removing primers



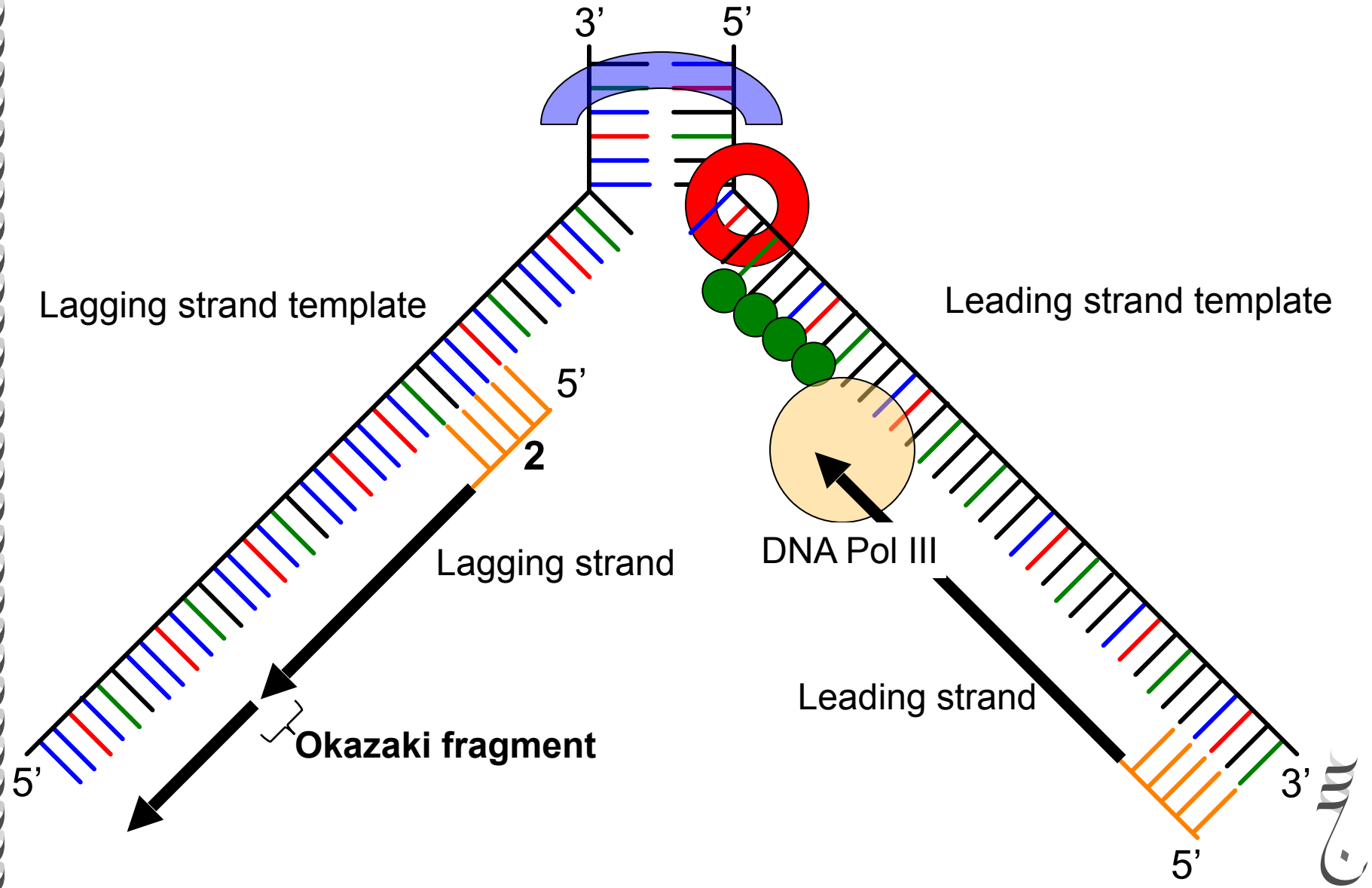
DNA replication process

7- **Removing primers**

DNA pol I on the lagging strand removers the RNA primer using its 5’-3’ exonuclease activity and replaces its location with nucleotides.

DNA replication process

8- **Okazaki fragments**



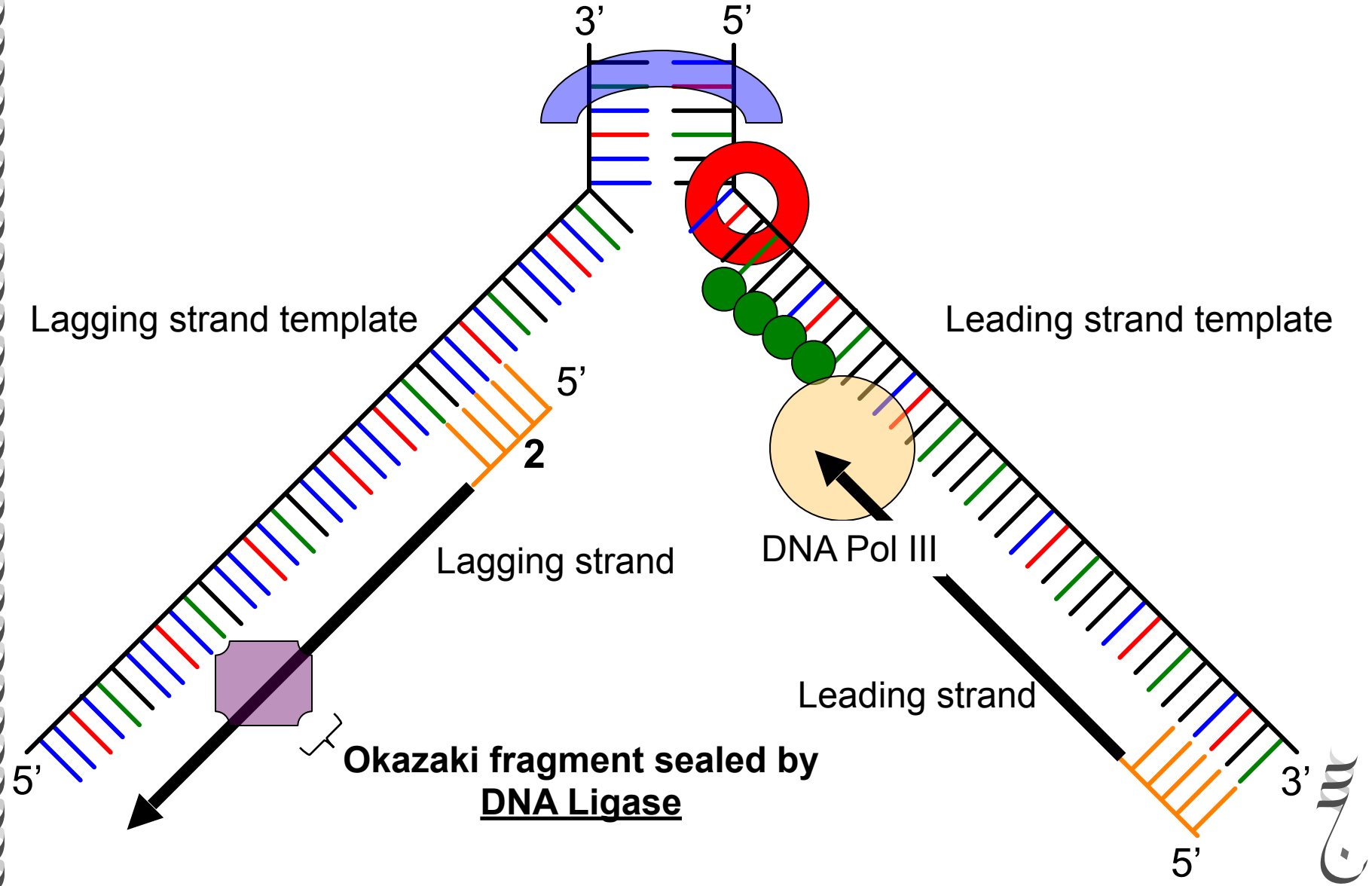
DNA replication process

8- **Okazaki fragments**

- The removal of the primer by DNA pol I generates a nick in the lagging strand where a phosphodiester bond is missing.
- Think a about it as a small cut in the strand.

DNA replication process

8- Okazaki fragments sealed by DNA ligase



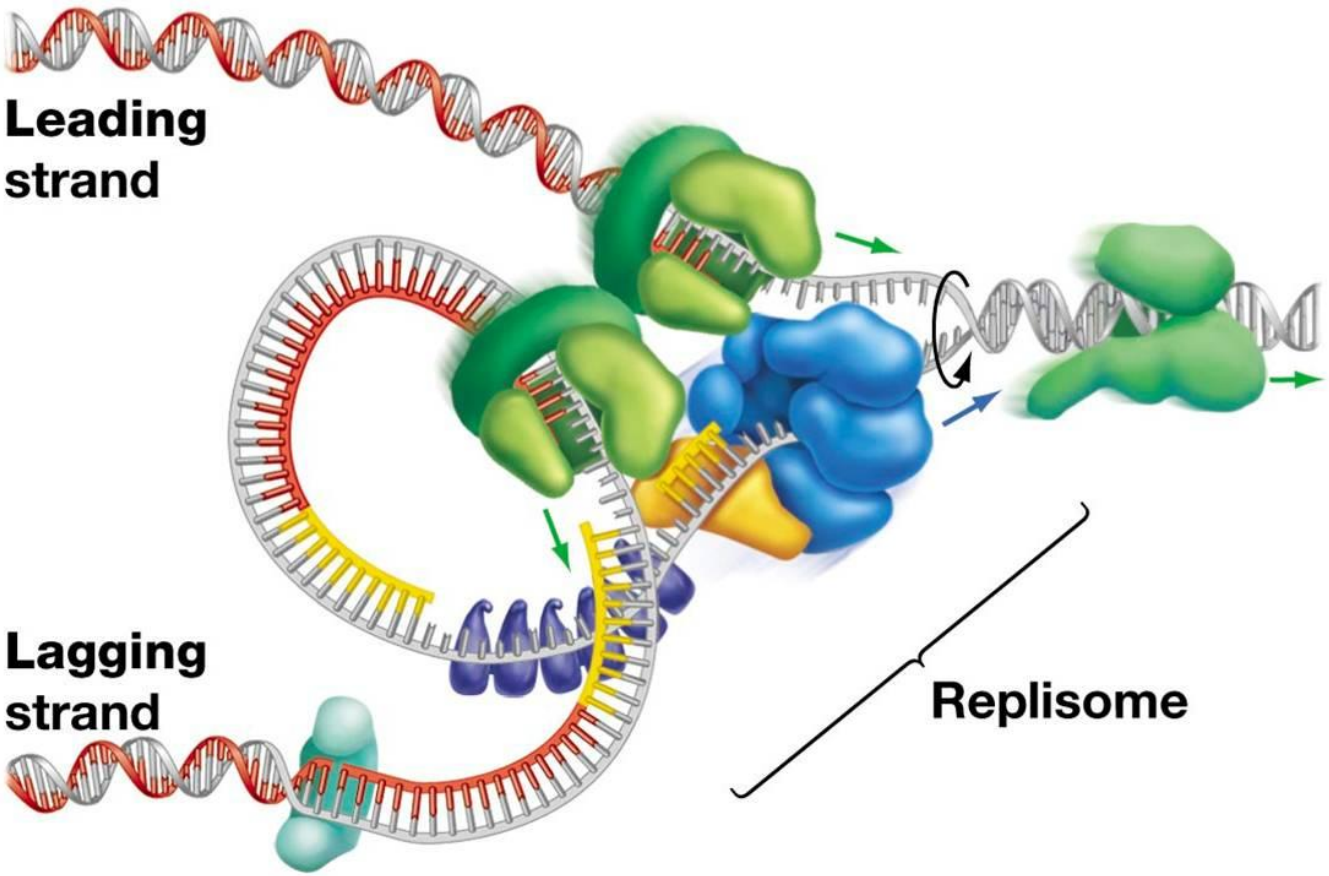
DNA replication process

8- **Okazaki fragments sealed by DNA ligase**

DNA ligase seals Okazaki fragments on the lagging strand.

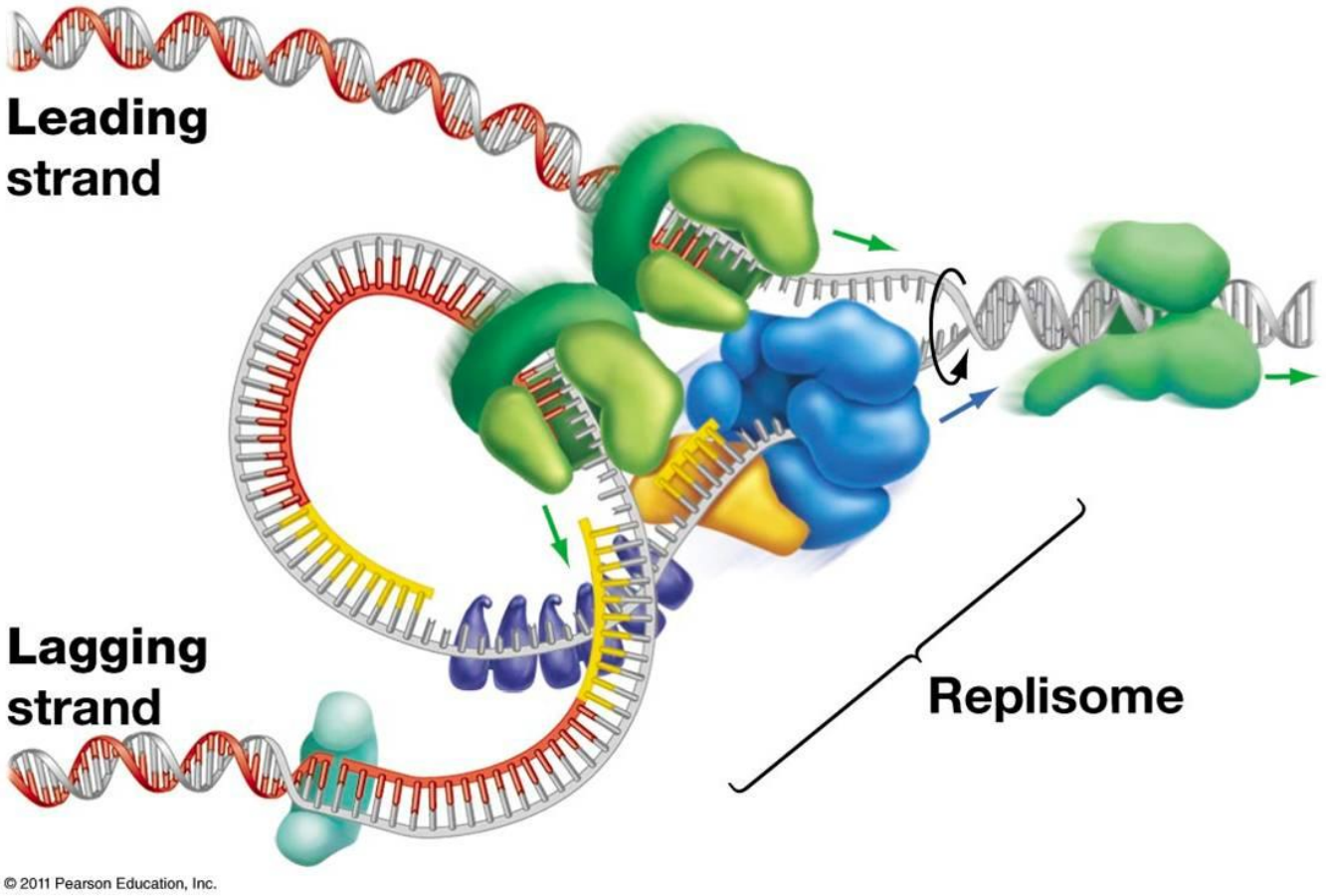
The replisome machine

For simplicity, we studied every enzyme and the reactions associated to be separate. In reality all units work as a one machine.



The replisome machine

All enzymes come together to form a machine called the **replisome.**



Quiz

The replication of the two strands is done in a …… way

a) Continuous

b) discontinuous

c) semi-continuous

d) semi-dis-continuous

e) None of the above

To study

Lagging strand template

DnaB

primer

DNA Ligase

Reannealing

S phase

Endonuclease

DNA primase

Helicase

Ori

Replication bubble

SSB

Leading strand

DnaC

DNA ligase

Cell cycle

Lagging strand

Okazaki fragments

Bidirectional replication

DnaA

Lagging strand template

Exonuclease

Gyrase

Replicator

DNA polymerase I

Replication fork

Denature

Origin of replication

DNA polymerase III

DNA melting

Replisome

Semi-dis-continuous replication

Expectations

- You know the process of DNA replication in prokaryotes.
- You know the names of the compartments of the replication fork.
- You know the sequence of events and where each enzyme/protein function.
- You know that all work together and breaking the events into multiple ones is only to make it easier to understand.

For a smile