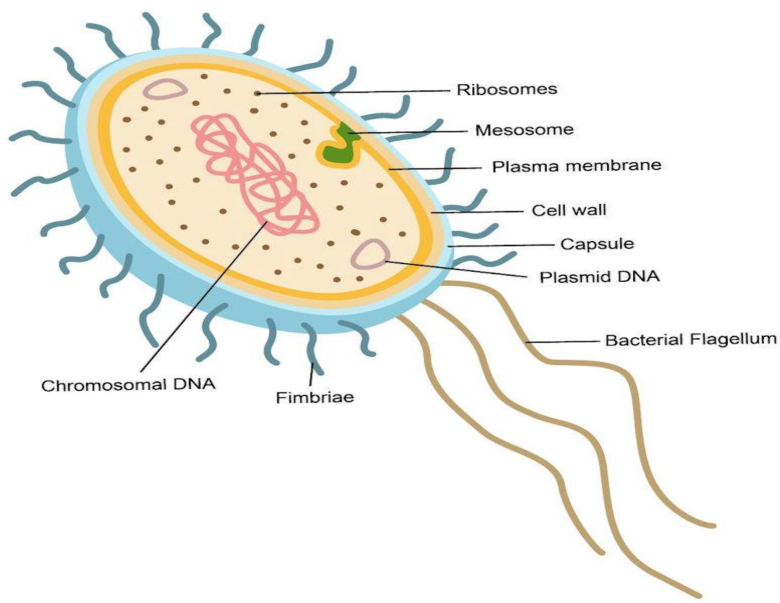
MICROBIAL GENETICS

There are three classical methods of DNA transfer in nature :

1. Transduction
2. Transformation
3. Conjugation

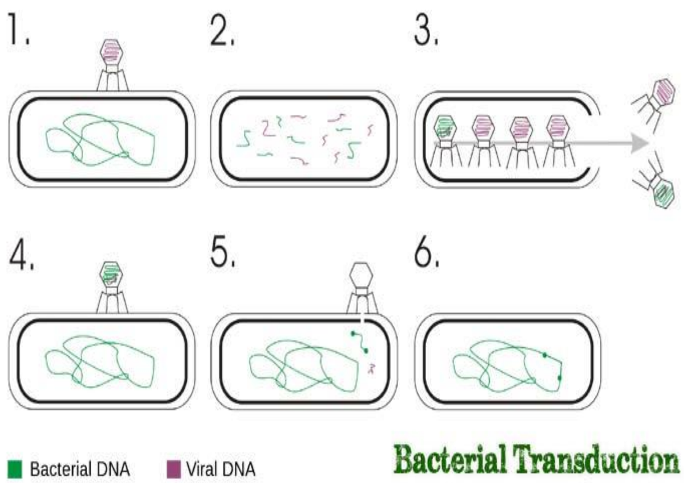
STRUCTURE OF A BACTERIAL CELL



TRANSDUCTION

- Transduction is the process by which DNA is transferred from one bacterium to another by a virus.
- Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNAase resistant.
- Transduction happens through either the lytic cycle or the lysogenic cycle.

- Transduction is especially important because it explains one mechanism by which antibiotic drugs become ineffective due to the transfer of antibiotic-resistance genes between bacteria.

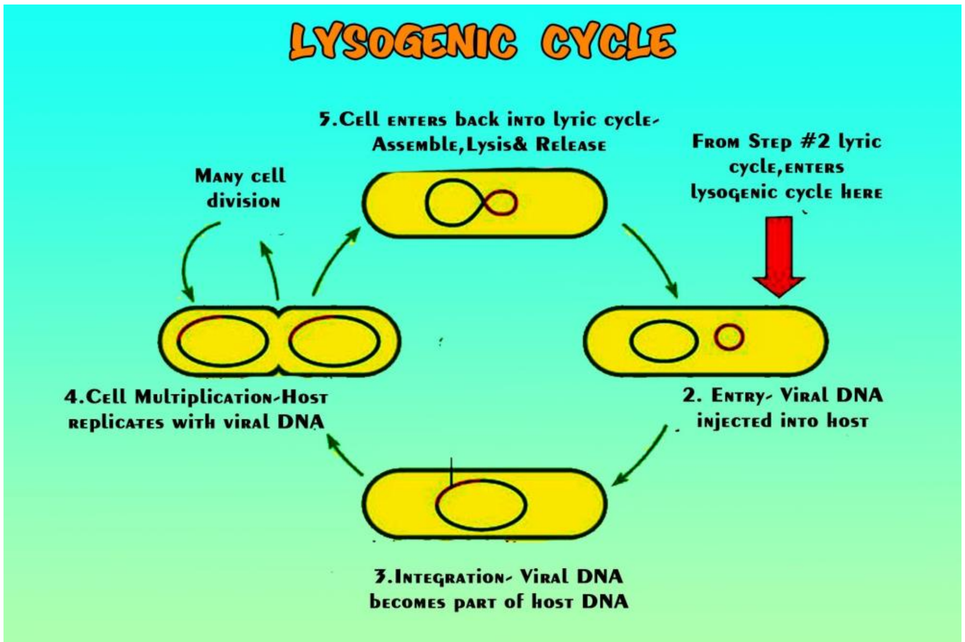


- It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector.
- Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.
- When bacteriophages (viruses that infect bacteria) infect a bacterial cell, their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.
- Transduction is especially important because it explains one mechanism by which antibiotic drugs become ineffective due to the transfer of antibiotic-resistance genes between bacteria.
- In addition, hopes to create medical methods of genetic modification of diseases such as Duchenne/Becker Muscular Dystrophy are based on these methodologies.

The Lytic Cycle and the Lysogenic Cycle

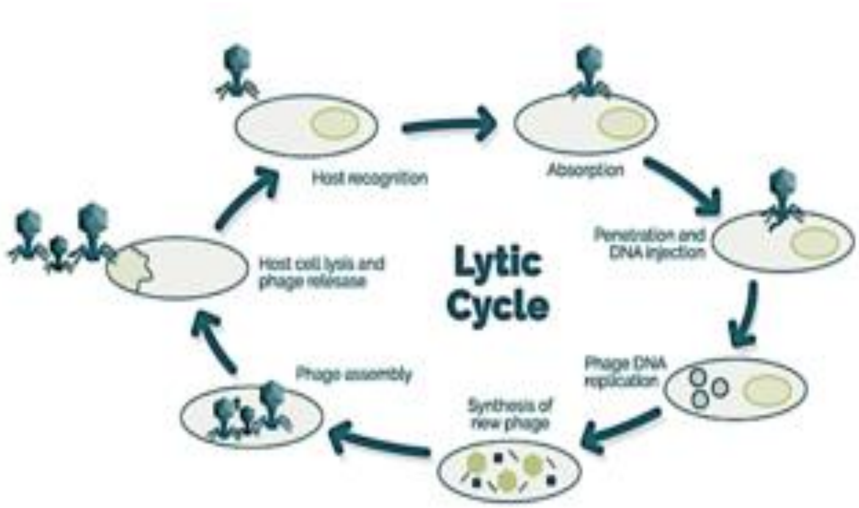
• Transduction happens through either the lytic cycle or the lysogenic cycle.

• If the lysogenic cycle is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, where it can remain dormant for thousands of generations.

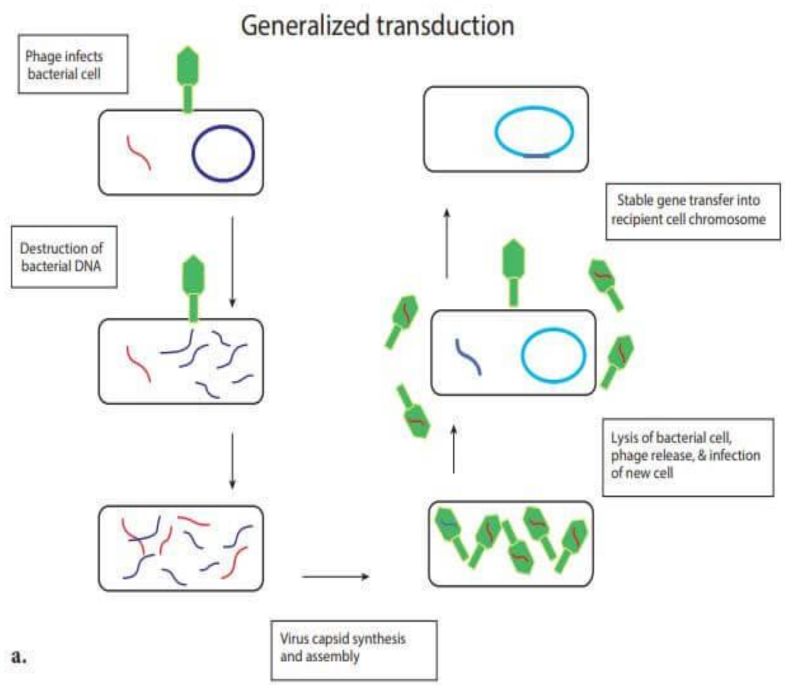


• If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles.

• The lytic cycle leads to the production of new phage particles which are released by lysis of the host.



- Transduction is a method for transferring genetic material.
- The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome.
- At the same time, some phage genes are left behind in the bacterial chromosome.
- There are generally three types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of recombination.
- *Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage, and typically carries only bacterial DNA and no viral DNA.*



- In essence, this is the packaging of bacterial DNA into a viral envelope. This may occur in two main ways, recombination and headful packaging.
- *If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA*.
- If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to generalized transduction.
- If the virus replicates using "headful packaging," it attempts to fill the nucleocapsid with genetic material.
- If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion.
- The new virus capsule, now loaded with part bacterial DNA, continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

Fates of DNA Inserted into the Recipient Cell

- When the new DNA is inserted into this recipient cell it can fall to one of three fates: the DNA will be absorbed by the cell and be recycled for spare parts; if the DNA was originally a plasmid, it will recirculate inside the new cell and become a plasmid again; if the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in conjugation.
- This type of recombination is random and the amount recombined depends on the size of the virus being used.
- Specialized transduction is the process by which a restricted set of bacterial genes are transferred to another bacterium.
- The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome.
- Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA.
- The excised DNA is then packaged into a new virus particle, which can then deliver the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage.
- When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote. "
- Example of specialized transduction is λ phages in Escherichia coli, which was discovered by Esther Lederberg.

Lytic cycle: The normal process of viral reproduction involving penetration of the cell membrane, nucleic acid synthesis, and lysis of the host cell.

Lysogenic cycle: A form of viral reproduction involving the fusion of the nucleic acid of a bacteriophage with that of a host, followed by proliferation of the resulting prophage.

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

transformation: In molecular biology transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s).

expression: Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product.

exogenous: Produced or originating outside of an organism.

translocase: An enzyme that assists in moving another molecule, usually across a membrane.

Genetic Alteration

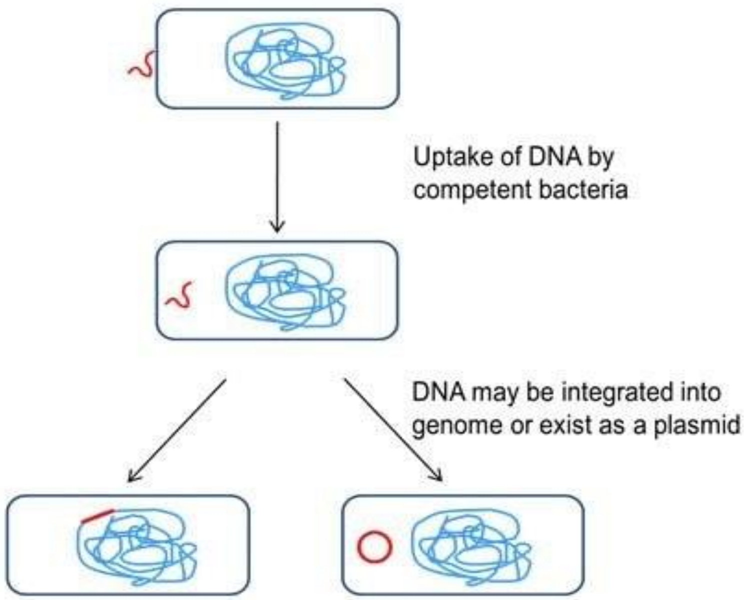
In molecular biology, transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s).

Transformation: Illustration of bacterial transformation. DNA from dead cells gets cut into fragments and exits the cell. The free-floating DNA can then be picked up by competent cells. The exogenous DNA is incorporated into the host cell's chromosome via recombination.

TRANSFORMATION

- Transformation occurs naturally in some species of bacteria, but it can also be effected by artificial means in other cells.

- For transformation to happen, bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density.
- Transformation is one of three processes by which exogenous genetic material may be introduced into a bacterial cell.



- "Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the term should be avoided for animal cells when describing introduction of exogenous genetic material.
- Introduction of foreign DNA into eukaryotic cells is often called "transfection".
- Bacterial transformation may be referred to as a stable genetic change, brought about by the uptake of naked DNA (DNA without associated cells or proteins).
- Competence refers to the state of being able to take up exogenous DNA from the environment.
- There are two forms of competence: natural and artificial.

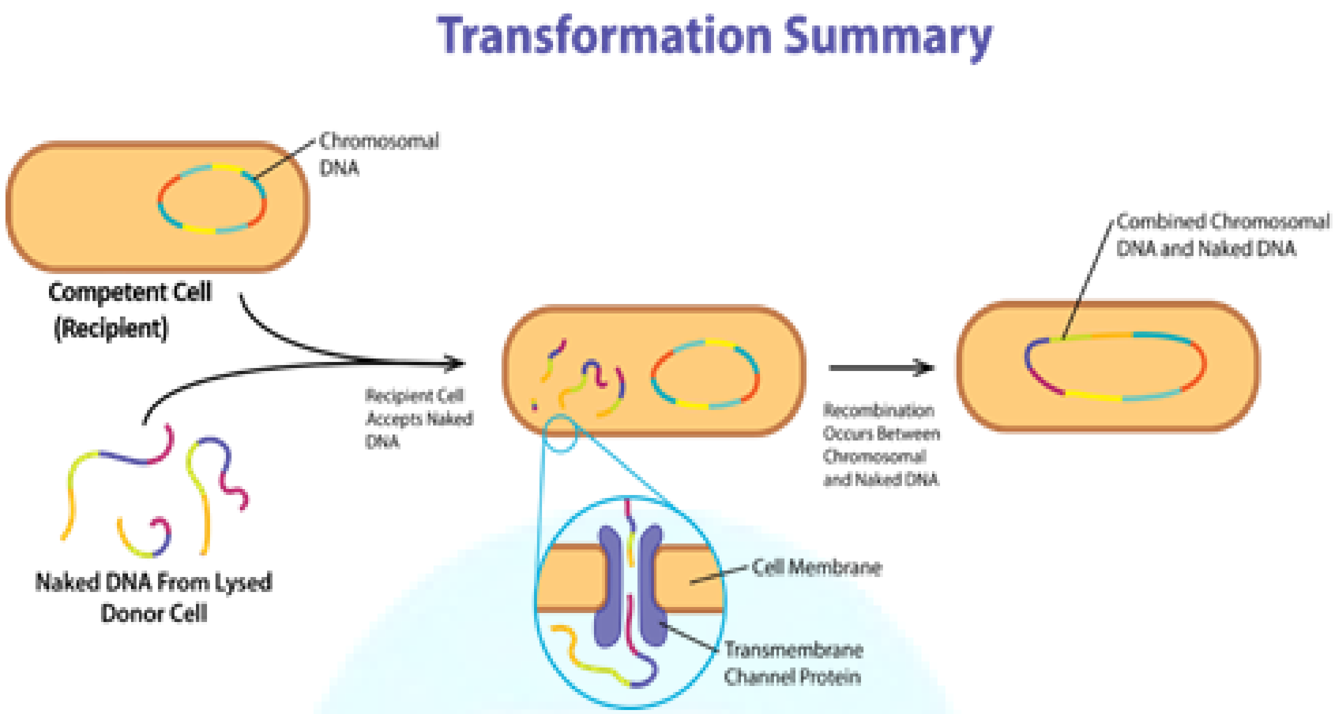
- About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; more may be able to take it up in their natural environments.
- DNA material can be transferred between different strains of bacteria in a process that is called horizontal gene transfer.
- Some species, upon cell death, release their DNA to be taken up by other cells; however, transformation works best with DNA from closely-related species.
- These naturally-competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s).
- The transport of the exogeneous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

GRAM-POSITIVE AND GRAM-NEGATIVE DIFFERENCES

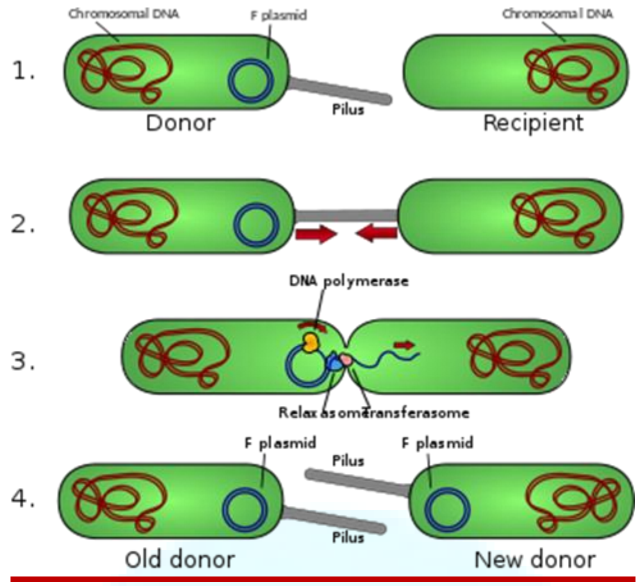
- Due to the differences in structure of the cell envelope between Gram-positive and Gram-negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells.
- However, most of them share common features that involve related proteins.
- The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase
- Only single-stranded DNA may pass through, one strand is therefore degraded by nucleases in the process, and the translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process.
- ***In Gram-negative cells***, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane. Pilin may be required for competence however, its role is uncertain.
- The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

ARTIFICIAL TRANSFER

- Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA, by exposing it to conditions that do not normally occur in nature.
- Typically, the cells are incubated in a solution containing divalent cations; most commonly, calcium chloride solution under cold condition, which is then exposed to a pulse of heat shock.
- However, the mechanism of the uptake of DNA via chemically-induced competence in this calcium chloride transformation method is unclear.
- The surface of bacteria such as E. coli is negatively-charged due to phospholipids and lipopolysaccharides on its cell surface, and the DNA is also negatively-charged.
- One function of the divalent cation therefore, would be to shield the charges by coordinating the phosphate groups and other negative charges, thereby allowing a DNA molecule to adhere to the cell surface.
- It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure of the cells making it more permeable to DNA.
- The heat-pulse is thought to create a thermal imbalance on either side of the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.
- Electroporation is another method of promoting competence.
- Using this method, the cells are briefly shocked with an electric field of 10-20 kV/cm which is thought to create holes in the cell membrane through which the plasmid DNA may enter.
- After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.



BACTERIAL CONJUGATION



- Bacterial conjugation is a way by which a bacterial cell transfers genetic material to another bacterial cell.
- The genetic material that is transferred through bacterial conjugation is a small plasmid, known as F-plasmid (F for fertility factor), that carries genetic information different from that which is already present in the chromosomes of the bacterial cell.
- In fact, the F-plasmid can replicate in the cytoplasm separately from the bacterial chromosome.
- A cell that already has a copy of the F-plasmid is called an F-positive, F-plus or F+ cell, and is considered a donor cell, while a cell that does not have a copy of the F-plasmid is called an F-negative, F-minus or F– cell, and is considered a recipient cell.
- The transfer of the F-plasmid takes place through a horizontal connection by which the donor cell and the recipient cell directly contact each other or form a bridge between the two through which the genetic material is transferred.
- In cases where the F-plasmid of a donor cell has been integrated in the cell's genome (i.e., in the chromosome), a part of the chromosomal DNA may also be transferred to the recipient cell together with the F-plasmid.

Bacterial Conjugation Steps

- In order to transfer the F-plasmid, a donor cell and a recipient cell must first establish contact.
- At this point, when the cells establish contact, the F-plasmid in the donor cell is a double-stranded DNA molecule that forms a circular structure.
- The following steps allow the transfer of the F-plasmid from one bacterial cell to another:

Step 1

- The F+ (donor) cell produces the pilus, which is a structure that projects out of the cell and begins contact with an F– (recipient) cell.

Step 2

- The pilus enables direct contact between the donor and the recipient cells.

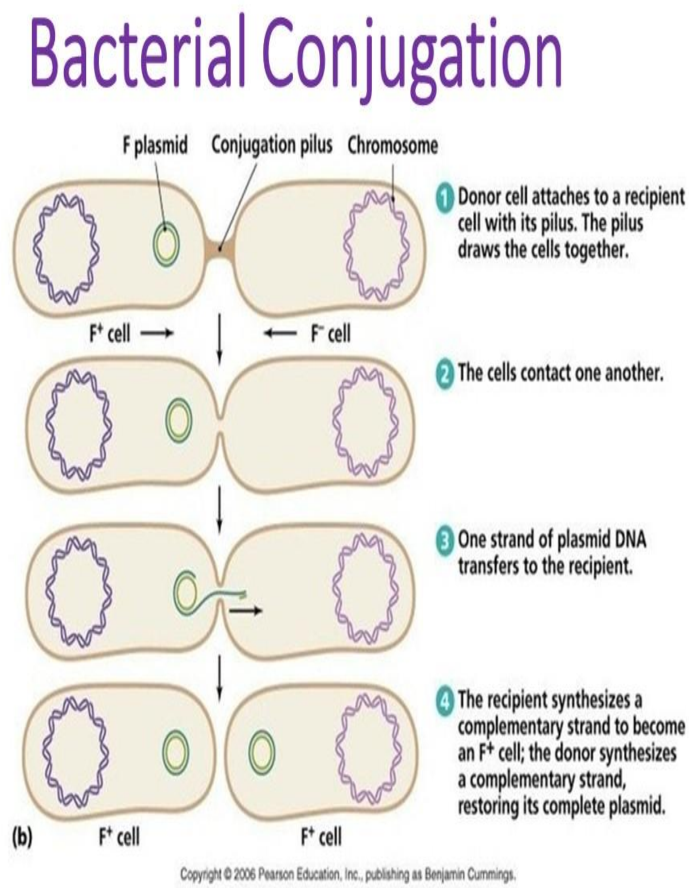
Step 3

- Because the F-plasmid consists of a double-stranded DNA molecule forming a circular structure, i.e., it is attached on both ends, an enzyme (relaxase, or relaxosome when it forms a complex with other proteins) nicks one of the two DNA strands of the F-plasmid and this strand (also called T-strand) is transferred to the recipient cell.

Step 4

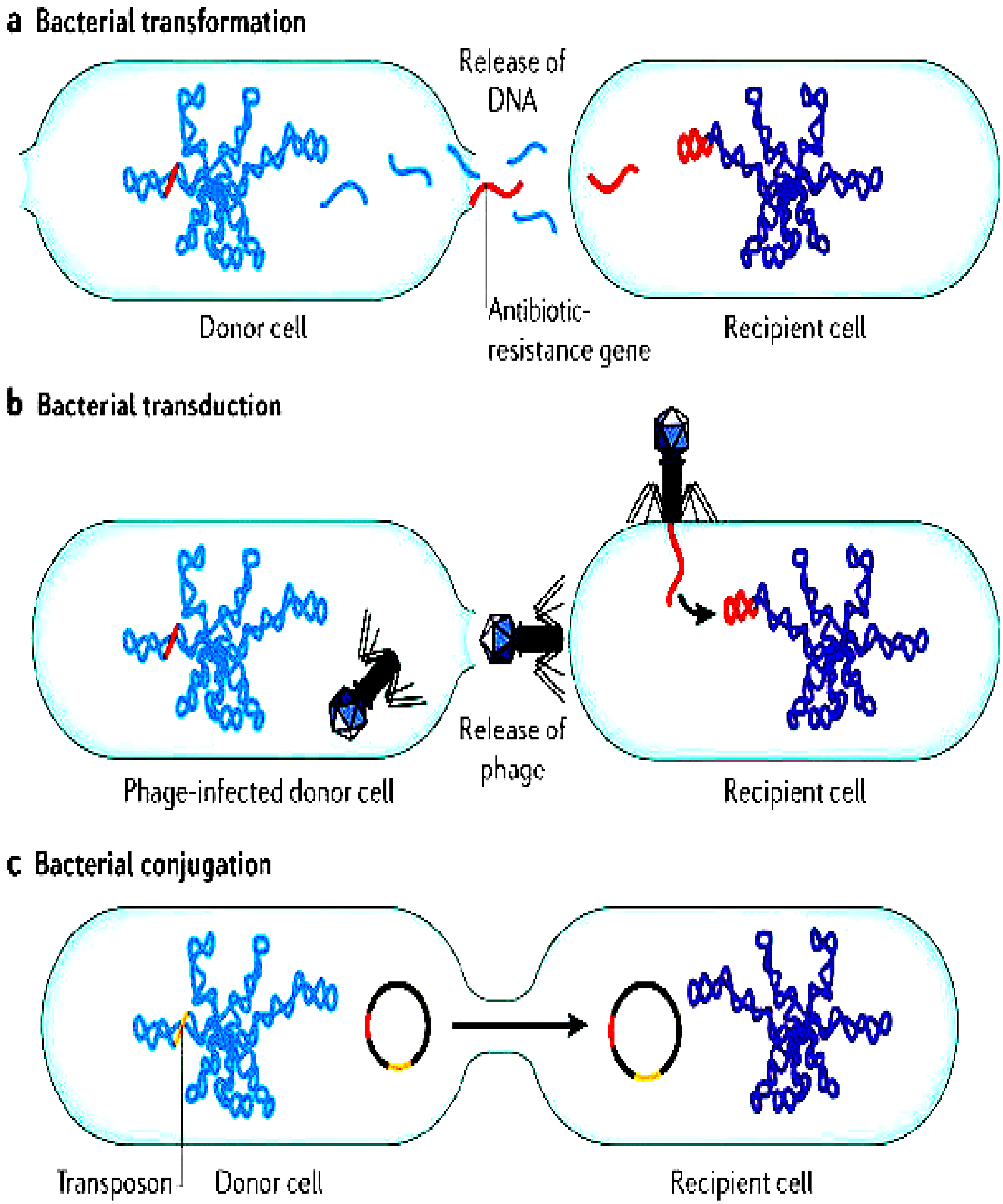
- In the last step, the donor cell and the recipient cell, both containing single-stranded DNA, replicate this DNA and thus end up forming a double-stranded F-plasmid identical to the original F-plasmid.

- Given that the F-plasmid contains information to synthesize pili and other proteins (see below), the old recipient cell is now a donor cell with the F-plasmid and the ability to form pili, just as the original donor cell was.
- Now both cells are donors or F+.



- In order to avoid transferring the F-plasmid to an F+ cell, the F-plasmid usually contains information that allows the donor cell to detect (and avoid) cells that already have one.
- In addition, the F-plasmid contains two main loci (tra and trb), an origin of replication (OriV) and an origin of transfer (OriT).

- The tra locus contains the genetic information to enable the donor cell to be attached to a recipient cell: the genes in the tra locus code for proteins to form the pili (pilin gene) in order to start the cell-cell contact, and other proteins to get attached to the F– cell and to start the transfer of the F-plasmid.
- The trb locus contains DNA that codes for other proteins, such as some that are involved in creating a channel through which the DNA is transferred from the F+ to the F– cell.
- The OriV is the site at which replication of the DNA occurs and the OriT is the site at which the enzyme relaxase (or the relaxosome protein complex) nicks the DNA strand of the F-plasmid .
- Although the DNA that is transferred in bacterial conjugation is that present in the F-plasmid, when the donor cell has integrated the F-plasmid into its own chromosomal DNA, bacterial conjugation can result in the transfer of the F-plasmid and of chromosomal DNA.
- When this is the case, a longer contact between the donor and the recipient cells results in a larger amount of chromosomal DNA being transferred.
- The advantages of bacterial conjugation make this method of gene transfer a widely used technique in bioengineering.
- Some of the advantages include the ability to transfer relatively large sequences of DNA and not harming the host's cellular envelope.
- Furthermore, conjugation has been achieved in laboratories not only between bacteria, but also between bacteria and types of cells such as plant cells, mammalian cells and yeast.


a Bacterial transformation
Release of DNA
Donor cell
Antibiotic-resistance gene
Recipient cell
b Bacterial transduction
Phage-infected donor cell
Release of phage
Recipient cell
c Bacterial conjugation
Transposon
Donor cell
Recipient cell