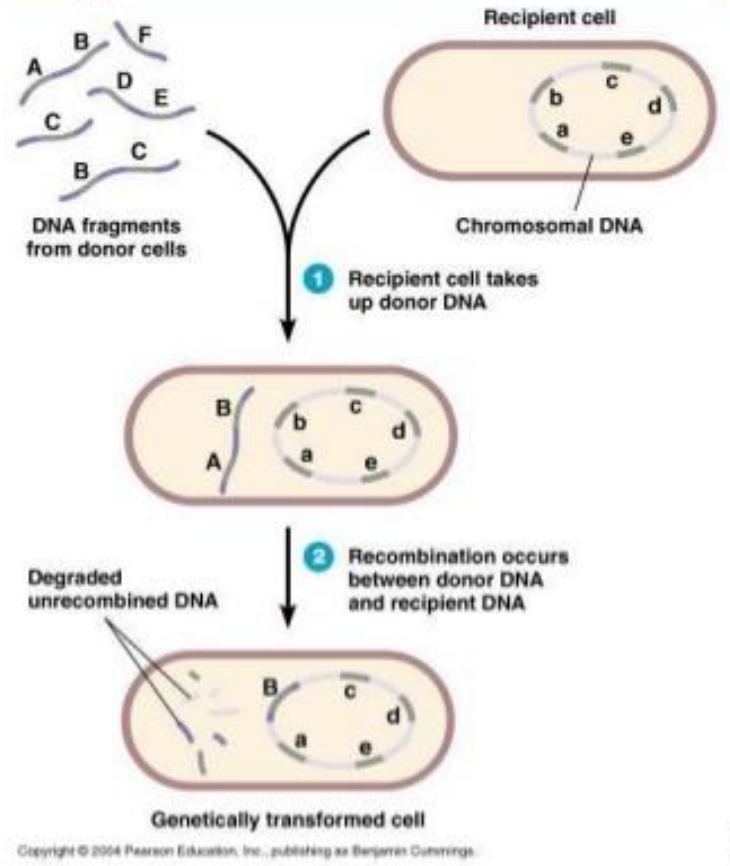
Transformation



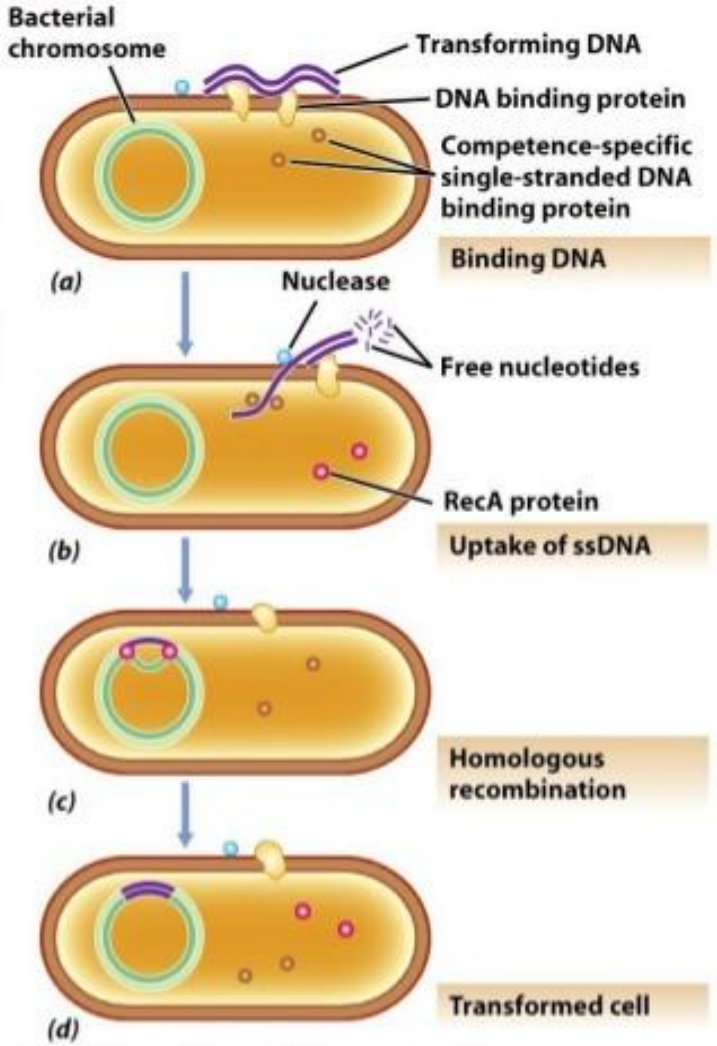
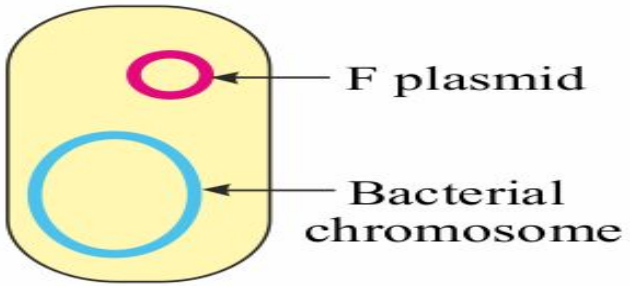
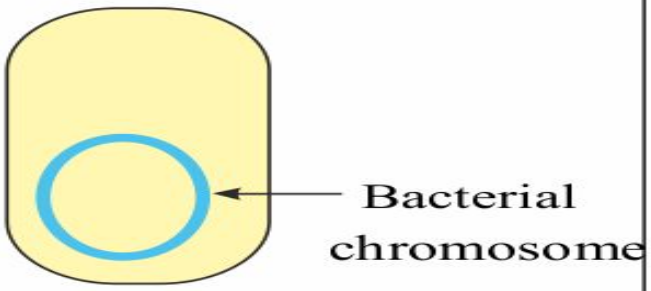
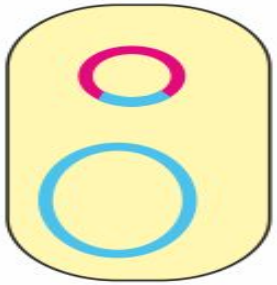
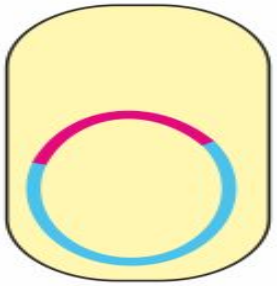
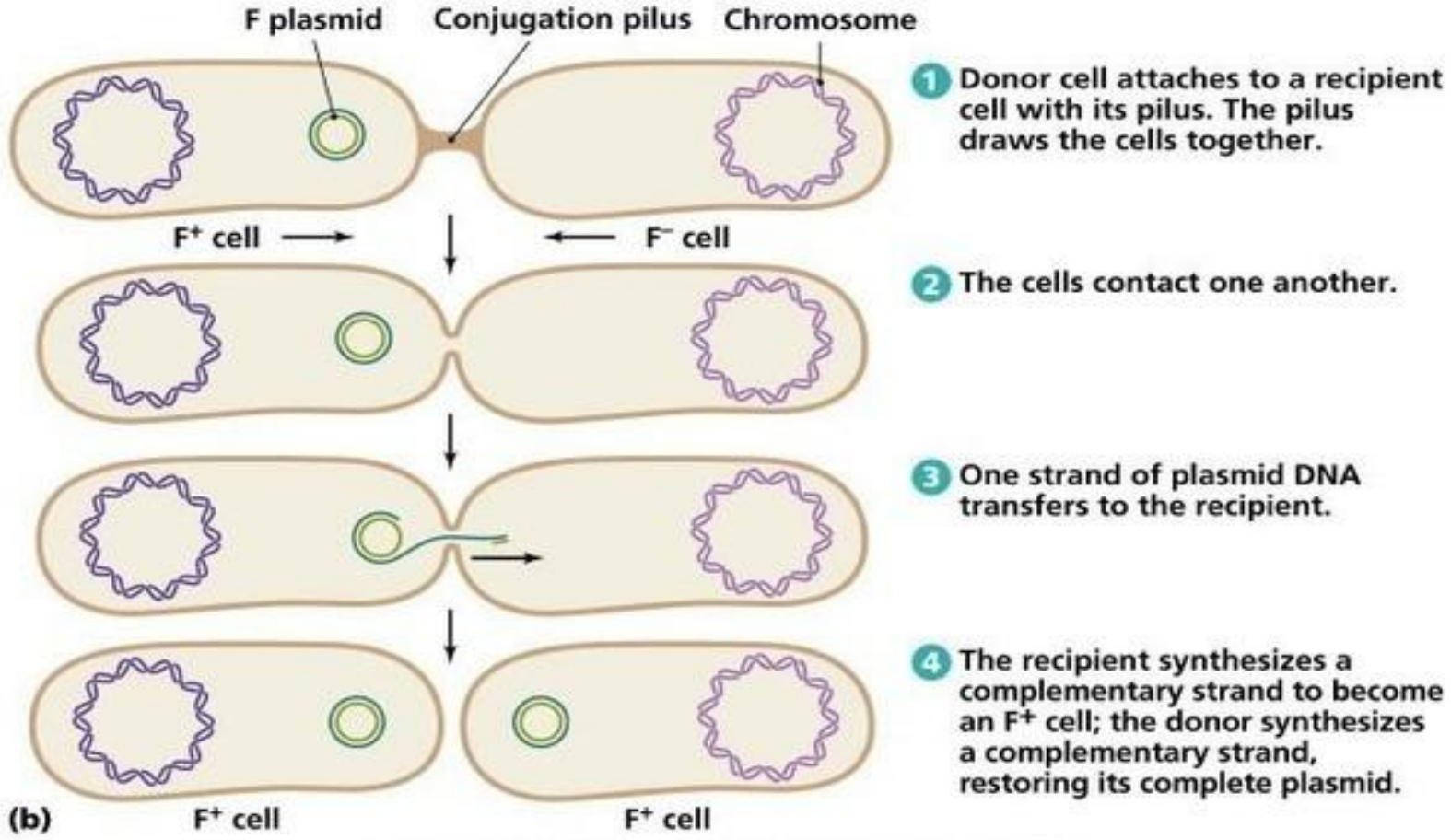


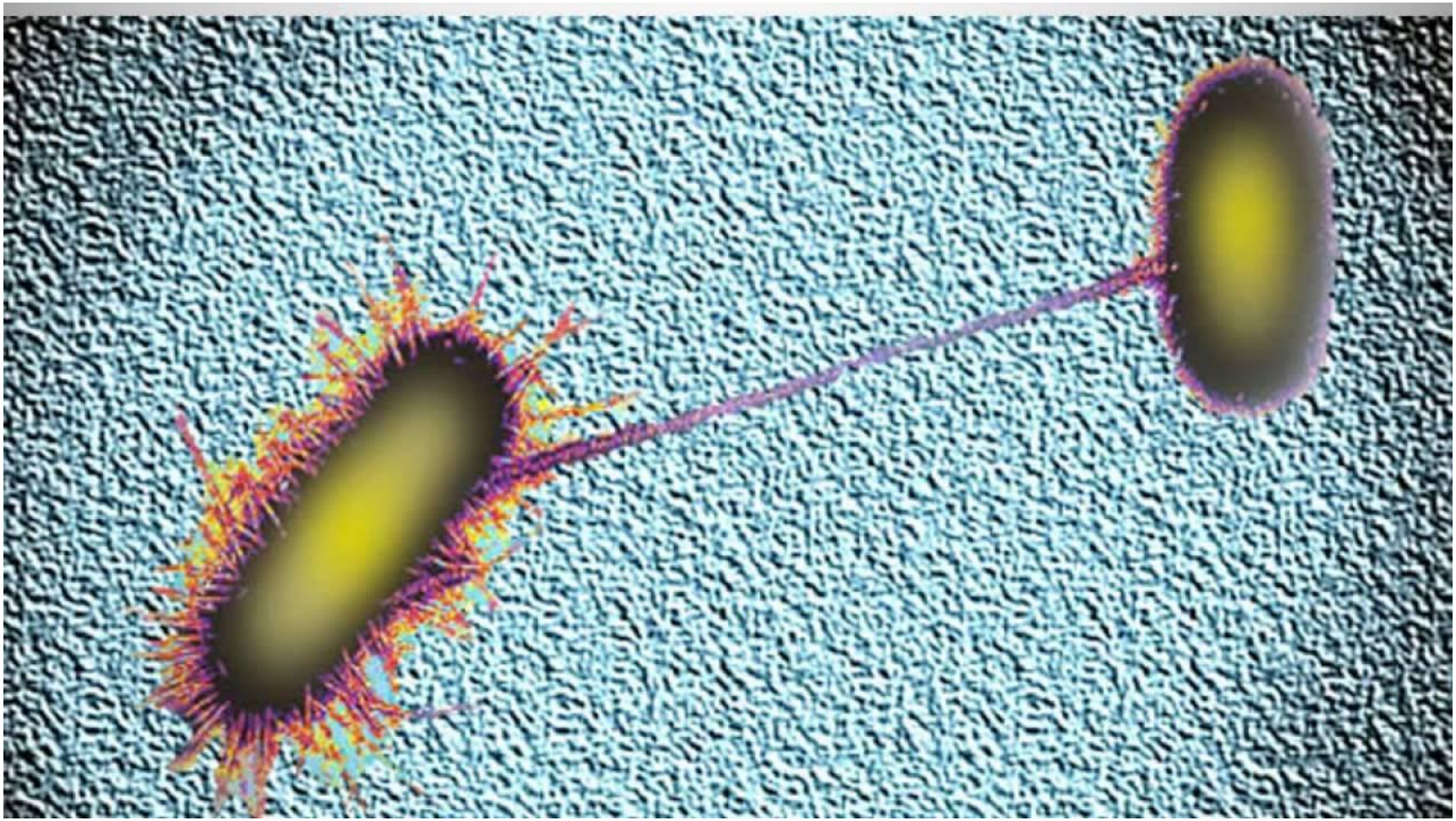
Figure 10-14 Brock Biology of Microorganisms 11/e

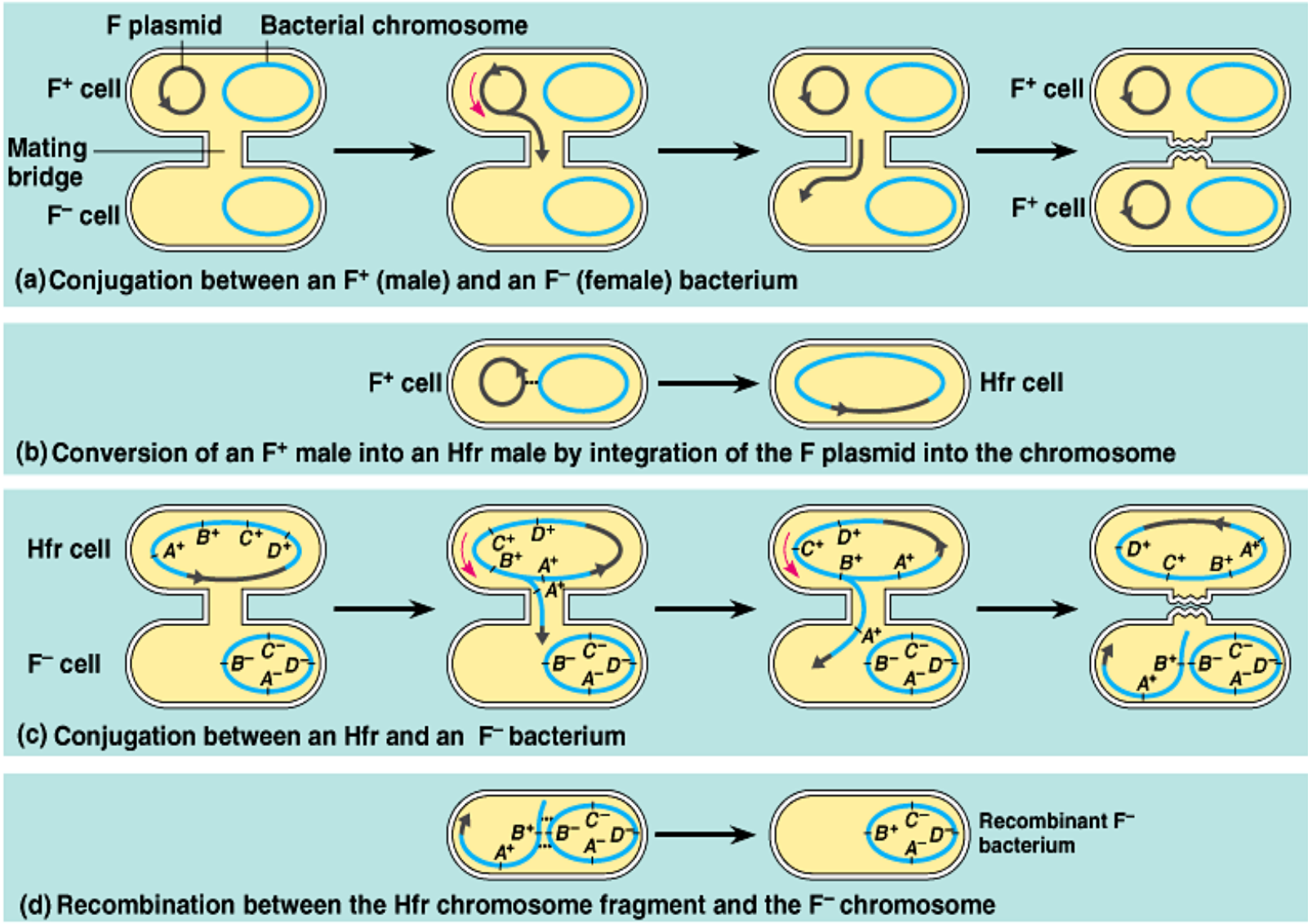
CONJUCATION

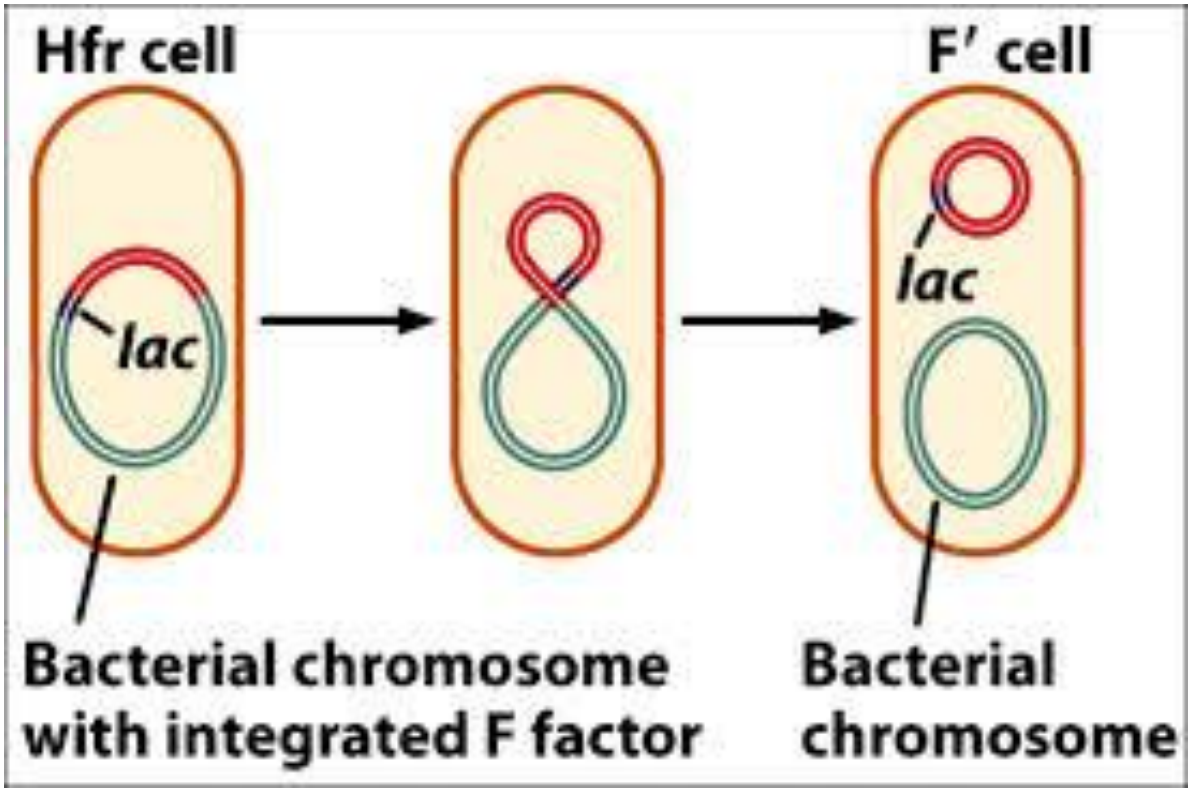
Types of cells involved in conjugation	
	
F^+ cell – with F plasmid	F^- cell – without F plasmid
	
F′ cell – with F plasmid containing few chromosomal genes	Hfr cell – F plasmid integrated in the bacterial chromosome

CONJUCATION



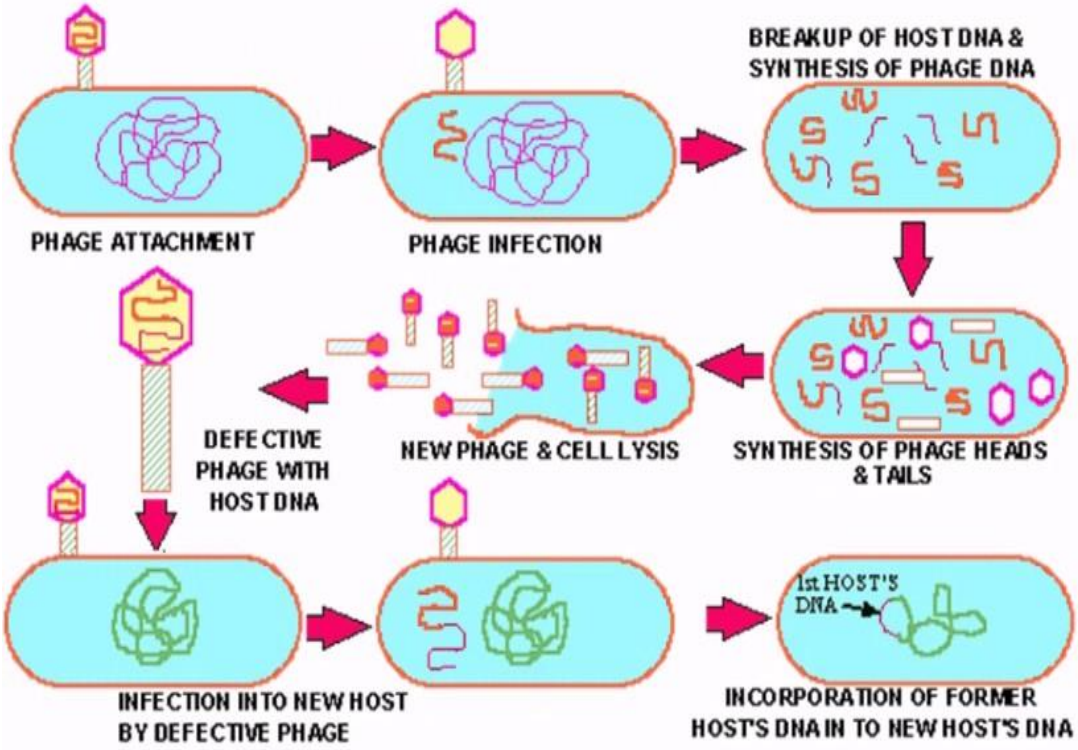





Hfr cell
F′ cell
lac
lac
Bacterial chromosome with integrated F factor
Bacterial chromosome

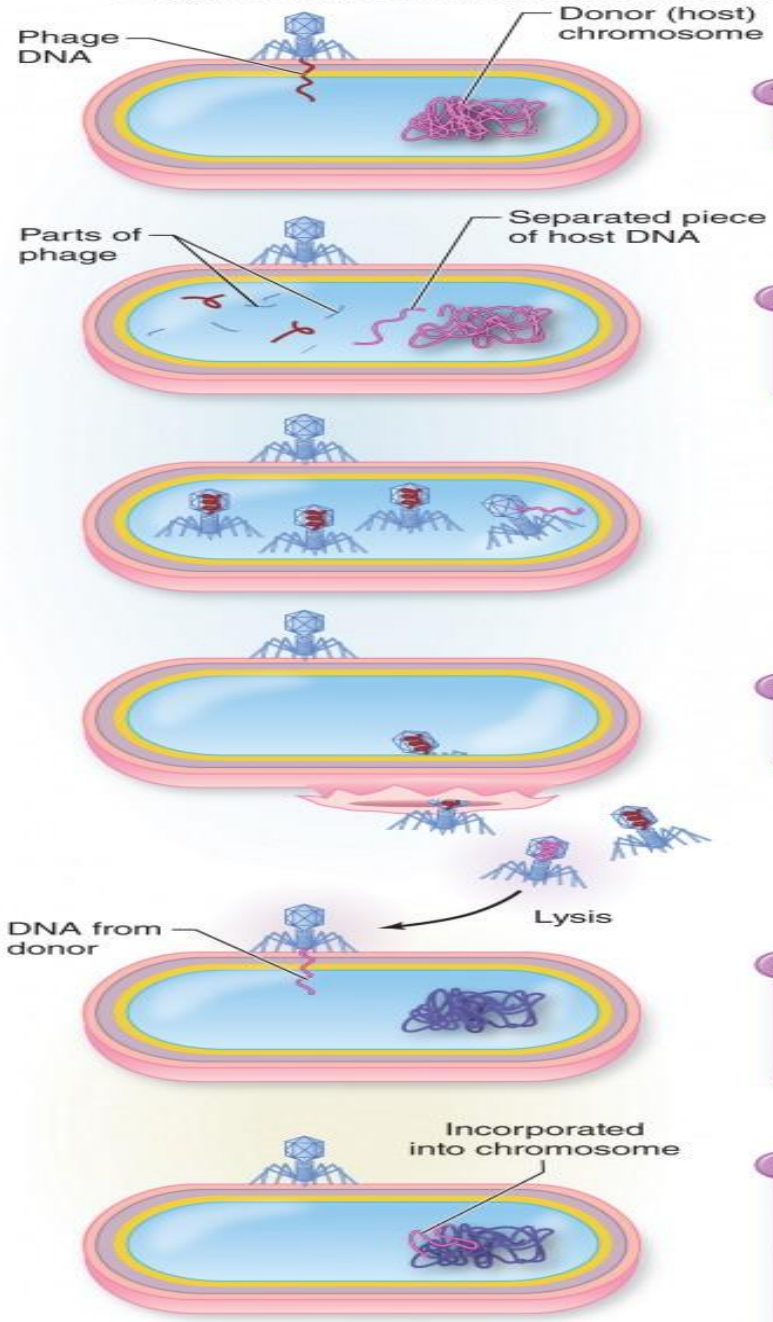
Transduction

Transduction is the process by which DNA is transferred from one bacterium to another by a virus



Does not need physical contact like conjugation

Dnase resistant



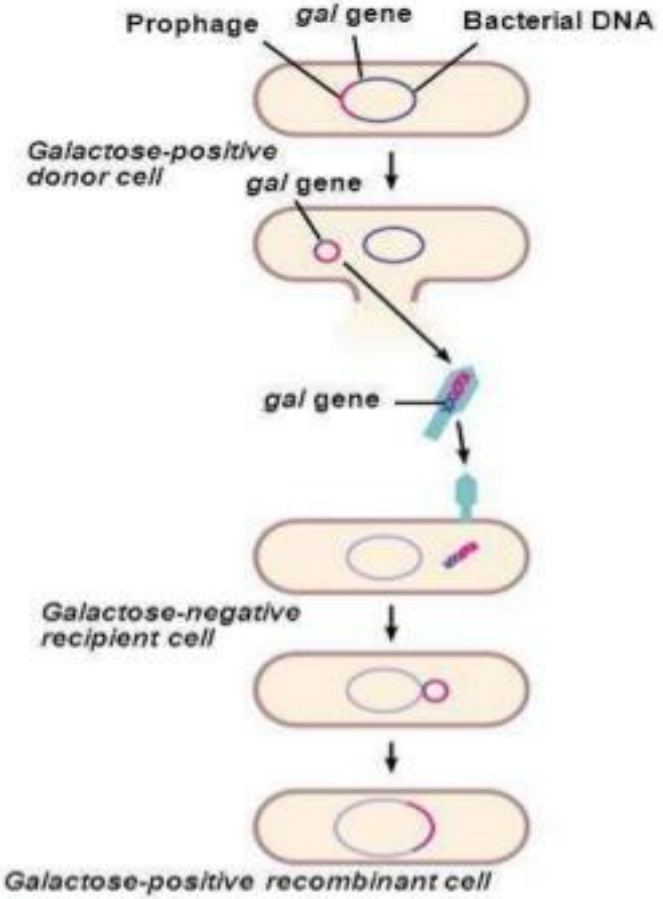
Cell A

1. A phage infects cell A (the donor cell) by normal means.
2. During replication and assembly, a phage particle incorporates a segment of bacterial DNA by mistake.
3. Cell A then lyses and releases the mature phages, including the genetically altered one.

Cell B

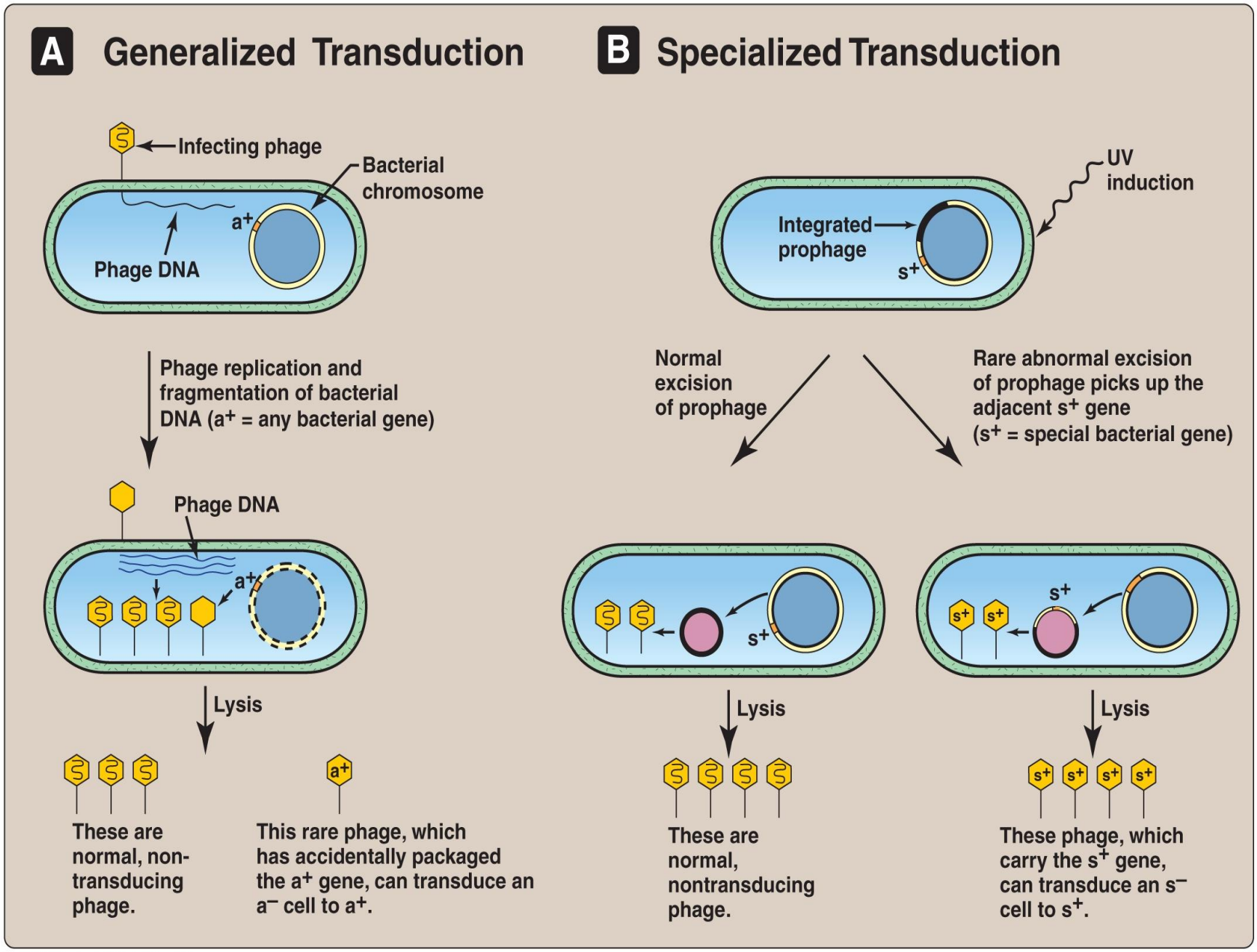
4. The altered phage absorbs to and penetrates another host cell (cell B), injecting the DNA from cell A rather than viral nucleic acid.
5. Cell B receives this donated DNA, which recombines with its own DNA. Because the virus is defective (biologically inactive as a virus), it is unable to complete a lytic cycle. The transduced cell survives and can use this new genetic material.

Specialized Transduction

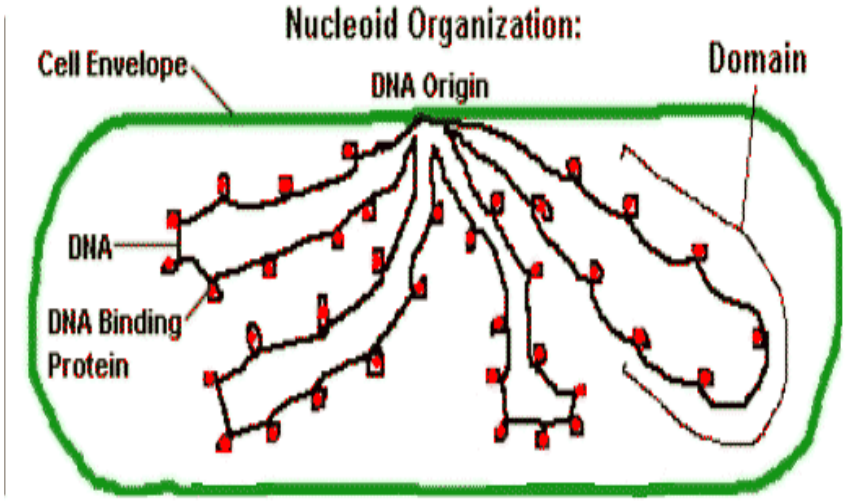


1. Prophage exists in galactose-using host (containing the *gal* gene).
2. Phage genome excises, carrying with it the adjacent *gal* gene from the host.
3. Phage matures and cell lyses, releasing phage carrying *gal* gene.
4. Phage infects a cell that cannot utilize galactose (lacking *gal* gene).
5. Along with the prophage, the bacterial *gal* gene becomes integrated into the new host's DNA.
6. Lysogenic cell can now metabolize galactose.

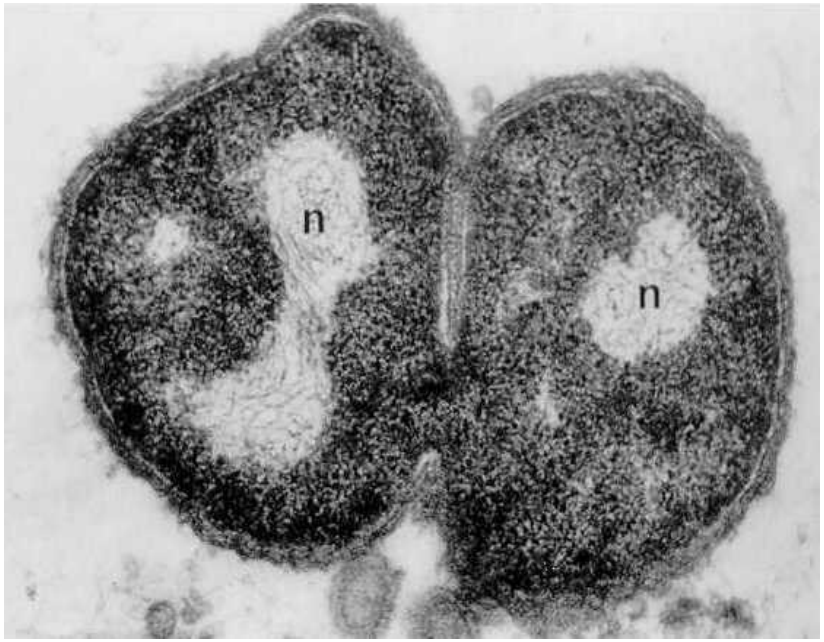
Figure 13.13

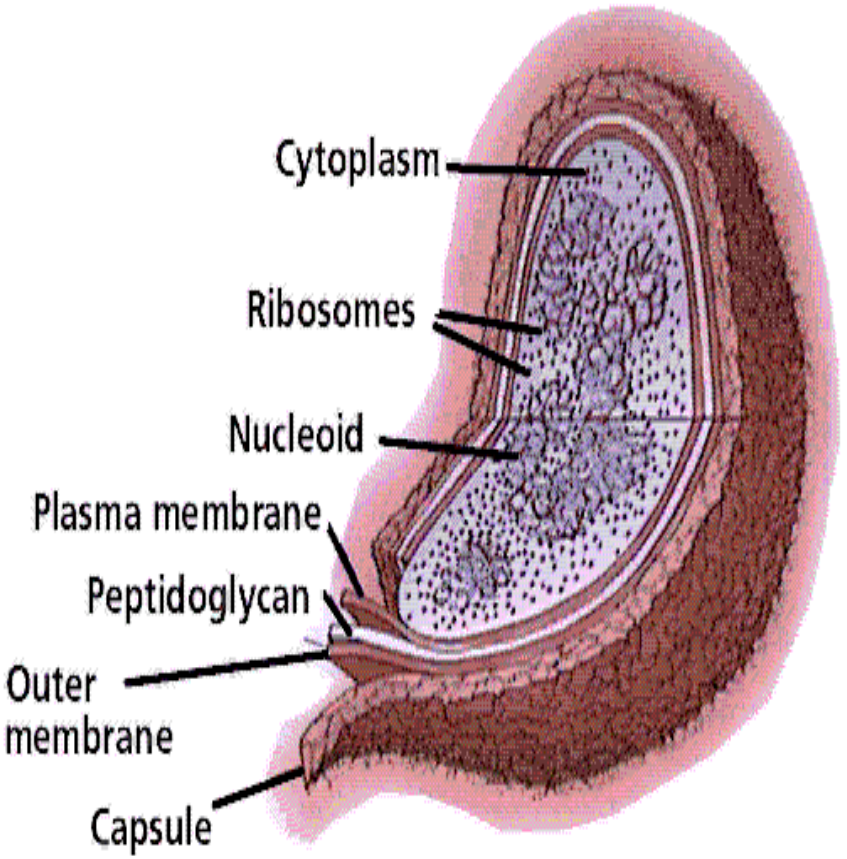


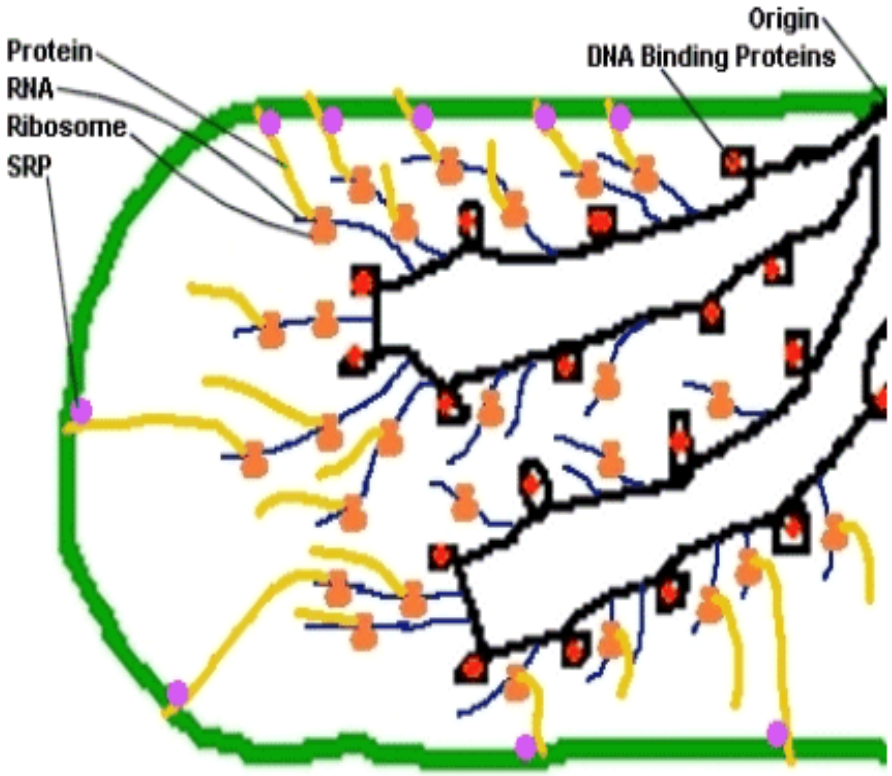
Bacterial Chromosome Structure



- **Prokaryotic cells** (bacteria) contain their chromosome as **circular DNA.**
- Usually the entire genome is a single circle, but often there are extra circles called **plasmids.**
- The DNA is packaged by **DNA-binding proteins**
- The bacterial DNA is packaged in loops back and forth.
- The bundled DNA is called the **nucleoid**.
- It concentrates the DNA in part of the cell, but it is not separated by a nuclear membrane (as in eukaryotes.)
- The DNA does form loops back and forth to a **protein core, attached to the cell wall**

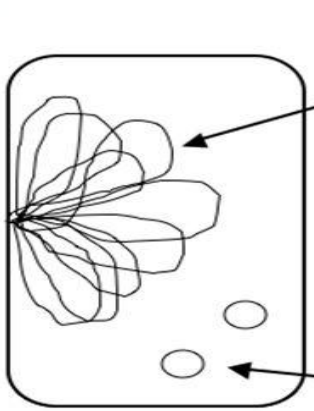

n
n


Cytoplasm
Ribosomes
Nucleoid
Plasma membrane
Peptidoglycan
Outer membrane
Capsule

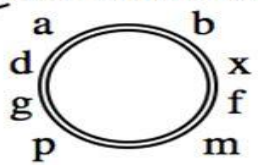


- The DNA is accessible to enzymes that make RNA and protein In the bacterial cell,
- the DNA gets transcribed to RNA, and
- the RNA gets translated to protein before it is completed

Genetic Information in Bacterial Cells



Bacterial Chromosome

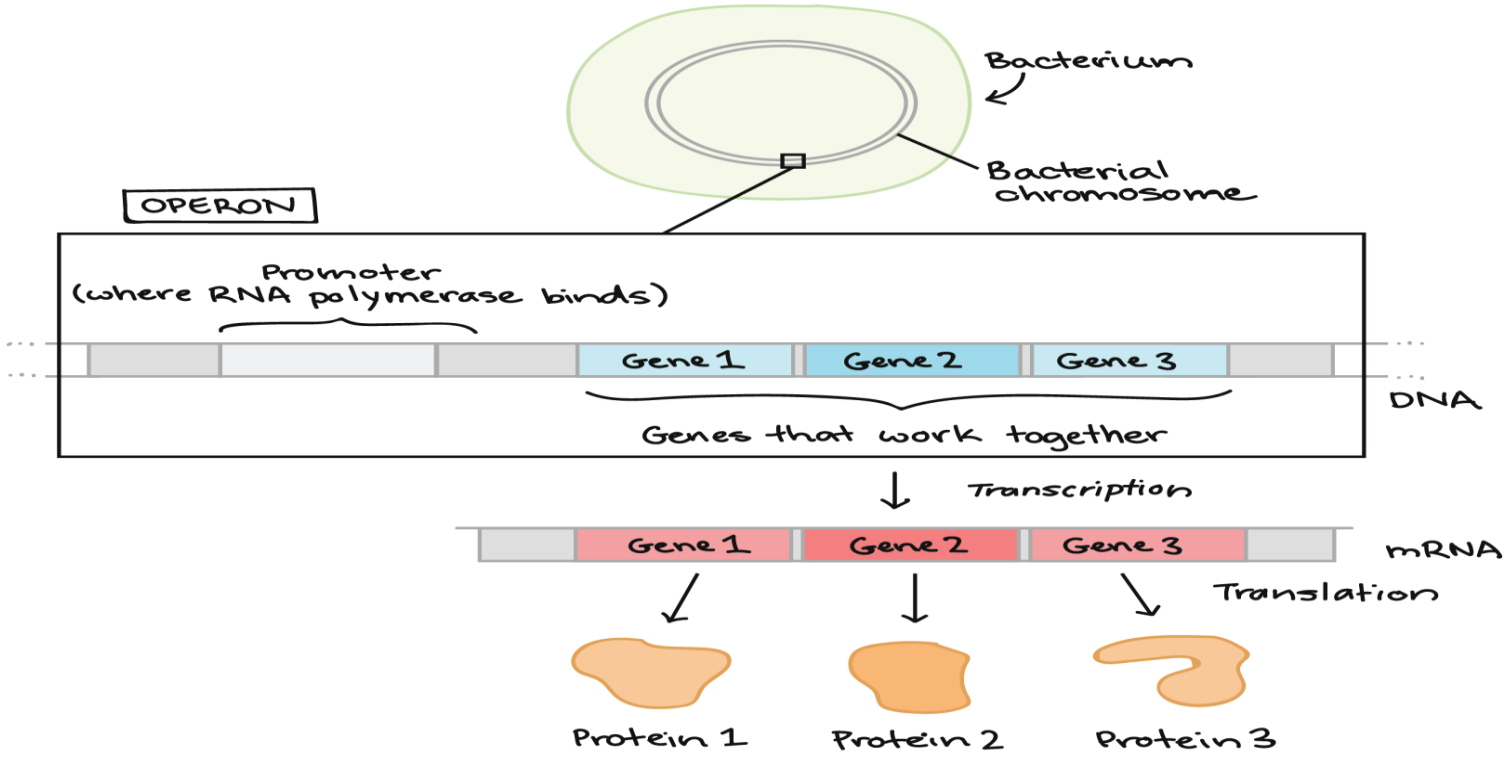


1 copy of each gene
supercoiled

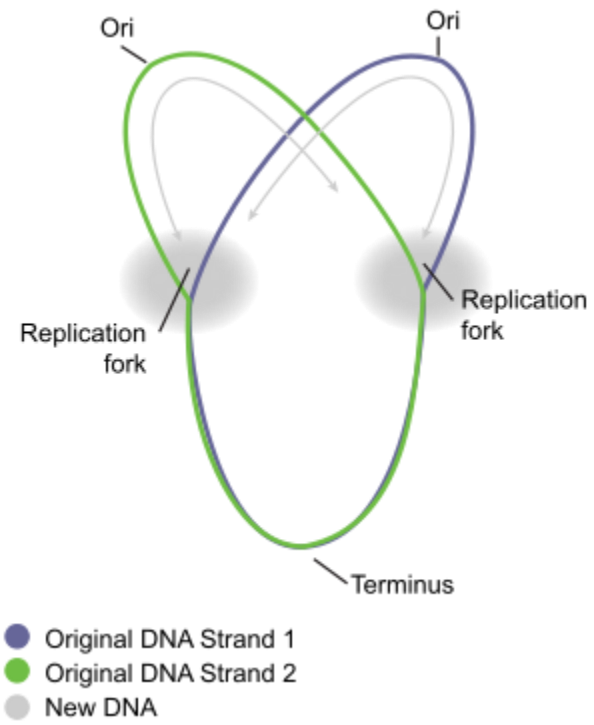
Plasmids (episomes)

circular DNA- 1-many
replicate autonomously
supercoiled

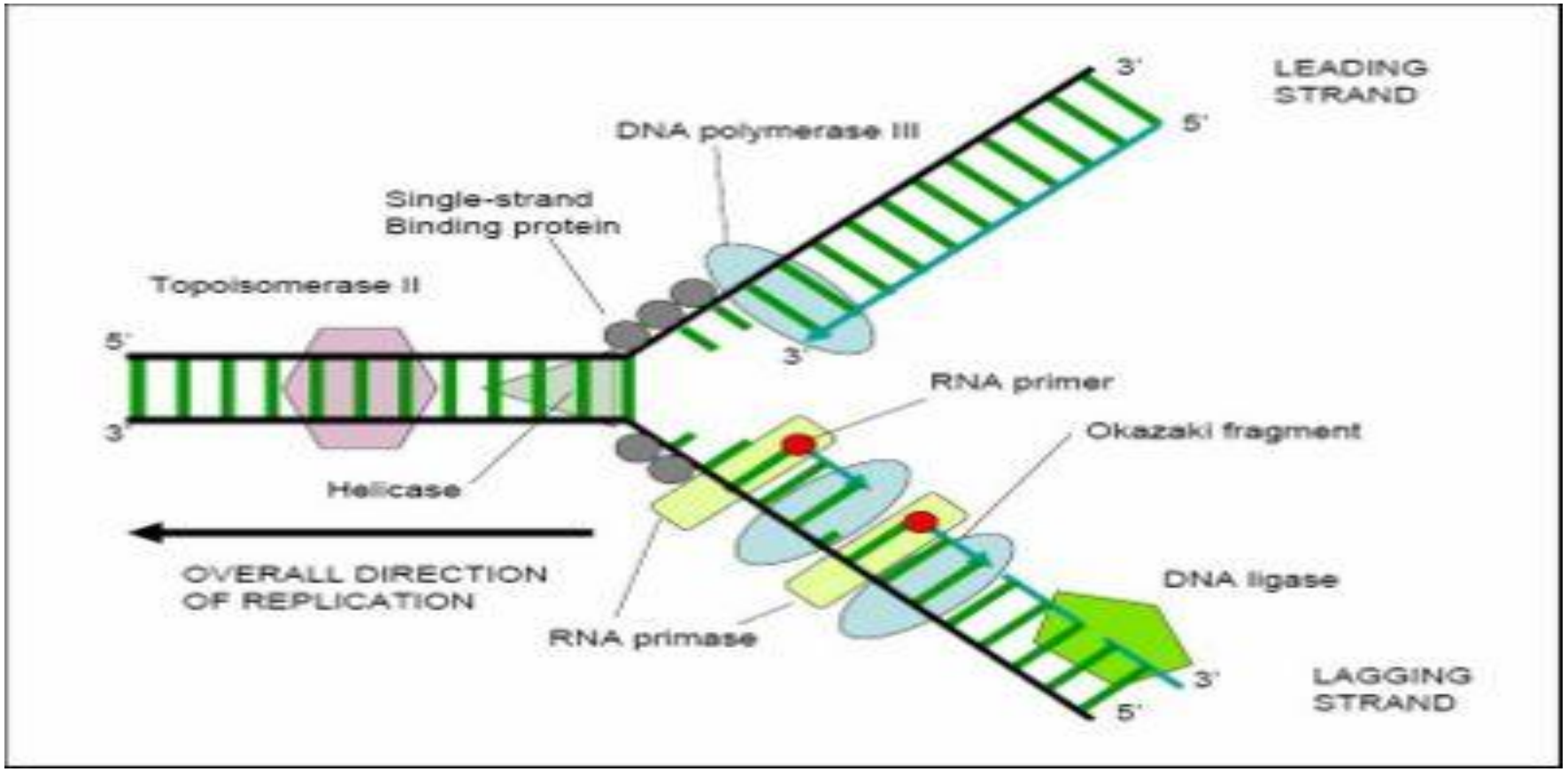
operon

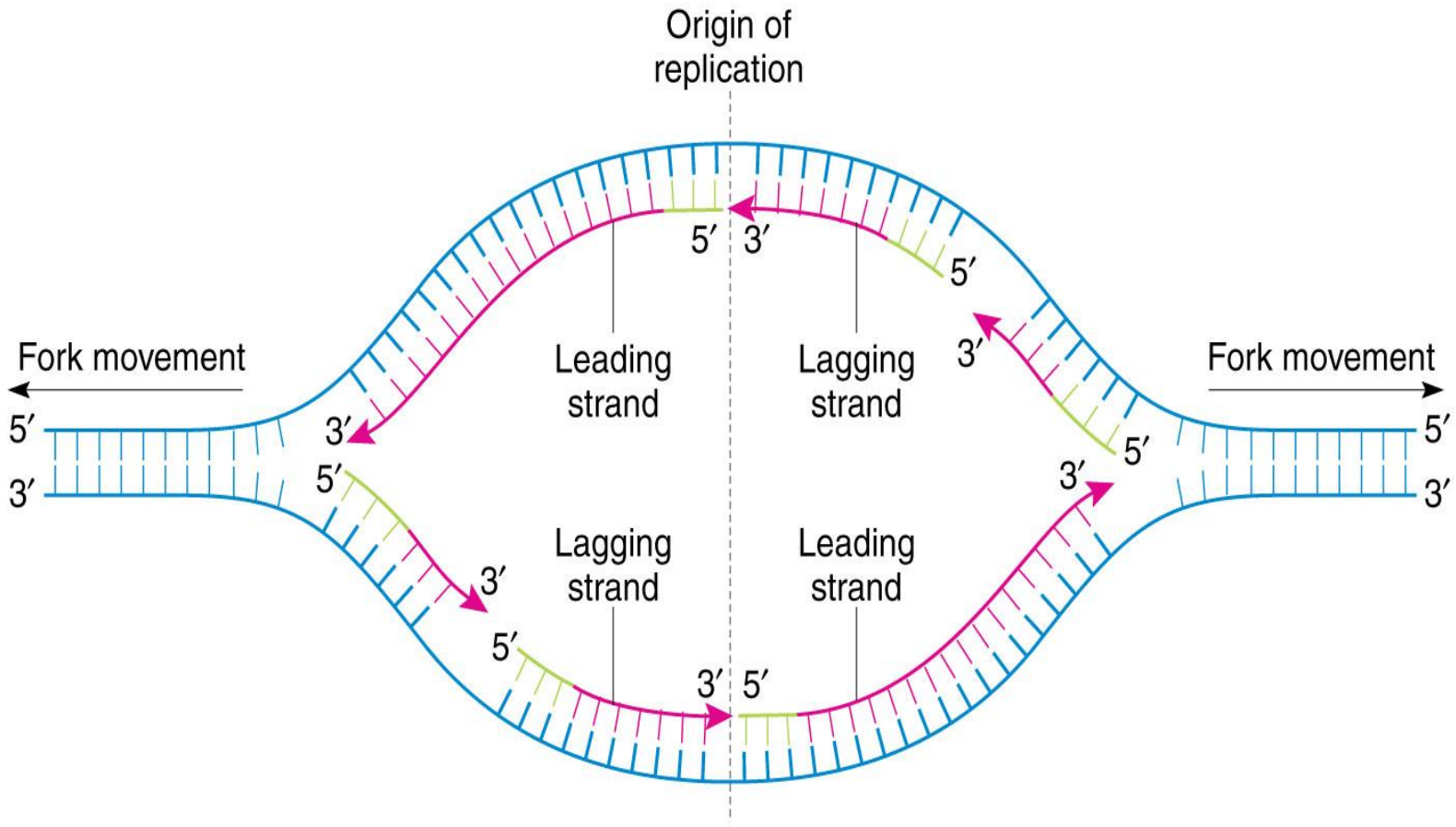

Bacterium
Bacterial chromosome
OPERON
Promoter (where RNA polymerase binds)
Gene 1
Gene 2
Gene 3
DNA
Genes that work together
Transcription
Gene 1
Gene 2
Gene 3
mRNA
Translation
Protein 1
Protein 2
Protein 3

Replication



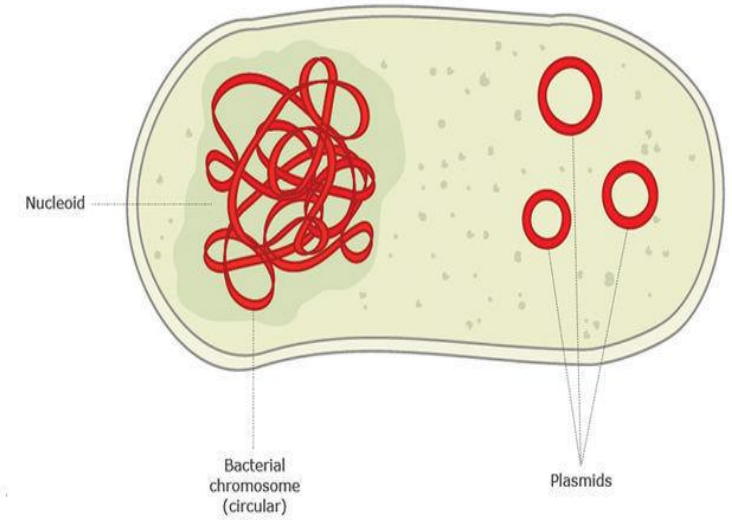
- A circular bacterial chromosome, showing DNA replication proceeding bidirectionally, with two replication forks generated at the "origin". Each half of the chromosome replicated by one replication fork is called a "replichore


3'
LEADING STRAND
5'
DNA polymerase III
Single-strand Binding protein
Topoisomerase II
5'
3'
3'
RNA primer
Okazaki fragment
Helicase
OVERALL DIRECTION OF REPLICATION
DNA ligase
RNA primase
3'
LAGGING STRAND
5'



plasmids

- A **plasmid** is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA.
- **Plasmids** naturally exist in **bacterial** cells, and they also occur in some eukaryotes.
- Often, the genes carried in **plasmids** provide **bacteria** with genetic advantages, such as antibiotic resistance

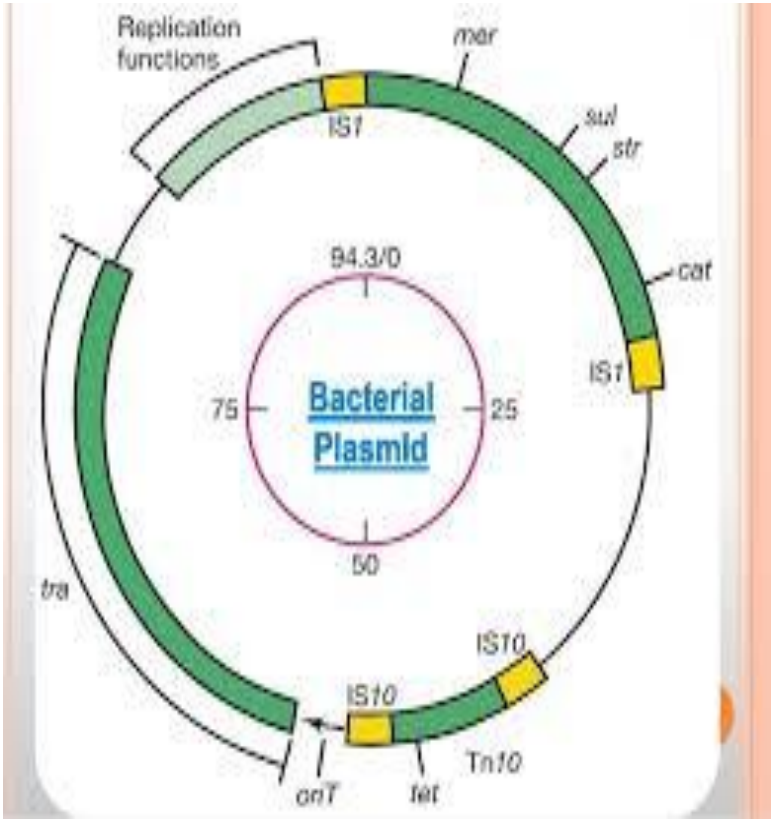


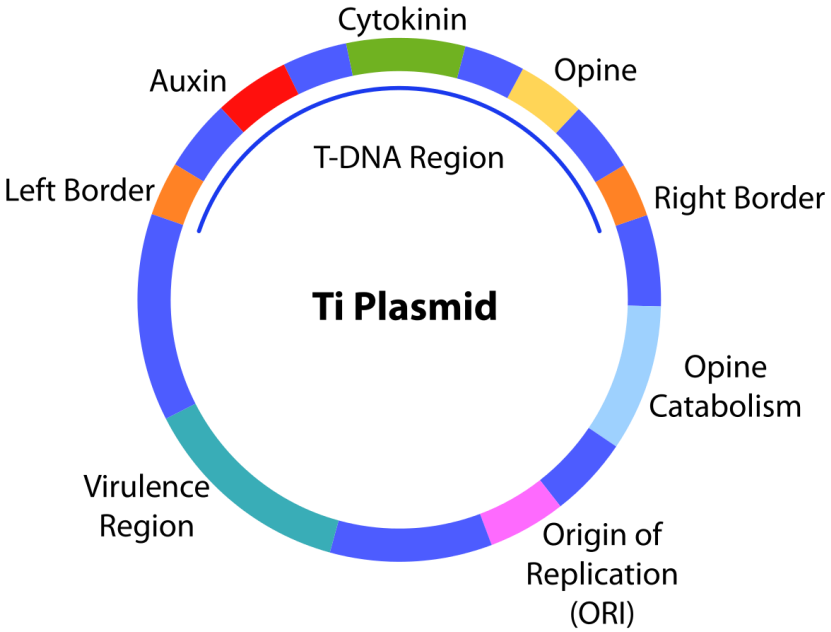
Conjugative and Non-Conjugative plasmids

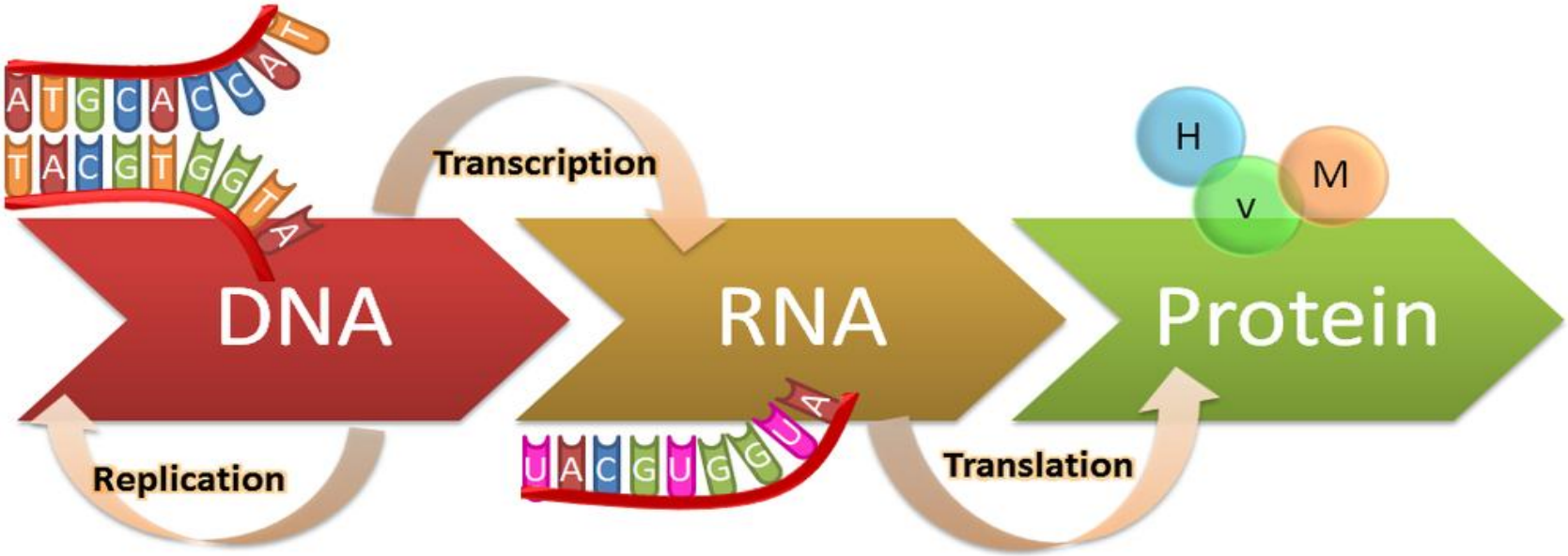
- Bacteria reproduce by sexual conjugation, which is the transfer of genetic material from one bacterial cell to another, either through direct contact or a bridge between the two cells.
- Conjucative plasmids contain genes called transfer genes that facilitate the beginning of conjugation.
- Non-conjugative plasmids cannot start the conjugation process, and they can only be transferred through sexual conjugation with the help of conjugative plasmids.

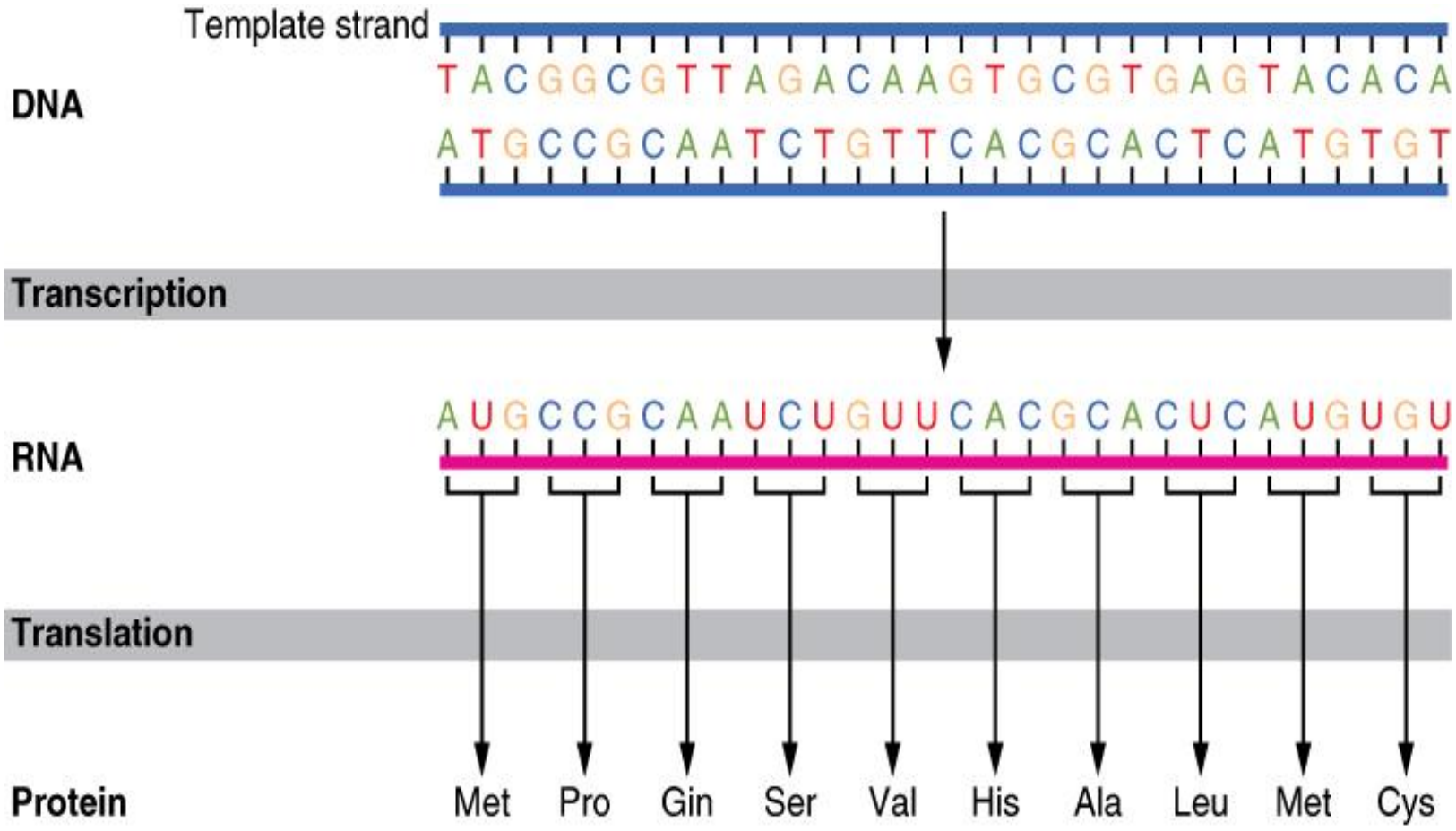
- **Incompatibility group**
- In a bacterium, different plasmids can co-occur if they are compatible with each other.
- An incompatible plasmid will be expelled from the bacterial cell.
- Plasmids are incompatible if they have the same reproduction strategy in the cell;
- this allows the plasmids to inhabit a certain territory within it without other plasmids interfering

plasmids

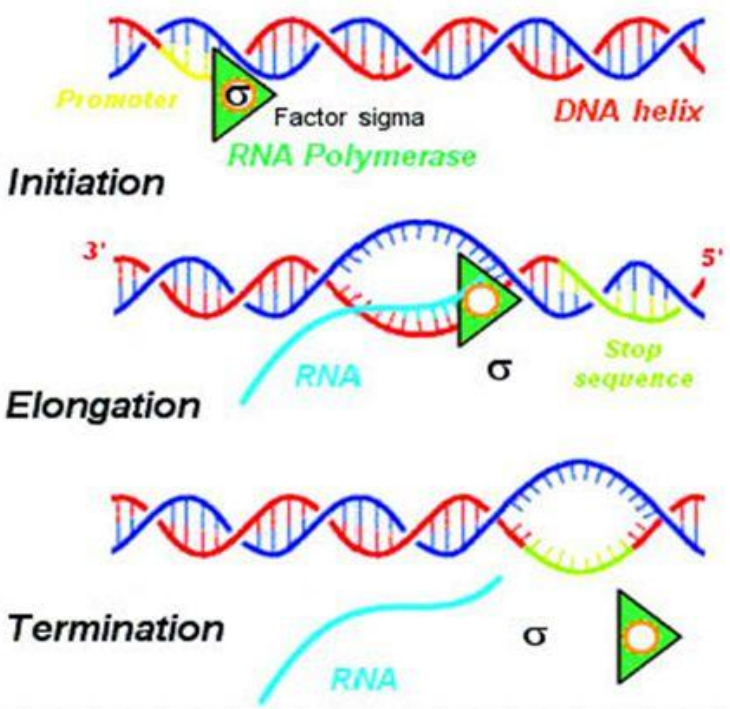

Replication functions
mer
IS1
sul
str
94.3/0
cat
IS1
75
Bacterial Plasmid
25
50
tra
IS10
IS10
Tn10
oriT
tet


Cytokinin
Auxin
Opine
T-DNA Region
Left Border
Right Border
Ti Plasmid
Opine Catabolism
Virulence Region
Origin of Replication (ORI)


ATGCACCAT
TACGTGGTA
Transcription
DNA
RNA
Protein
H
M
v
UACGUGGUA
Replication
Translation

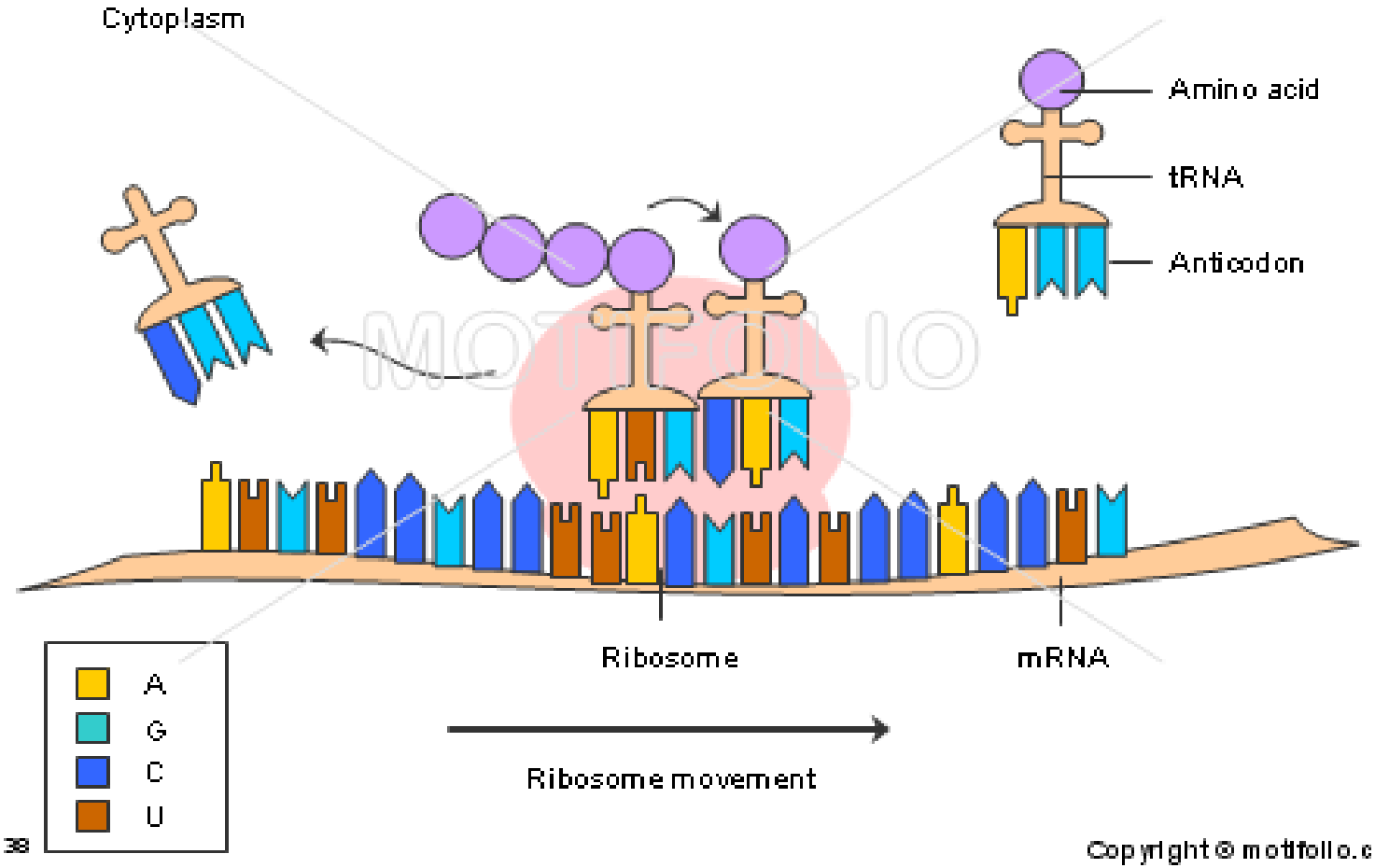

Template strand
DNA
T A C G G C G T T A G A C A A G T G C G T G A G T A C A C A
A T G C C G C A A T C T G T T C A C G C A C T C A T G T G T
Transcription
RNA
A U G C C G C A A U C U G U U C A C G C A C U C A U G U G U
Translation
Protein
Met Pro Gin Ser Val His Ala Leu Met Cys

Transcription



- The process of making a mRNA strand from a DNA template using base pairing rules. Occurs in the nucleus.
  - Initiation- RNA polymerase binds to the promoter.
  - Elongation- RNA polymerase makes a copy of the coding region using base pairing rules. The bond that forms between adjacent RNA nucleotides is a phosphodiester bond.
  - Termination- RNA polymerase makes mRNA until it reaches the termination site where it stops.

The translation process in protein synthesis



- The science of using living systems to benefit humankind is called **biotechnology**
- Technically , domestication of plants and animals through farming and **breeding** practices is a type of biotechnology

- In a contemporary sense, we associate biotechnology with the direct alteration of an organism's genetics to achieve desirable traits through the process of **genetic engineering**.

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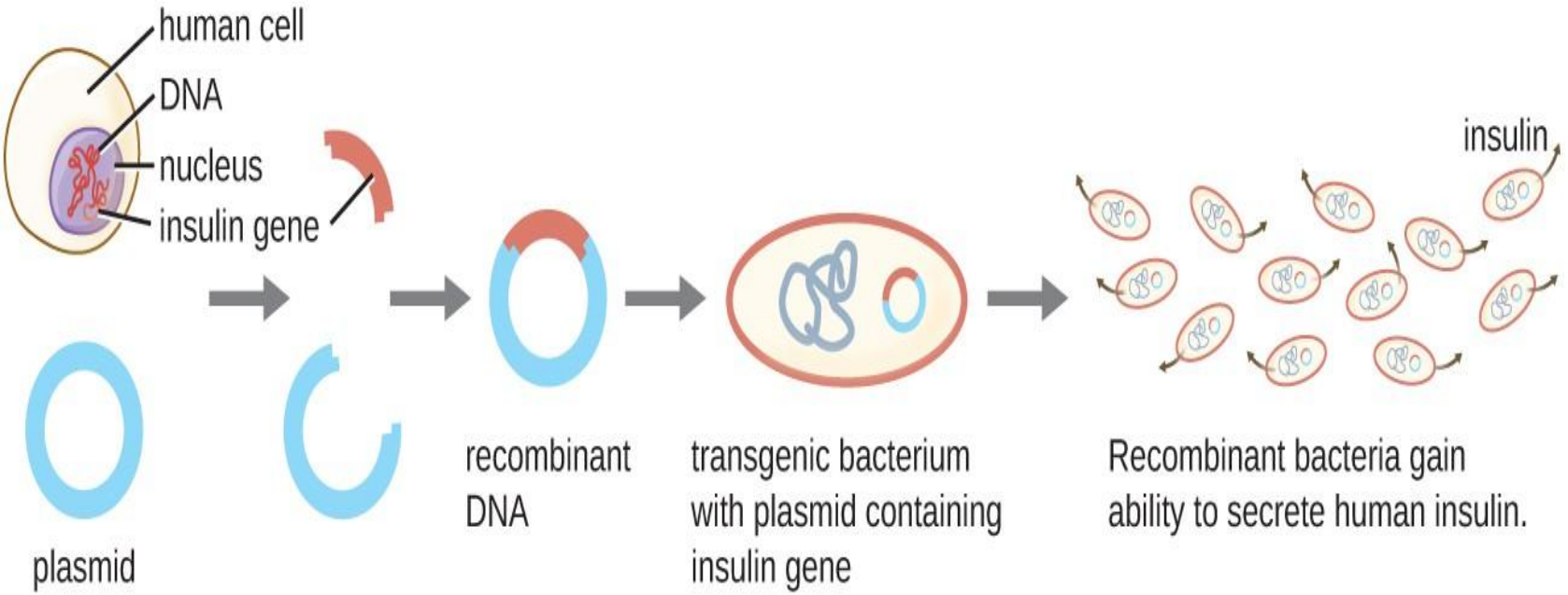
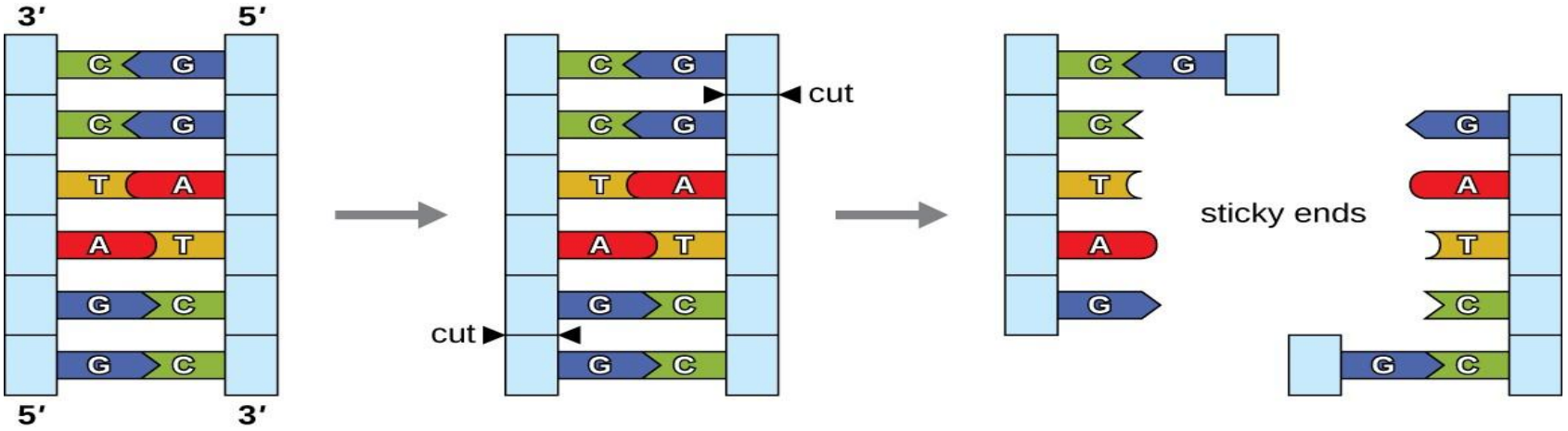
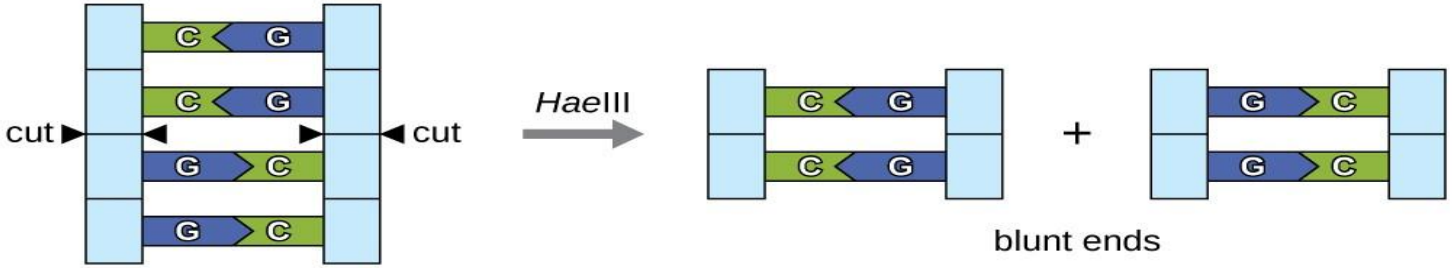
Recombination



Restriction endonuclease

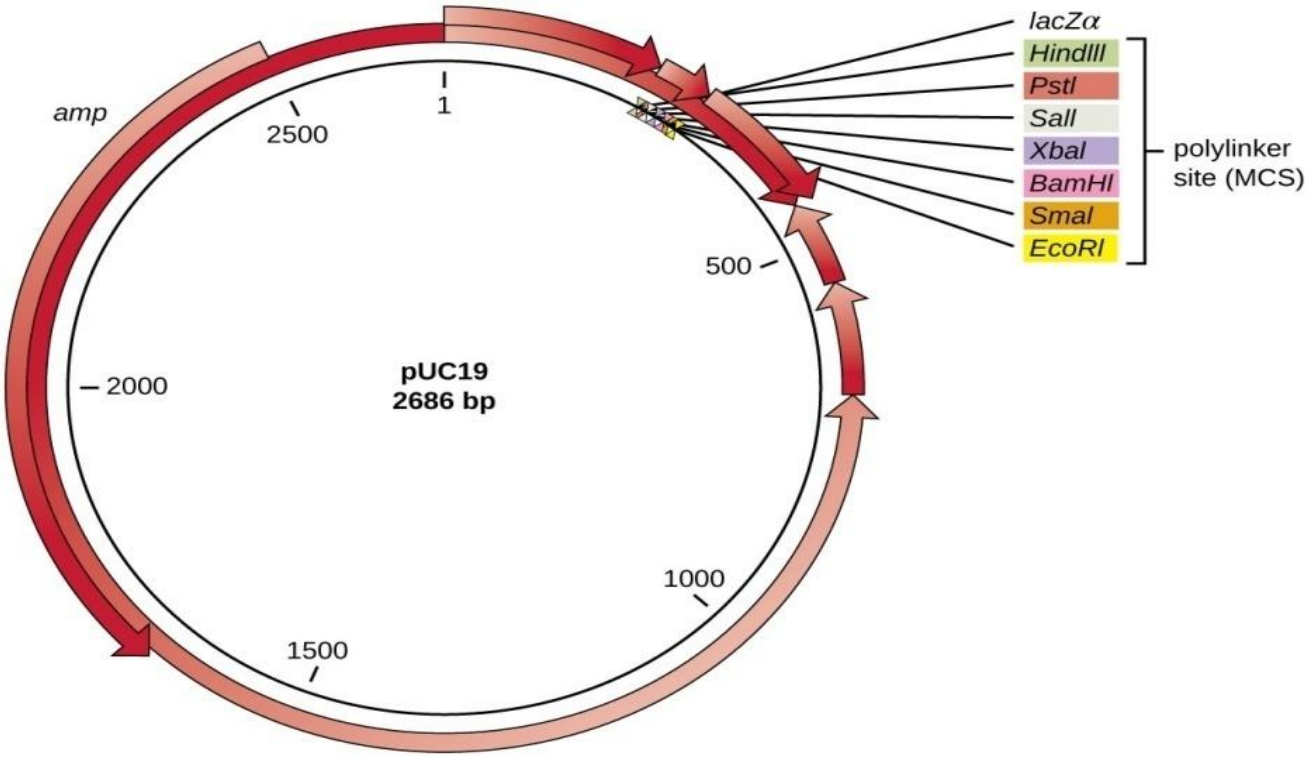


(a) sticky end cutting

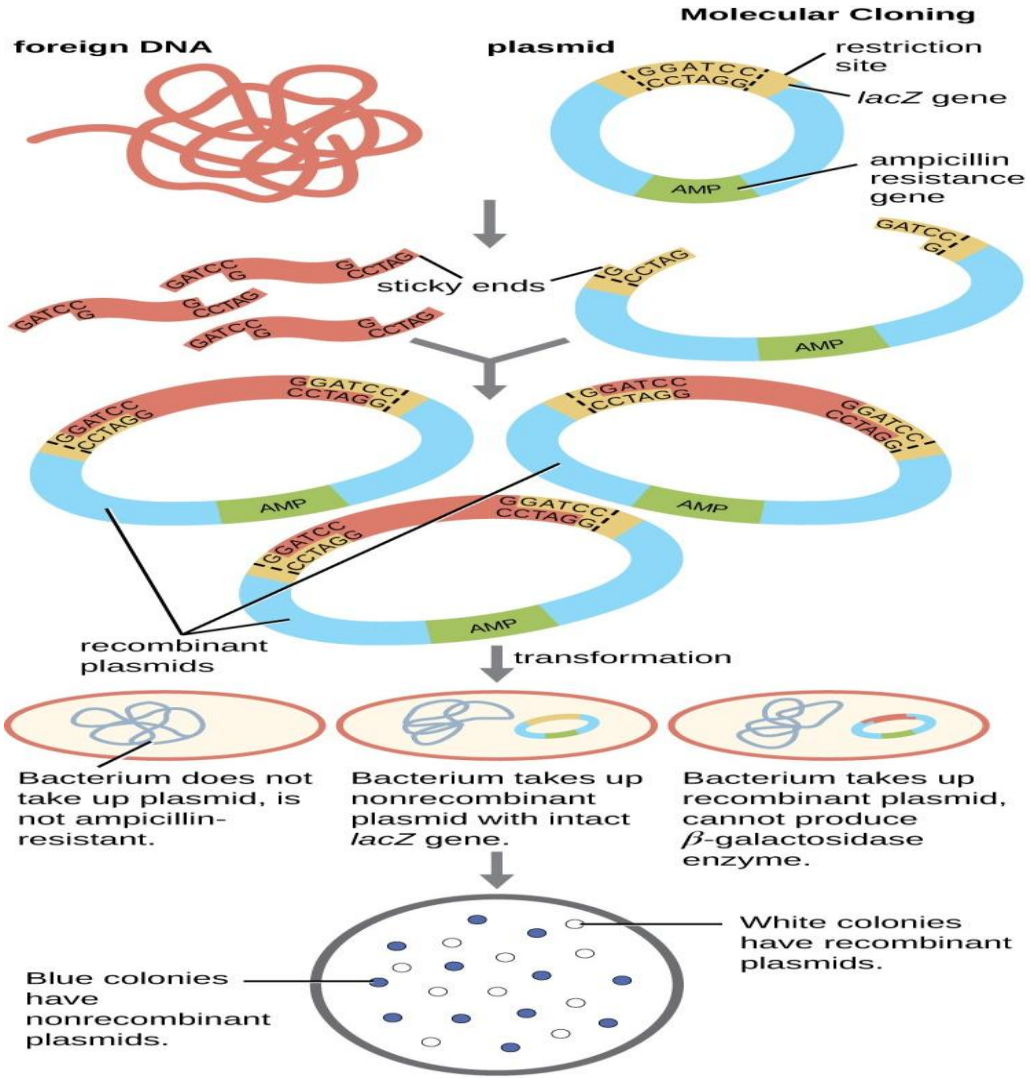


(b) blunt end cutting

Plasmid


lacZα
HindIII
PstI
SalI
XbaI
BamHI
SmaI
EcoRI
polylinker
site (MCS)
amp
1
2500
500
2000
pUC19
2686 bp
1000
1500

Molecular cloning-Transformation



1. Both foreign DNA and a plasmid with an ampicillin resistance gene are cut with the same restriction enzyme. In the plasmid, the restriction site occurs in the middle of a single copy of the *lacZ* gene in the plasmid. When functional, the *lacZ* gene will lead to the production of an enzyme β-galactosidase. Cutting the *lacZ* gene prevents the eventual production of the enzyme β-galactosidase.

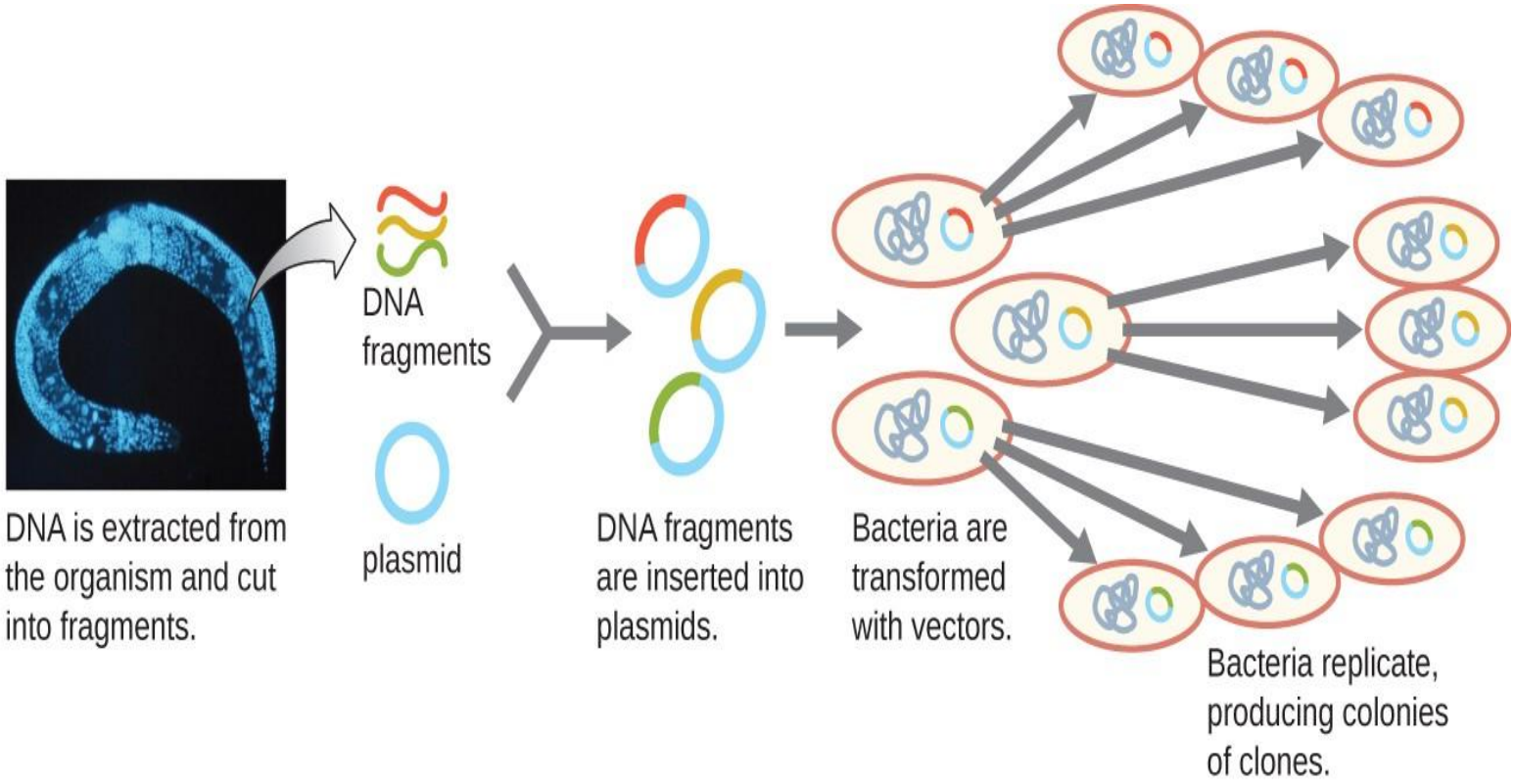
2. The restriction enzyme leaves complementary sticky ends on the foreign DNA fragment and the plasmid. This allows the foreign DNA to be inserted into the plasmid when the sticky ends anneal.

3. Adding DNA ligase reattaches the DNA backbones. These are recombinant plasmids.

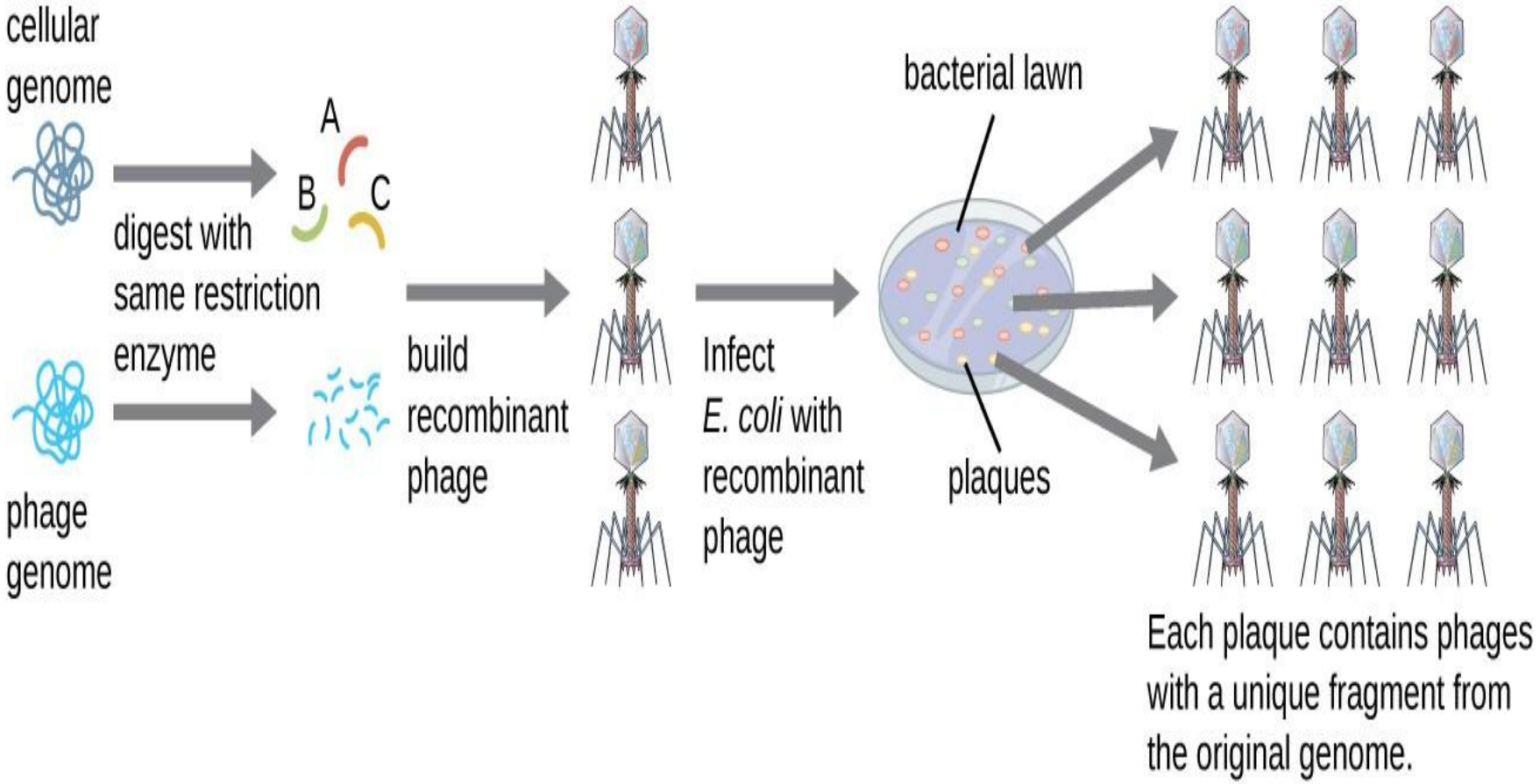
4. The plasmids are combined with a culture of actively growing bacteria. Some cells do not take up plasmids, others take up nonrecombinant plasmids, and a few take up the recombinant plasmids.

5. Bacteria are cultured on a plate with ampicillin and a substance that changes color when exposed to the β-galactosidase enzyme. Cells that did not take up plasmids are killed by ampicillin. Cells with nonrecombinant plasmids grow colonies that change color. Cells with recombinant plasmids grow white colonies.

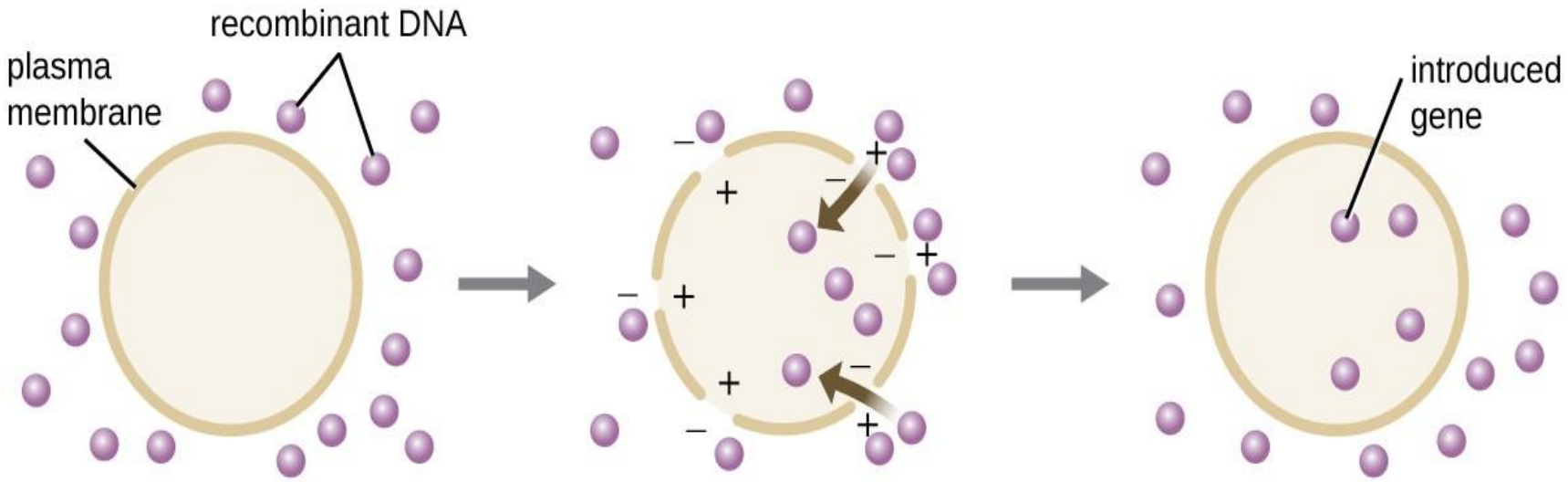
Genomic Library- plasmid



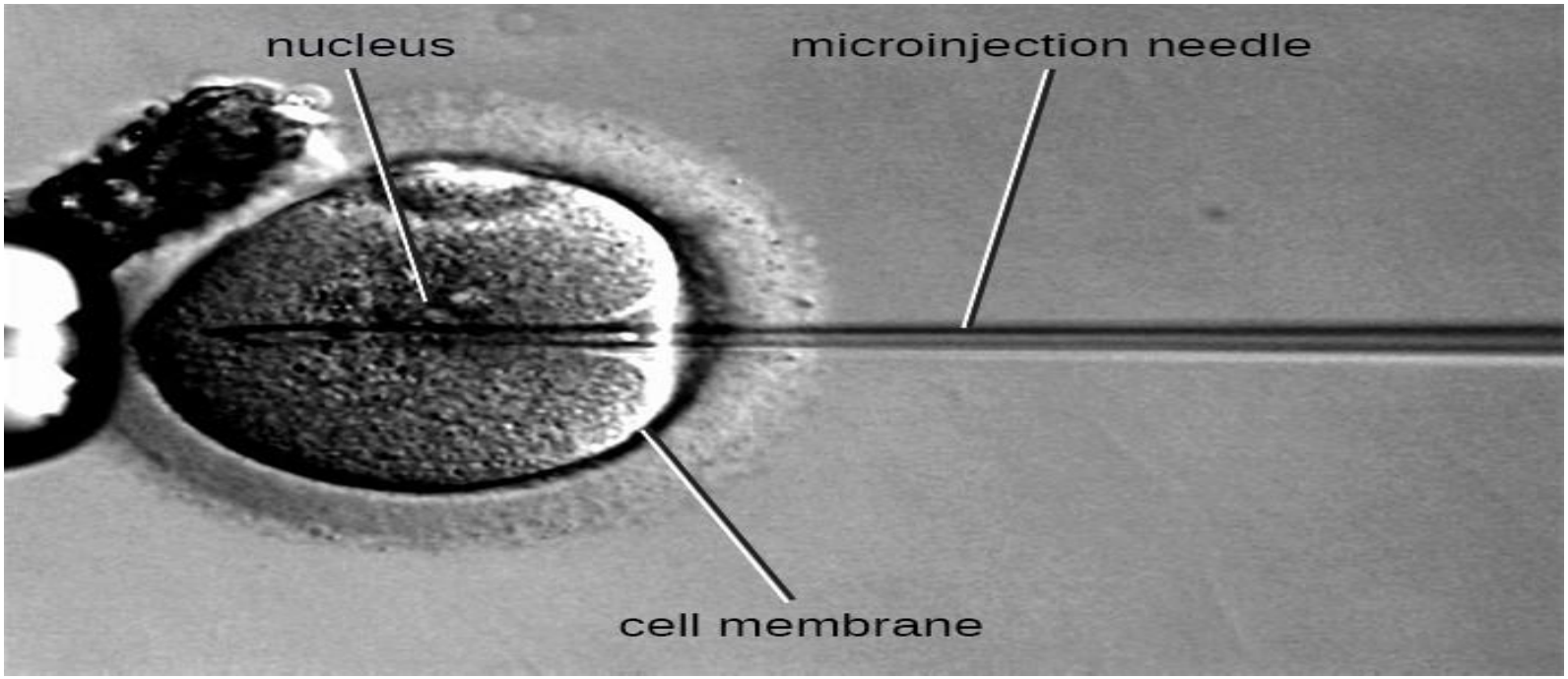
Genomic Library- lambda phage



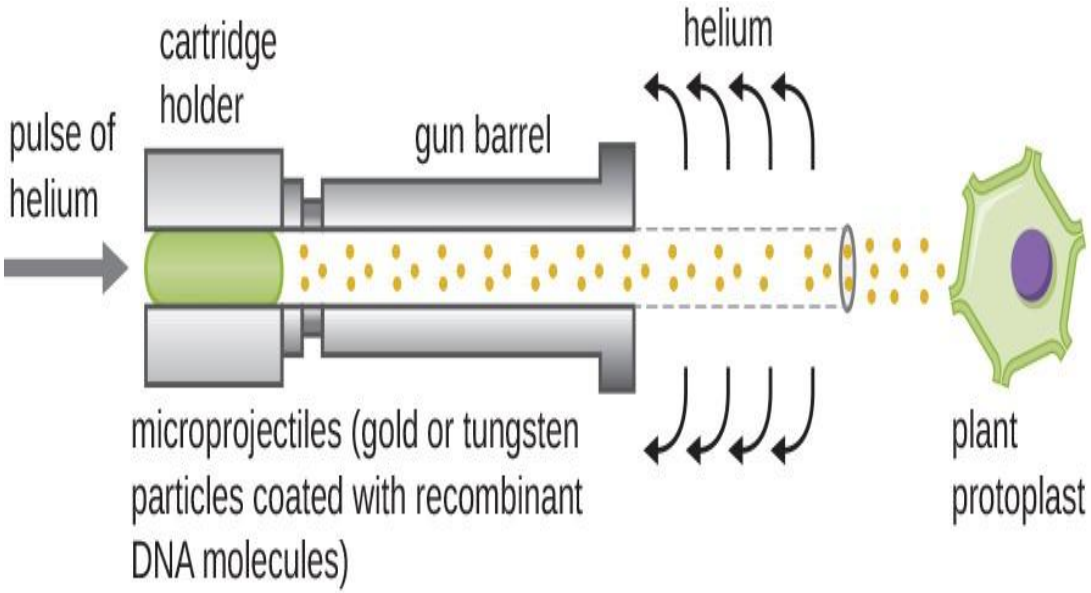
Introducing Recombinant Molecules into Eukaryotic Hosts--electroporation



Microinjection



Gene Guns


cartridge holder
helium
pulse of helium
gun barrel
microprojectiles (gold or tungsten particles coated with recombinant DNA molecules)
plant protoplast

(a)

(b)

Shuttle Vectors-Ti plasmid