INTRODUCTION

DNA replicate by using a semi-conservative method that results in the formation of double-stranded DNA with one parental strand and a new daughter strand. It is a process in which each strand of DNA molecule acts as template for the synthesis of its complementary strands. Newly synthesized DNA strands transferred into two different daughter cells. Replication occurs before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material. Replication process was studied in the modal organism E. coli. It is a bi-directional process and originates at a single origin of replication (OriC) site occurring in DNA.

DNA replication process complete in three steps:

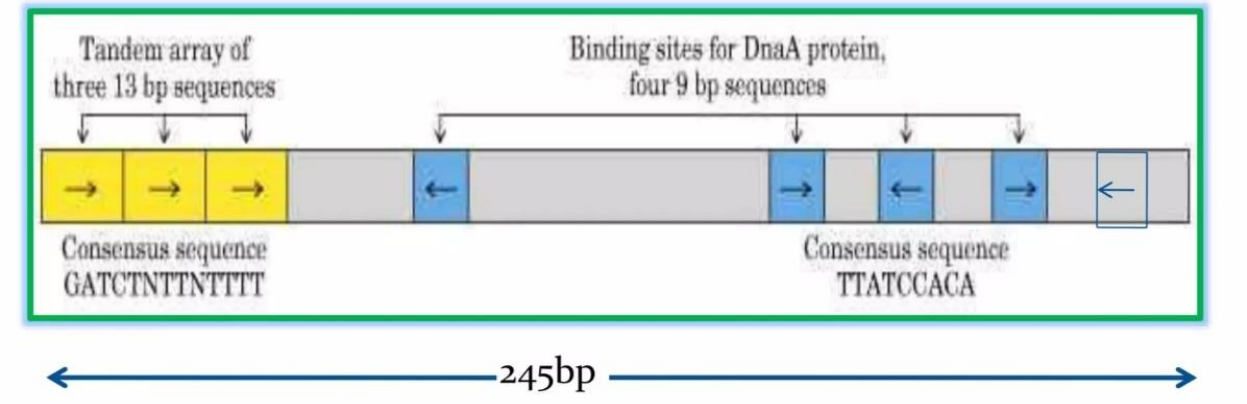
1) INITIATION

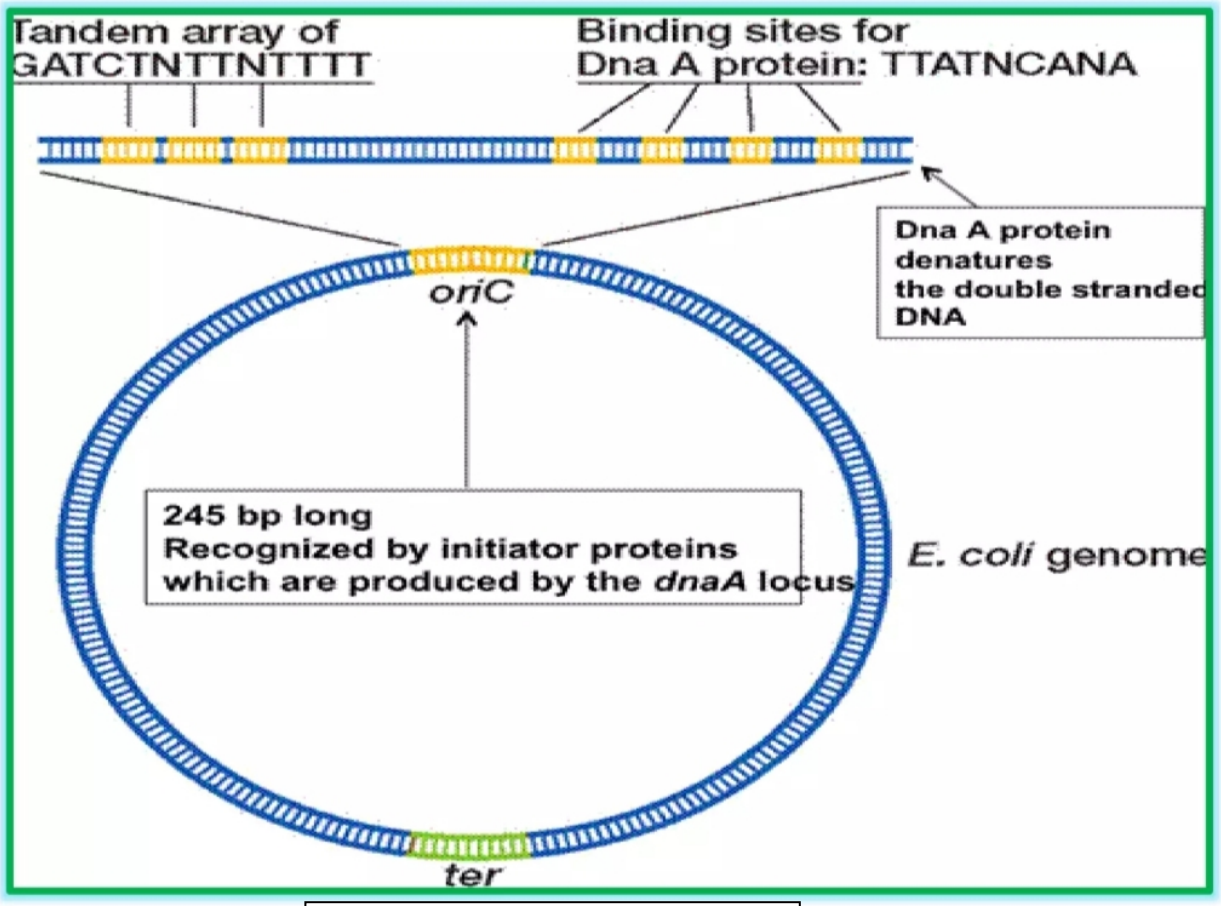
2) ELONGATION

3) TERMINATION

INITIATION

In E.coli the process of replication is initiated from the origin of replication. The origin of replication in E.coli is called as oriC. oriC consists of a 245bp long AT-rich sequence which is highly conserved in almost all prokaryotes. Mostly two types of sequences present in this region, three repeats of 13bp called as a 13mer and five repeats of 9bp called as a 9mer.





E.Coli oriC Replication Site

Prokaryotic Replication Proteins

A few proteins play an important role in DNA replication:

DnaA- It recognizes oriC sequences for initiation of replication.

DanB- Unwinds DNA double helix. It is actually work as a helicase.

DnaC- At the origin of replication it helps helicase (DnaB) to recognize the site for its action.

DnaG- It is actually act as a primase. It synthesizes new RNA primer.

DNA gyrase- It is a DNA topoisomerase II which helps in the unwinding of DNA.

DNA pol I - Removes RNA primer and replaces it with newly synthesized DNA.

DNA pol III- Main enzyme that adds nucleotides in the 5'-3' direction.

Helicase- Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases. (Dna B)

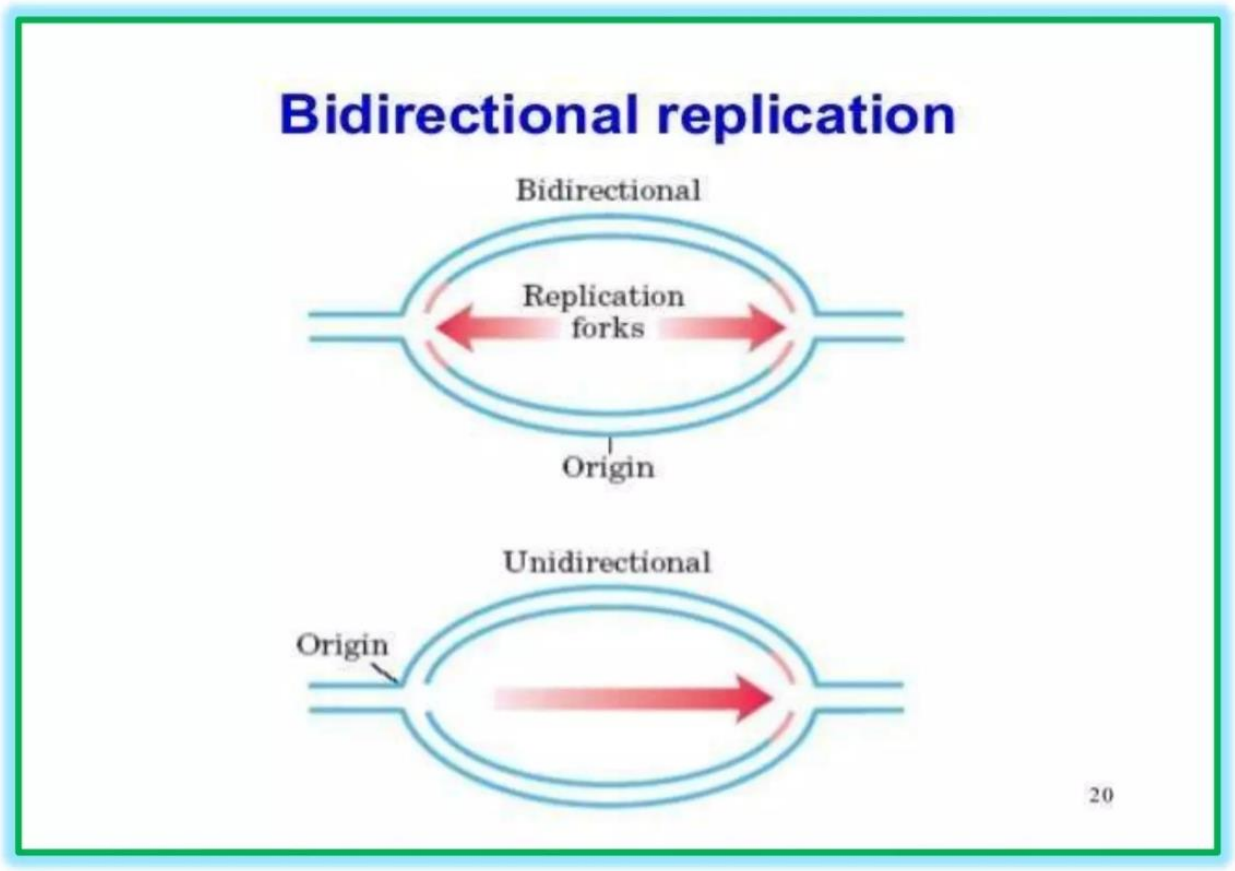
Ligase- Seals the gaps between the Okazaki fragments to create one continuous DNA strand.

Primase - Synthesizes RNA primers needed to start replication.

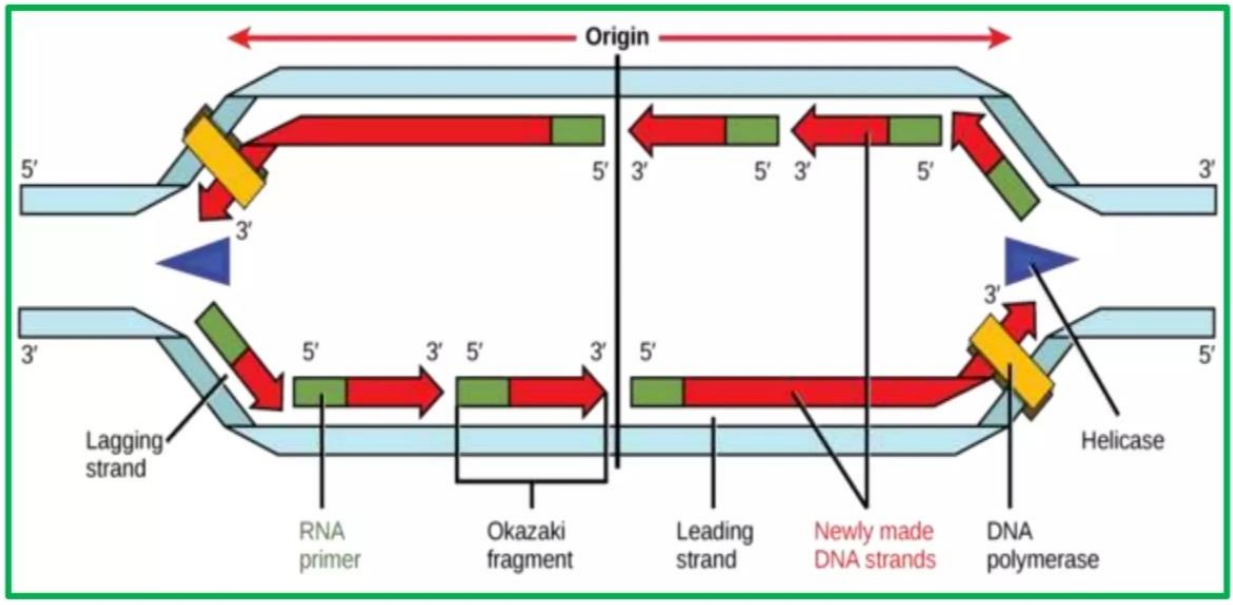
Sliding Clamp - Helps to hold the DNA polymerase in place when nucleotides are being added.

Topoisomerase- Helps relieve the strain on DNA when unwinding by causing breaks, and then resealing the DNA

Single-strand binding proteins (SSB) - Binds to single-stranded DNA to prevent DNA from rewinding back.



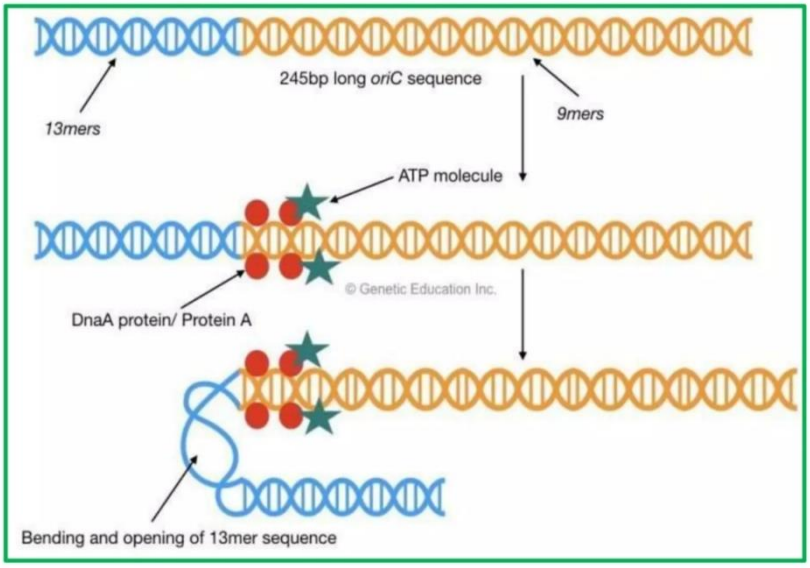
Bidirectional replication

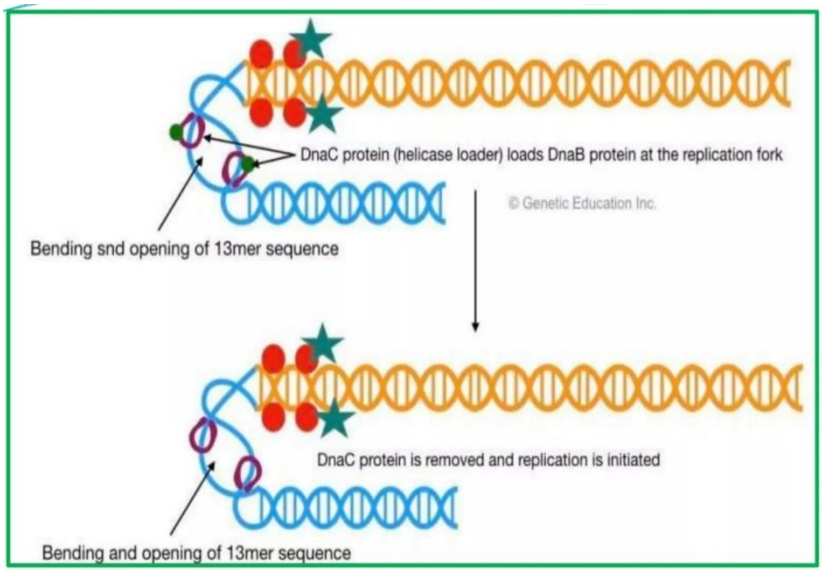


Replication Fork

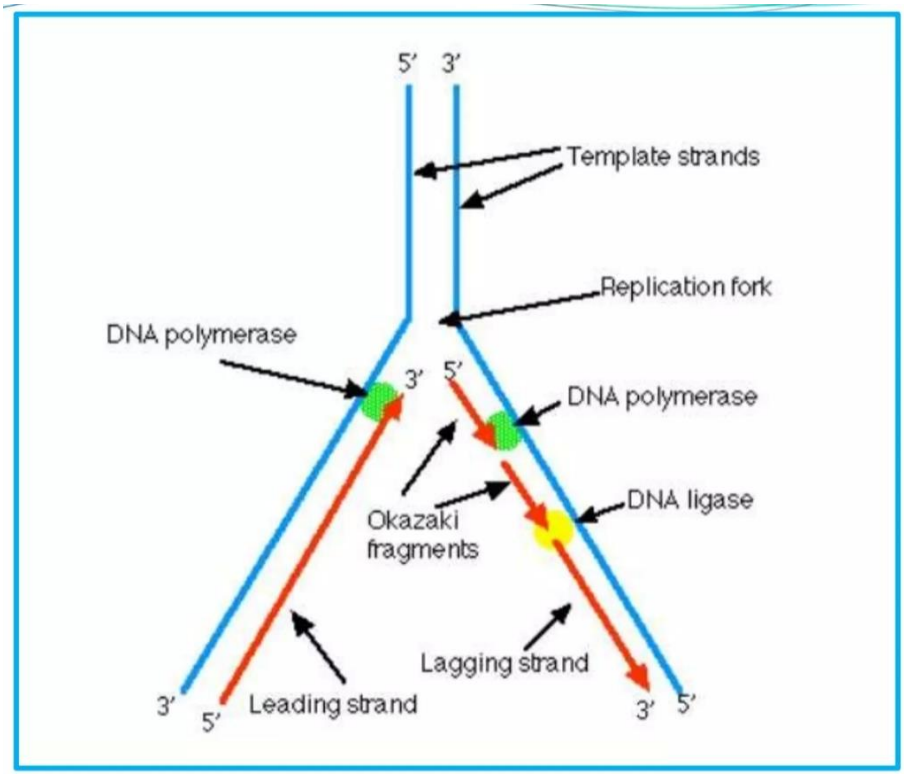
Here as the oriC is recognized, the opening of the DNA strand creates a replication fork. The replication fork is a Y shaped structure and it is important because it facilitates polymerase to work properly. As the chromosome of bacteria is circular, two different replication forks are developed in two different directions and it runs towards each other. When helicase unwinds DNA, SSB proteins bind the single strand near helicase. The single-stranded binding protein binds to the single strand of DNA and protects it from rejoining. SSB is a tetrameric structure that protects the DNA and facilitates the helicase activity constantly. It also prevents single-stranded DNA from the attack of nuclease. As the helicase Starts unwinding, polymerase starts adding nucleotide in the elongation stage.

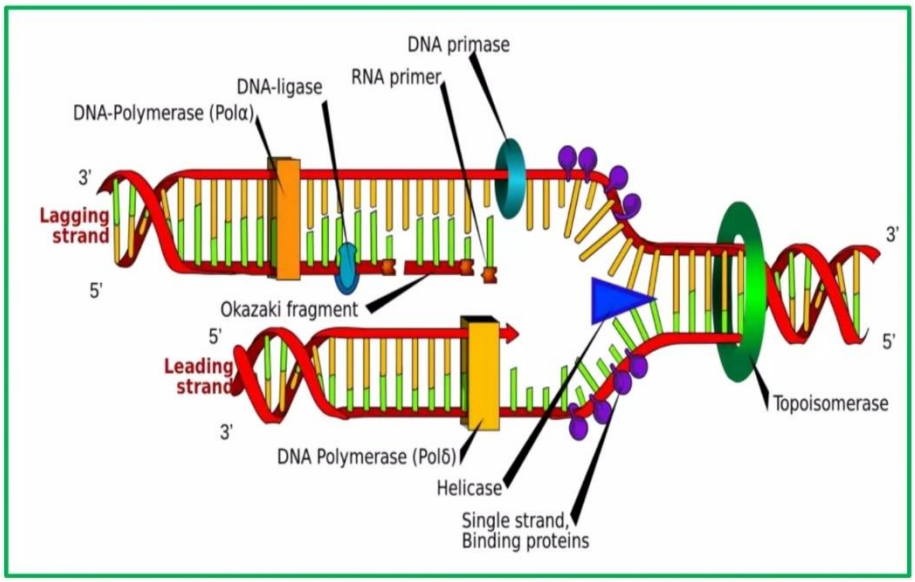
Mechanism of Initiation





Protein A (Dna A protein) with ATP, binds to the 9mer sequences of oriC. This binding allows the opening of 13mer sequences of oriC by bending the DNA. DnaB proteins bind to 13mer repeats which are recognized by DnaC. Once DnaB is settled on oriC (at 13mer) the DnaC protein will be released. DnaB helps in the unwinding of DNA and generates two single-stranded DNA molecules, one with 5'-P end and another with 3'-OH end. Helicase uses ATP as an energy source for the unwinding of DNA. The activity of helicase (Dna B) generates tension on the remaining double strand of DNA. DNA Gyrase helps in the unwinding of DNA and releases the tension on the DNA strand by negative supercoiling. Tensioned or supercoiled DNA decreases the rate of replication.


5′ 3′
Template strands
Replication fork
DNA polymerase
3′ 5′
DNA polymerase
DNA ligase
Okazaki fragments
Lagging strand
3′ 5′
Leading strand
3′ 5′


DNA primase
DNA-ligase
RNA primer
DNA-Polymerase (Polα)
3'
Lagging strand
5'
3'
5'
Okazaki fragment
5'
Leading strand
3'
Topoisomerase
DNA Polymerase (Polδ)
Helicase
Single strand, Binding proteins

ELONGATION

Before the start of elongation, primase synthesized short 10bp long RNA primer. The DNA polymerase cannot able to add nucleotide without an RNA primer. It provides a start site as a free 3'-OH group for the polymerase to work. The elongation is a step in which the DNA synthesis is initiated. once the dsDNA becomes single-stranded, the polymerase settles on the junction of DNA-RNA primer. The first DNA polymerase is isolated by Arthur Kornberg, in 1959 and he was the first person who synthesized the first DNA molecule in vitro.

Three different types of DNA polymerase helps in prokaryotic DNA replication.

DNA polymerase I: removes RNA primer by exonuclease activity and proofreads the DNA

DNA polymerase II: DNA repair function. The DNA polymerase II mainly involved in the DNA repair pathway.

DNA polymerase III: Adds nucleotide from 5' to 3' direction.

Once a primosome complex is created (primosome complex is a primer - Helicase complex) polymerase recognized it and starts the polymerization process at the leading strand. The process of replication progressed from 3' to 5' direction while the polymerase III adds nucleotide from 5' to 3' direction.

The activity of DNA polymerase:

Two DNA polymerase synthesized both leading stand as well as lagging stand at once. It is possible with the help of two subunits of DNA polymerase III. Both subunits have different properties as one is specific for leading strand synthesis and one is specific for lagging strand synthesis. Two sliding clamps and a polymerase clamp loader help polymerase to settle on each DNA strand. The clamp loader places the sliding clamp on to the DNA. The sliding clamp holds DNA properly and prevents them from floating off.

Leading strand synthesis:

A 3'-OH end of single-stranded DNA is called a leading stand. Primer is first bound to this end, after the recognization of primer binding site, as the helicase moves, polymerase III adds nucleotides from 5' to 3' direction on growing leading strand. During the process of replication 1000 nucleotides are added per second. The leading strand DNA synthesis is very straightforward. It starts at primosome and continuously adds nucleotides until the termination sequences. Termination sequences are unique conserved sequences which are recognized by polymerase as the end of replication.

Lagging strand DNA synthesis:

The lagging strand of DNA has the 5'-P open end, hence the polymerase cannot synthesize new DNA from this direction. The lagging strand DNA synthesis is a little different from the leading strand. Here primase synthesizes multiple primers. The lagging strand is synthesized in short DNA fragments called as Okazaki fragments. Subsequently, each new primer is added after 1000 nucleotide. After the synthesis of Okazaki fragment, the sliding clamp is removed. Once again, the new primer is synthesized by primase and binds to DNA, the clamp is further loaded by sliding clamp loader and the synthesis of new Okazaki fragment begins.

TERMINATION

After the synthesis of leading and lagging strand, the polymerase is detached from the site of replication. In the termination step, firstly, the multiple primers at lagging strand are cleaved by RNase H and removed by DNA polymerase I. It also fills the gap between two Okazaki fragments by the addition of nucleotides. Furthermore, DNA polymerase proofreads the sequence for avoiding error in replication. Finally, the enzyme DNA ligase fills the gap (creates a phosphodiester bond between Okazaki fragments and newly added nucleotides). At the last stage of termination, two replication fork meets at terminator recognizing sequences, called as a Ter. Ter sequences with TUS protein create a complex which arrests the replication fork and prevent them to escape. At this complex, the process of replication is completed and all other proteins and enzymes leave this site. Only DNA topoisomerase II remains in action, it cuts both strands, dissociates Ter-TUS complex and two different circular DNA is generated.