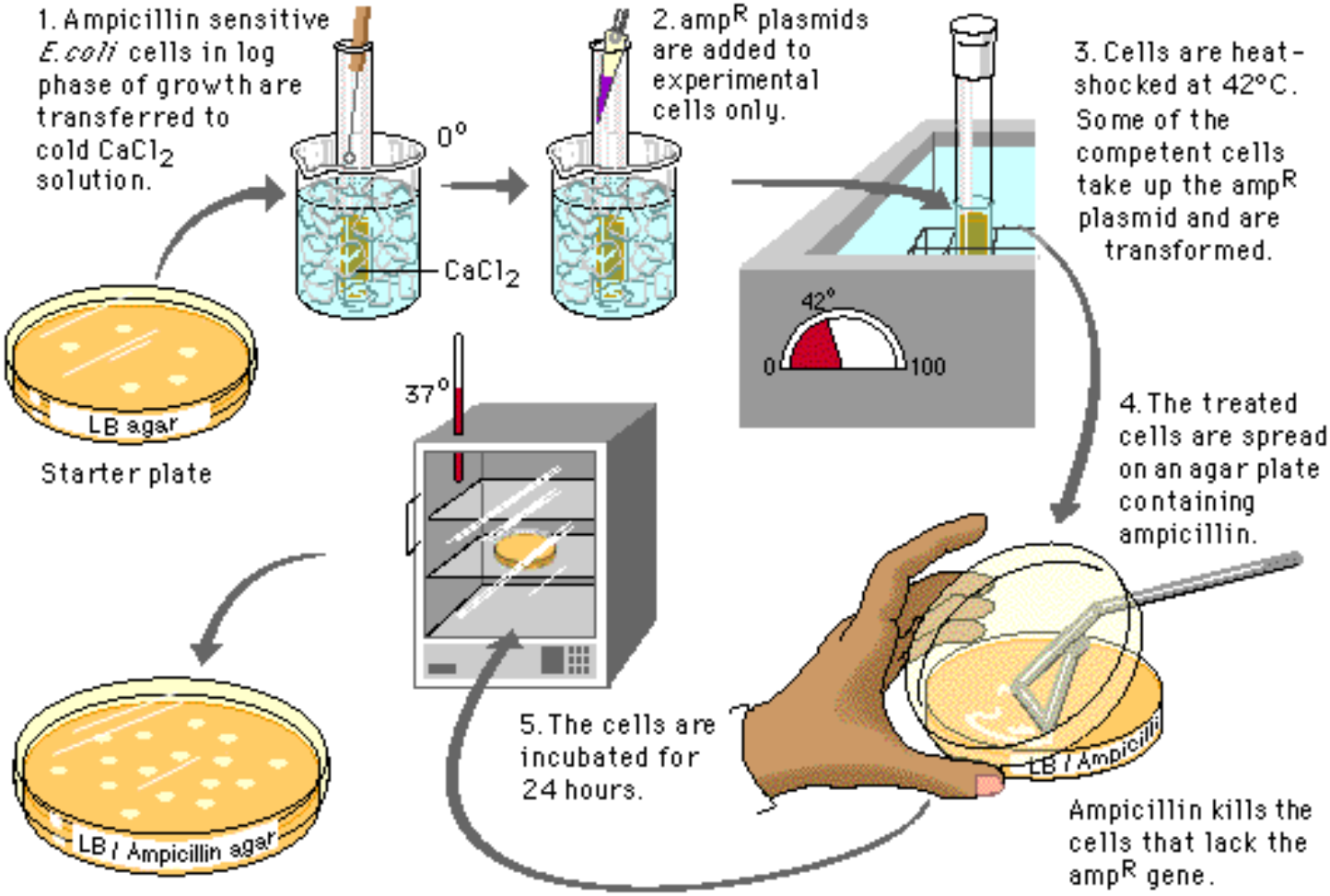
- Transformation is one of three basic mechanisms for genetic exchange in bacteria.

- One of the easiest ways to get large amounts of DNA is to place the desired DNA into bacteria.

- Grow the bacteria, harvest the bacteria, and then isolate the DNA.


1. Ampicillin sensitive *E.coli* cells in log phase of growth are transferred to cold $CaCl_2$ solution.
0°
$CaCl_2$
2. amp$^R$ plasmids are added to experimental cells only.
3. Cells are heat-shocked at 42°C. Some of the competent cells take up the amp$^R$ plasmid and are transformed.
42°
0
100
LB agar
Starter plate
37°
4. The treated cells are spread on an agar plate containing ampicillin.
LB / Ampicillin
Ampicillin kills the cells that lack the amp$^R$ gene.
5. The cells are incubated for 24 hours.
LB / Ampicillin agar
6. Only colonies of *E.coli* that have been transformed by the amp$^R$ gene will grow.

- Bacteria can maintain DNA as plasmids: circles of DNA that usually contain a gene that allows the bacterium to grow in the presence of an antibiotic.

❖**HOW DO WE INTRODUCE A PLASMID INTO A BACTERIUM?**

- This can be done through a process called transformation.

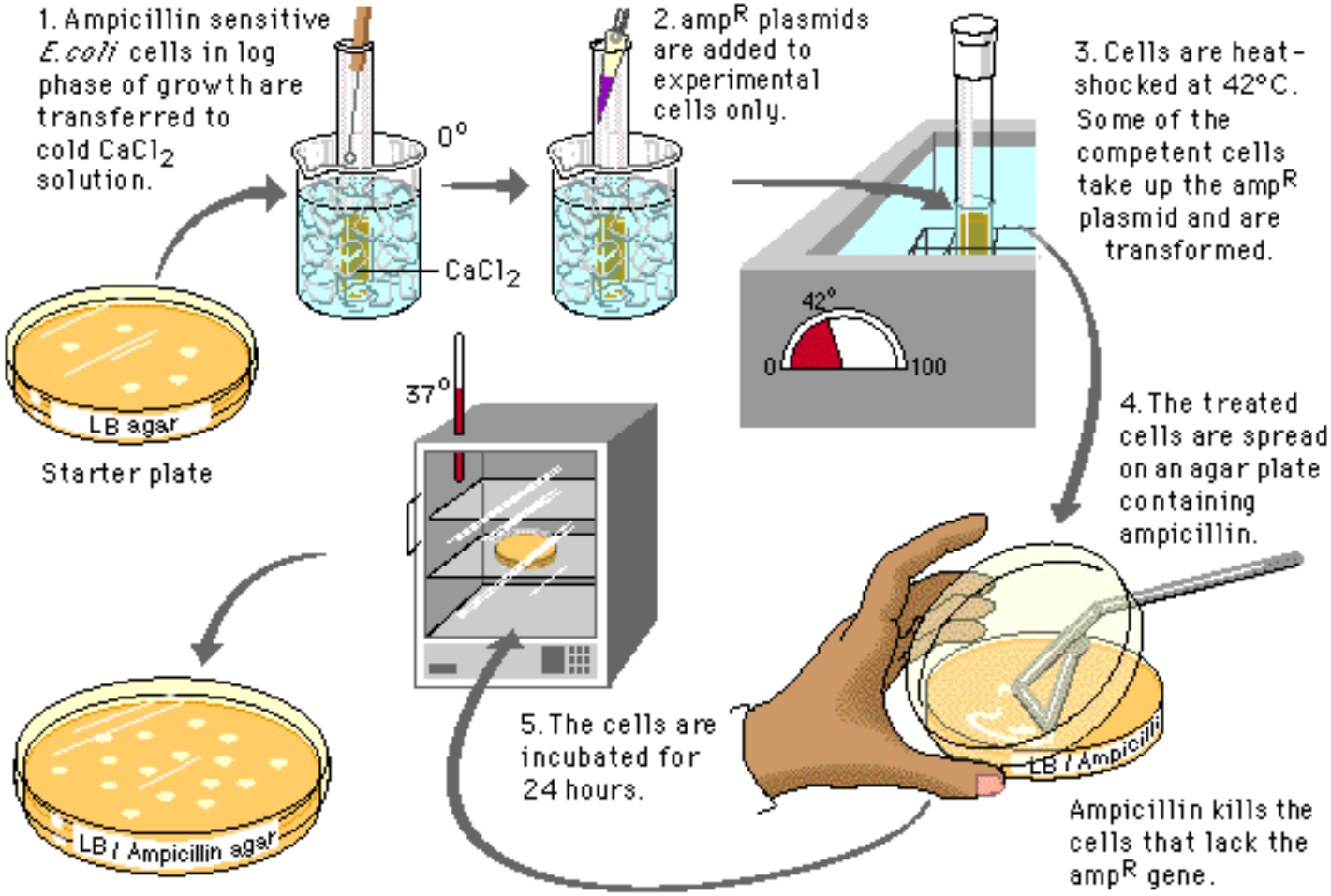
- Bacterial transformation is the process by which bacterial cells take up exogenous DNA (DNA that is outside the host cell) molecules through the cell membrane and can integrate into the bacterial chromosome. OR,

- It is a mechanism for the transfer of genetic material in which free DNA of one genotype is taken in through the cell surface of bacteria of another genotype and is incorporated into the recipient cell chromosome.

Bacteria are treated so they will take the plasmid up into their cells.

- These are called **competent cells**.

- Subjecting the bacterial cells in extreme cold temperatures will cause pores (small holes) to appear in the bacterial membrane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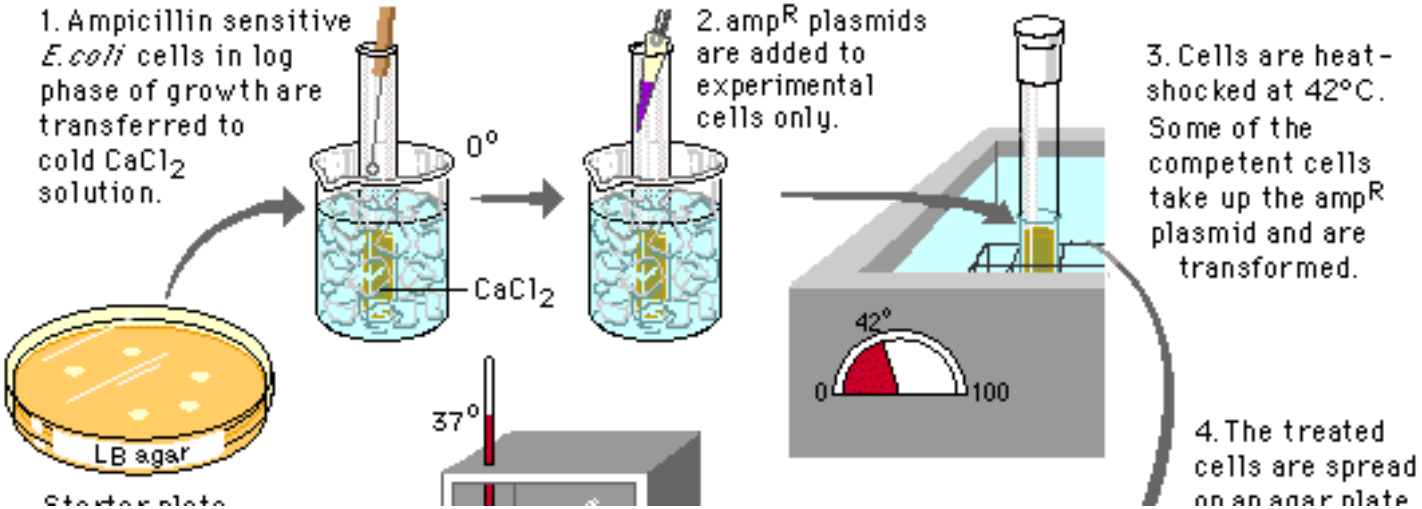
6. Only colonies of *E.coli* that have been transformed by the $amp^R$ gene will grow.

- Transformation involves mixing competent bacteria with plasmid DNA and then selecting bacteria containing the plasmid using agar plates that contain an antibiotic.



# Natural and Artificial Transformation

- There appear to be two basic mechanisms by which bacteria can become competent for transformation.

- These are **Natural** and **Artificial Transformation.**

-

- Natural transformation is a physiological process that is genetically encoded in a wide range of bacteria.

- Most bacteria must shift their physiology in order to transform DNA; that is, they must become "competent" for taking up exogenous DNA.

- In some bacteria, including *Streptococcus pneumoniae* and *Bacillus subtilis*, competence is externally regulated.

- These bacteria produce and secrete a small protein called competence factor that accumulates in the growth medium.

- When the bacterial culture reaches a sufficient density, the concentration of competence factor reaches a level high enough to bind receptors on the outside of the cell.

- This event causes an internal signal to turn on the expression of the genes needed for transformation.

- Therefore, competence development is controlled by cell density.

- In other bacteria, including *Haemophilus influenzae* and *Pseudomonas stutzeri*, competence development is internally regulated.

- When there is a shift in the growth dynamics of the bacterium, an internal signal triggers competence development.

- Once competence is induced, three additional steps are required for natural transformation.

- After induction of competence, double-stranded DNA is bound to specific receptors on the surface of the competent cells.

- These receptors are lacking in noncompetent cells.

- The double-stranded DNA is nicked and one strand is degraded while the other strand enters the cell.

- This process is called DNA uptake.

- Finally, the recombination enzymes of the recipient cell will bind the single-strand DNA that has entered it, align it with its **homologous** DNA on the recipient

- chromosome, and recombine the new DNA into the chromosome, incorporating any genetic differences that exist on the entering DNA.

## ❖Artificial Transformation

- While a wide variety of bacteria can transform naturally, many species cannot take up DNA from an outside source.

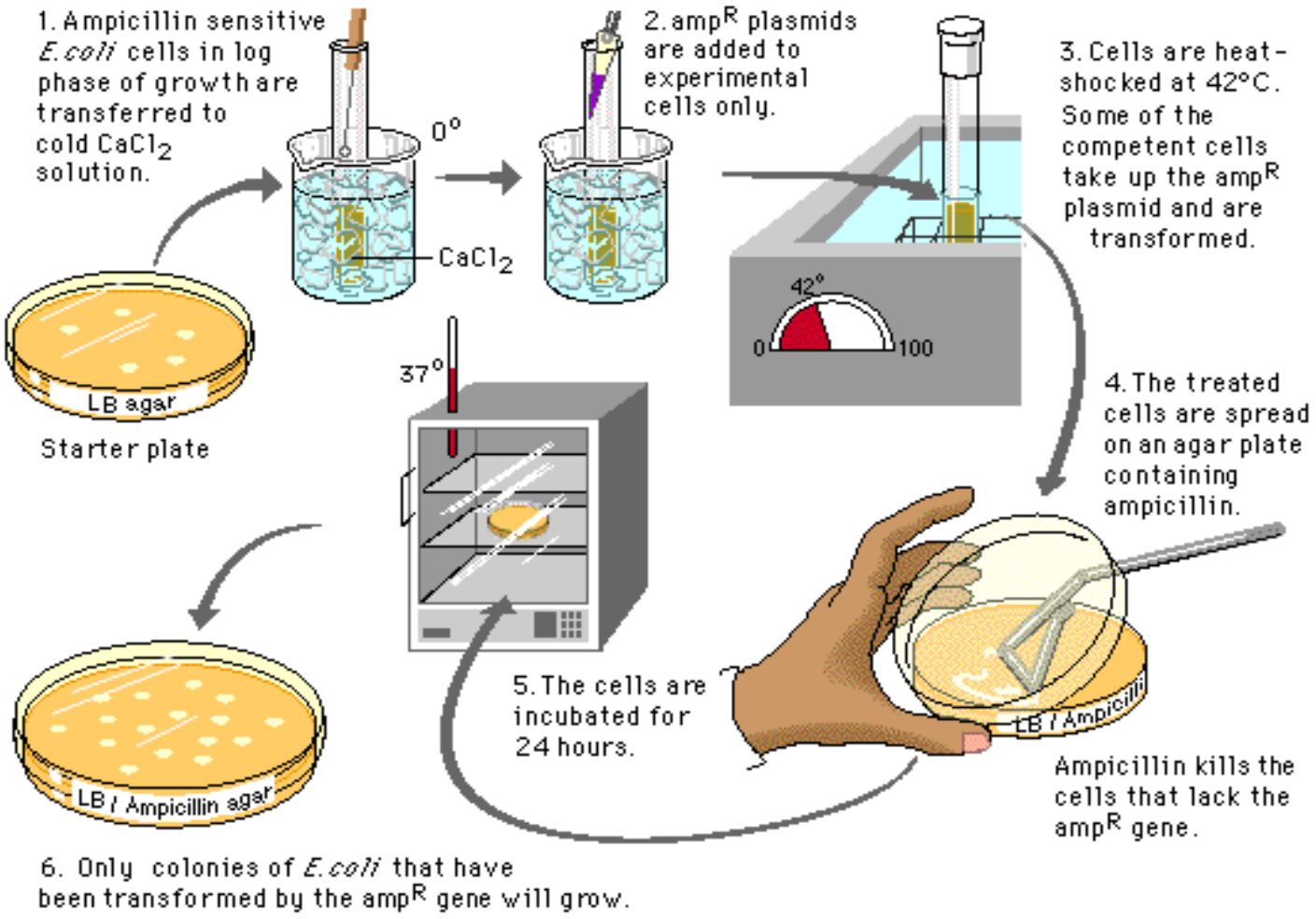
- In some cases DNA can be forced into these cells by chemical, physical, or enzymatic treatment.

- This is especially important in genetic engineering, as artificial transformation is essential for the introduction of genetically altered sequences into recipient cells.

- In both cases, exogenous DNA, is taken into a recipient cell where it is incorporated into the recipient genome, changing the genetic makeup of the bacterium.

- One of the two most common methods is a chemical process where cells are heat-shocked, then treated with the DNA and a high concentration of calcium ions.

- The calcium ions precipitate the DNA on the surface of the cell, where the DNA is forced into the recipient.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Ampicillin kills the cells that lack the $amp^R$ gene.
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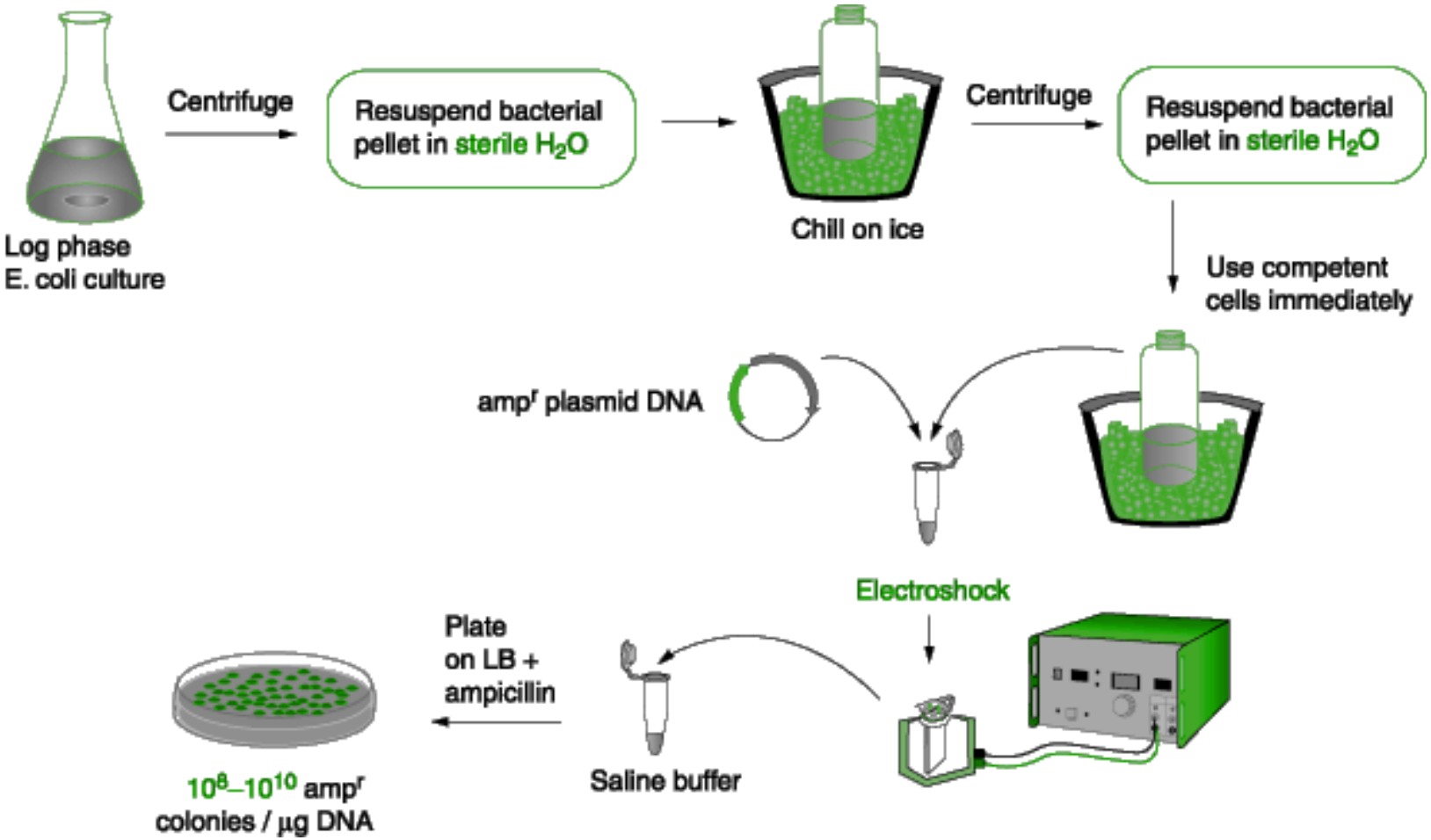
6. Only colonies of *E.coli* that have been transformed by the $amp^R$ gene will grow.

- More recently a new method, called **electroporation**, has been used to introduce DNA by artificial transformation.

- In this process a suspension of recipient bacteria and transforming DNA is placed in a container with metal sides.

- A high-voltage electrical current is passed through the sample, temporarily creating small pores, or channels, in the membranes of the bacteria.

- The DNA enters the cells and the pores close and therefore the exogenous DNA is introduced into the recipient.


Log phase
E. coli culture
Centrifuge
Resuspend bacterial pellet in sterile $H_2O$
Chill on ice
Centrifuge
Resuspend bacterial pellet in sterile $H_2O$
Use competent cells immediately
amp$^r$ plasmid DNA
Electroshock
Saline buffer
Plate on LB + ampicillin
$10^8$–$10^{10}$ amp$^r$ colonies / µg DNA

- Because exogenous DNA is not enclosed within cell walls, it is susceptible to enzymes that degrade DNA, called **DNases**.

- A hallmark of transformation is that it is sensitive to DNase, while the other two processes of genetic exchange, **transduction** and **conjugation**, are DNase resistant.

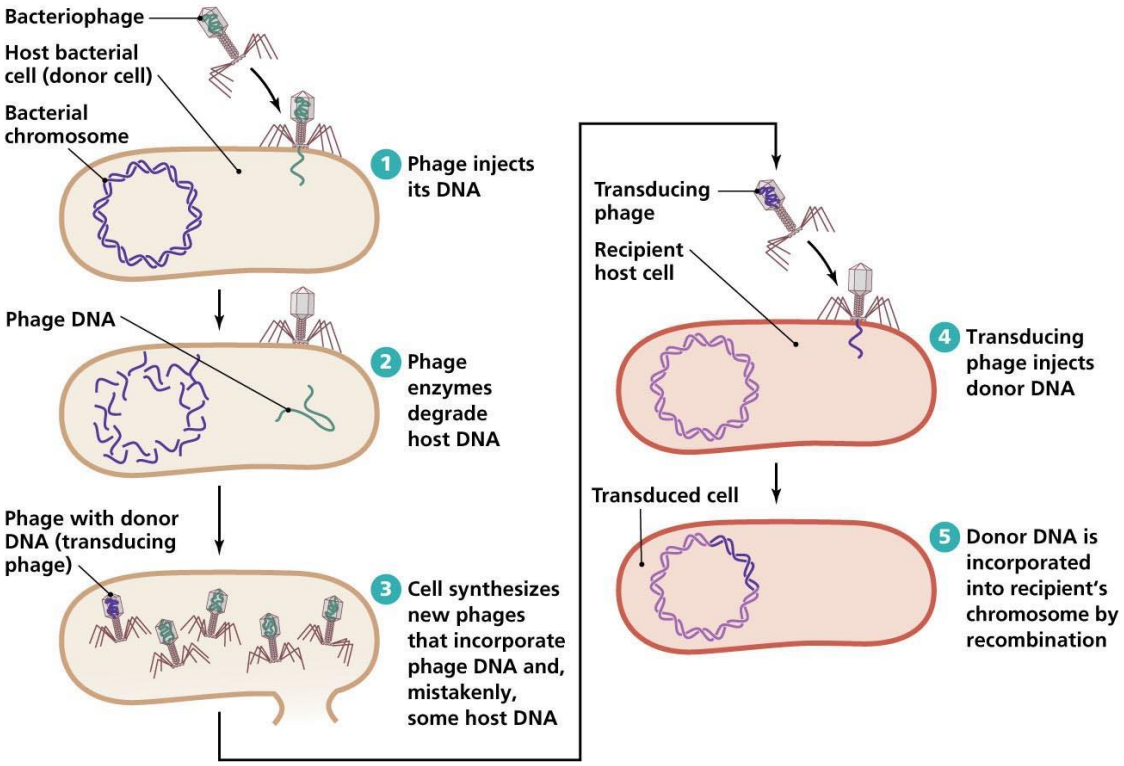
❖READ MORE ON THE EXPERIMENT OF GRIFFITH ON TRANSFORMATION

# Transduction

- It is the phenomenon or process in which the DNA from one bacterium is transferred to other bacterium with the help of a viral vector. OR

- It is a process of genetic recombination in bacteria in which genes from a host cell are incorporated or integrated into the genome of a bacterial virus (bacteriophage) and then…

- carried to another host cell when the bacteriophage initiates another cycle of infection.

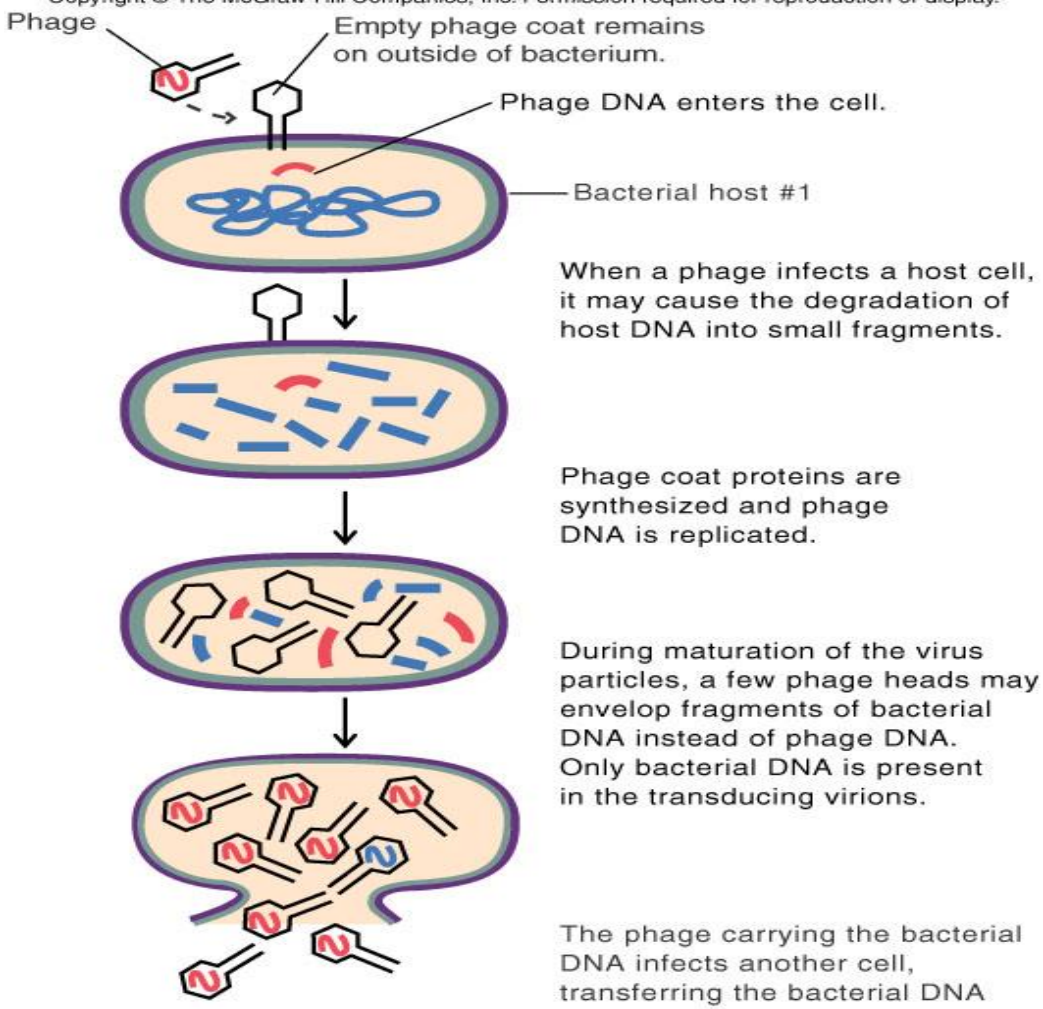


- Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNAase resistant (transformation is susceptible to DNAase).

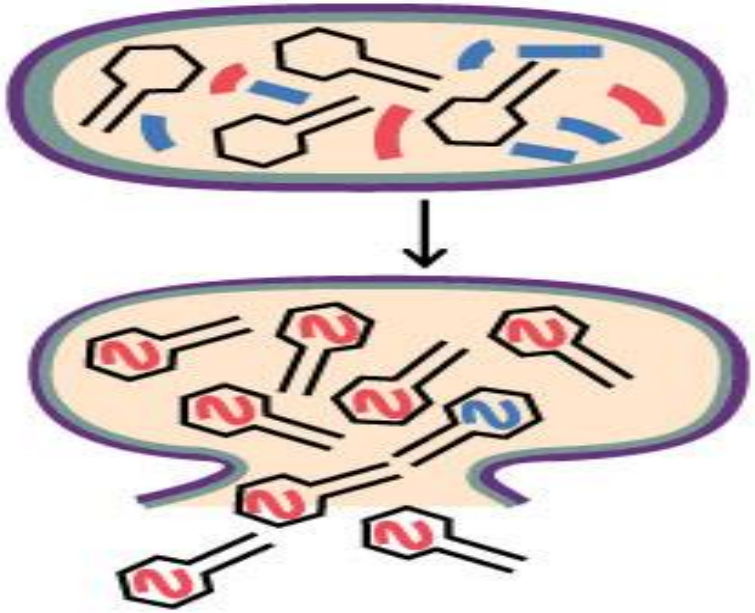
- Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.

- Bacteriophage are viruses that parasitize bacteria and use their machinery for their own replication.

- During the process of replication inside the host bacteria the bacterial chromosome or plasmid is erroneously packaged into the bacteriophage capsid.



- Therefore newer progeny of phages may contain fragments of host chromosome along with their own DNA or entirely host chromosome.



- When such phage infects another bacterium, the bacterial chromosome in the phage also gets transferred to the new bacterium.

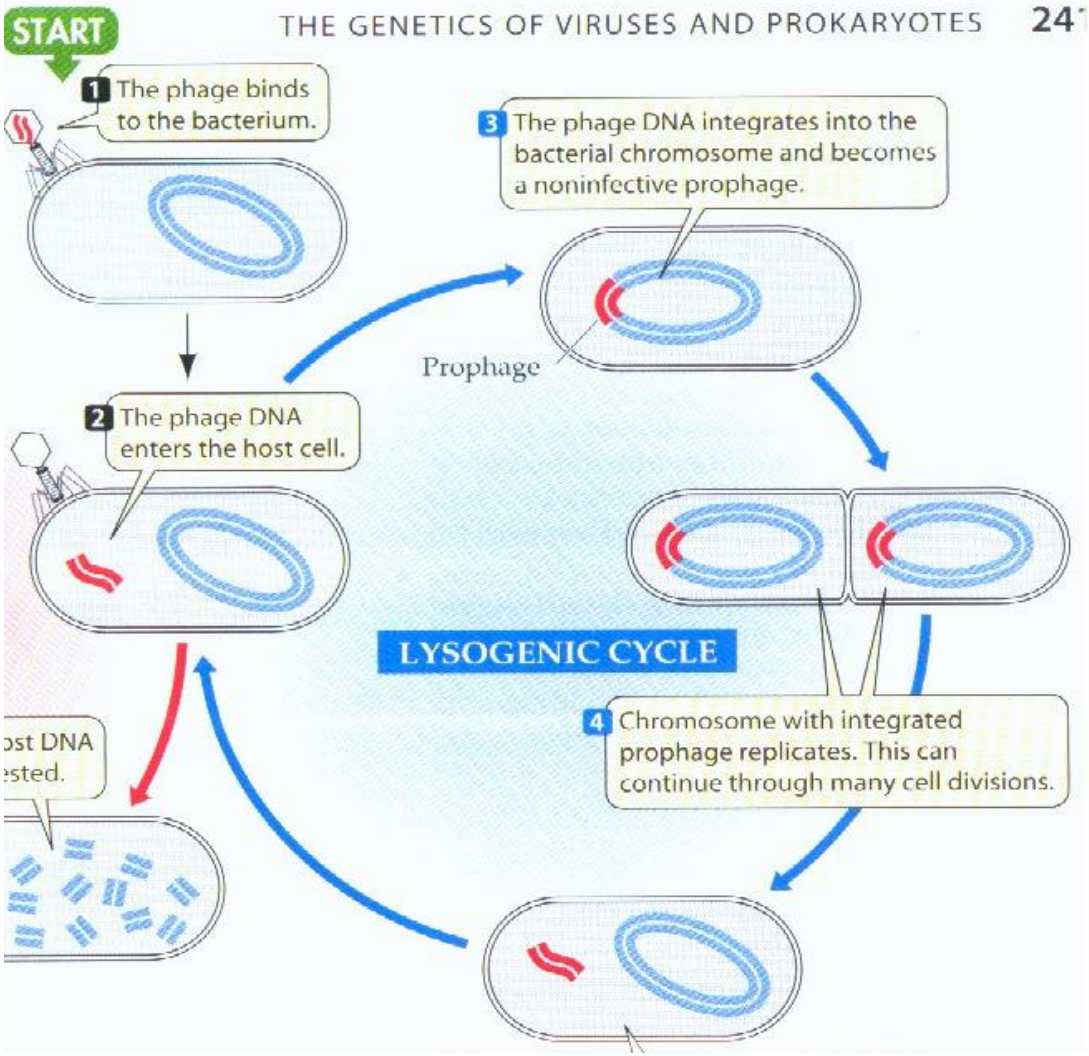
- This fragment may undergo recombination with the host chromosome and confer new property to the bacterium

- Life cycle of a bacteriophage may either be by lytic or lysogenic.

- In the former, the parasitized bacterial cell is killed with the release of mature phages while in the latter the phage DNA gets incorporated into the bacteria chromosome as prophage.

- In the case of temperate phages that undergo lysogenic cycle, the phage DNA gets incorporated into the bacterium chromosome.

- This is called a prophage and it behaves as if it were a part of bacterial chromosome.


START
1 The phage binds to the bacterium.
2 The phage DNA enters the host cell.
3 The phage DNA integrates into the bacterial chromosome and becomes a noninfective prophage.
Prophage
4 Chromosome with integrated prophage replicates. This can continue through many cell divisions.
LYSOGENIC CYCLE

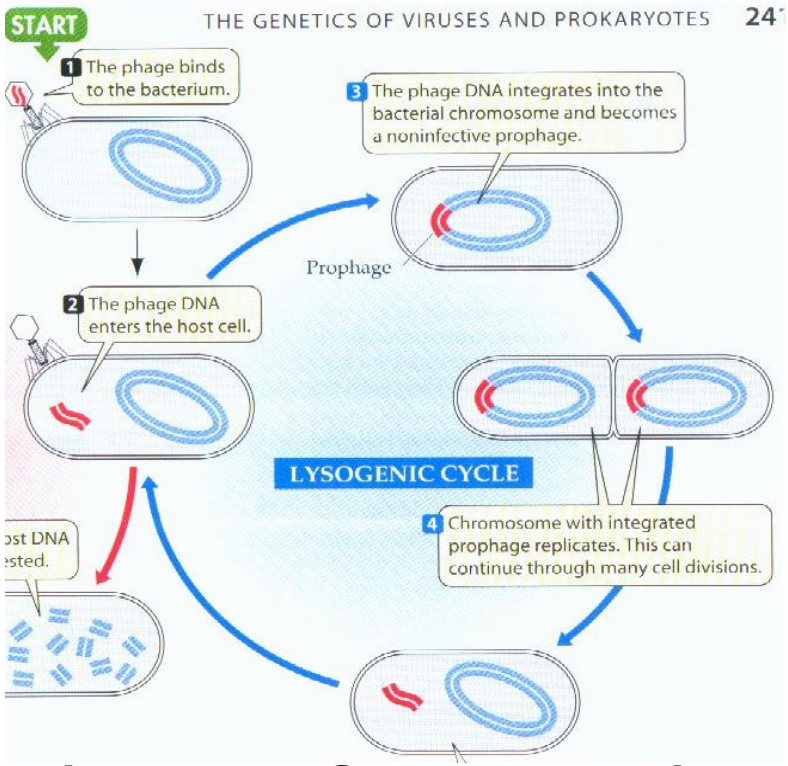
- This process is known as lysogenic conversion and the bacteria are called lysogenic bacteria.

- The genes present in the phage DNA also get expressed in the bacterium.

- The prophage sometimes disassociates itself from the host chromosome during multiplication of lysogenic bacteria, and in doing so;

- it sometimes carries along with itself fragments of bacterial chromosome.

- The separated prophage then initiates lytic cycle and the subsequent phage progeny may have a piece of chromosomal DNA.




- When such phage infects another bacterium, newer characteristics coded by that chromosomal gene are conferred or imparted to the infected bacterium.

- Two types of transduction are known; **restricted** (**Specialised**) transduction and **Generalized** transduction.

- Generalized transduction can transfer any part of bacterial gene to the recipient.

- This process may occur with phages (lytic phages) that degrade their host DNA into pieces about the size of the viral genomes.

- If these pieces are erroneously packaged into phage particles, they can be delivered to another bacterium in the next phage infection cycle.

- Phages P22 of *Salmonella typhimurium* and P1 and μ of *E. coli* carry out generalized transduction.

- In restricted transduction only those chromosomal genes that lie adjacent to the prophage are transmitted.

- The lambda phage that infects *E.coli* always transfers gal+ gene (responsible for galactose fermentation).

- Specialized transduction is only effective in transducing a few special bacterial genes while generalized transduction can transduce any bacterial gene

# Conjugation

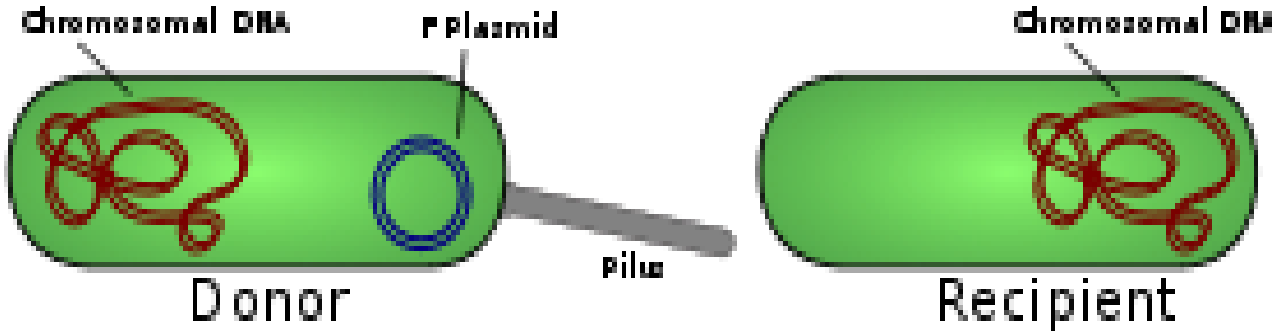
- Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells.

- Conjugation is a mechanism of horizontal gene transfer, as are transformation and transduction, though these two other mechanisms do not involve cell-to-cell contact.

- Bacterial conjugation is often incorrectly regarded as the bacterial equivalent of sexual reproduction or mating.

- Bacteria conjugation is merely a transfer of gene from one bacterium to other unlike the fusion of gametes in sexual reproduction in organisms.

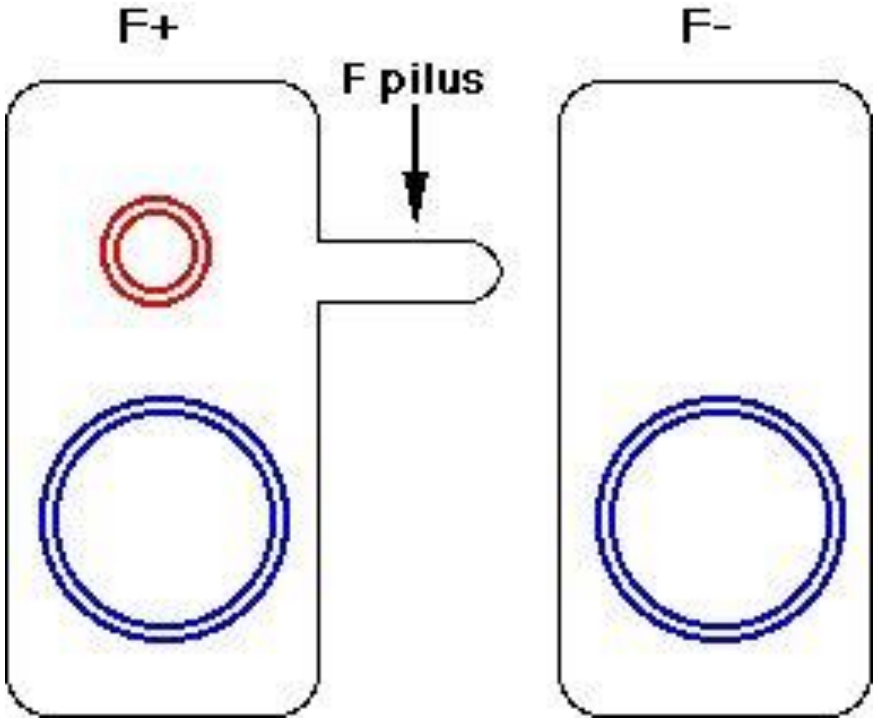
- The contact between the cells is via a protein tube called an **F** or **sex pilus**, which is also the conduit for the transfer of the genetic material.



- The basic conjugative plasmid is the **F-plasmid**, or F-factor.

- The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome) with a length of about 100,000 base pairs.

- Basic conjugation involves two strains of bacteria: **F+** and **F-**.



- The difference between these two strains is the presence of a **Fertility factor** (or F factor) in the F+ cells.

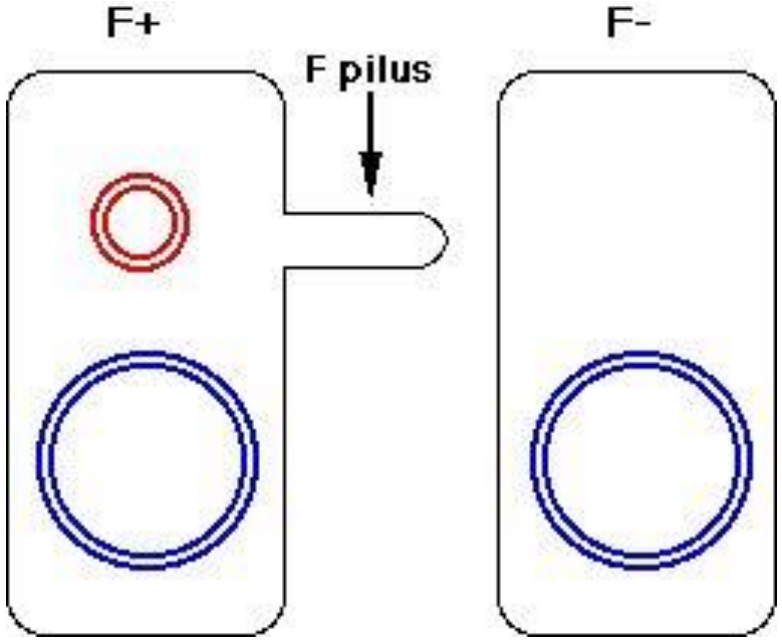
- The F factor contains about 19 genes and confers the ability to conjugate upon its host cell.

- There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted $F^+$).

- Cells that lack F plasmids are called *F-negative* or *F-minus* ($F^-$) and can function as recipient cells.

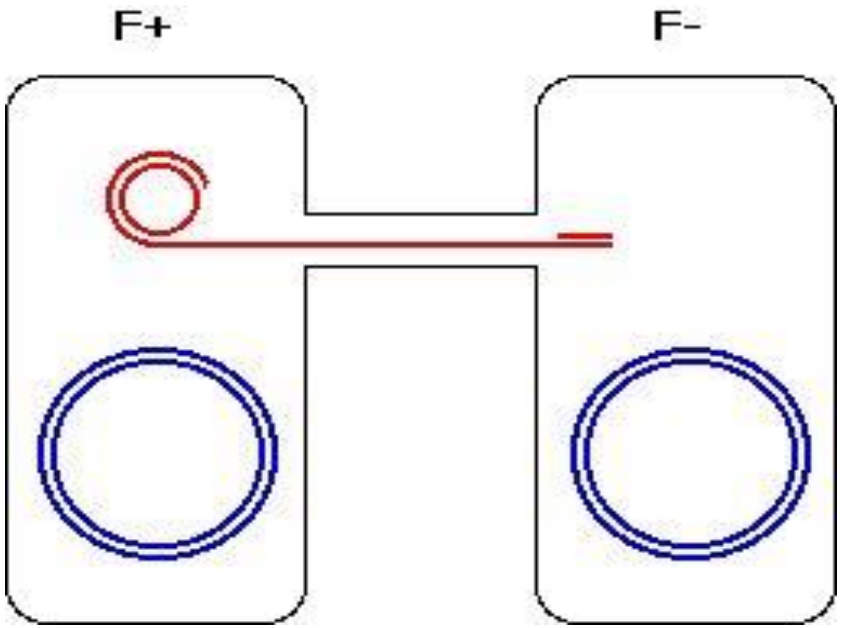
- Genetic transfer in conjugation is from an F+ cell to an F- cell, and the genetic material transferred is the F factor itself.

- The F+ cell initiates conjugation by extending an F pilus toward the F- cell.



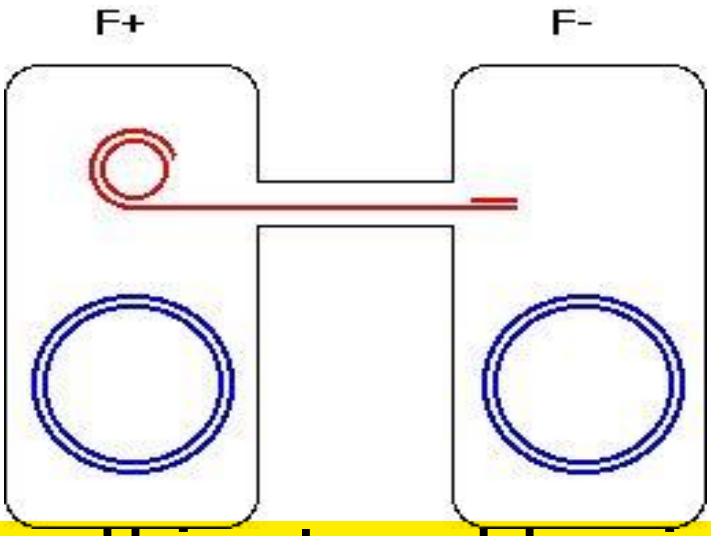

- Among the genes present on the F factor are the genes encoding the proteins required for pilus construction.

- The F pilus, when finished, temporarily connects the two cells.



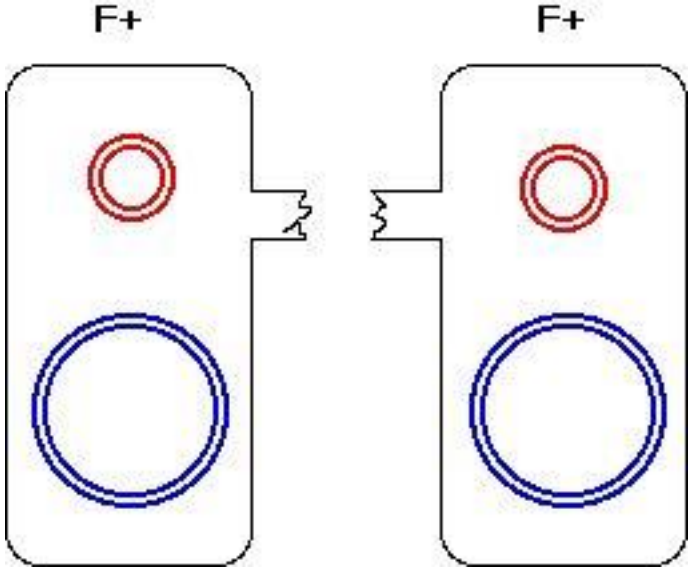

- On strand of the F factor is nicked, and begins unwinding from the other strand.

- The nicked strand begins to transfer through the F pilus to the F- cell.




- As it does so, this strand begins to be replicated, as does circular strand remaining behind in the F+ cell.

- Eventually, the nicked strand completely passes through to the recipient cell, and is completely replicated.

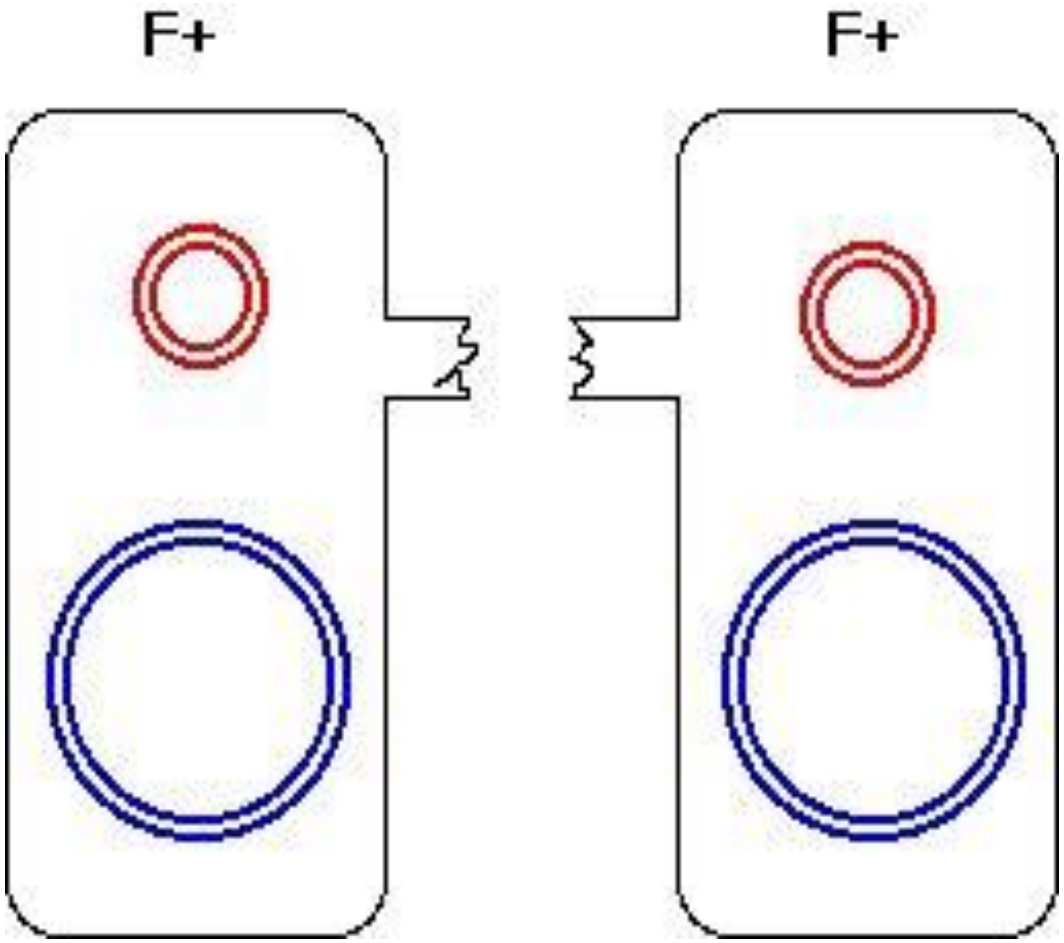



- This process produces a new F factor in the recipient cell.

- The pilus is broken, severing the connection between the two cells.

- Since both cells now contain an F factor, both cells are F+.

- The new F+ cell (which was the F- cell, can now initiate conjugation with another F- cell.


F+
F+

# DISTINGUISHING CHARACTERISTICS OF CONJUGATION

- DNA transfer requires cell-cell contact.
- DNA transfer occurs via a conjugal pore.
- DNA transfer occurs in one direction - from donor to recipient not vice vers.
- DNA transfer does not require protein synthesis in donor.

# Benefits of Conjugation

- The genetic information transferred is often beneficial to the recipient cell.

- Benefits may include antibiotic resistance, xenobiotic tolerance, or the ability to utilize a new metabolite.