



Lecture 9:

DNA replication in prokaryotes

The process of replication

Course 281

Lessons for life

THREE SIMPLE RULES IN LIFE

1. IF YOU DO NOT GO
AFTER WHAT YOU WANT,
YOU'LL NEVER HAVE IT.
2. IF YOU DO NOT ASK,
THE ANSWER WILL
ALWAYS BE NO.
3. IF YOU DO NOT STEP
FORWARD, YOU
WILL ALWAYS BE IN
THE SAME PLACE.

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. DNA 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.
-

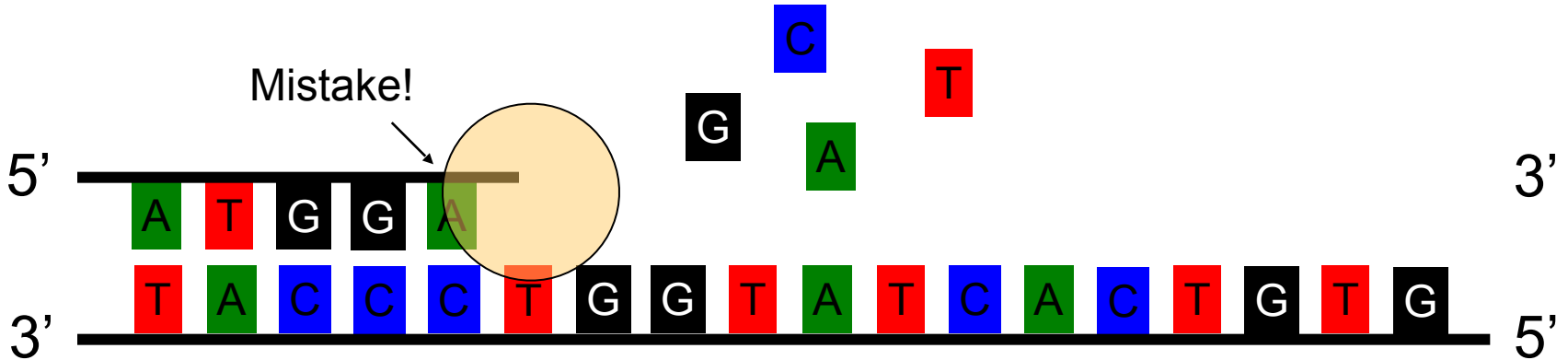
A bit of review

DNA Polymerase Exonuclease activity

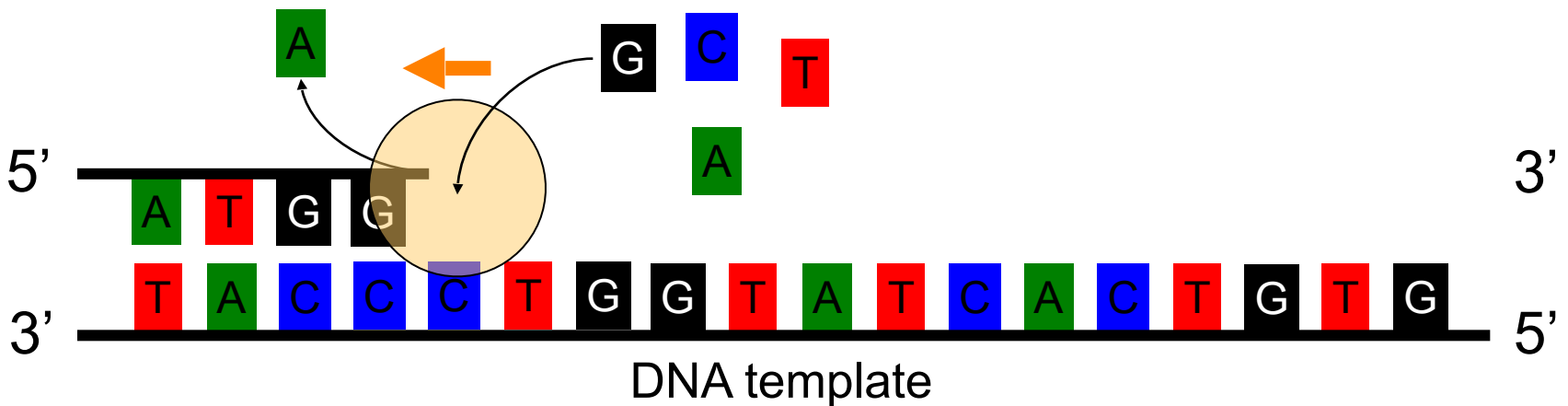
- **DNA Pol I:**
 - 3'→5' exonuclease activity
 - 5'→3' exonuclease activity
- **DNA Pol III:**
 - 3'→5' exonuclease activity

A bit of review

DNA Polymerase 3'→5' Exonuclease activity

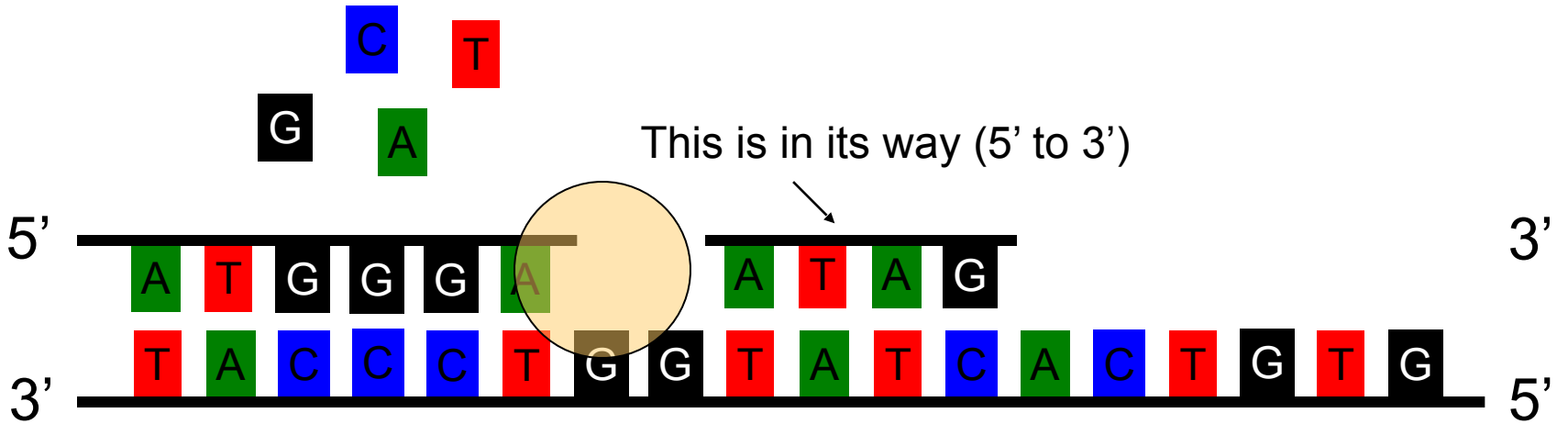


DNA polymerase goes back (3' to 5') and removes wrong nucleotide then adds the correct one (**proofreading**)

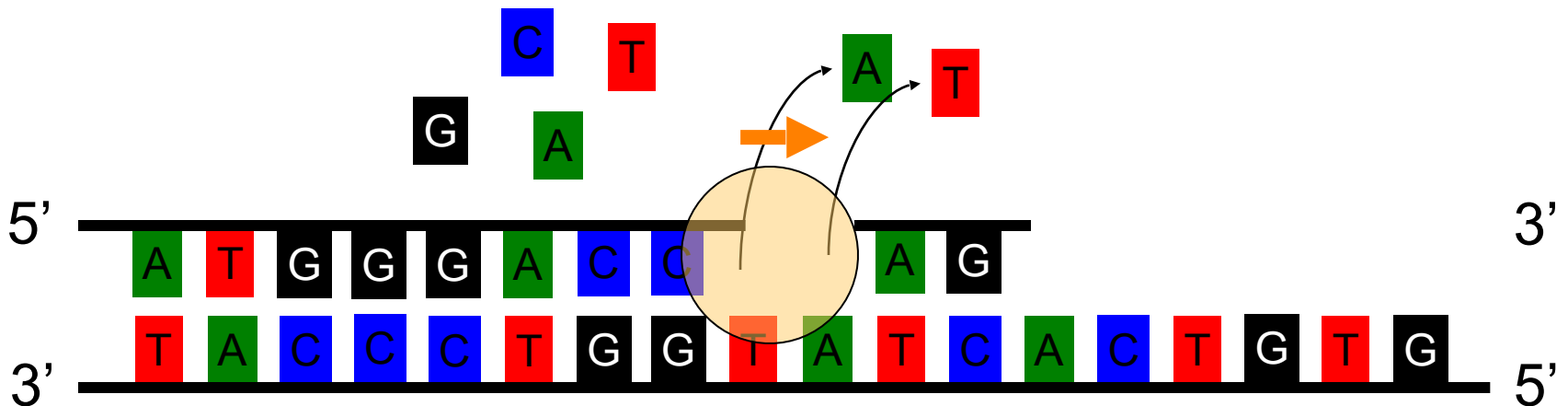


A bit of review

DNA Polymerase I 5'→3' Exonuclease activity

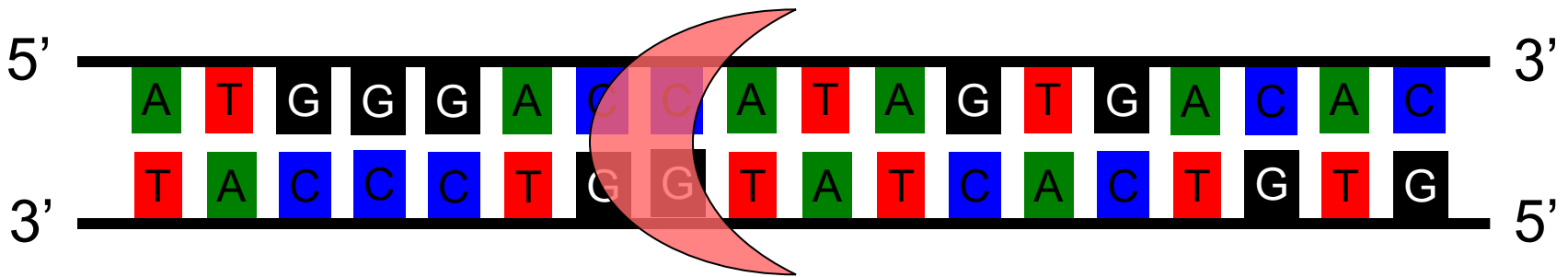


DNA polymerase I removes forward (5' to 3') the nucleotides in its way and adds new ones

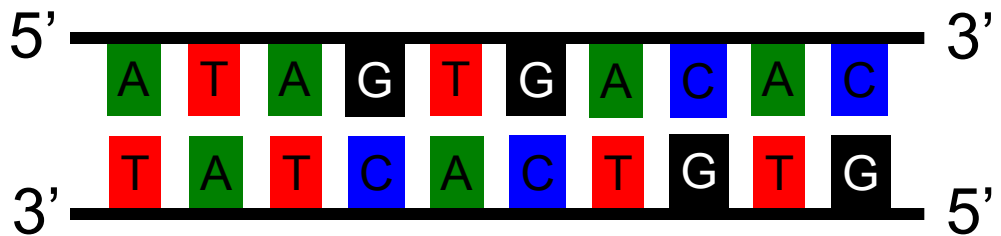
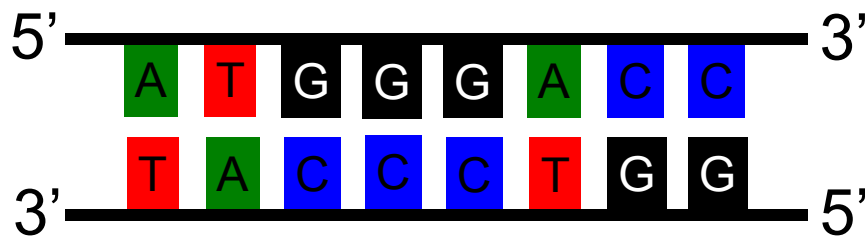


A bit of review

For later in the course (Endonuclease)

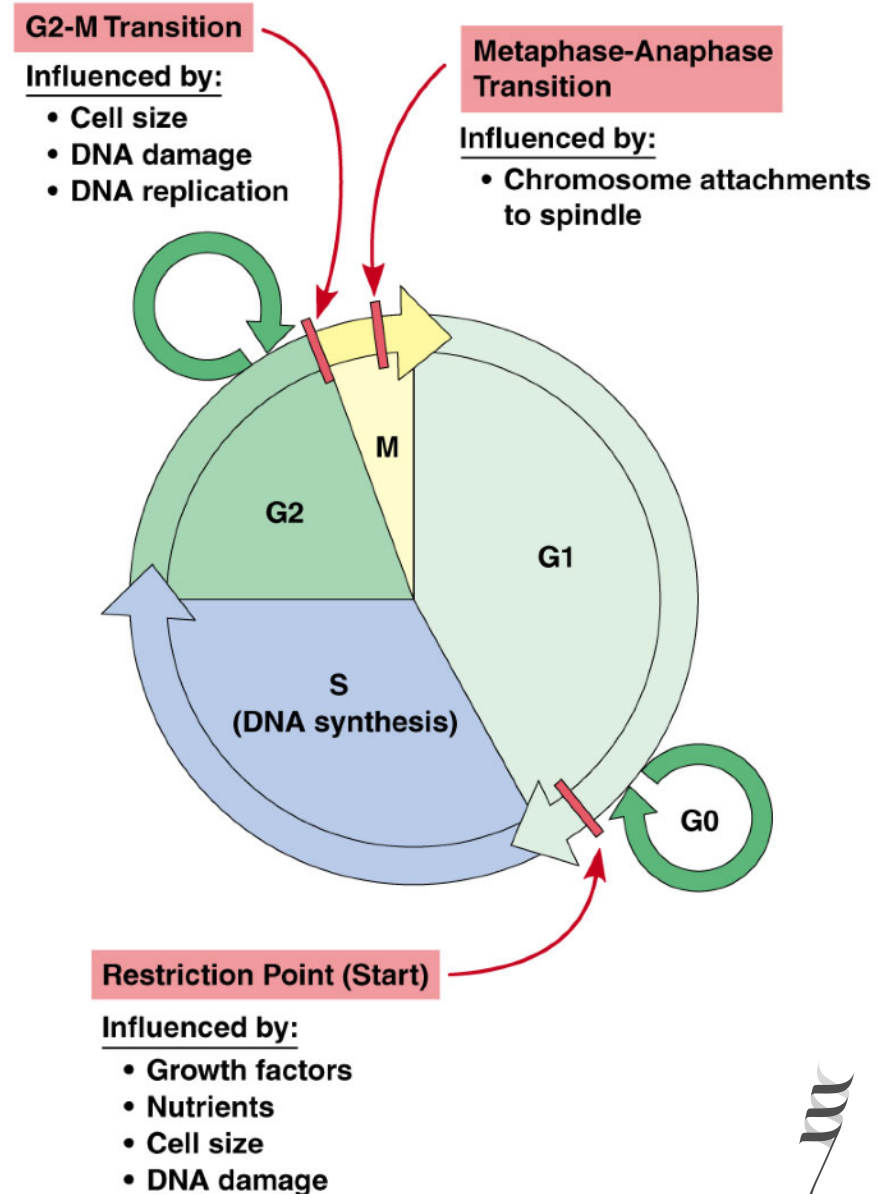


Endonuclease cuts the molecule in the middle



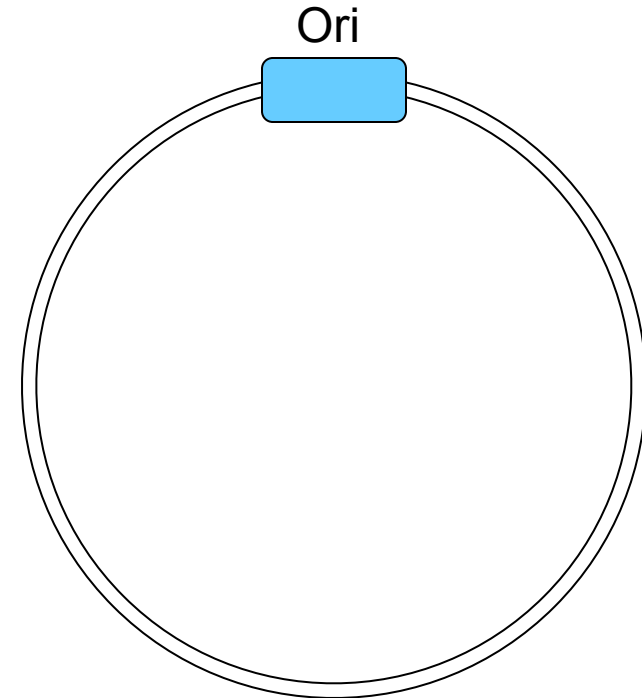
DNA synthesis in the cell cycle

- 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?

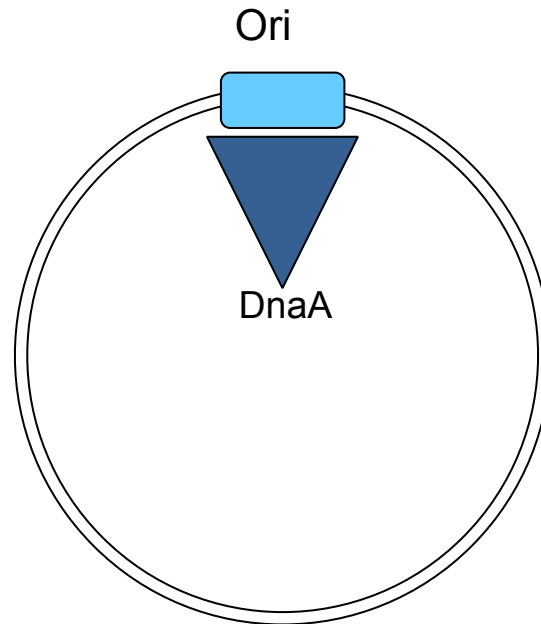
- **Initiation of replication start at a specific location:**
 - Replicator.
 - Origin of replication (Ori).
- **Origin of replication has specific characteristics:**
 1. About 245 bp.
 2. A-T rich region.



Remember: A-T hydrogen bonds!

DNA replication process

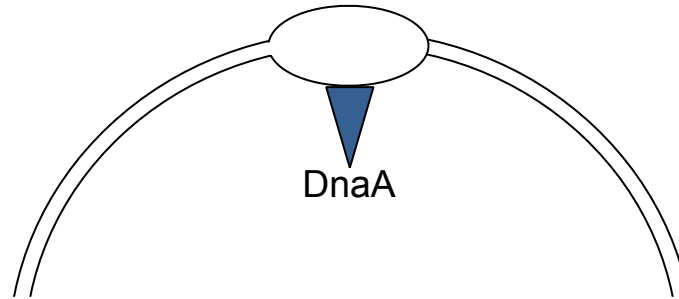
1- DNA denaturation



- 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

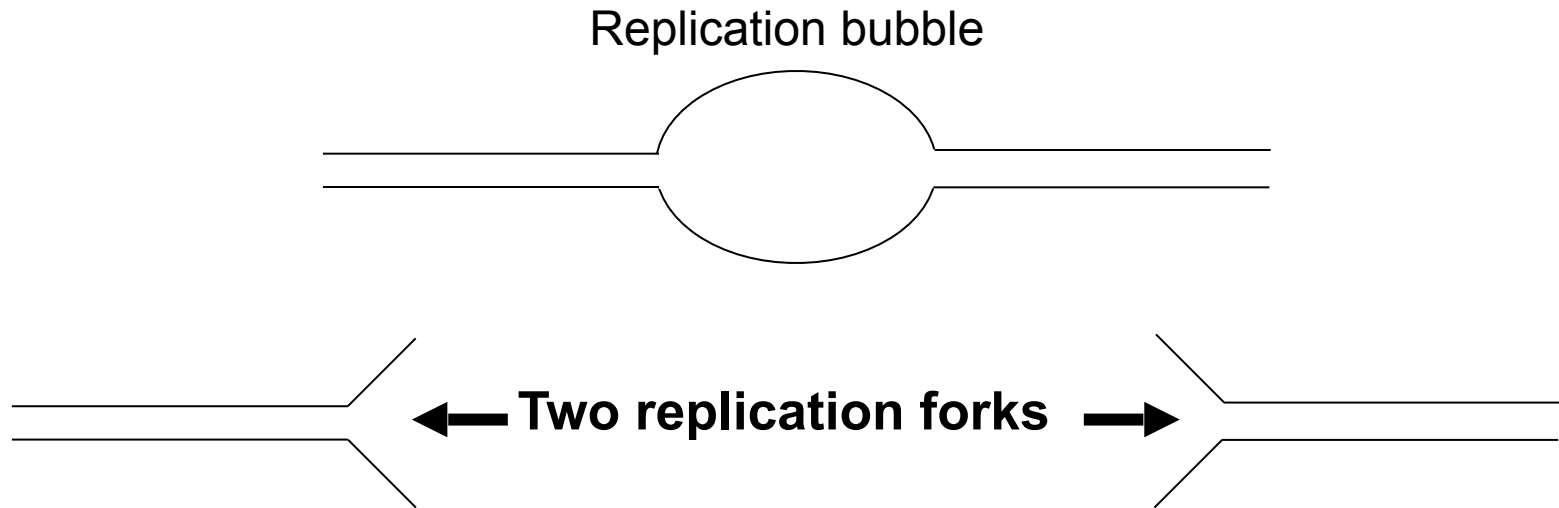
1- DNA denaturation



- 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

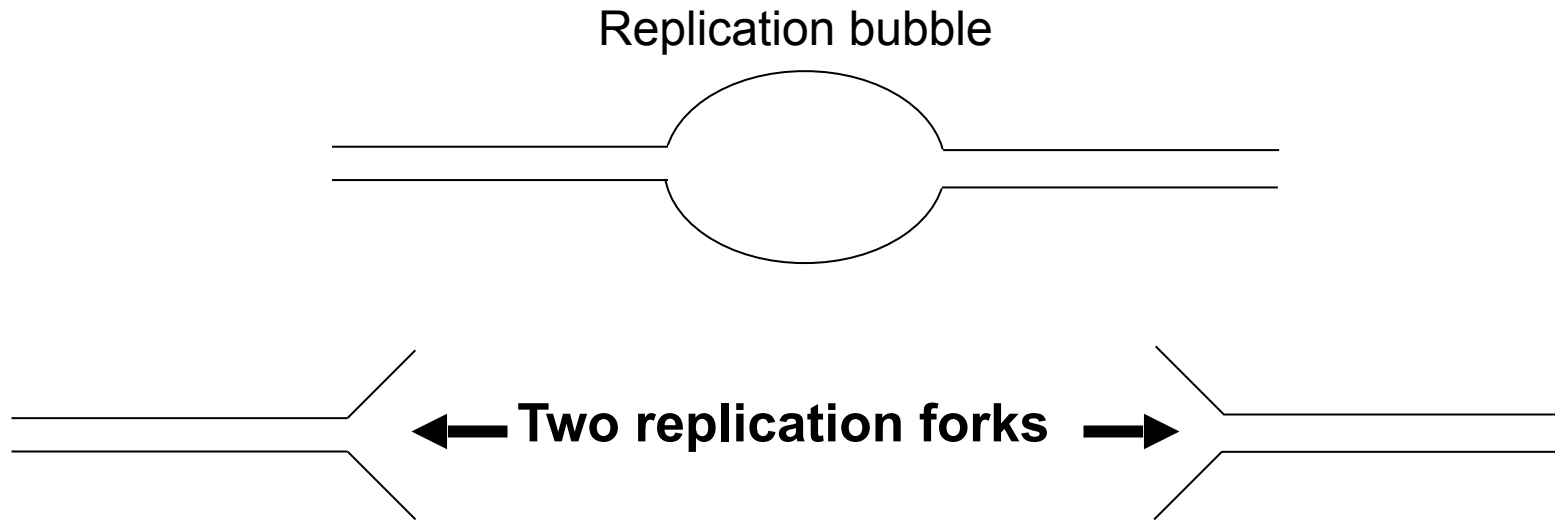
1- DNA denaturation/DNA melting



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

DNA replication process

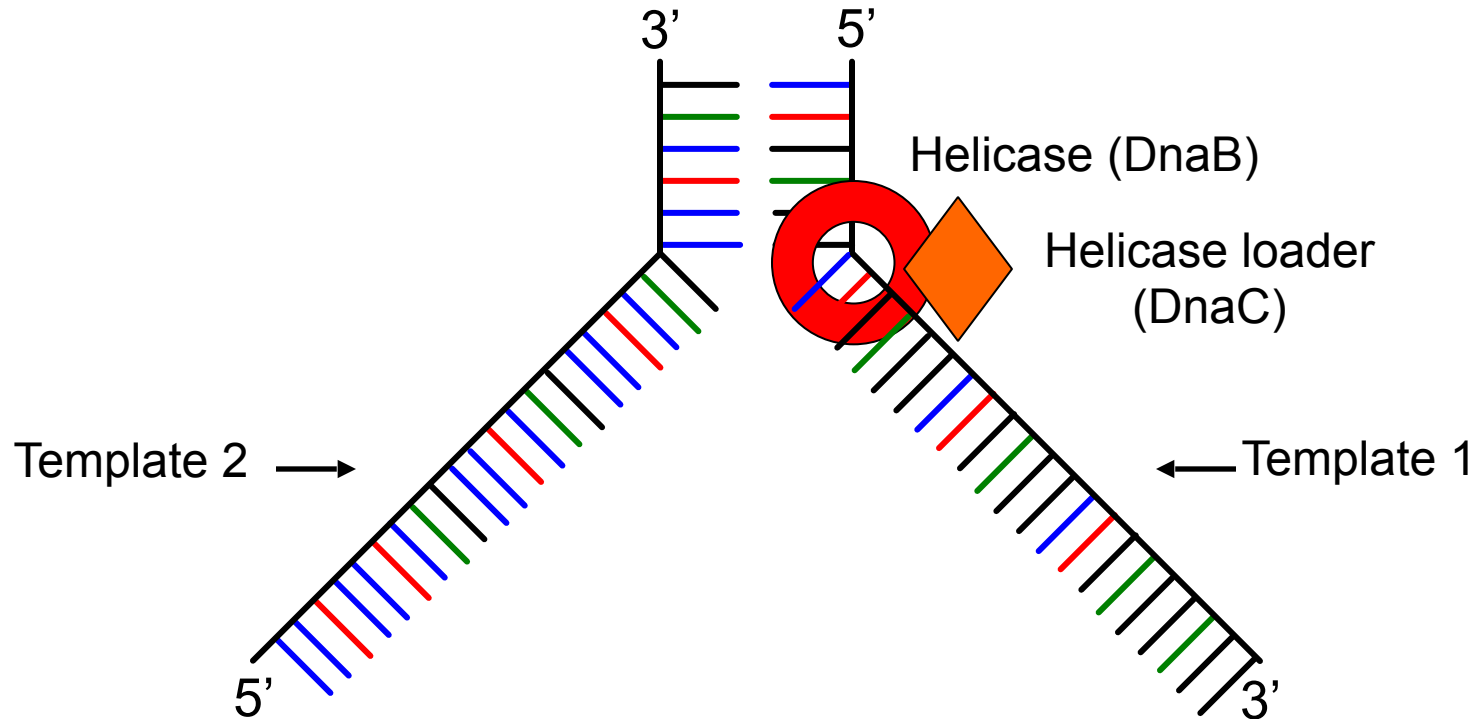
1- DNA denaturation/DNA melting



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

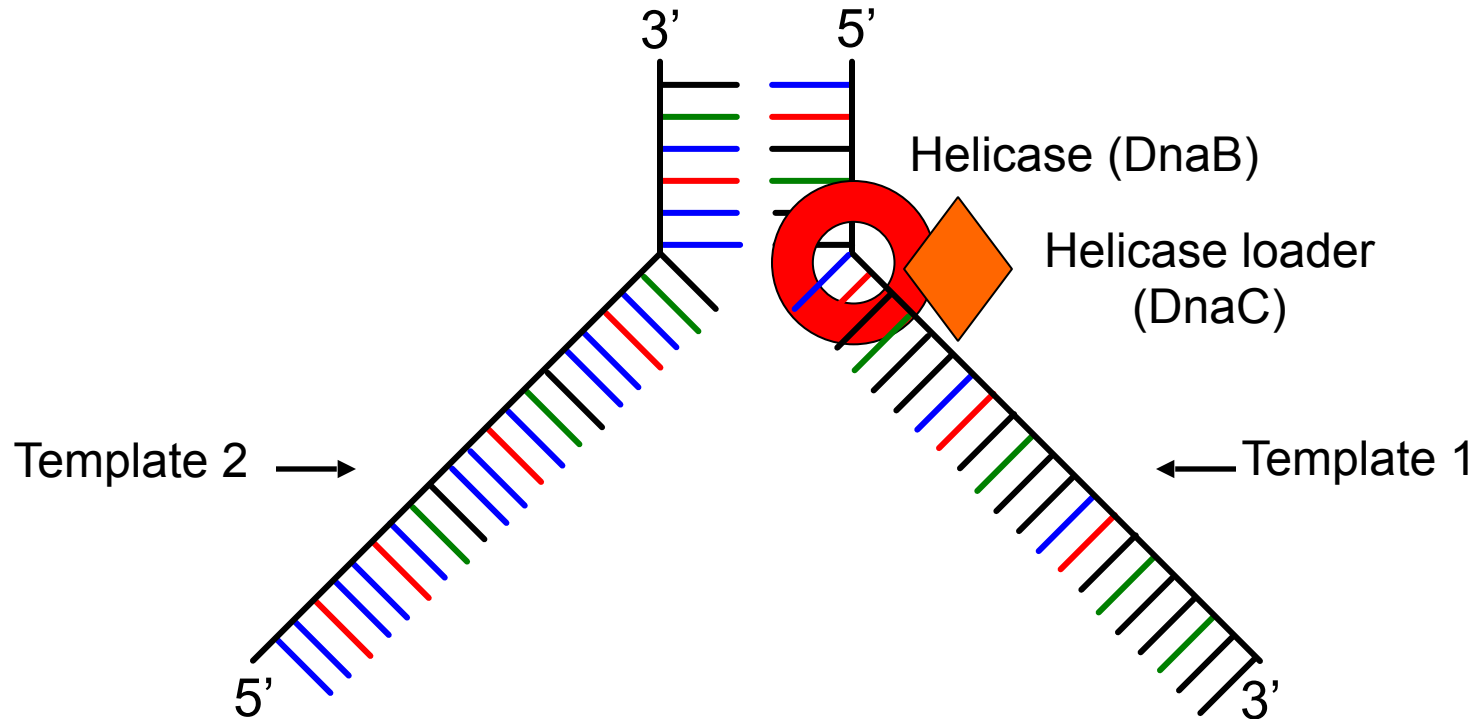
2- Helicase loading



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

DNA replication process

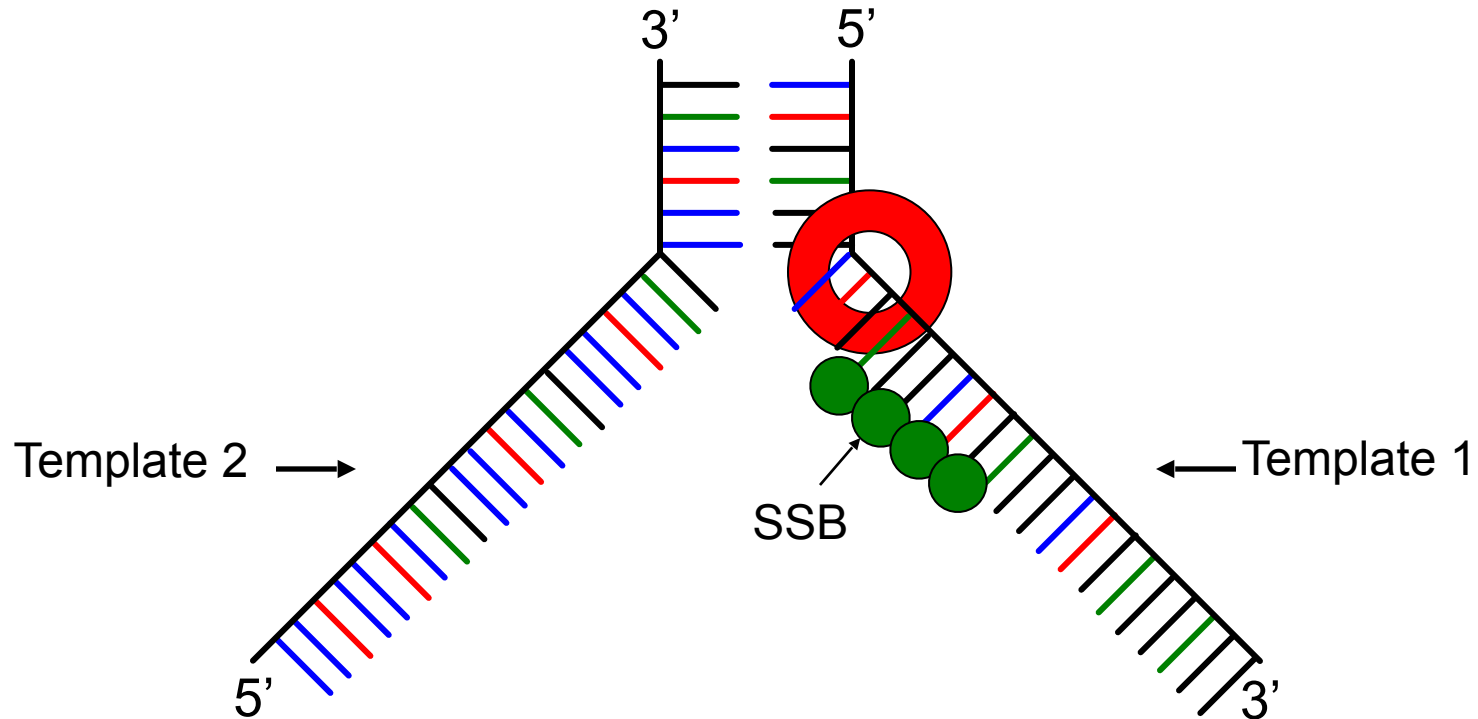
2- Helicase loading



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

DNA replication process

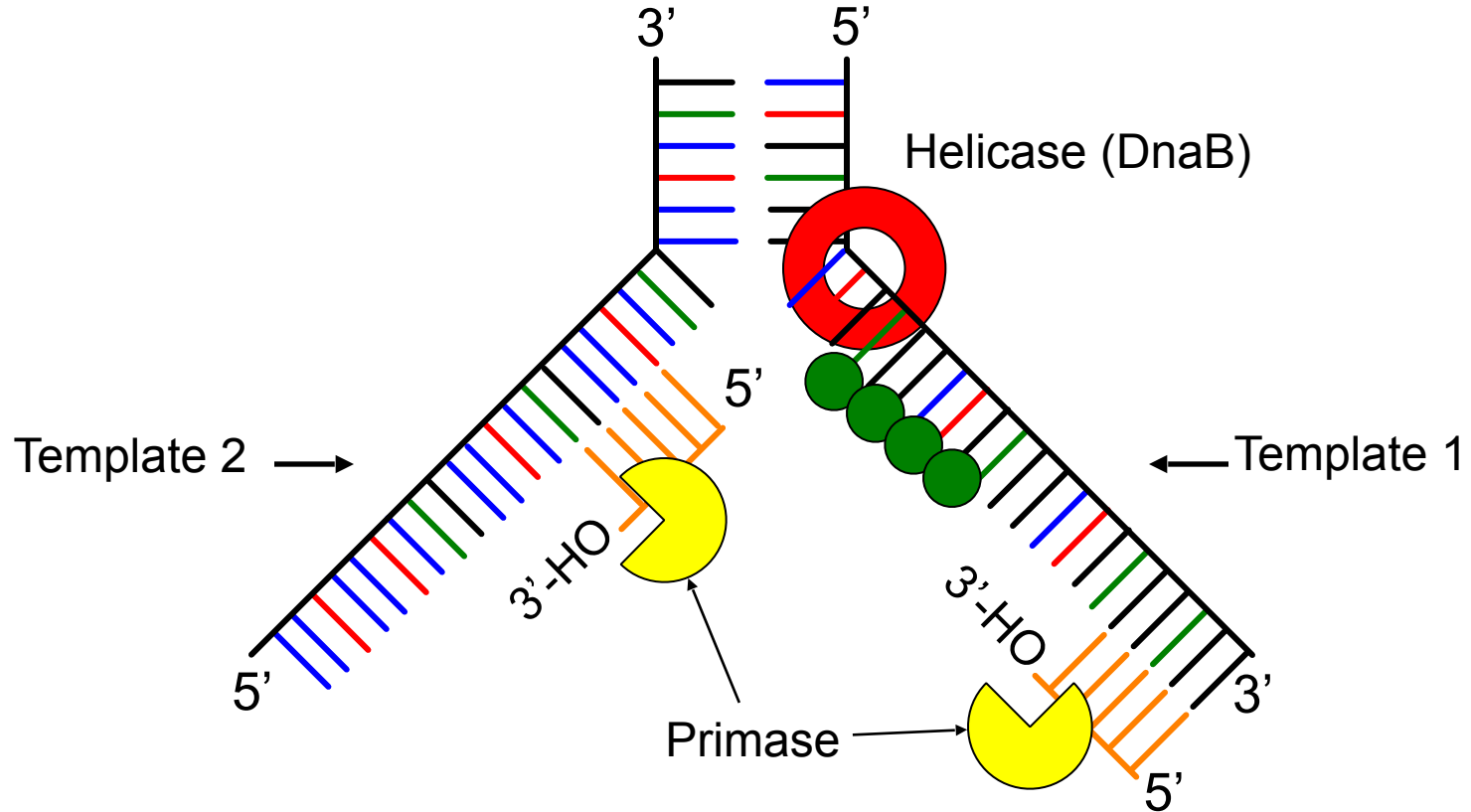
3- Single strand DNA binding proteins(SSB)



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

DNA replication process

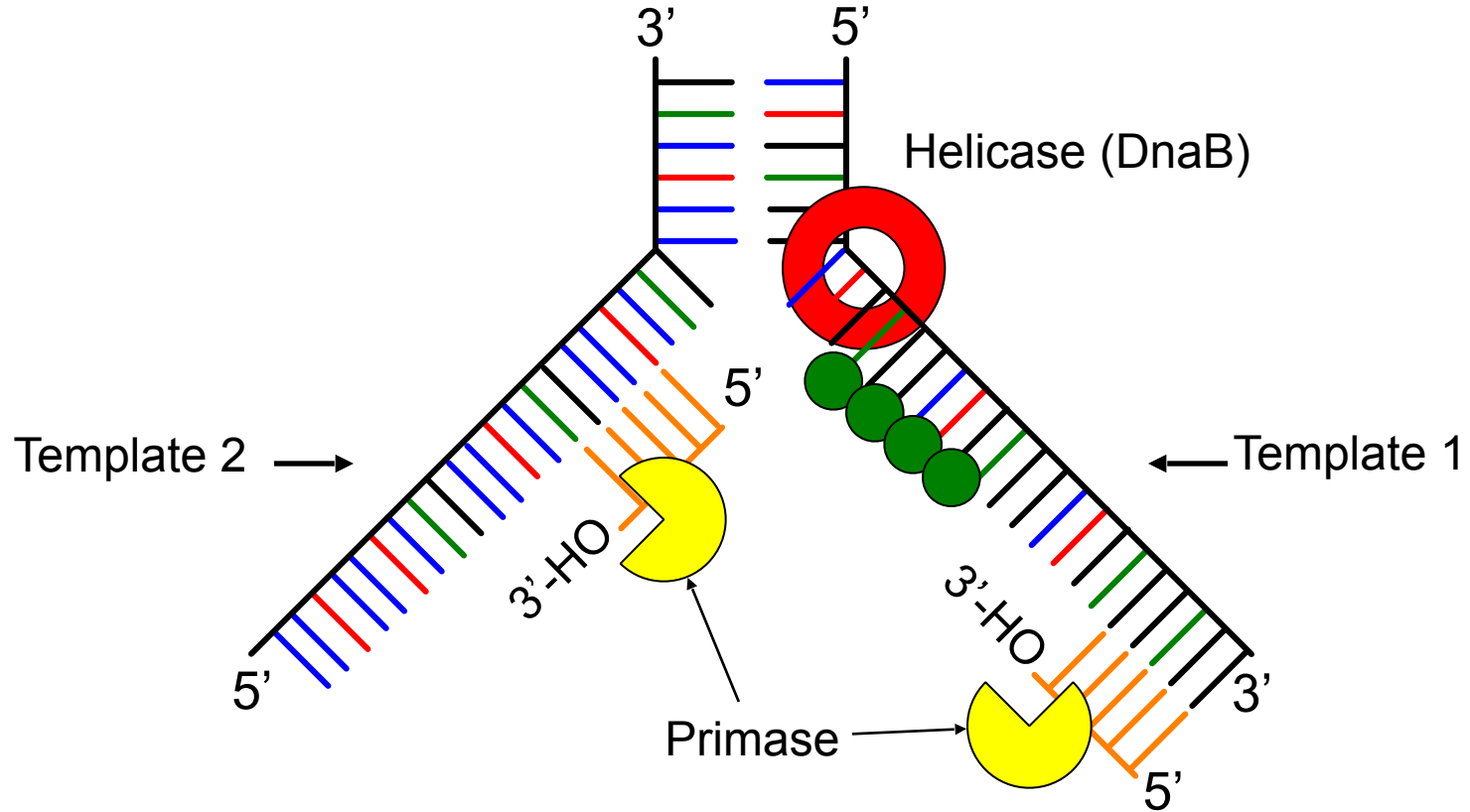
4- DNA primase



DNA 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

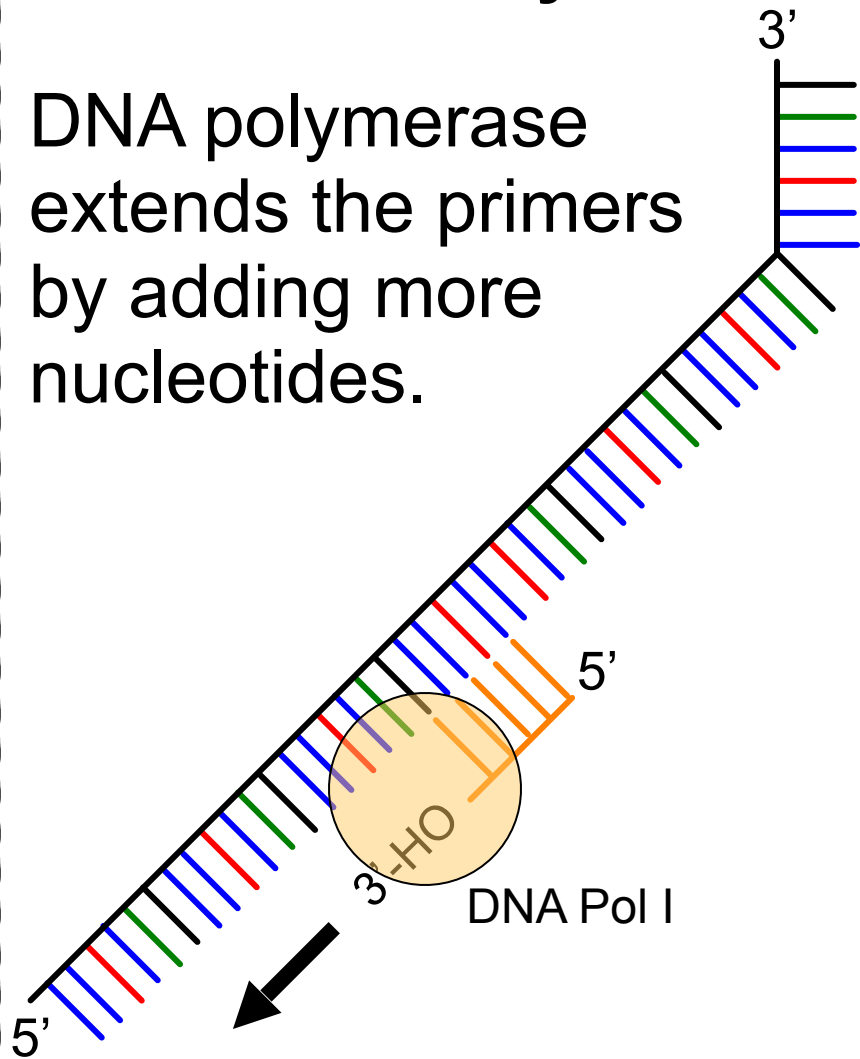


Primase add a single primer on the leading strand and multiple primers on the lagging strand.

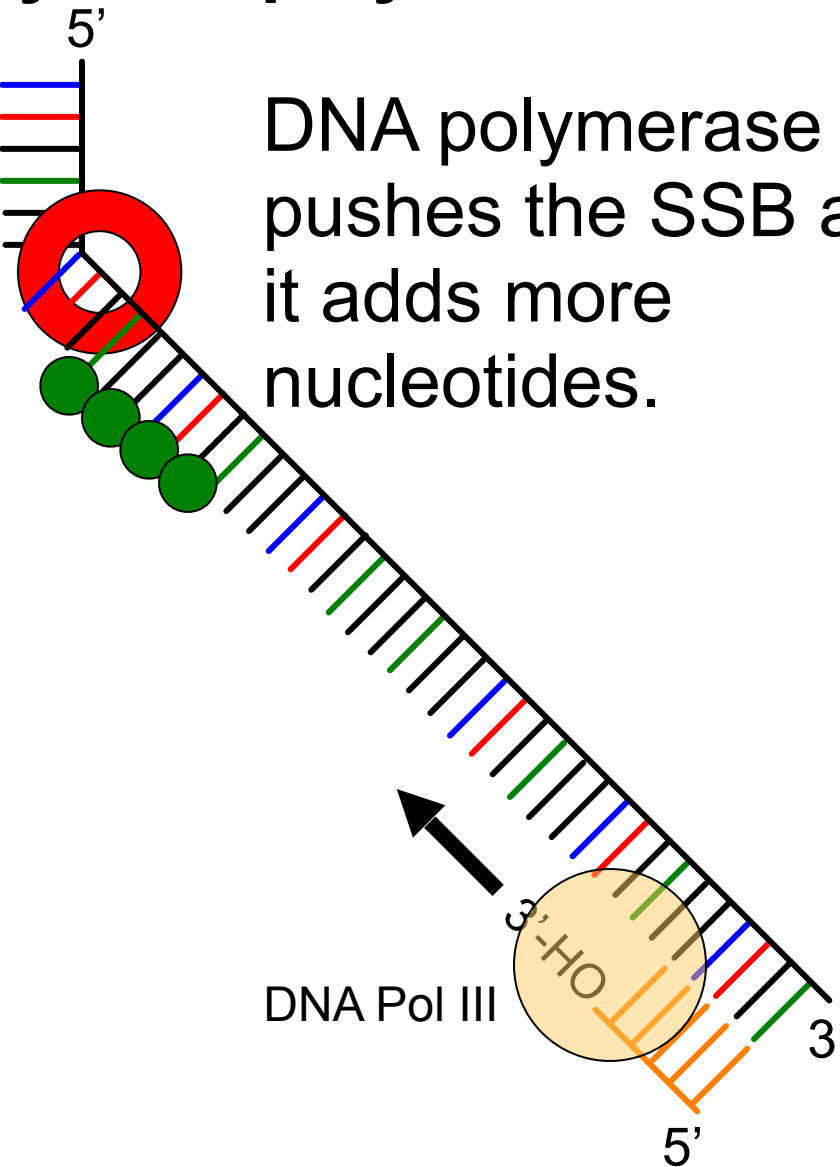
DNA replication process

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

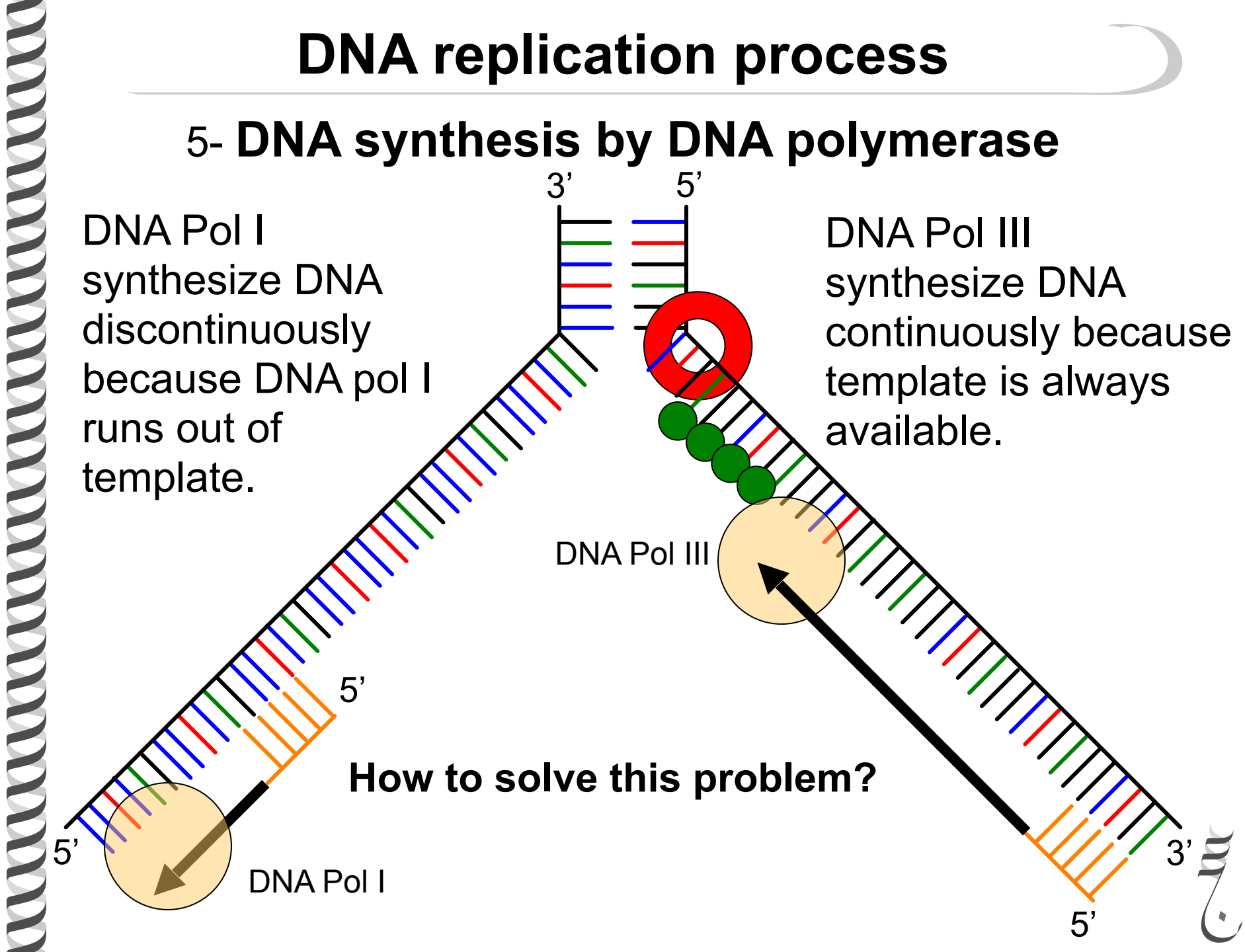
DNA Pol I
synthesize DNA
discontinuously
because DNA pol I
runs out of
template.

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

DNA Pol III

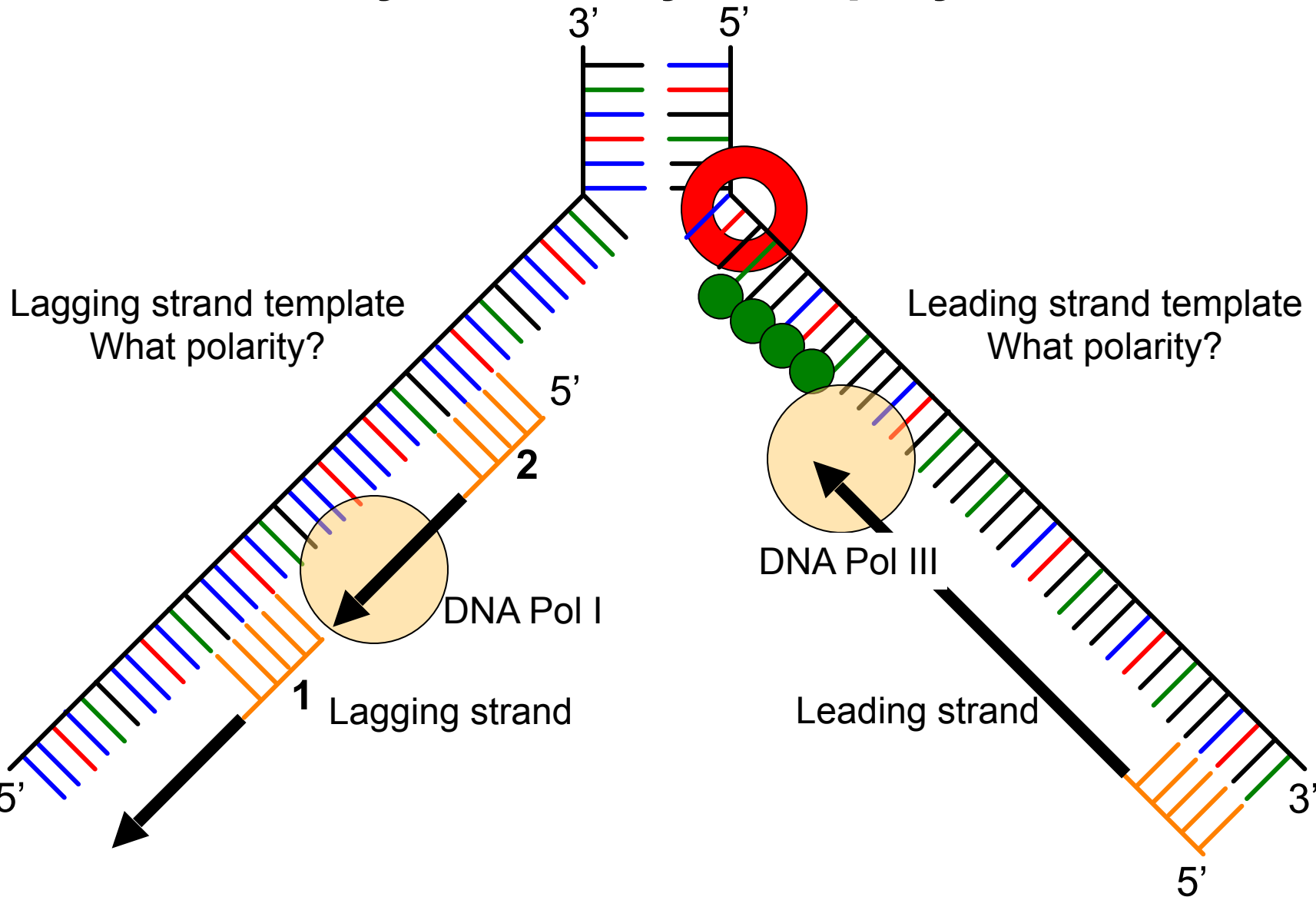
How to solve this problem?

DNA Pol I



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

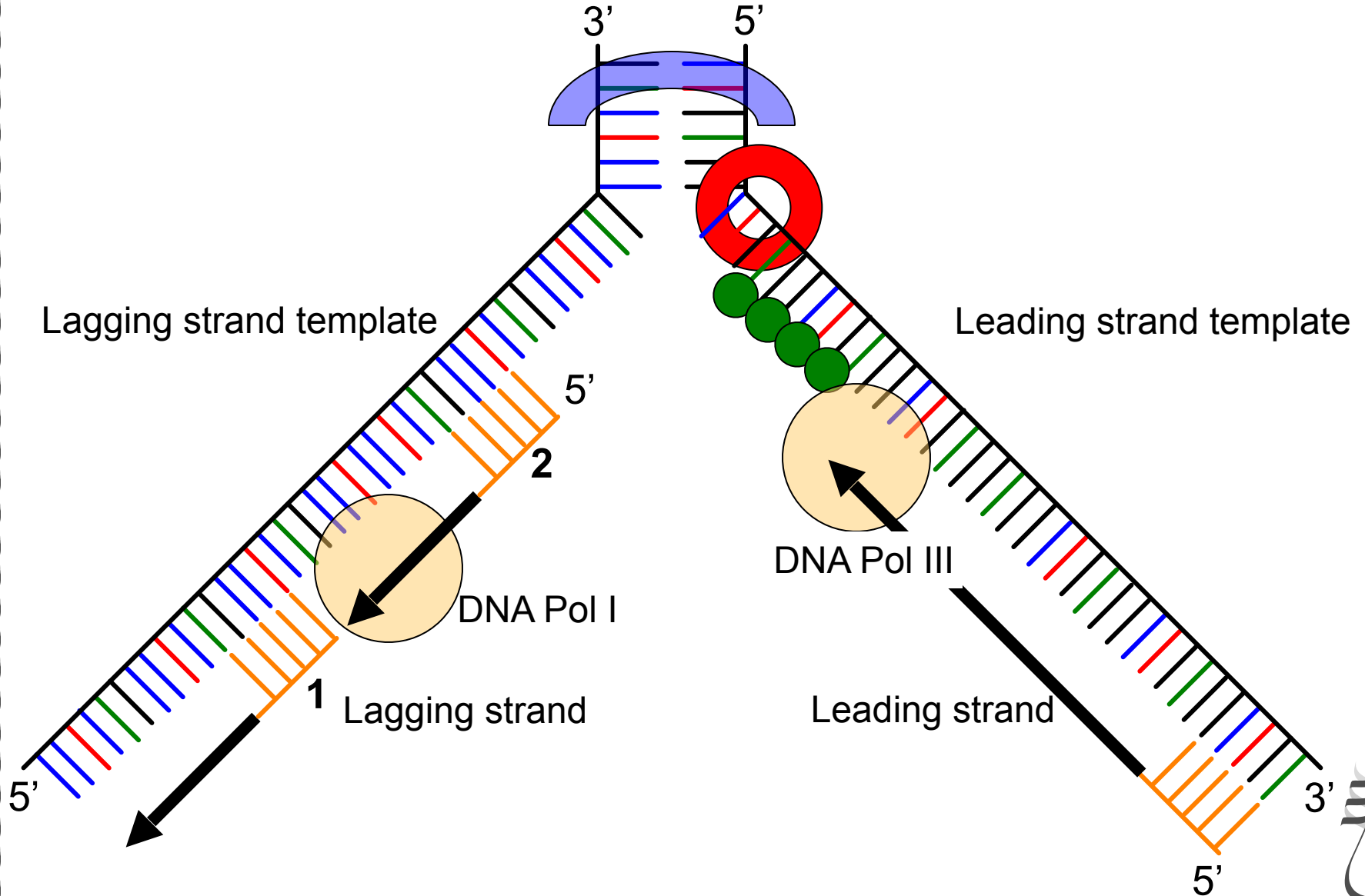


5- DNA synthesis by DNA polymerase

So DNA is replicated in
Semi-dis-continuous way

DNA replication process

6- DNA gyrase

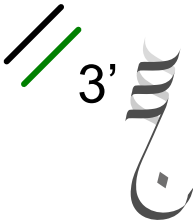


DNA replication process



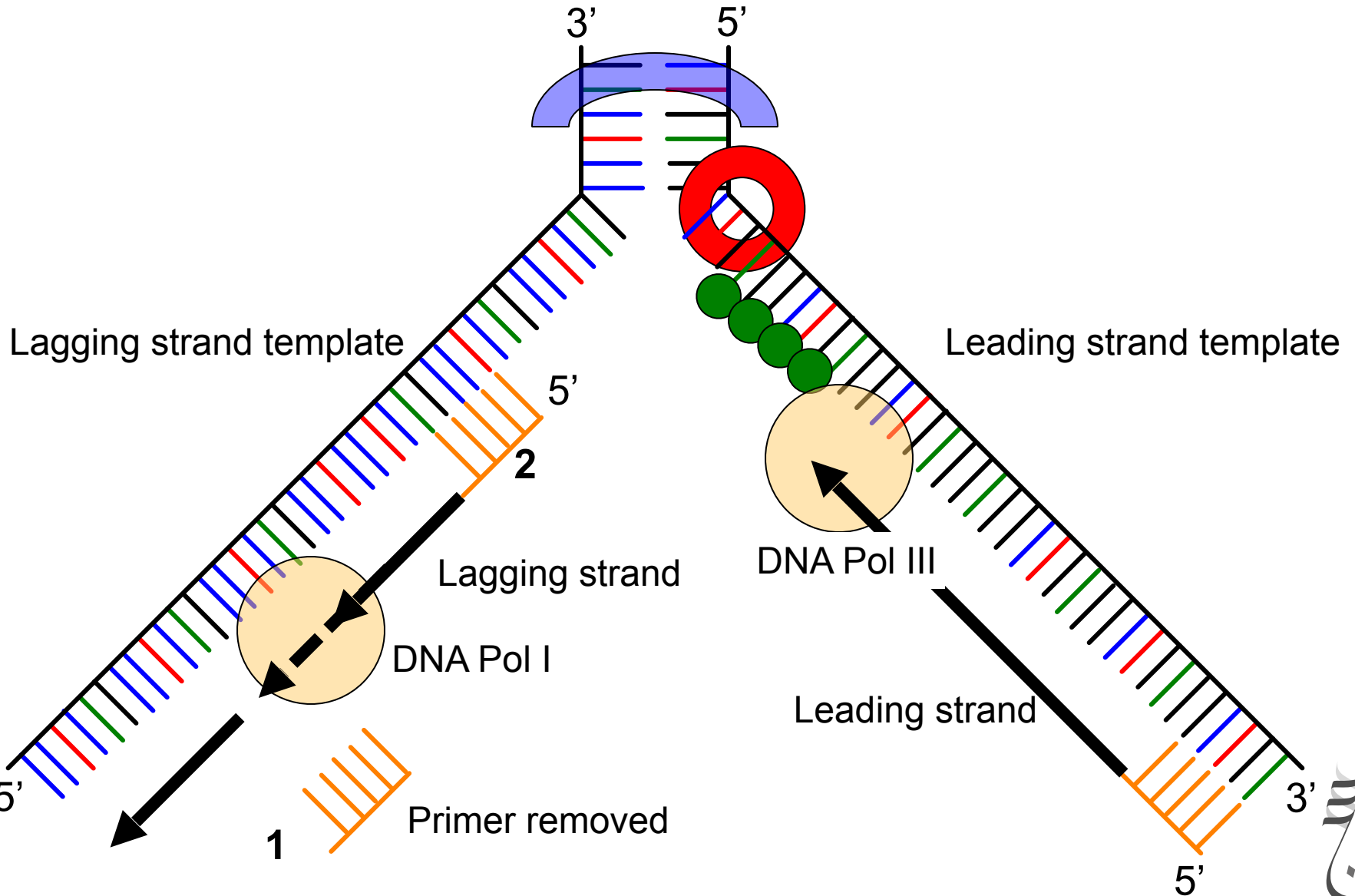
6- DNA gyrase

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

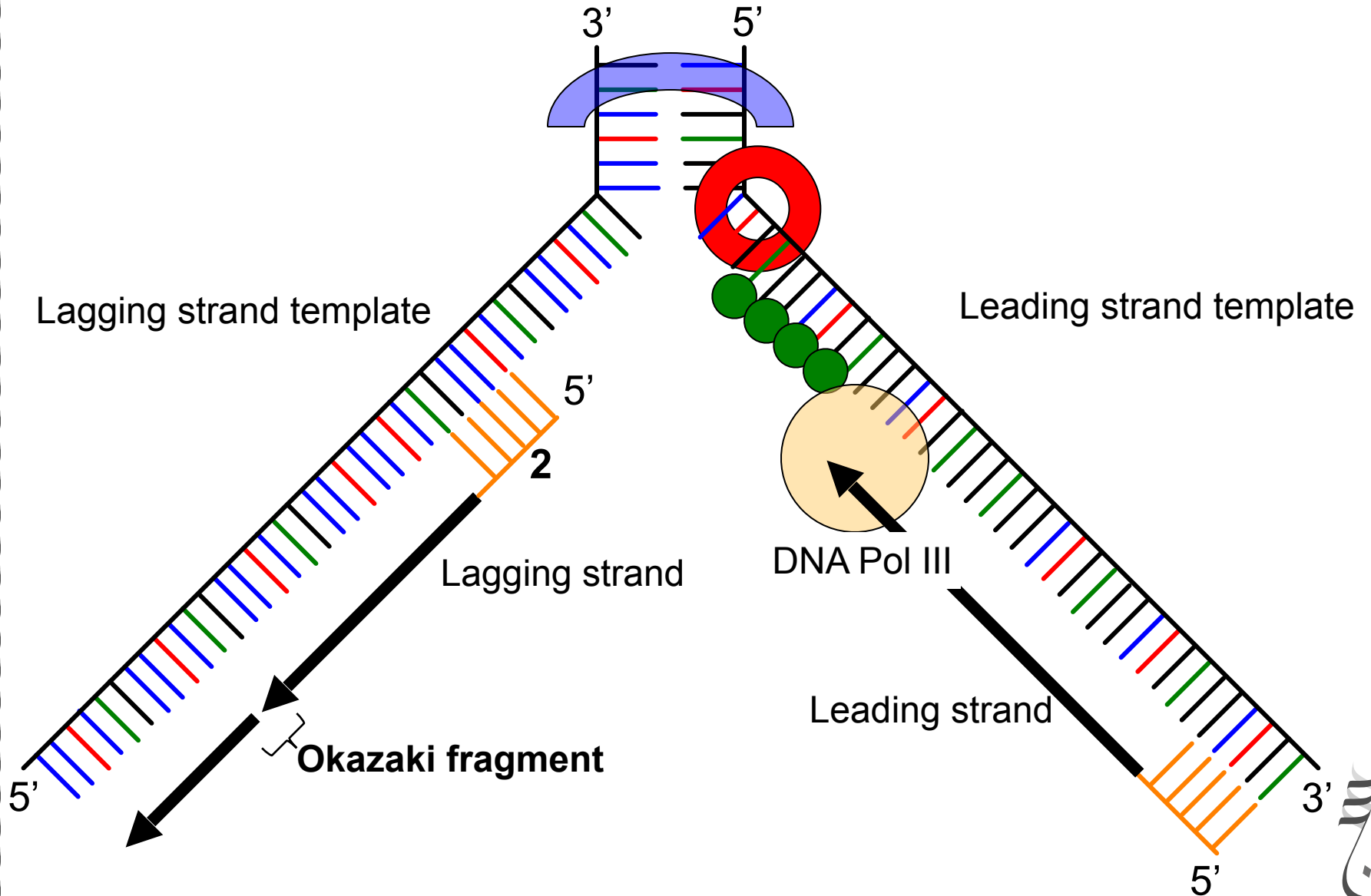


7- Removing primers

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

DNA replication process

8- Okazaki fragments



DNA replication process

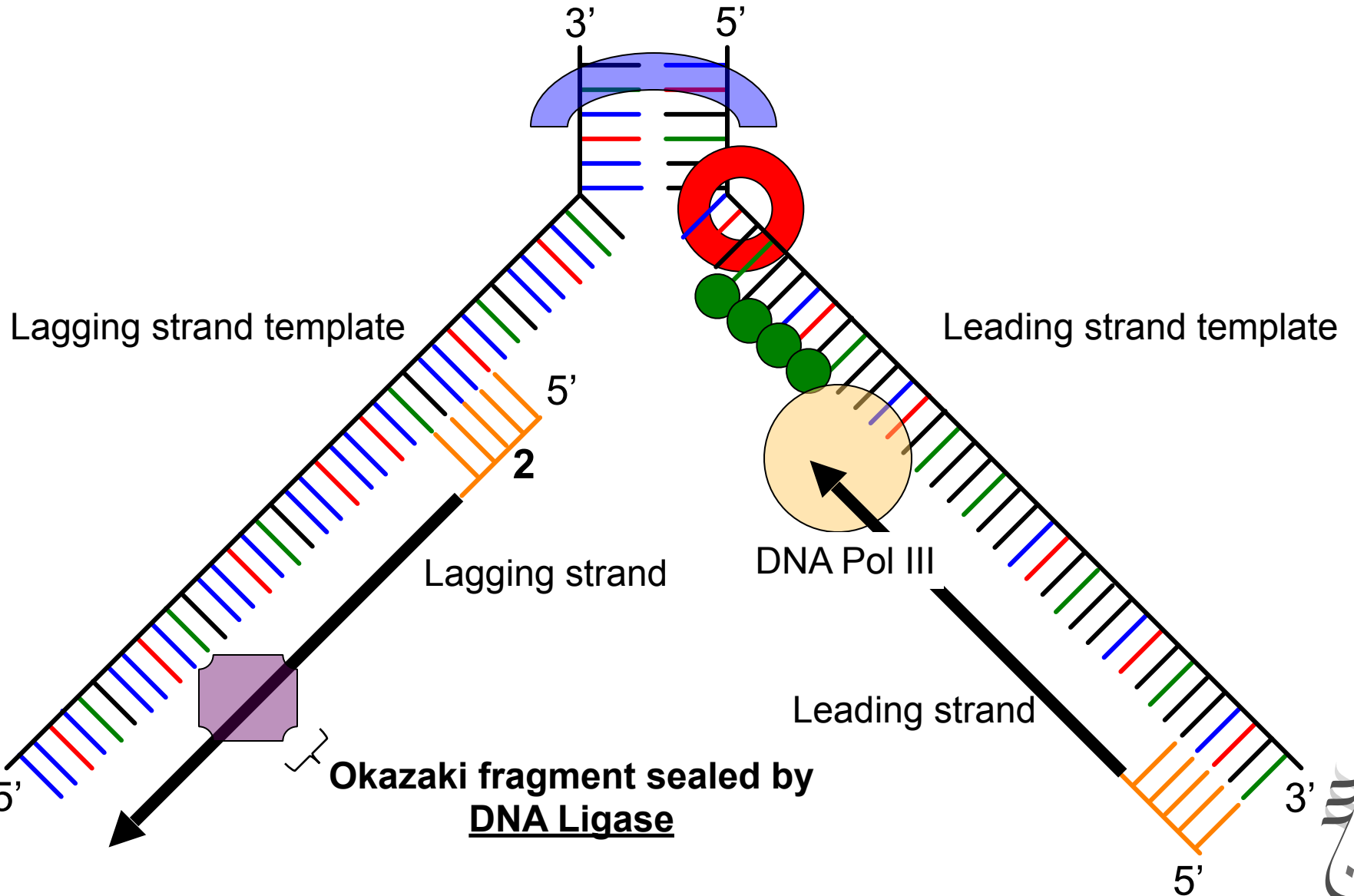


8- Okazaki fragments

- 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

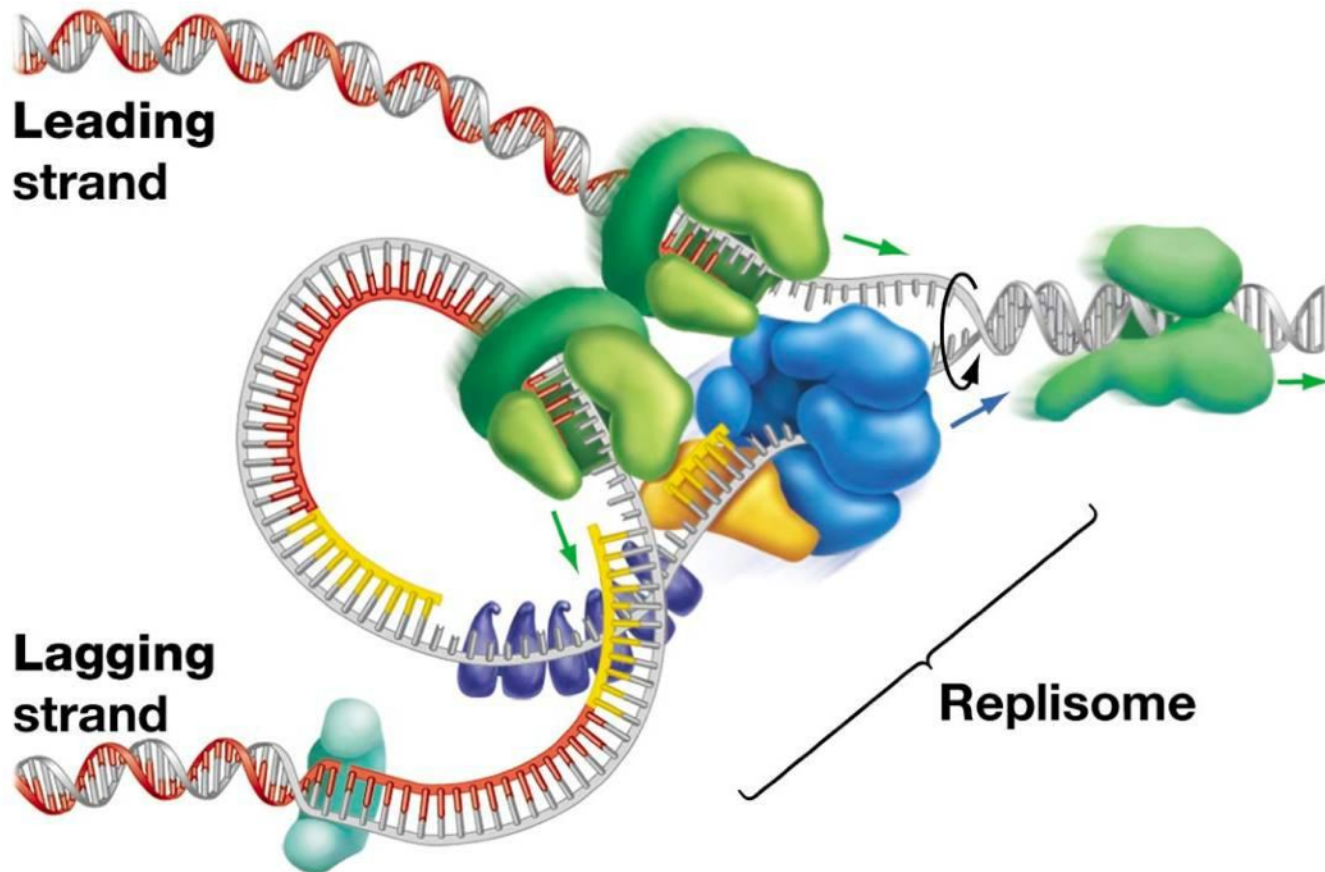


8- Okazaki fragments sealed by DNA ligase

DNA ligase seals Okazaki fragments on the lagging strand.

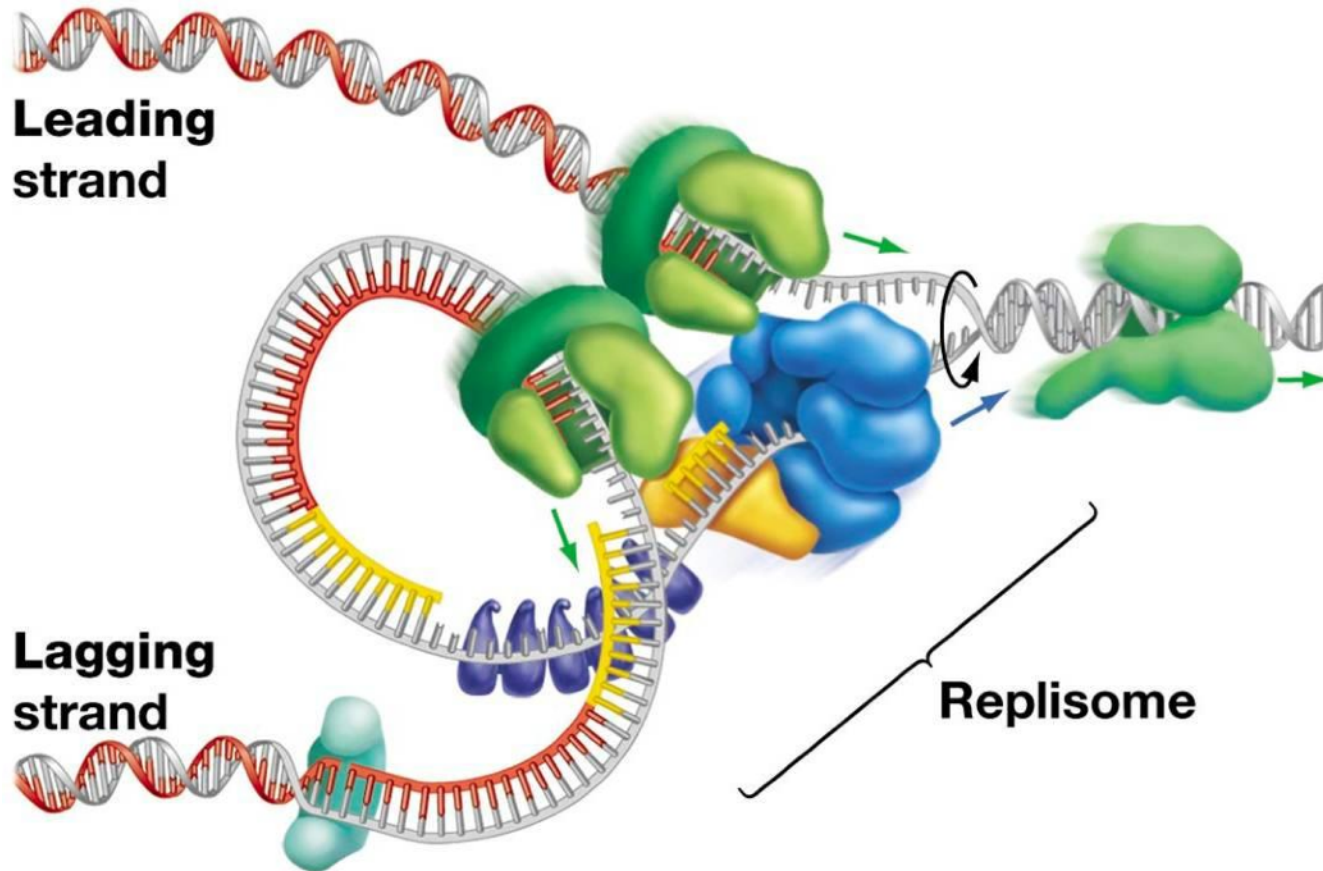
The replisome machine

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**.



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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

Replication bubble Ori SSB Leading strand

Lagging strand DNA ligase Cell cycle DnaC

Okazaki fragments Bidirectional replication

Exonuclease DnaA Lagging strand template

DNA polymerase I Gyrase Replicator

DNA melting Origin of replication Denature Replication fork

Semi-dis-continuous replication Replisome DNA polymerase III



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

