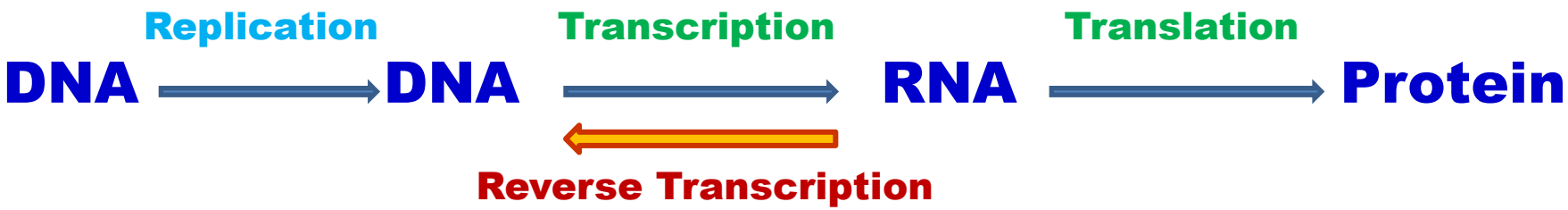
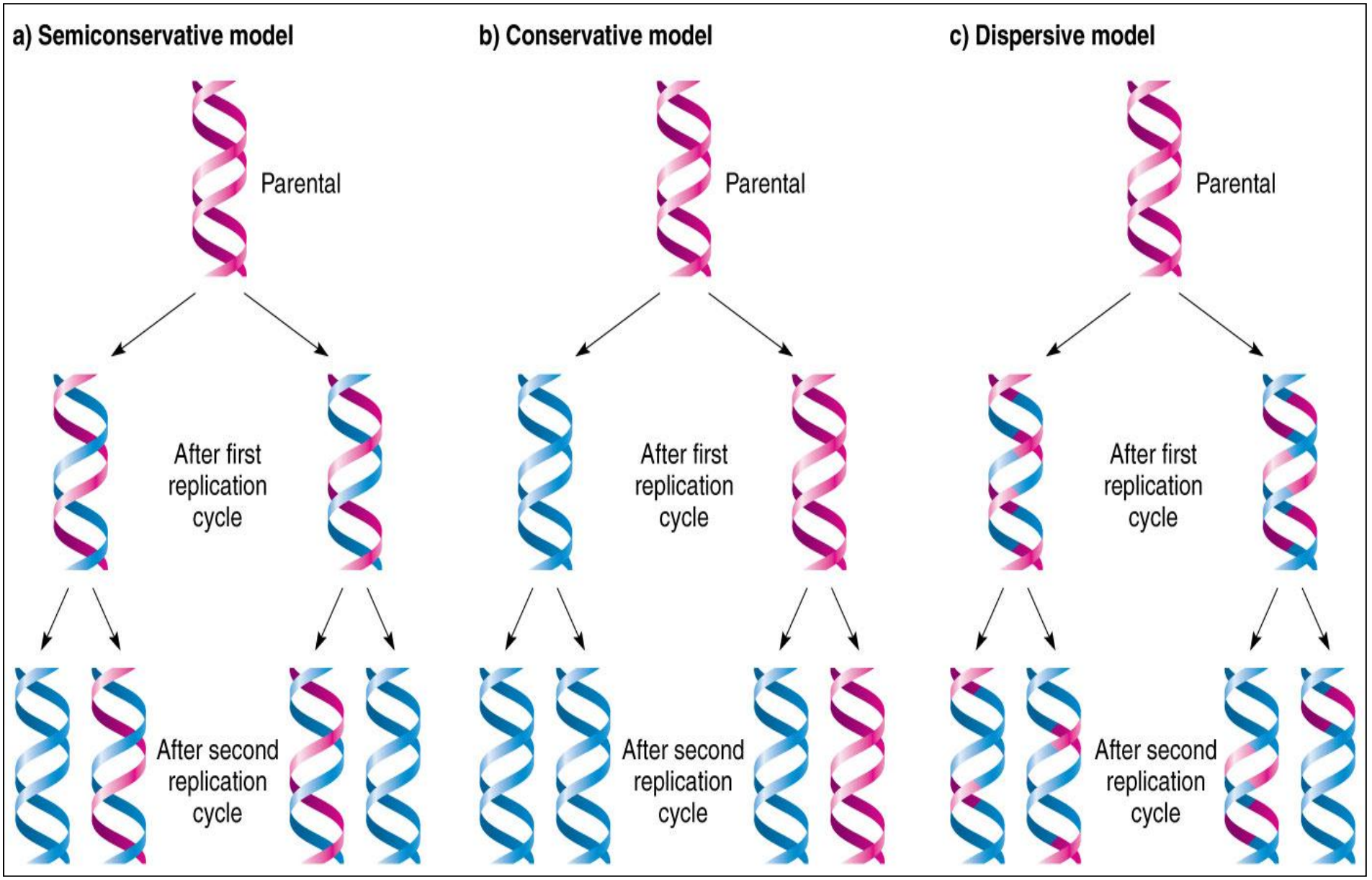
Central Dogma

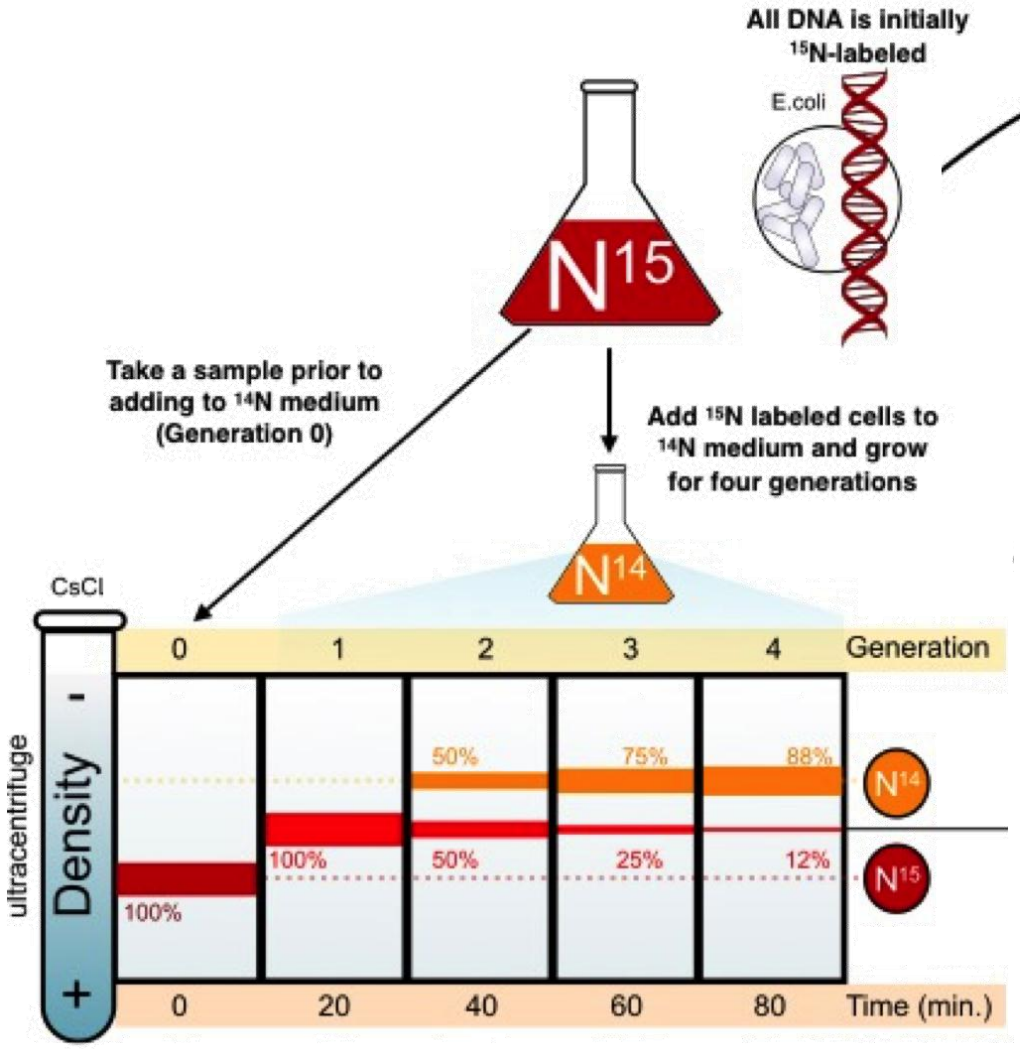


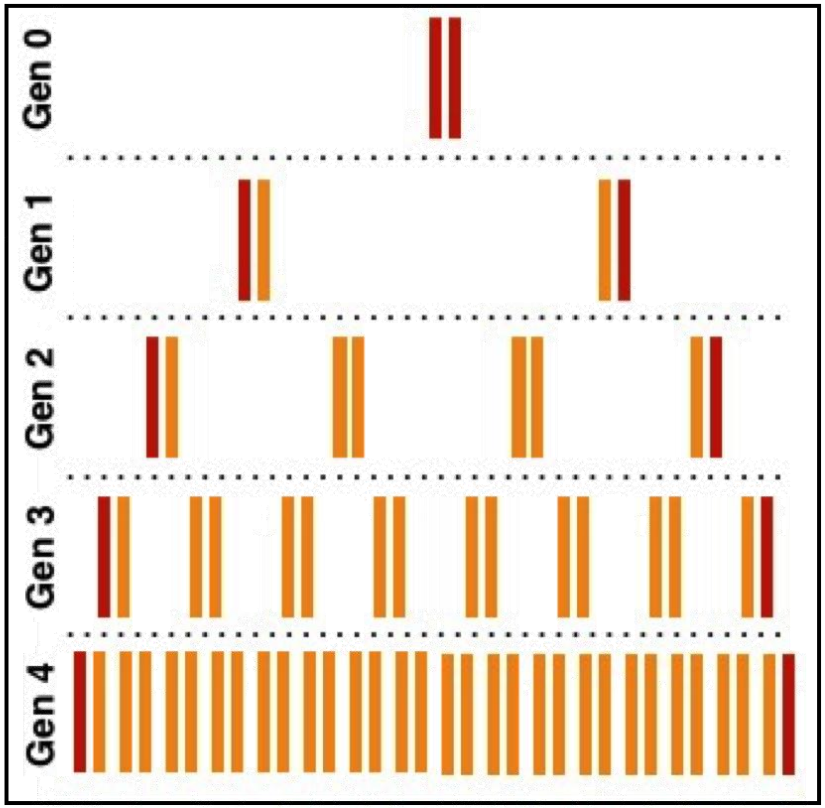
DNA REPLICATION

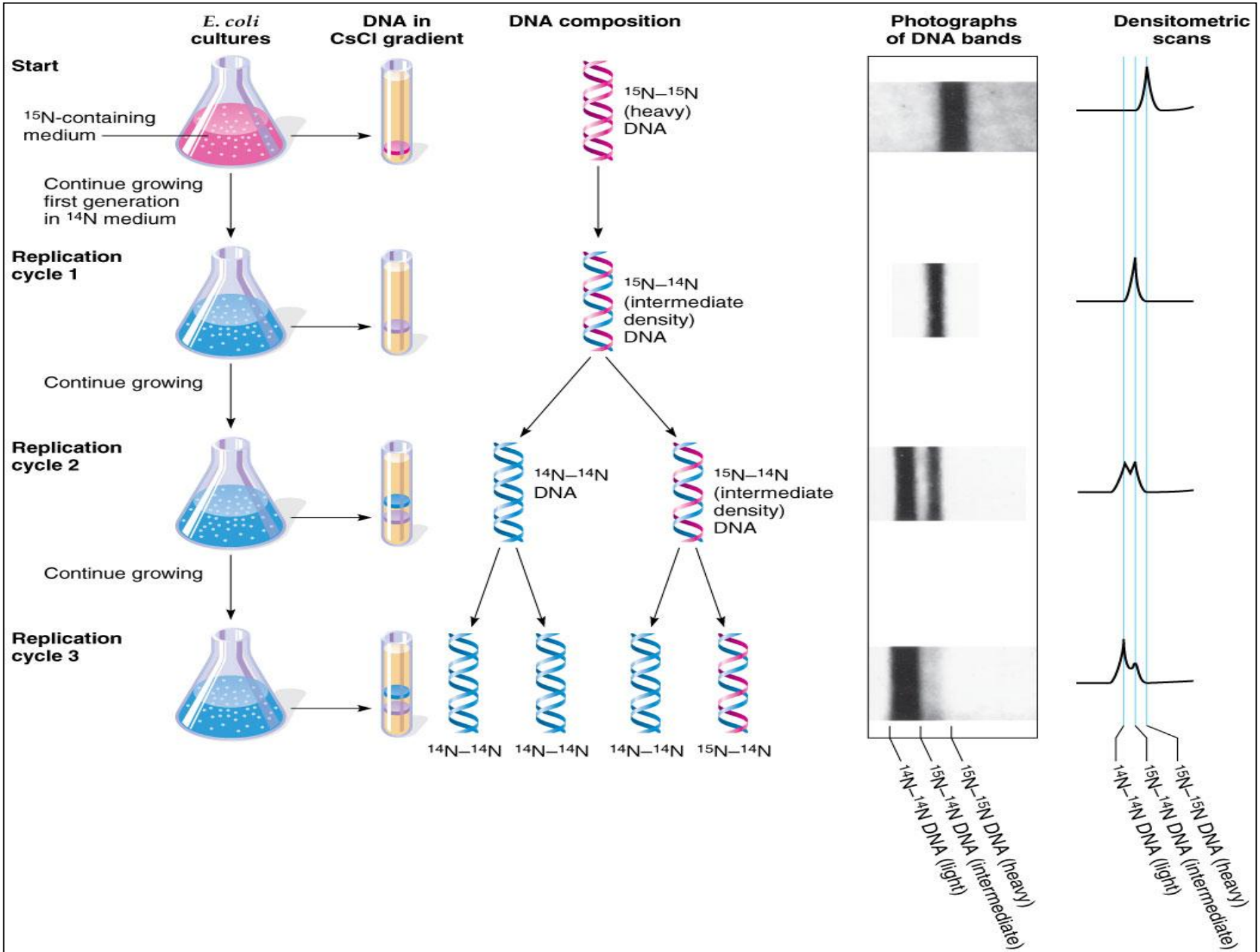
- Occur before cell division at interphase stage.
- A combination of many individual process like origin, strand separation, initiation, polymerization, proof-reading, termination.
- Semiconservative, Bidirectional, Semi-discontinuous process.

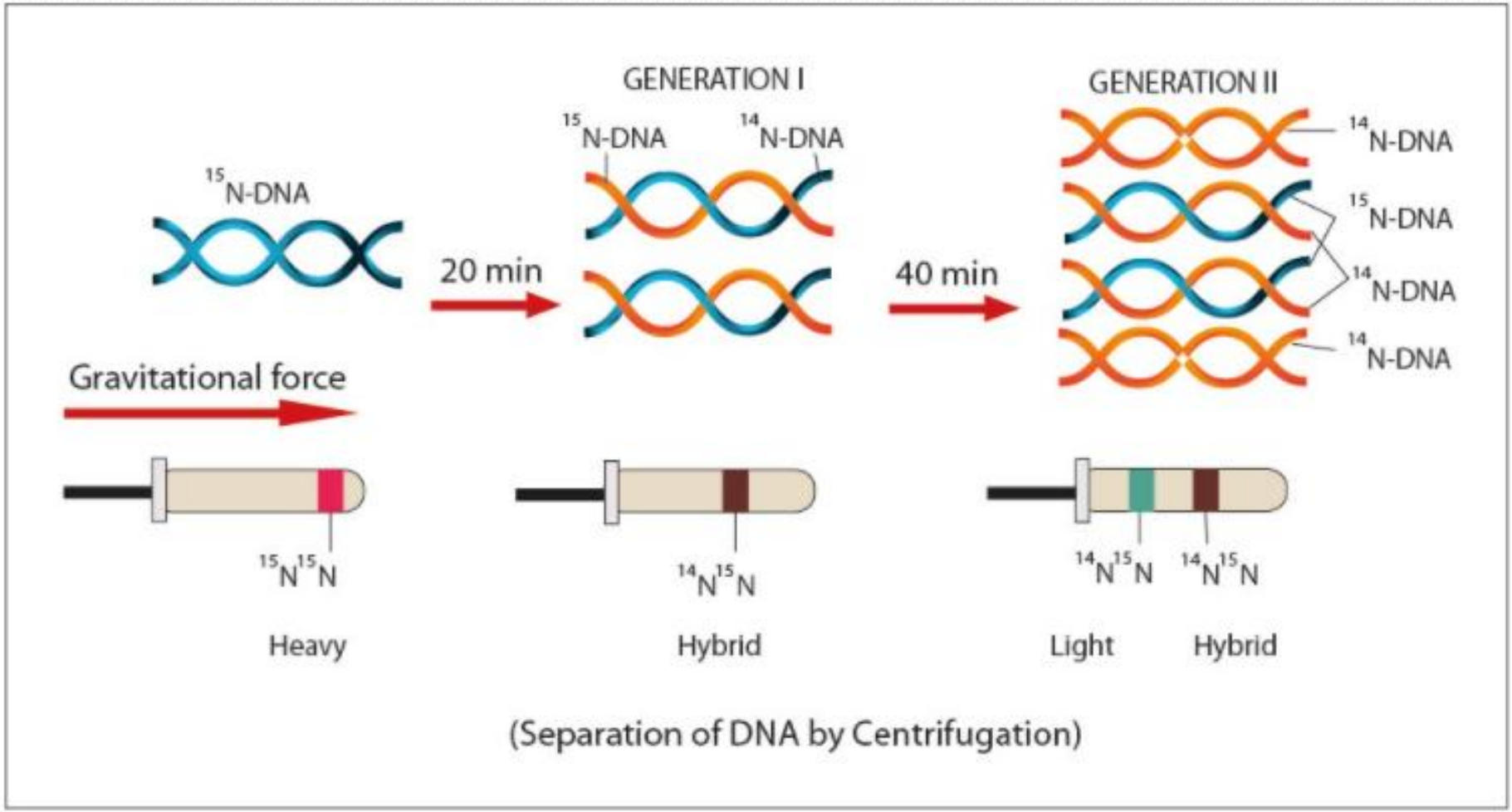

a) Semiconservative model
Parental
After first replication cycle
After second replication cycle
b) Conservative model
Parental
After first replication cycle
After second replication cycle
c) Dispersive model
Parental
After first replication cycle
After second replication cycle

Meselson and Stahl Experiment to prove semiconservative DNA replication






E. coli cultures
DNA in CsCl gradient
DNA composition
Photographs of DNA bands
Densitometric scans
Start
15N-containing medium
Continue growing first generation in 14N medium
Replication cycle 1
Continue growing
Replication cycle 2
Continue growing
Replication cycle 3
15N–15N (heavy) DNA
15N–14N (intermediate density) DNA
14N–14N DNA
15N–14N (intermediate density) DNA
14N–14N
14N–14N
14N–14N
15N–14N
14N–14N DNA (light)
15N–14N DNA (intermediate)
15N–15N DNA (heavy)
14N–14N DNA (light)
15N–14N DNA (intermediate)
15N–15N DNA (heavy)



(Separation of DNA by Centrifugation)

Molecular details:

Requirements:

1. Template
2. Free 3’ – OH group
3. Nucleotides (Building Blocks)
4. Enzymes
5. Energy

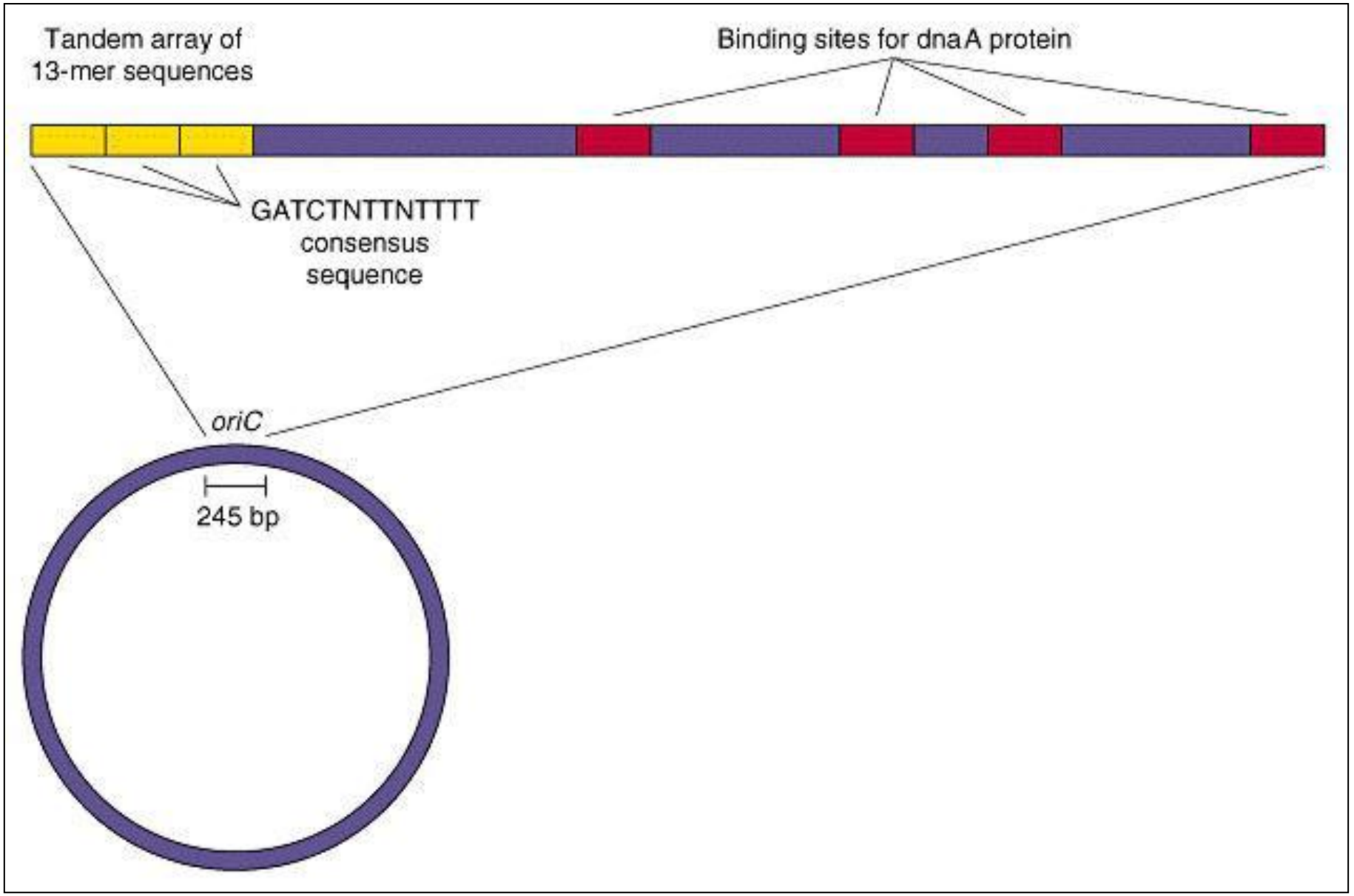
Enzymes and Proteins:

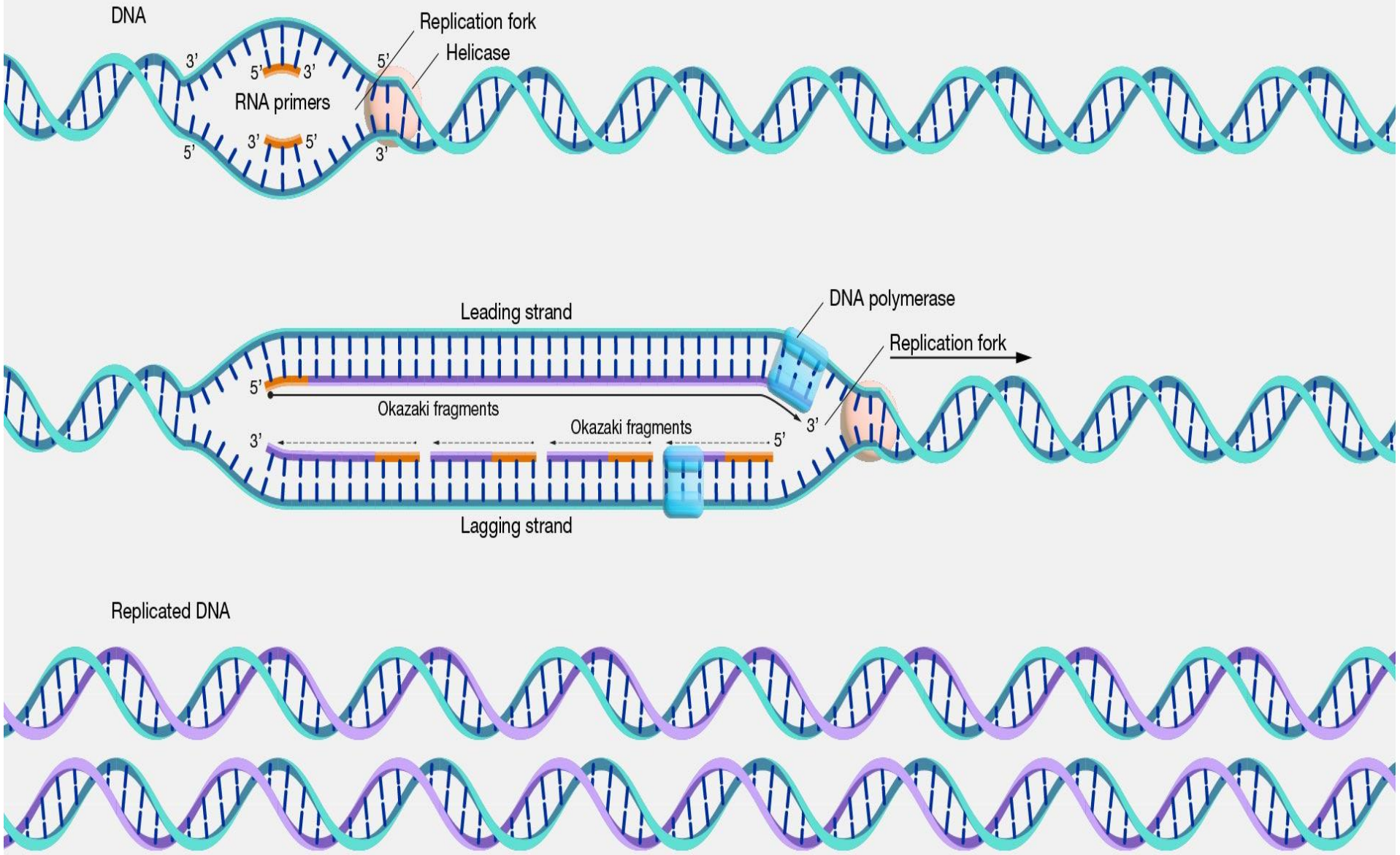
Require 20 or more different enzymes and proteins -

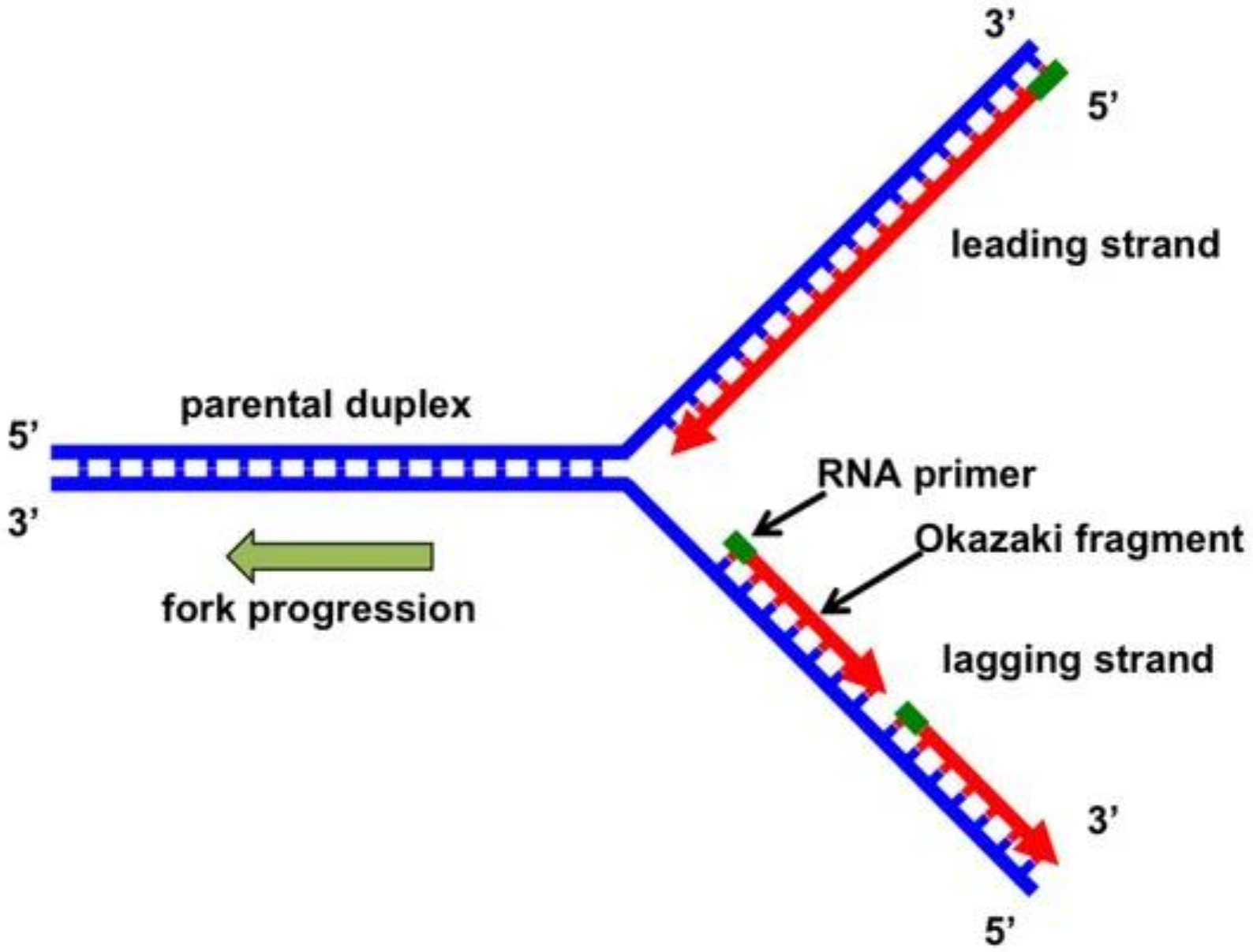
1. DNA Polymerase I
2. DNA Polymerase II
3. DNA Polymerase III
4. DNA Helicase
5. DNA Gyrase
6. Primase
7. Ligase
8. DBP
9. SSB

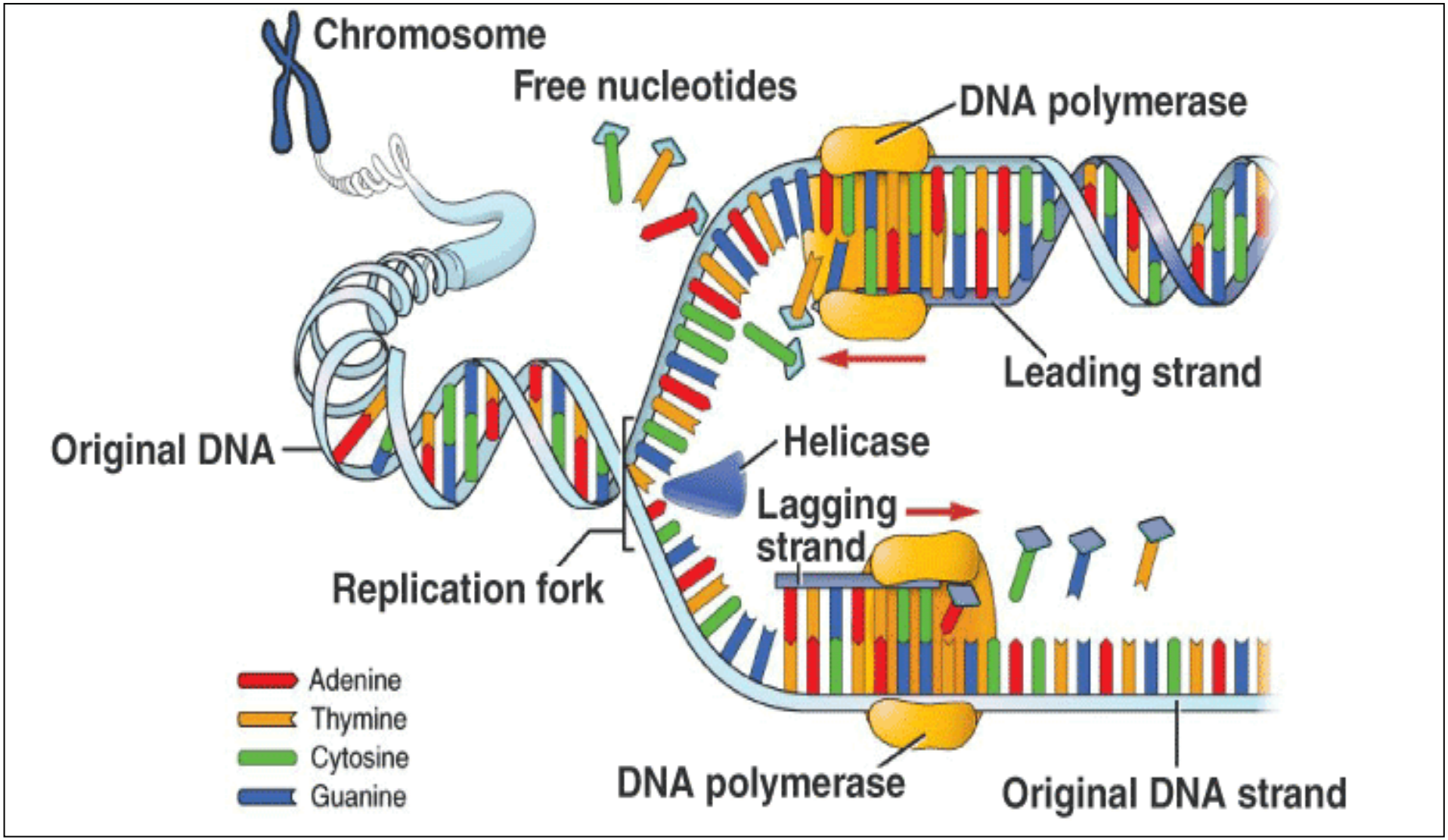
Activities	DNA Polymerase		
	I	II	III
5’ to 3’ polymerization	yes	yes	yes
3’ to 5’ exonuclease	yes	yes	yes
5’ to 3’ exonuclease	yes	no	no

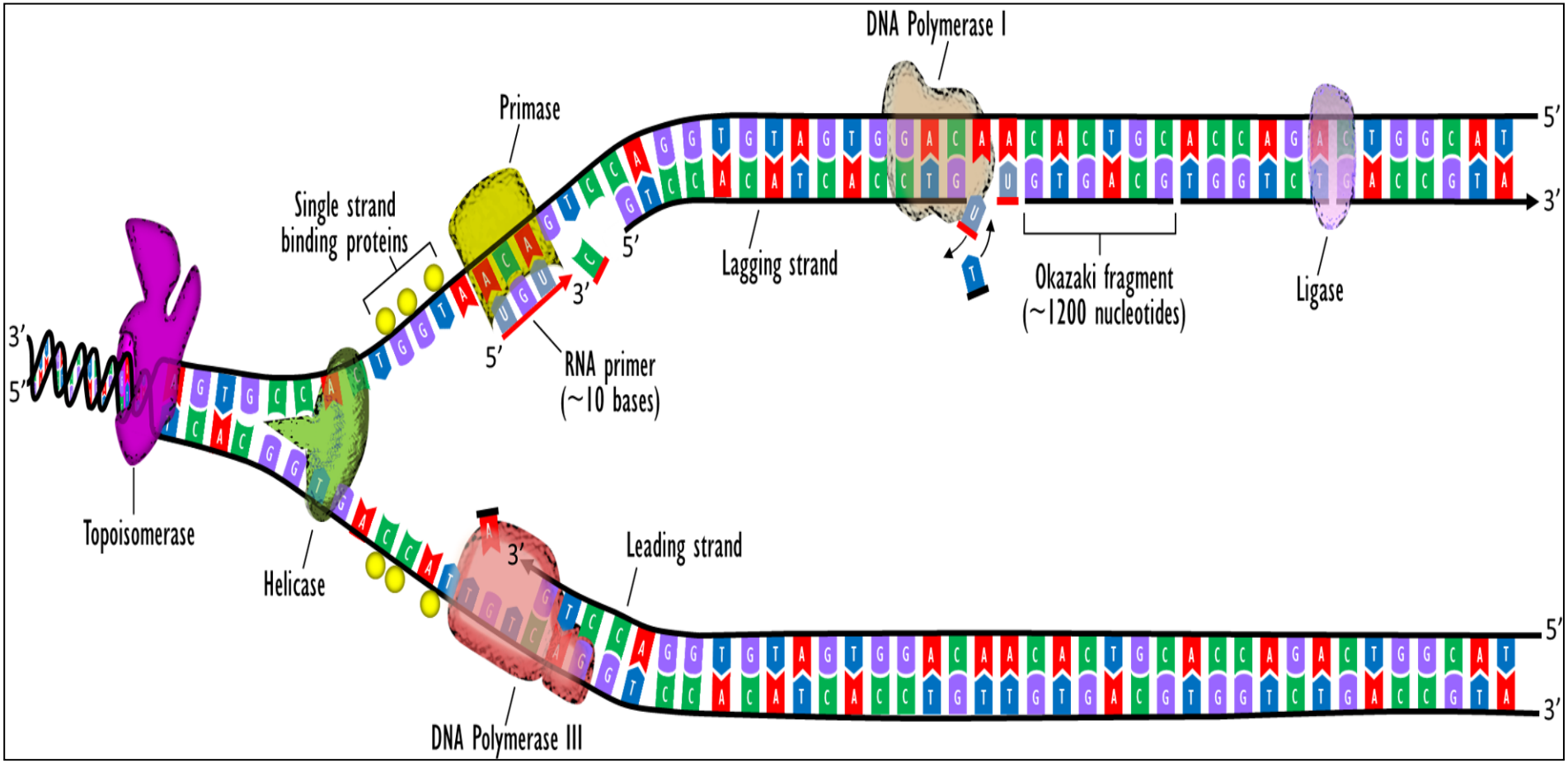
Origin of *E.coli* DNA replication

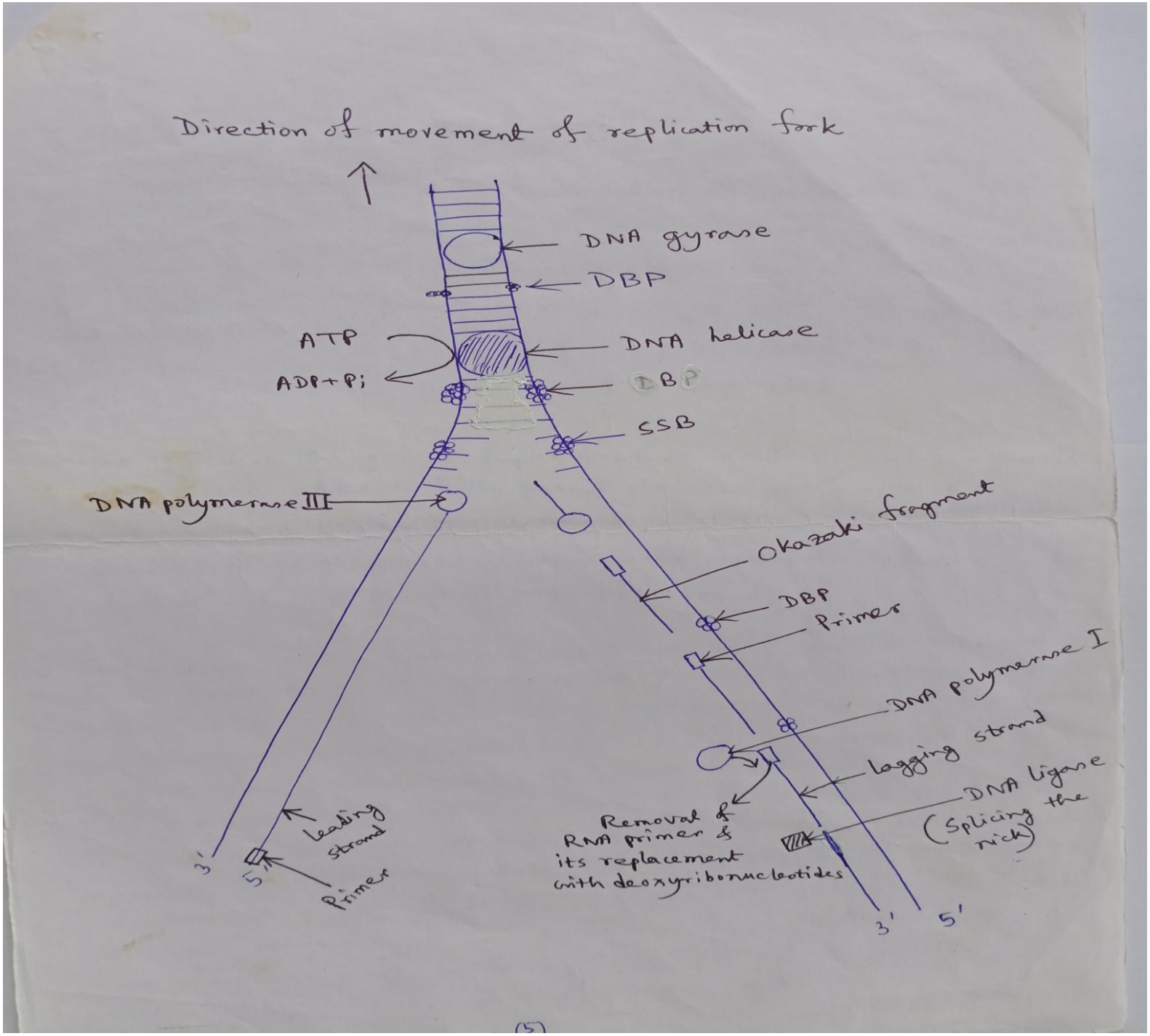



DNA
Replication fork
Helicase
RNA primers
3'
5'
5'
3'
3'
5'
5'
3'
Leading strand
DNA polymerase
Replication fork
5'
3'
Okazaki fragments
Okazaki fragments
3'
5'
Lagging strand
Replicated DNA


3’
5’
leading strand
parental duplex
5’
3’
RNA primer
Okazaki fragment
fork progression
lagging strand
3’
5’


Chromosome
Free nucleotides
DNA polymerase
Leading strand
Original DNA
Helicase
Lagging strand
Replication fork
Adenine
Thymine
Cytosine
Guanine
DNA polymerase
Original DNA strand


DNA Polymerase I
Primase
Single strand
binding proteins
Lagging strand
Okazaki fragment
(~1200 nucleotides)
Ligase
5′
3′
RNA primer
(~10 bases)
Topoisomerase
Helicase
Leading strand
DNA Polymerase III


Direction of movement of replication fork
DNA gyrase
DBP
ATP
ADP+Pi
DNA helicase
DBP
SSB
DNA polymerase III
Okazaki fragment
DBP
Primer
DNA polymerase I
Lagging strand
DNA ligase
(Splicing the nick)
Removal of RNA primer & its replacement with deoxyribonucleotides
3'
5'
Leading strand
Primer
3'
5'

Proof reading :