Microbial World
Part II

- B. Sc. BOTANY
- SEM I

Reproduction In Bacteria

Asexual Reproduction

Budding

- Certain bacteria (eg. Caulobacter, Rhodomicrobium) can also reproduce by budding.
- The bacterial cell gives out many outgrowth.
- These outgrowth are called buds.
- These buds get separated from the body of bacterial cell and grow into new bacterial cell.

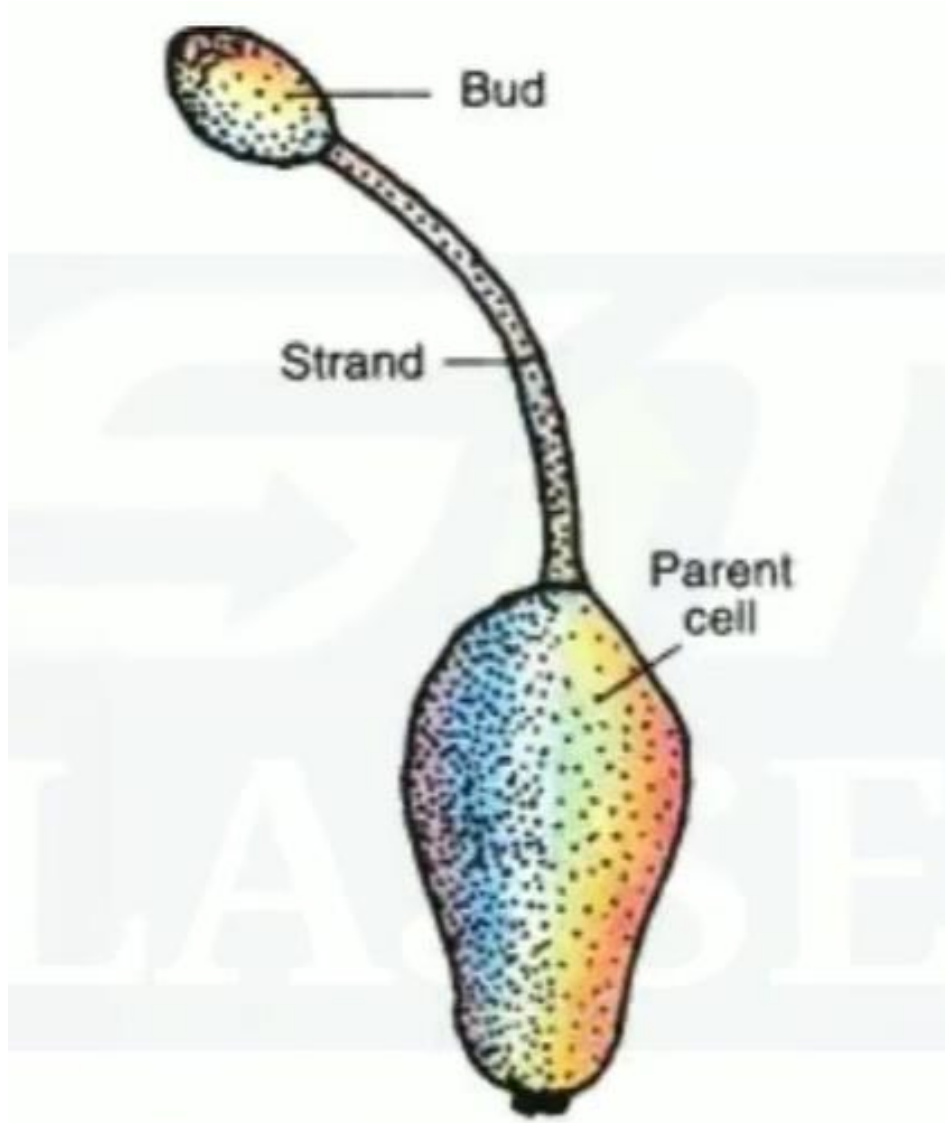


Fig. 18.9. *Bacteria.*

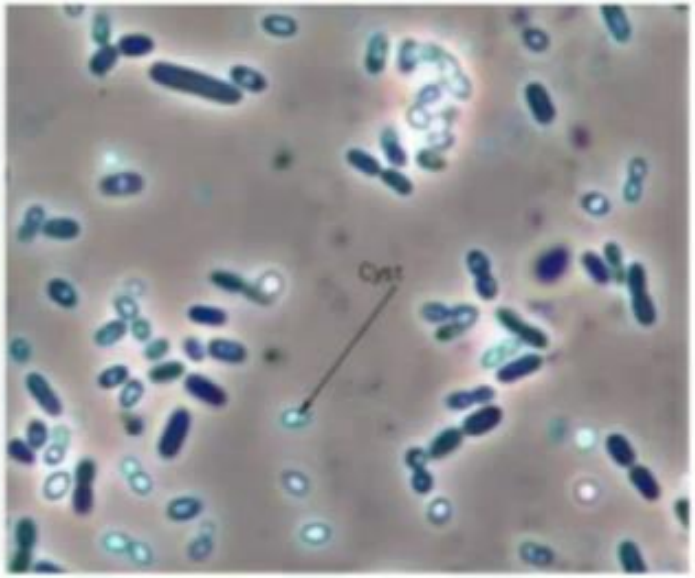
Cyst formation

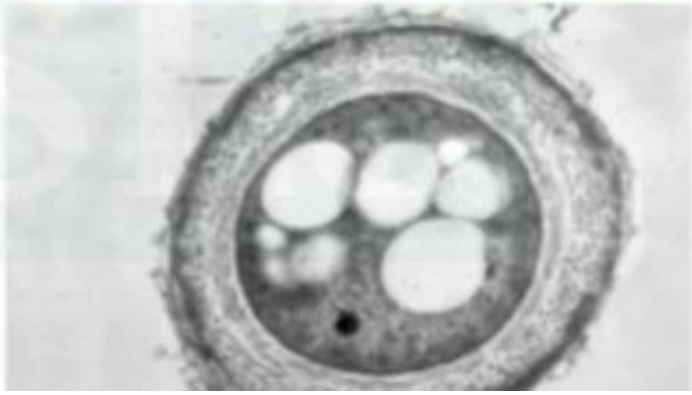
In some bacteria (eg.Azotobacter) thick walled ,spherical bodies called cyst are formed

These cyst release from the body and develop into new bacterial cells after germination.

Azotobacter Cysts

- Bacteria of the genus *Azotobacter* are nitrogen-fixing, Gram-negative organotrophs.
- At the end of exponential growth, some cells undergo a final division and initiate the formation of cysts.
- These differentiated cells are strikingly different from endospores formed by *Bacillus* spp.
- ***A. vinelandii*** has been the object of intensive research on its growth, differentiation and genetic properties.

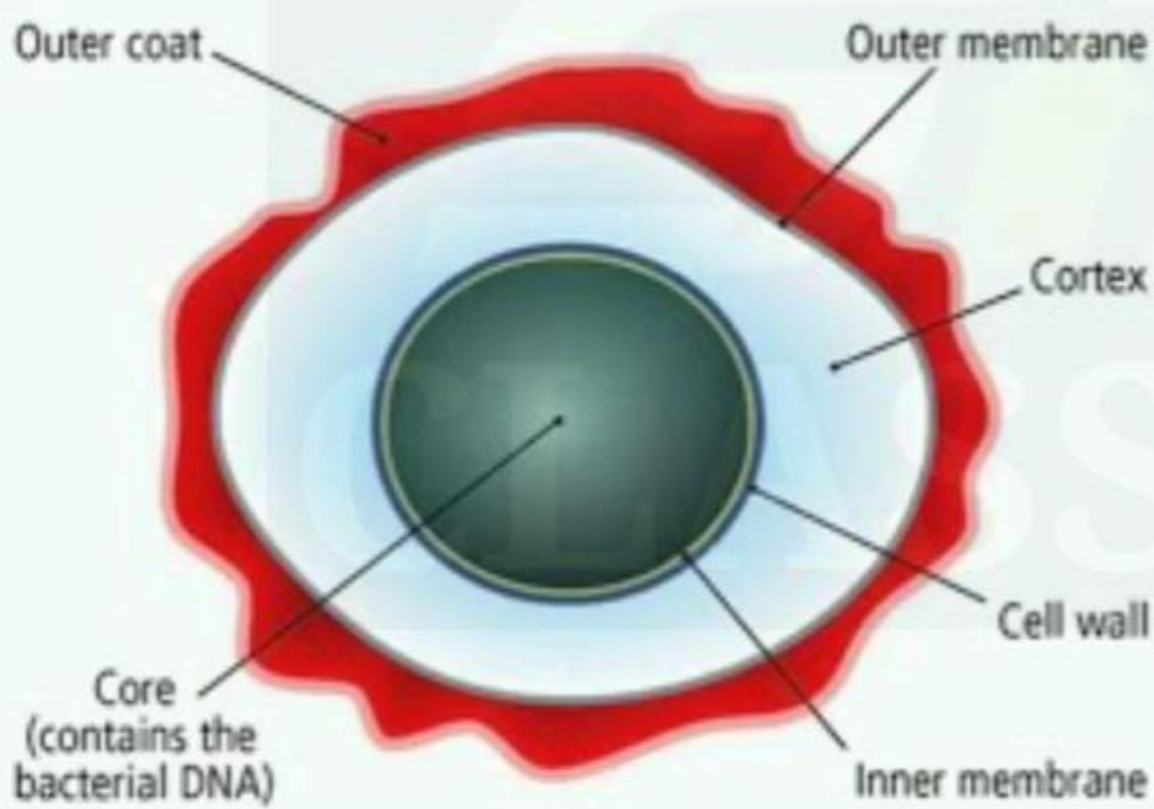




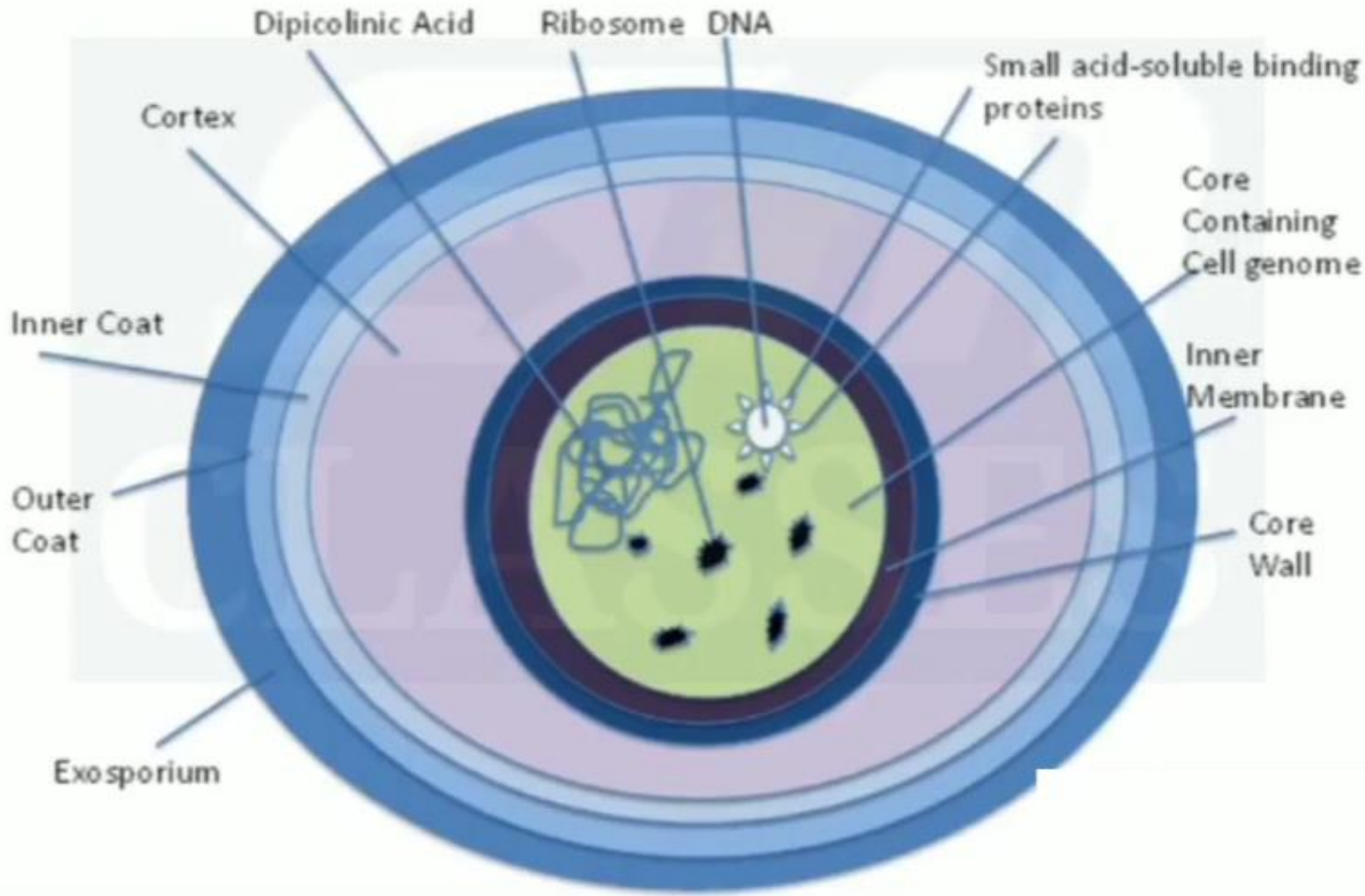
Endospores formation

- These are found in only a few bacili like Bacillus and Clostridium.
- But they are never found in cocci or spirilla.
- In this case single spore are formed inside the parental cell .they are called endospores.
- Strictly speaking endospores formation is not a method of reproduction because there is no increase in the number of cells.
- It is only a method of perennation during unfavourable condition.

Endospore

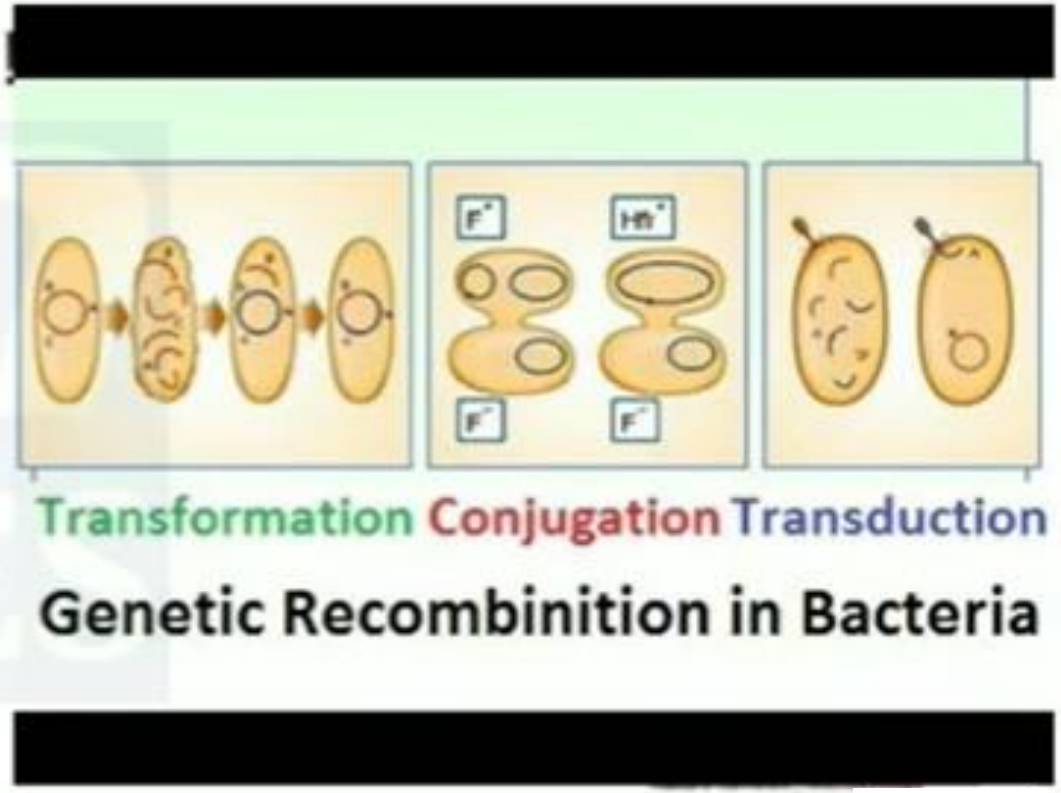

Outer coat
Outer membrane
Cortex
Cell wall
Core
(contains the
bacterial DNA)
Inner membrane

Structure of endospore



Sexual Reproduction In Bacteria

- Genetic variability – ESSENTIAL!!!
  - Survival of the species
- Diploid Eukaryotes
  - Crossing over
  - Meiosis
- Haploid Prokaryotes
  - Transduction
  - Transformation
  - Conjugation



Bacterial Recombination

- Genetic recombination refers to the exchange of genes between two DNA molecules to form new combinations of genes on a chromosome.

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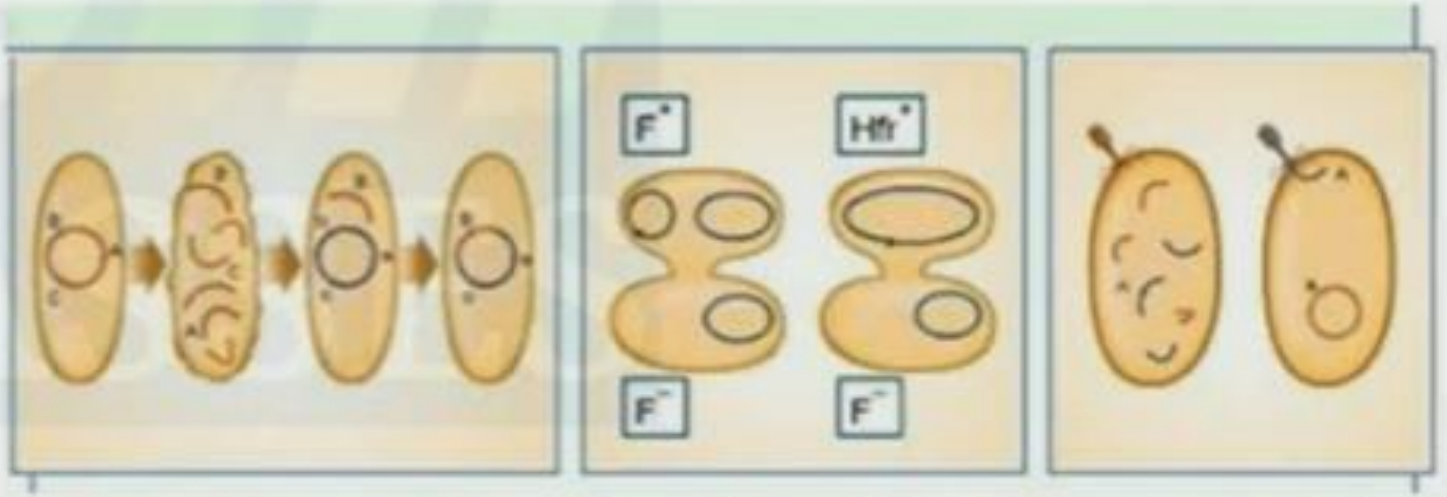
- Transduction
- Transformation
- Conjugation



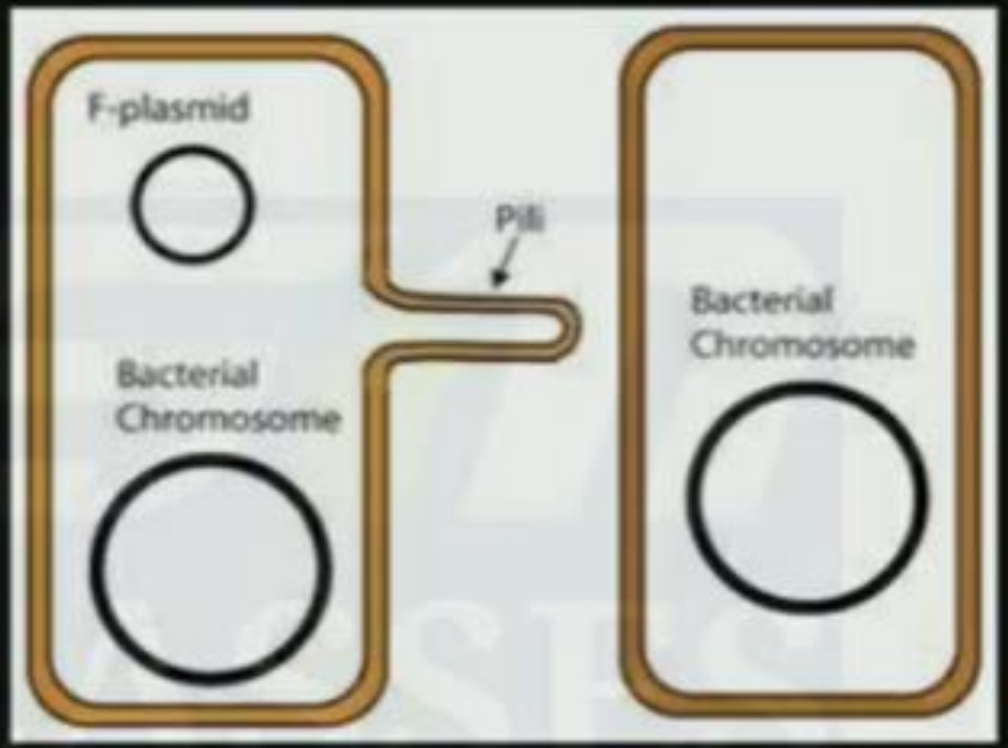
Genetic Recombinition in Bacteria

Bacterial Conjugation

- **Conjugation is the process by which one bacterium transfers genetic material to another through direct contact.**
- **Bacterial conjugation was first postulated by J. Lederberg and Edward Tatum.**
- **During conjugation, one bacterium serves as the donor of the genetic material, and the other serves as the recipient.**

CONJUGATION

BACTERIA A

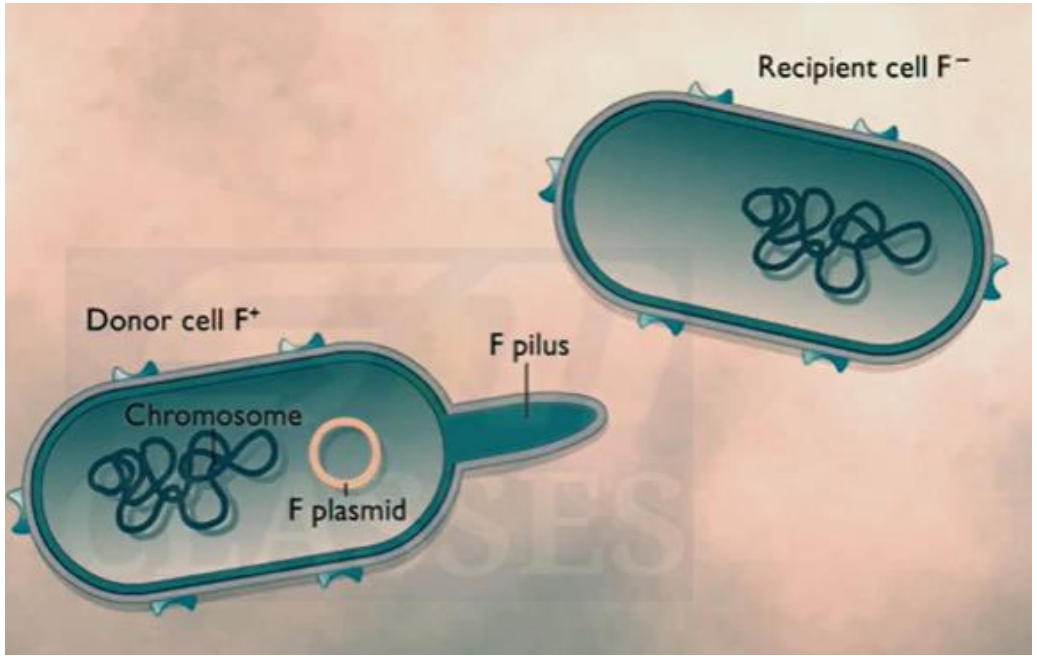


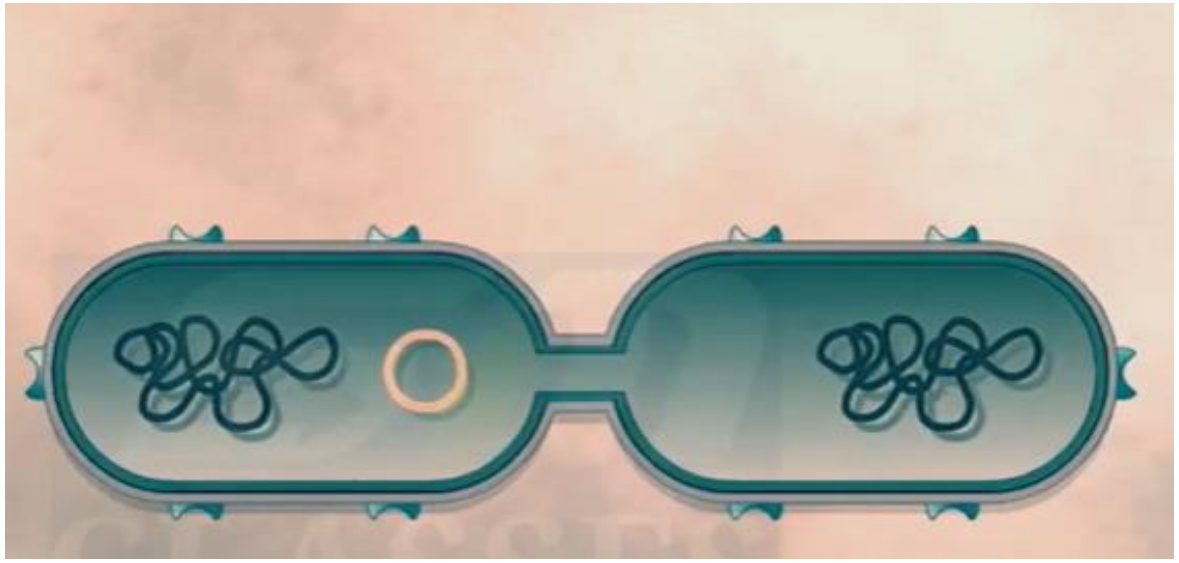
BACTERIA B

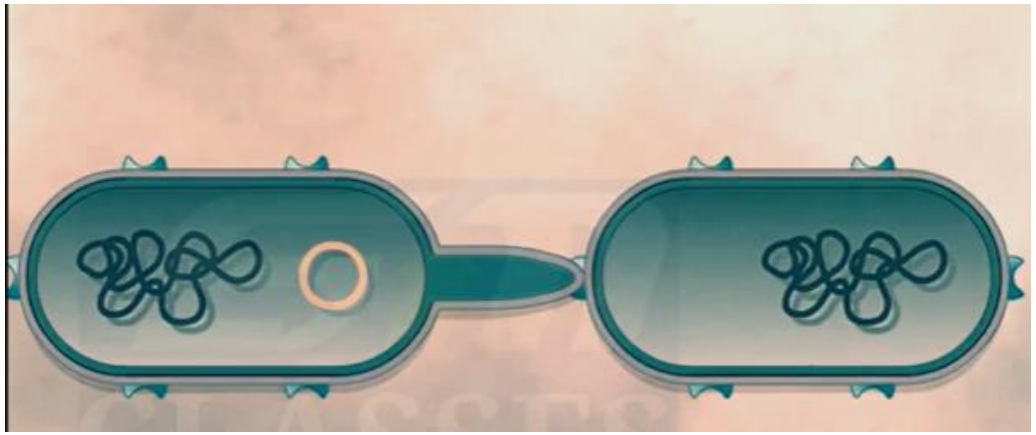
TRANSFER OF GENETIC MATERIAL FROM BACTERIA A TO BACTERIA B BY MATING OR CONTACT IS CALLED CONJUGATION.

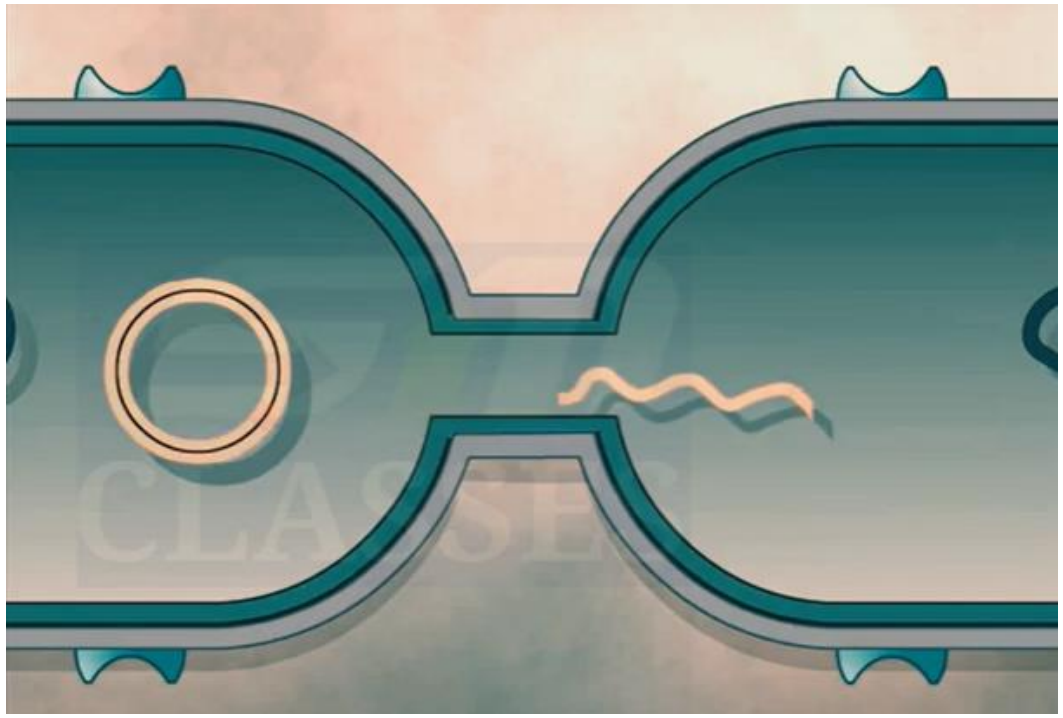
Steps of Bacterial Conjugation

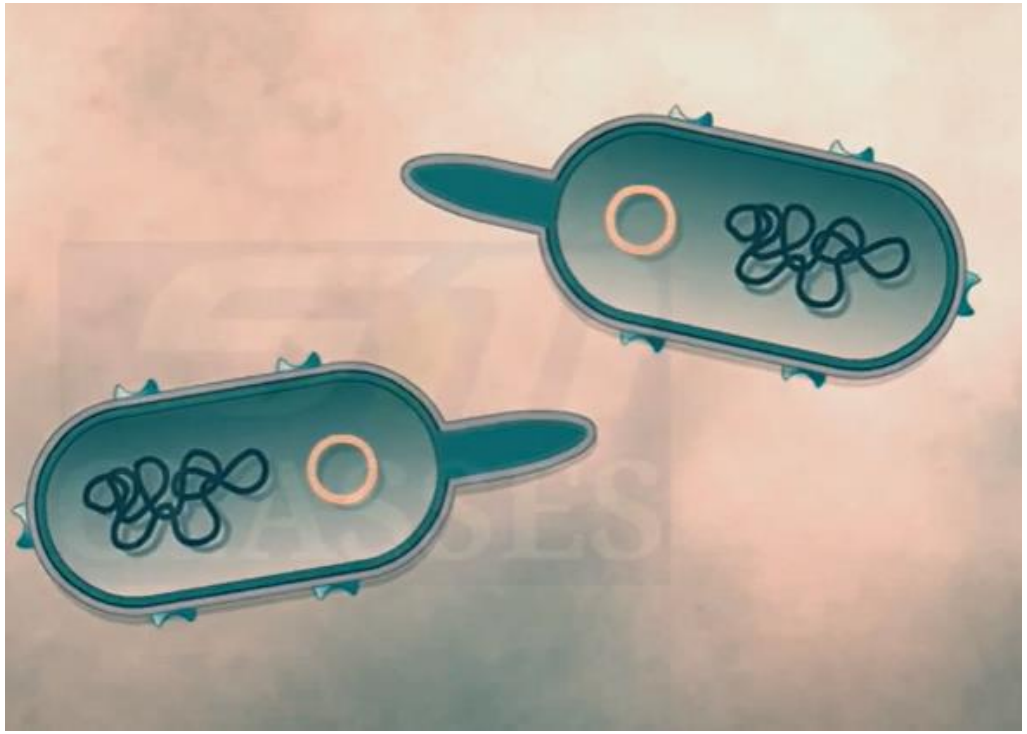
1. Pilus formation
2. Physical contact between donor cell and recipient cell
3. Transfer of F- plasmid
4. Complementary strand synthesis (maturation)


Recipient cell F⁻
Donor cell F⁺
F pilus
Chromosome
F plasmid

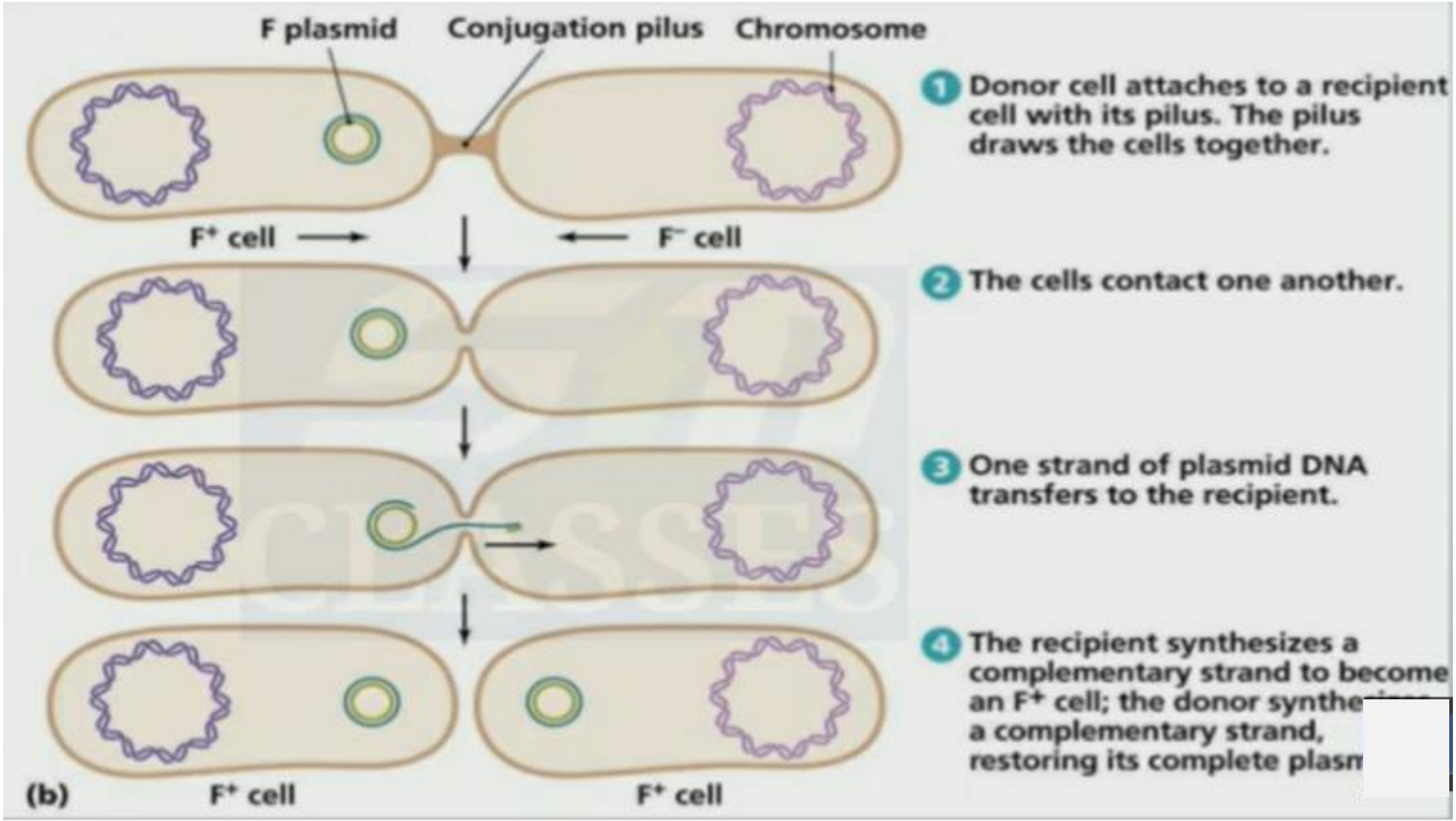








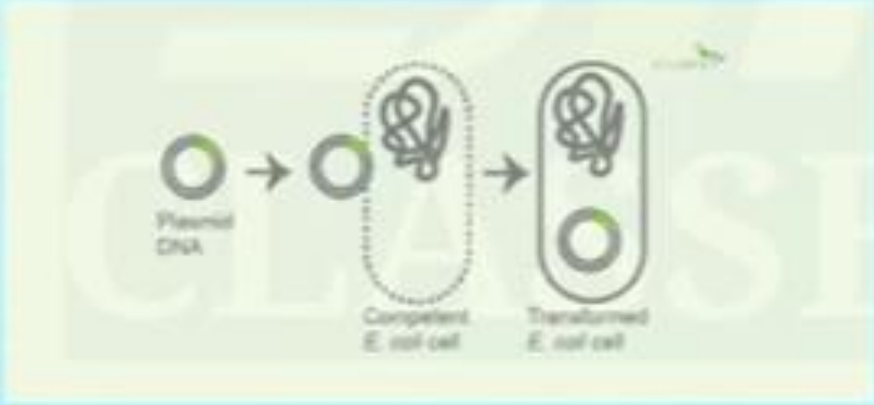
- The donor bacterium carries a DNA sequence called the fertility factor, or F factor.
- The donor cell (F^+), gives up DNA; and the recipient cell (F^-), receives the DNA.
- The F-factor allows the donor to produce a thin, tube like structure called a pilus.
- This sex pilus joins the donor and recipient during the transfer.


F plasmid
Conjugation pilus
Chromosome
F+ cell
F− cell
1 Donor cell attaches to a recipient cell with its pilus. The pilus draws the cells together.
2 The cells contact one another.
3 One strand of plasmid DNA transfers to the recipient.
4 The recipient synthesizes a complementary strand to become an F+ cell; the donor synthe
a complementary strand,
restoring its complete plasm
(b)
F+ cell
F+ cell

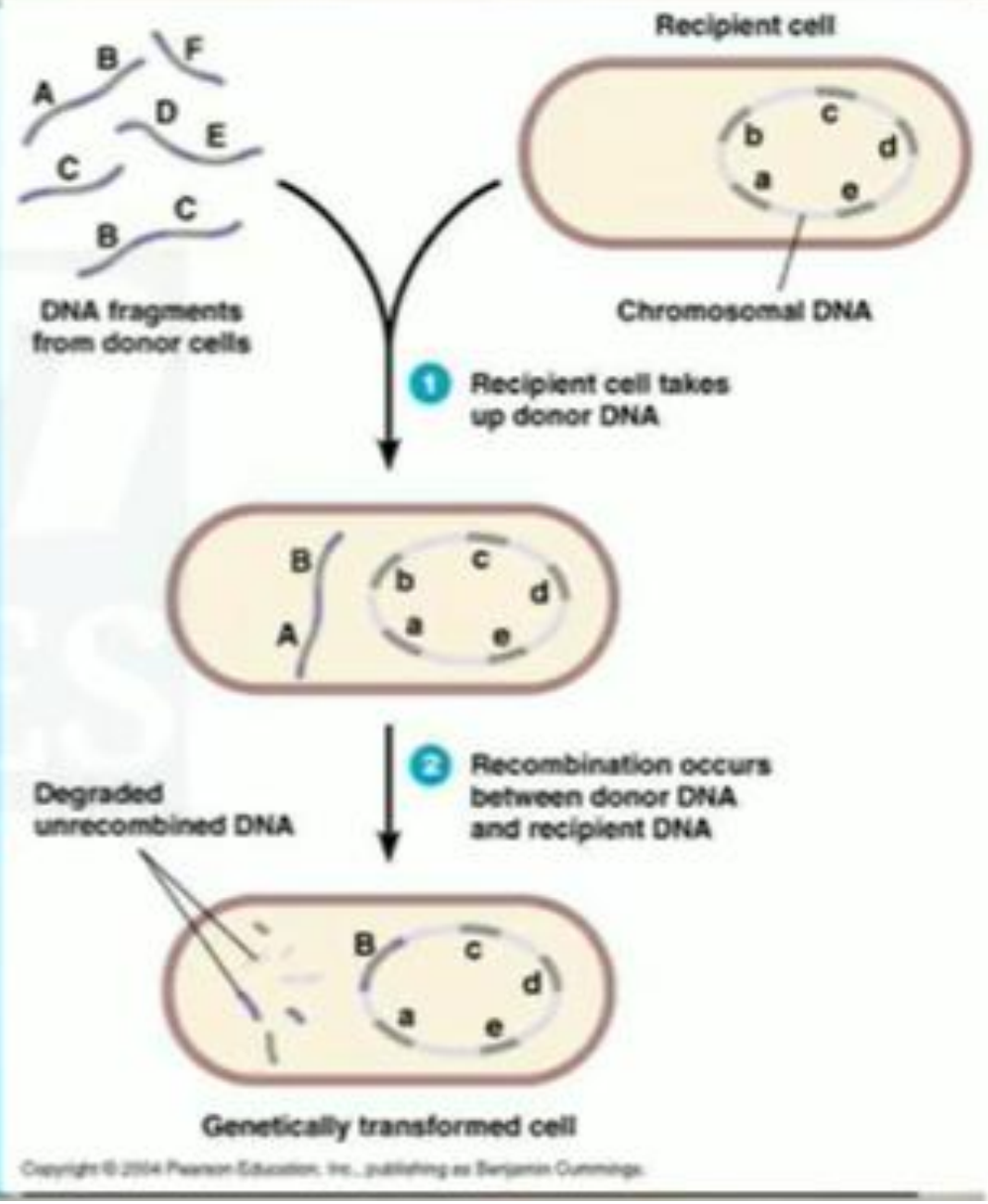
Terminology

A high-frequency recombination cell (Hfr cell) (also called an Hfr strain) is a bacterium with a conjugative plasmid (for example, the F-factor) integrated into its chromosomal DNA. The integration of the plasmid into the cell's chromosome is through homologous recombination. A conjugative plasmid capable of chromosome integration is also called an episome (a segment of DNA that can exist as a plasmid or become integrated into the chromosome)

What is transformation?

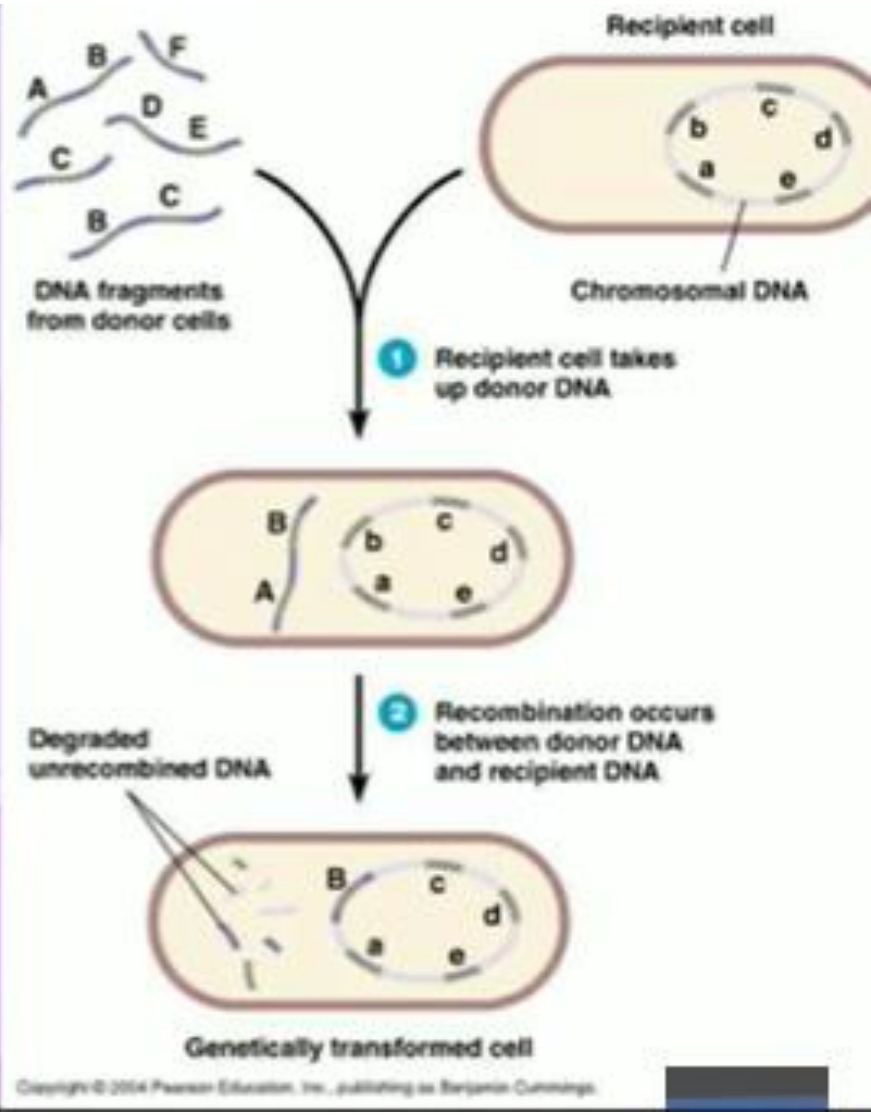
- Genetic transformation is the incorporation of naked DNA from the extracellular environment.
- 

- Cells that can be used for transformation are called competent.



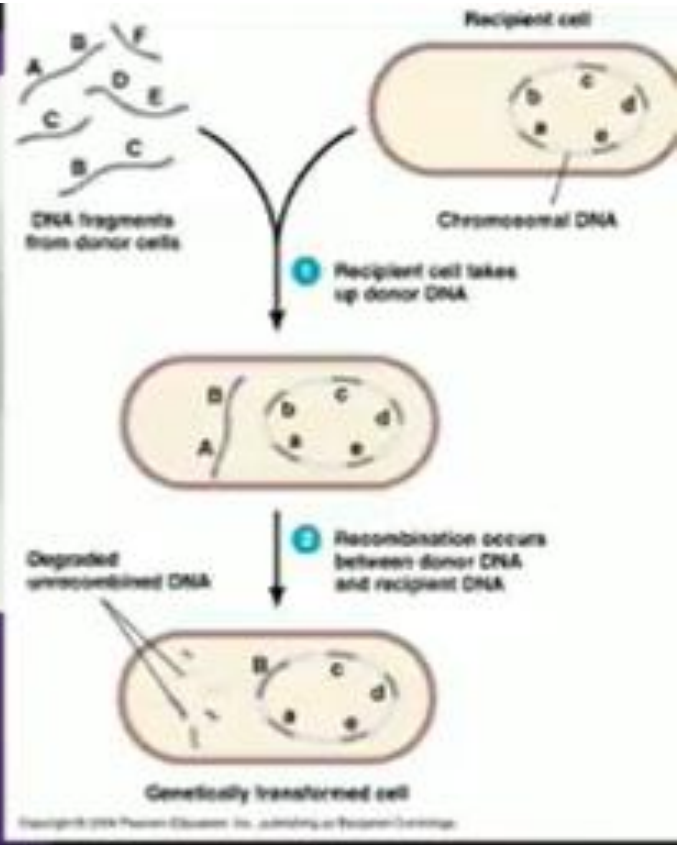
Introduction

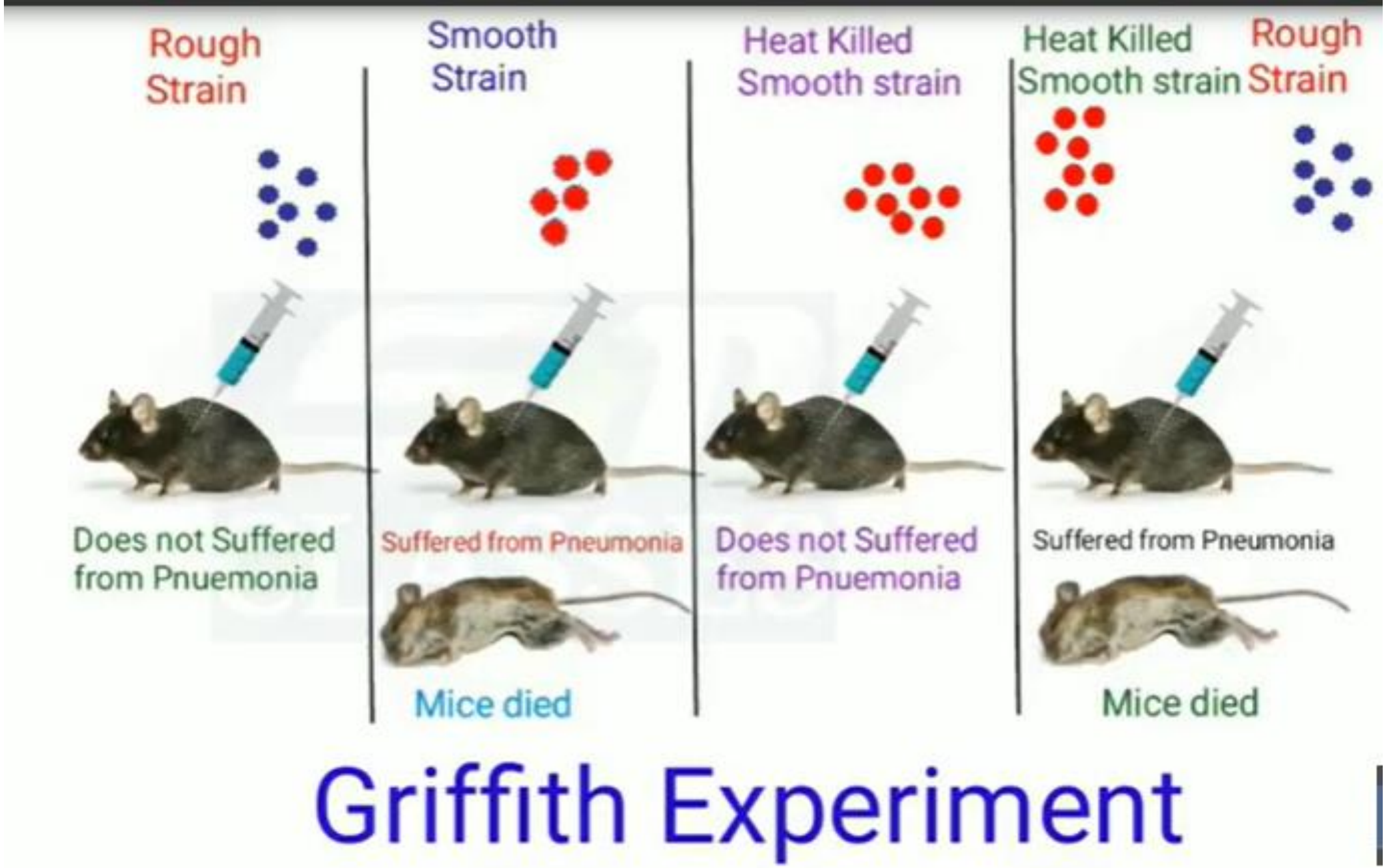
- The DNA can move between bacteria by a process called DNA transformation was first discovered by Fred Griffith in 1928.



Frederick Griffith - 1928

- He put forward the concept of Genetic Transformation with is Experiment.
- A non-virulent strain (R- Strain) of bacteria can become virulent (S - Strain) when mixed with their heat killed pathogenic counterpart.





Griffith Experiment

Frederick Griffith

British medical officer

1928

Performed an experiment on

Bacterium

Streptococcus pneumonia

Diplococcus pneumonia

Causes Pneumonia

In Humans & other Mammals

Streptococcus pneumonia has 2 strains

S strain

Smooth strain
Virulent
Pathogenic
Encapsulated

R strain

Rough strain
Non virulent
Non pathogenic
Non encapsulated

Four experiments on these Bacteria

First

Rough Strain

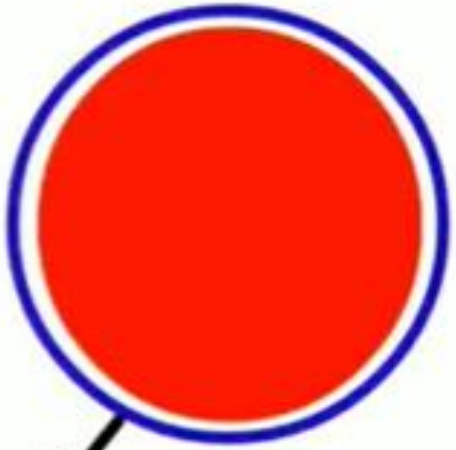
Mice does not suffered from Pneumonia

Survived

Second

S strain

Mice developed Pneumonia



Polysaccharide Capsule

Died

Third

Mice survived

Heat Killed S strain

Due to heating

Lost its
Polysaccharide coat

Fourth experiment

Heat Killed
S strain

Mixed

+

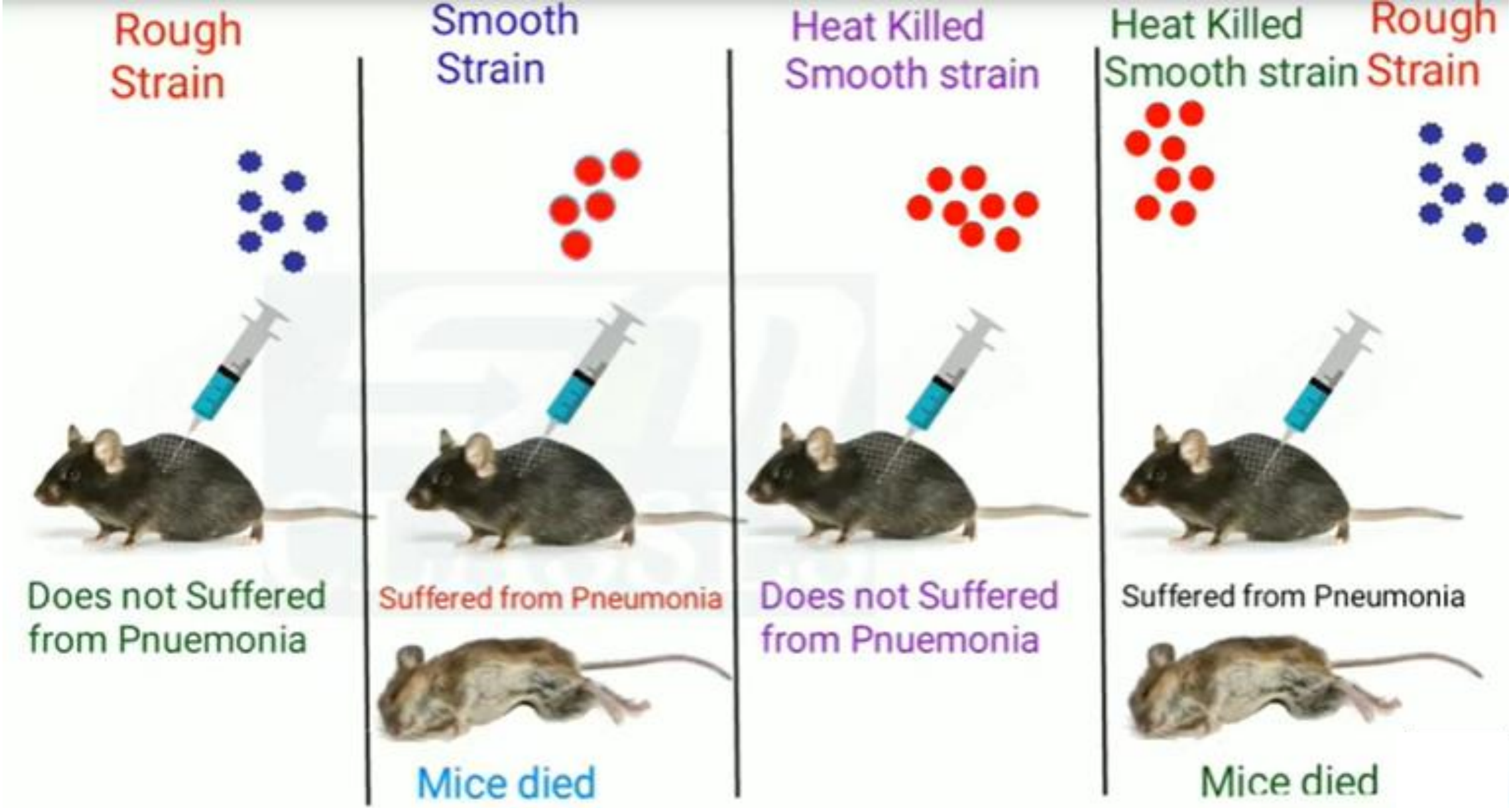
R strain

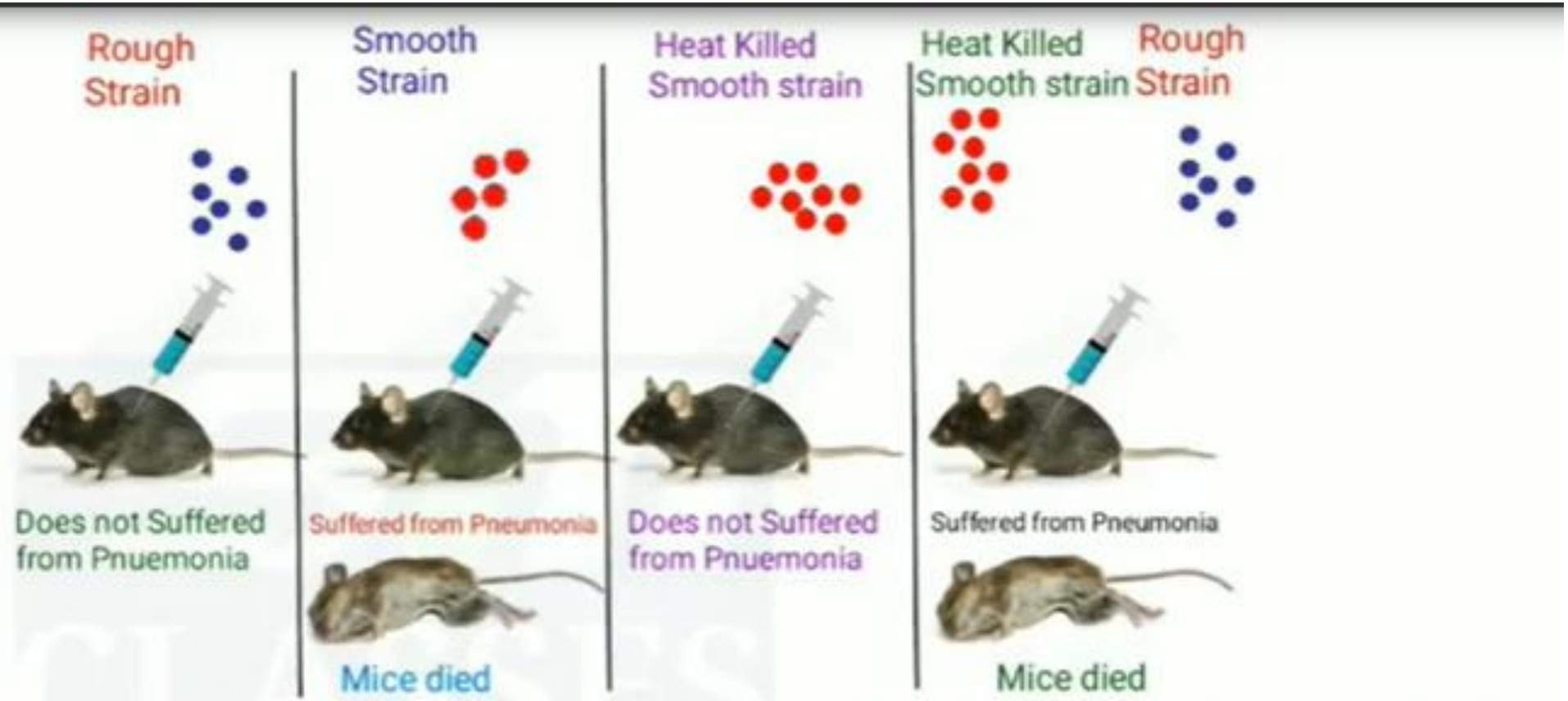
Mice suffered from Pneumonia

Died

Blood

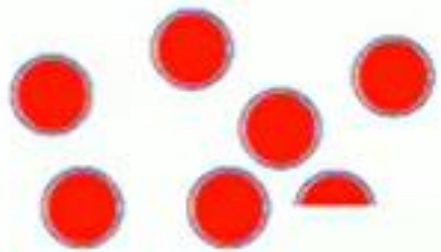
Recovered large number of
live S strain


Rough Strain
Smooth Strain
Heat Killed Smooth strain
Heat Killed Smooth strain
Rough Strain
Does not Suffered from Pnuemonia
Suffered from Pneumonia
Mice died
Does not Suffered from Pnuemonia
Suffered from Pneumonia
Mice died



Harmless R strain

Deadly S strain bacterium



Griffith showed that the change was Genetic

Heat killed
S strain

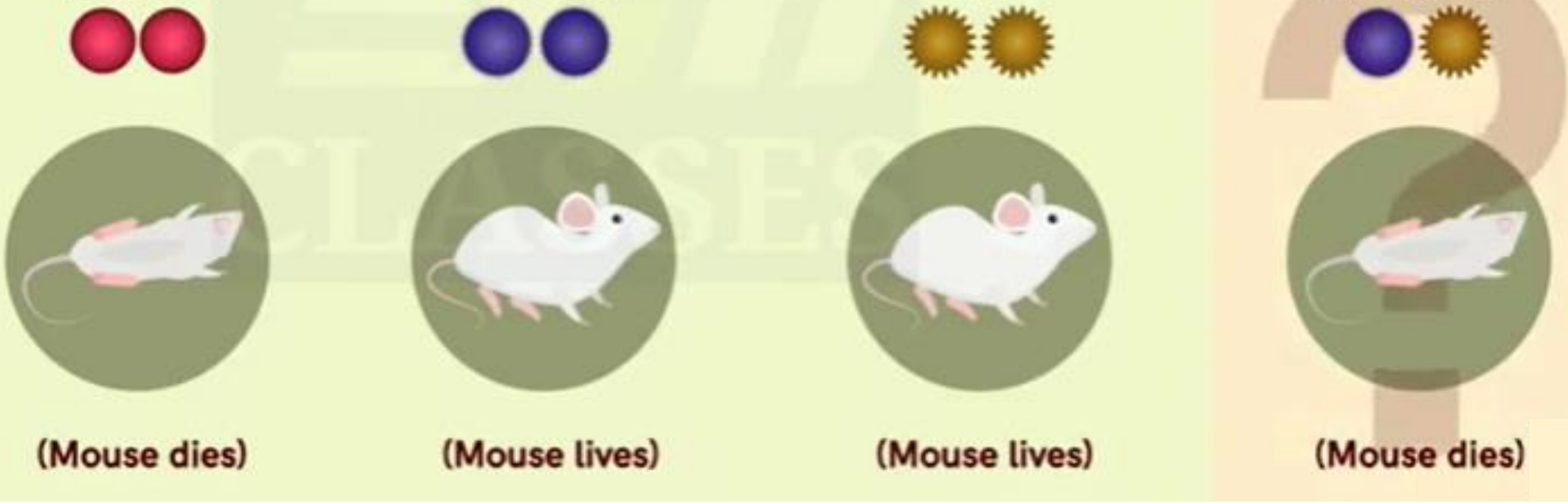
Genetic Material
Some how changed

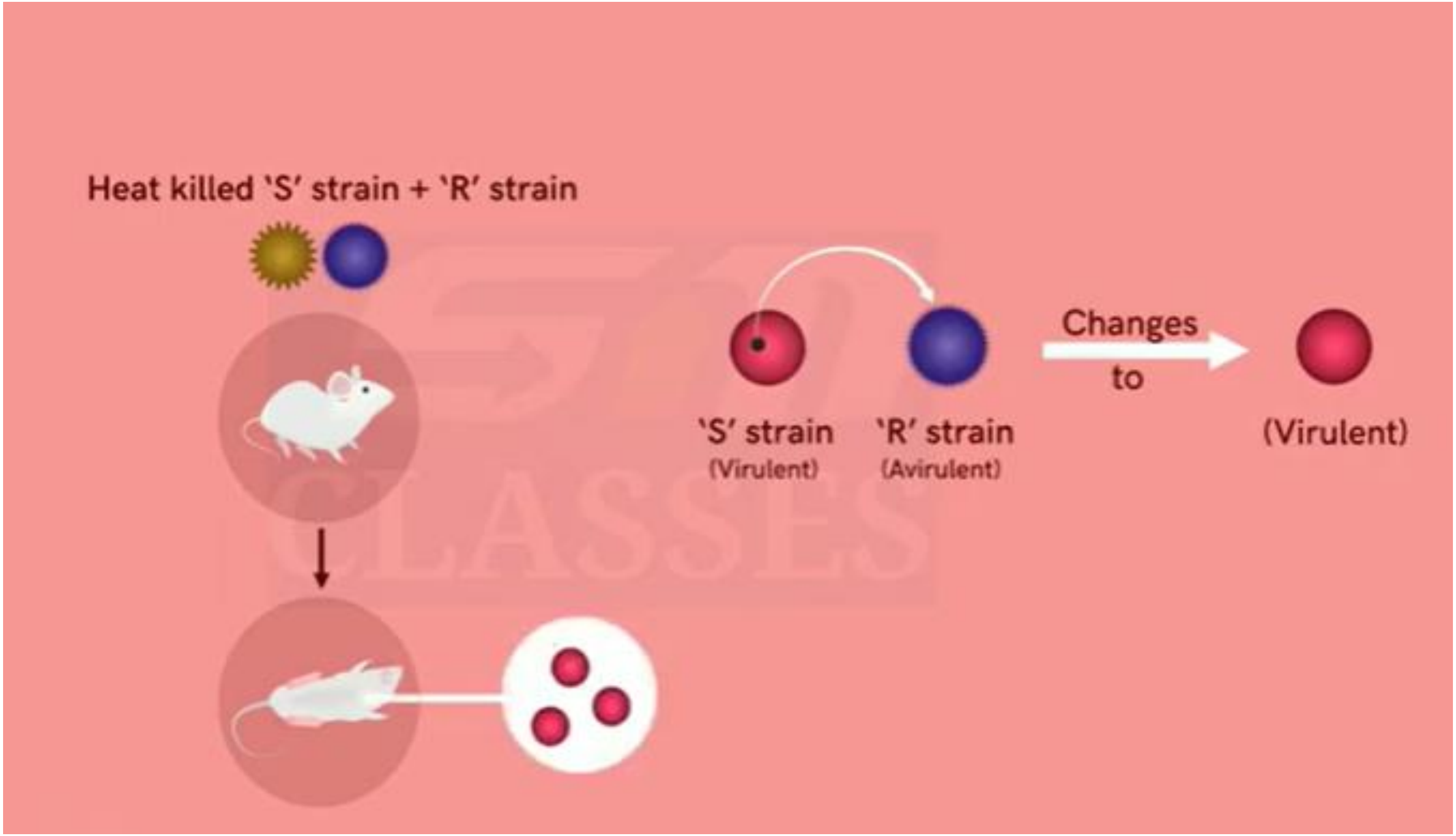
Living R strain

S strain
Bacterium

Pneumococcal strains

Smooth strain → Rough strain → Heat killed Smooth strain




Heat killed 'S' strain + 'R' strain
'S' strain
(Virulent)
'R' strain
(Avirulent)
Changes
to
(Virulent)

Types of Transformation

There are two types of transformation:

1.Natural transformation

2.Artificial transformation

Natural Transformation

- In this case DNA take-up occurs without outside help.
- **Naturally competent bacterium** – They can take up DNA from the environment without requiring special treatment.
- About 40 species have been found to be naturally competent or transformable.
- **Examples**: *Bacillus subtilis, Streptococcus pneumoniae, Haemophilus influenzae, Neisseria gonorrhoeae, Helicobacter pylori, Acinetobacter baylyi,* and some species of marine cyanobacteria.

Artificially induced competence

- Bacteria can be sometimes be made competent by certain chemical treatments or DNA can be forced into bacteria by a strong electric field in a process called electroporation.

1. Chemical Treatment (with calcium ions).

- Chemically induced transformation is usually inefficient, and only a small percentage of the cells are ever transformed.
- The cells must be plated under conditions, selective for the transformed cells.
- Therefore, the DNA used for the transformation should contain a selectable gene such as encoding resistance to an antibiotic.

Artificially induced competence

2. Electroporation

- The bacteria are mixed with DNA and briefly exposed to a strong electric field.
- The bacteria first be washed extensively in buffer with very low ionic strength such as distilled water.
- The brief electric field across the cellular membranes might create artificial pore of H_2O lined by phospholipid head groups. DNA can pass through these temporary hydrophilic pores.

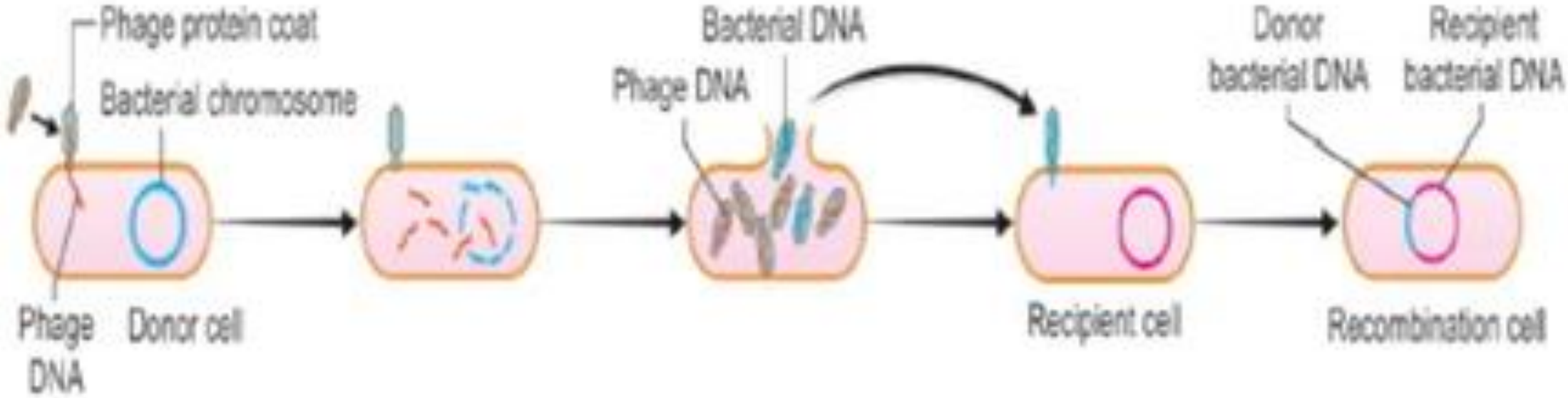
Transduction

- Transmission of a portion of DNA from one bacterium to another by a **bacteriophage (VIRUS)** is known as transduction

1. Bacteriophages are viruses that parasitise bacteria and multiply in it.

2. During the assembly of bacteriophage progeny inside infected bacteria, errors may happen occasionally.
3. Besides its own nucleic acid, host DNA may accidentally be incorporated into the bacteriophage. This is known as **'packaging error'**

4. Hence, when this bacteriophage infects another bacterium, host DNA is transferred and the recipient cell acquires new characters coded by donor DNA.



1 A phage infects the donor bacterial cell

2 Phage replication and the bacterial chromosome is degraded into pieces

3 Phage assembly- Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. The donor cell lyses and releases phage particles containing bacterial DNA

4 A phage carrying bacterial DNA infects another cell and delivers donor cell DNA

5 Integration of donor DNA and recombination can occur producing a recombinant cell

- In Transduction → transfer can occur of **chromosomal DNA as well as episomes and plasmids**

- **Eg.** Penicillin resistance in staphylococci is due to the plasmids transferred from one bacterium to another by transduction.

Role of Transduction

- Transduction provides an excellent tool for the **genetic engineering**

- **Transduction appears to be the most widespread mechanism of gene transfer among prokaryotes**

TRANSDUCTION IN BACTERIA

Transduction is the process by which bacterial DNA is moved from one bacterium to another by a **virus**.

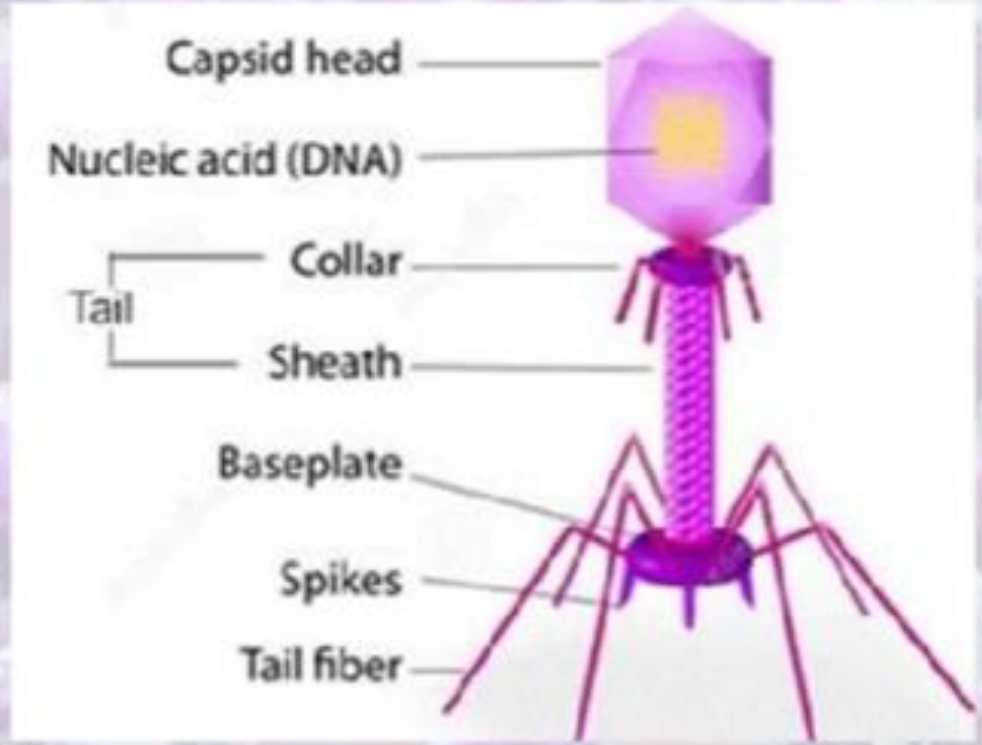
- It was first discovered by Joshua Lederberg in **1952**.

Features

1. The most striking feature is the transfer of genetic material from cell to cell by **viruses**.
2. The second feature is the fact that only a **small** part of the total genetic material of any one bacterial cell is carried by any particular transducing particle.

BACTERIOPHAGE:

Bacteriophage is a virus that infects a **bacterial cell.**



TRANSDUCING PHAGE:

A phage virus that **transfers genetic material (DNA)** from one bacterial cell to another is called transducing phage.

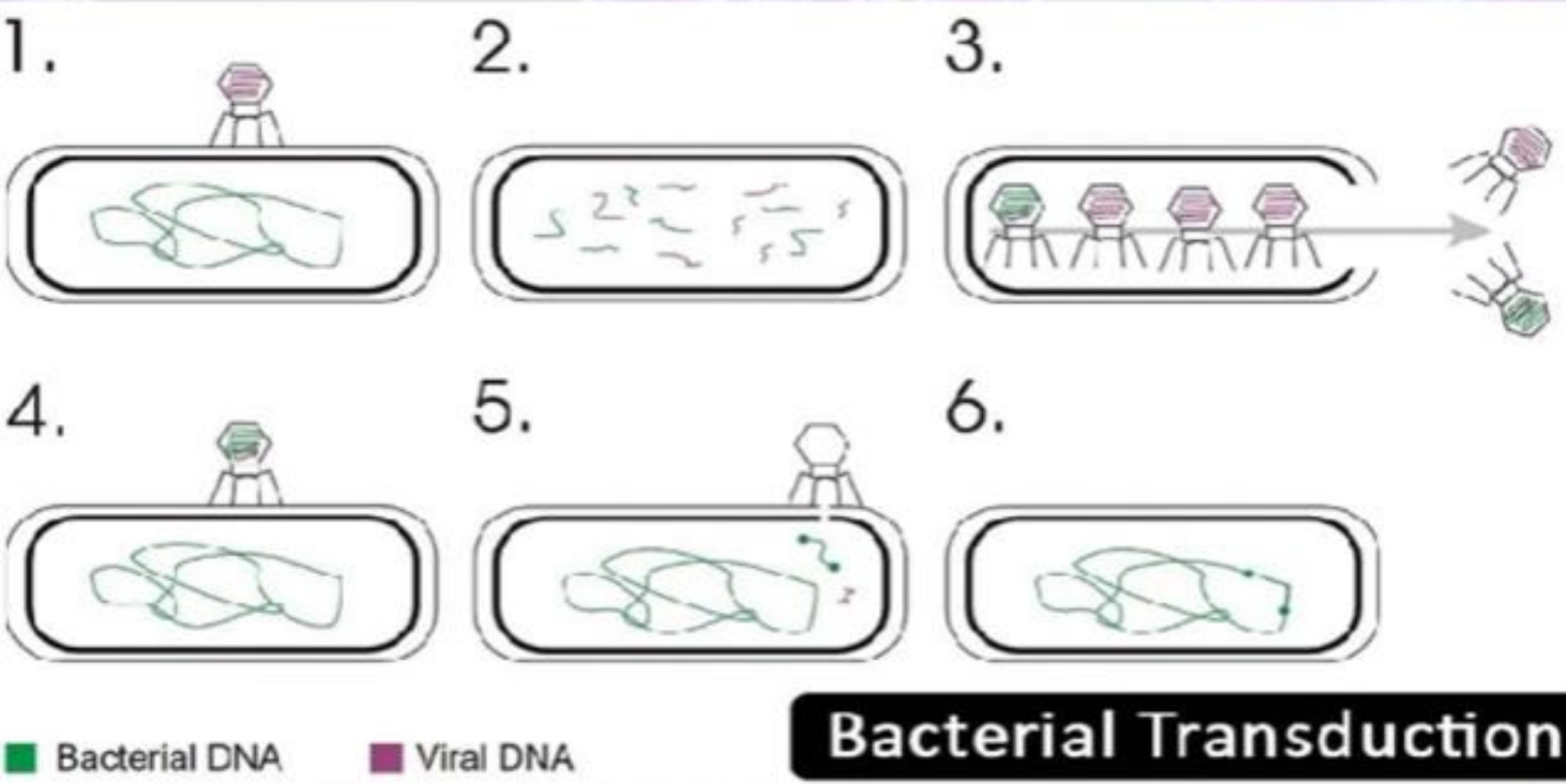
Virulent phage

1. Virulent phages are a type of bacteriophages that replicate only via the lytic cycle
2. Do not form prophage stage.
3. Lyrics cycle.
4. Generalized transduction

Temperate phage

1. Temperate phages are bacteriophages that replicate using both lytic and lysogenic cycles
2. Form prophage stage.
3. Both lytic and lysogenic cycle
4. Specialized transduction

MECHANISM OF TRANSDUCTION:



TYPES OF TRANSDUCTION:

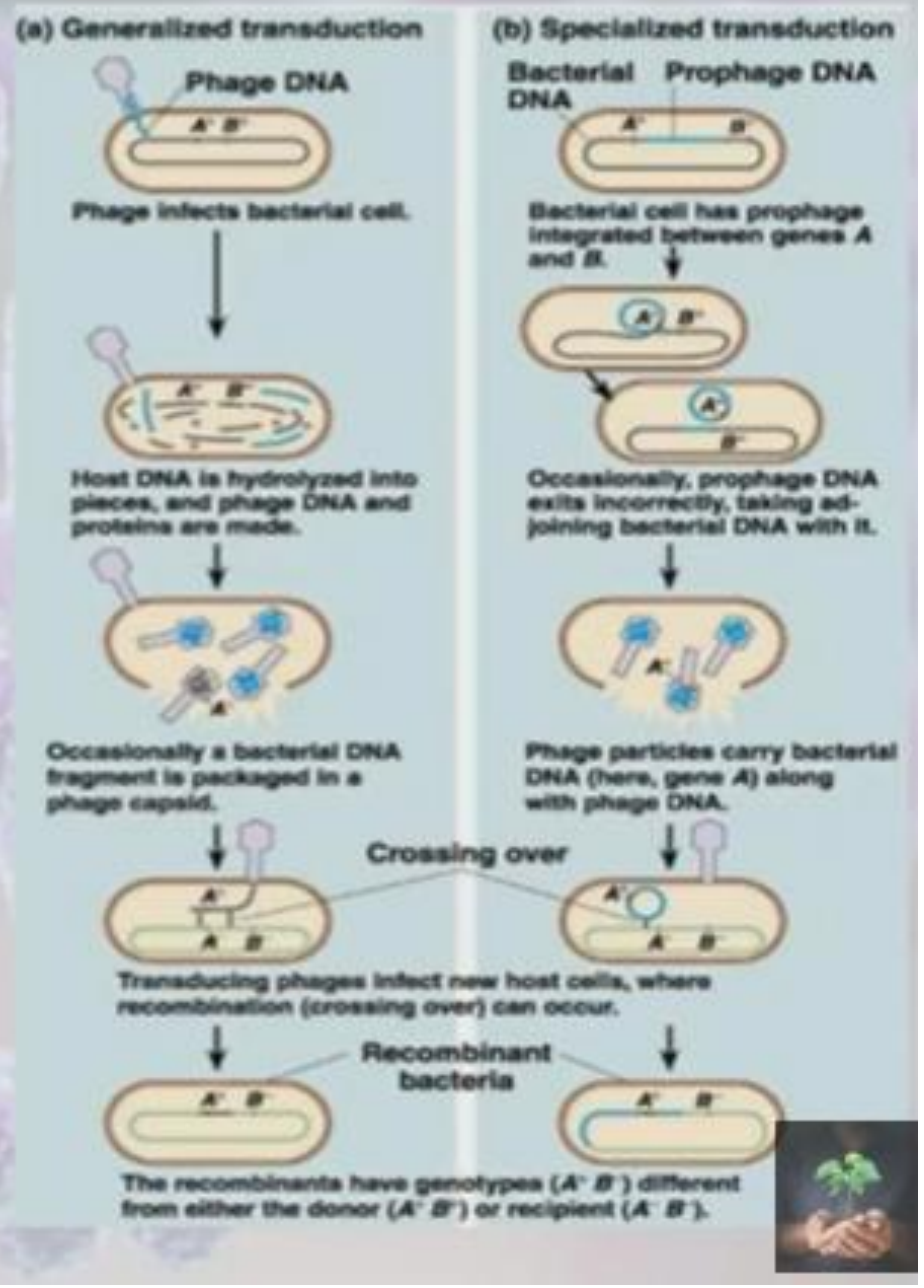
1. Generalized transduction.
2. Specialized transduction.
3. Restricted transduction.

MECHANISM OF TRANSDUCTION:

1. Bacteriophages (viruses that infect bacteria) infect a bacterial cell. Their normal mode of reproduction is to use the **DNA replication machinery** of the host bacterial cell.
2. Thus this bacteriophage makes **numerous copies of its DNA or RNA.**
3. These copies of bacteriophage DNA or RNA are then packaged into newly synthesized copies of bacteriophage virions.
4. However, packaging of bacteriophage DNA is not fool-proof. Small pieces of **bacterial DNA also pack into a bacteriophage virion** along with bacteriophage genome.
5. Viruses with RNA genomes are not able to package DNA. Thus they don't usually make this mistake.

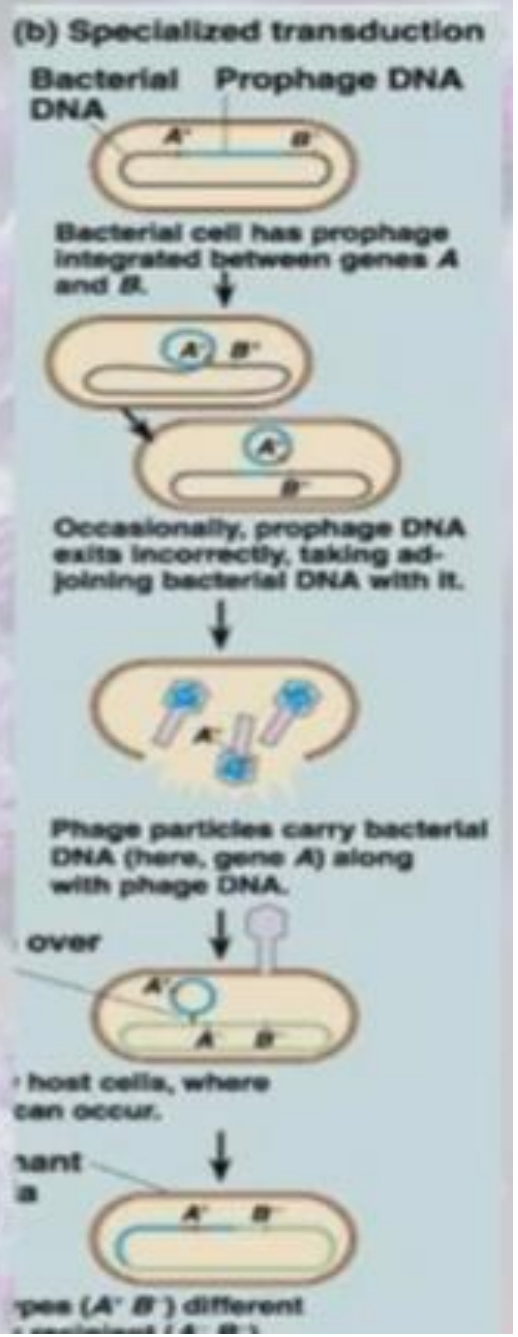
GENERALIZED TRANSDUCTION:

- It occurs in the **lytic cycle** of the phage virus.
- DNA of phages virus enter into E.coli bacteria. This DNA replicates and develops many new DNA and capsids.
- The DNA of bacteria is broken. Some pieces of DNA also enter into capsid of virus. Bacteria burst and release new phage viruses. Now this **phage enters into recipient bacteria** and transfer DNA of donor bacteria into the DNA of recipient bacteria.
- **Bacterial endonucleases enzymes** destroy the phage virus. Now these bacteria incorporate genes of donor bacteria and replicates..



SPECIALIZED TRANSDUCTION:

- It occurs in **Lysogenic cycle** of phage virus.
- In this cycle **viral DNA incorporate into bacterial DNA** as prophage. It remains peacefully there.
- But sometime, it **becomes lytic**. It comes out of bacterial DNA. Some part of bacterial DNA remain attach with it. Viral DNA with a piece of bacterial DNA replicates and develops new capsids.
- **Bacteria burst**. Virus infects other bacteria and transfer genes. of donor bacteria to recipient bacteria



RESTRICTED TRANSDUCTION:

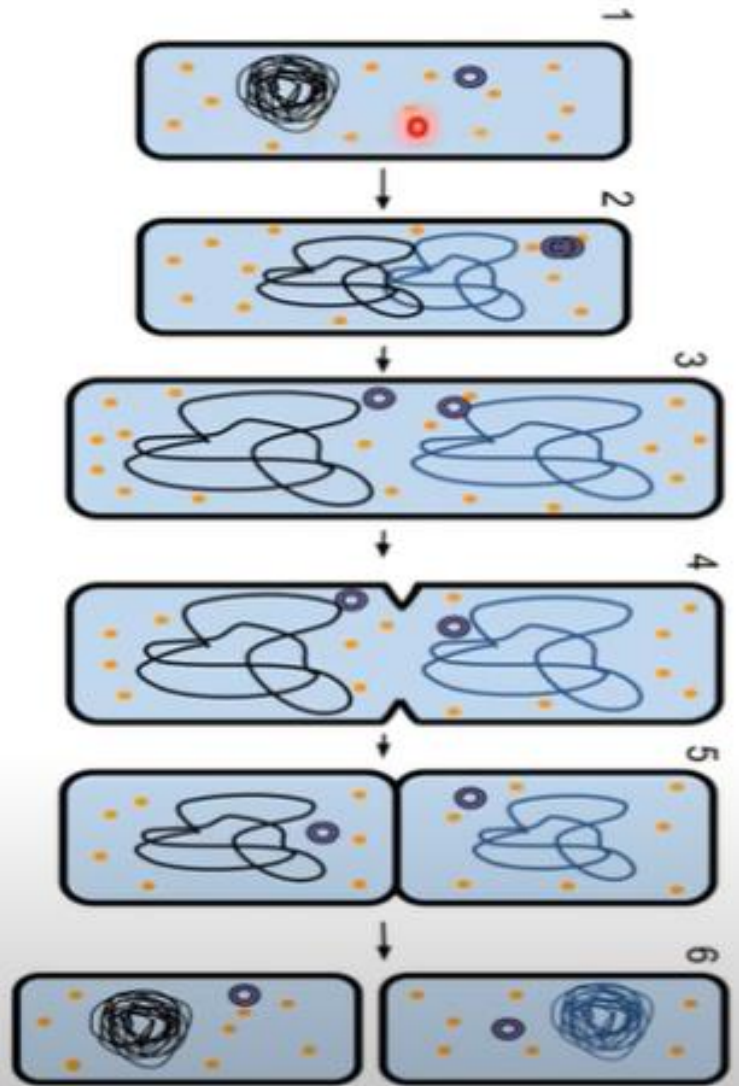
- Certain phages carry out a more restricted kind of transduction. They carry only a specific section of bacterial genetic material.
- They transduce only a few genes. Retroviruses carry out specific or restricted transduction. These viruses can cause the formation of **tumors** (oncogenes is) in animals. It is now known that these viruses exchange a small portion of their genome for a mutant cellular gene.
- This it has a role in gene replication. These viruses carrying mutant genes to in carrying. They **transform these cells into tumors cells.**

Bacterial Growth

What do you mean by Bacterial growth

Microbes growth represents Increasing in number of bacteria NOT a by SIZE

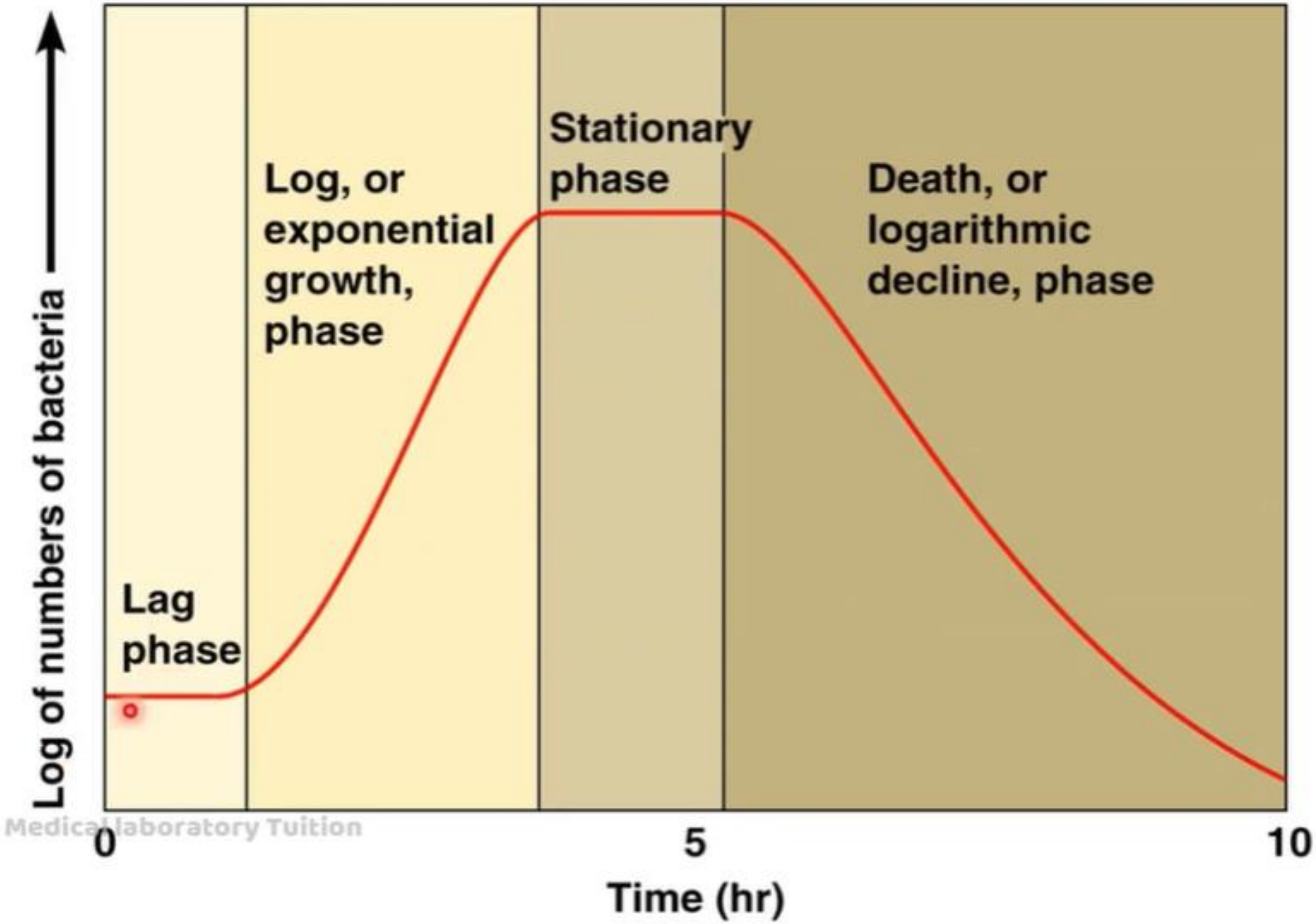
Binary fission



Phases of Growth

4 phase

1) Lag phase
2) Log phase (logarithmic)
3) Stationary phase
4) Decline phase or death phase



LAG Phase

- Adaptation phase
- Bacteria produce enzyme for digestion of nutrients.

LOG Phase

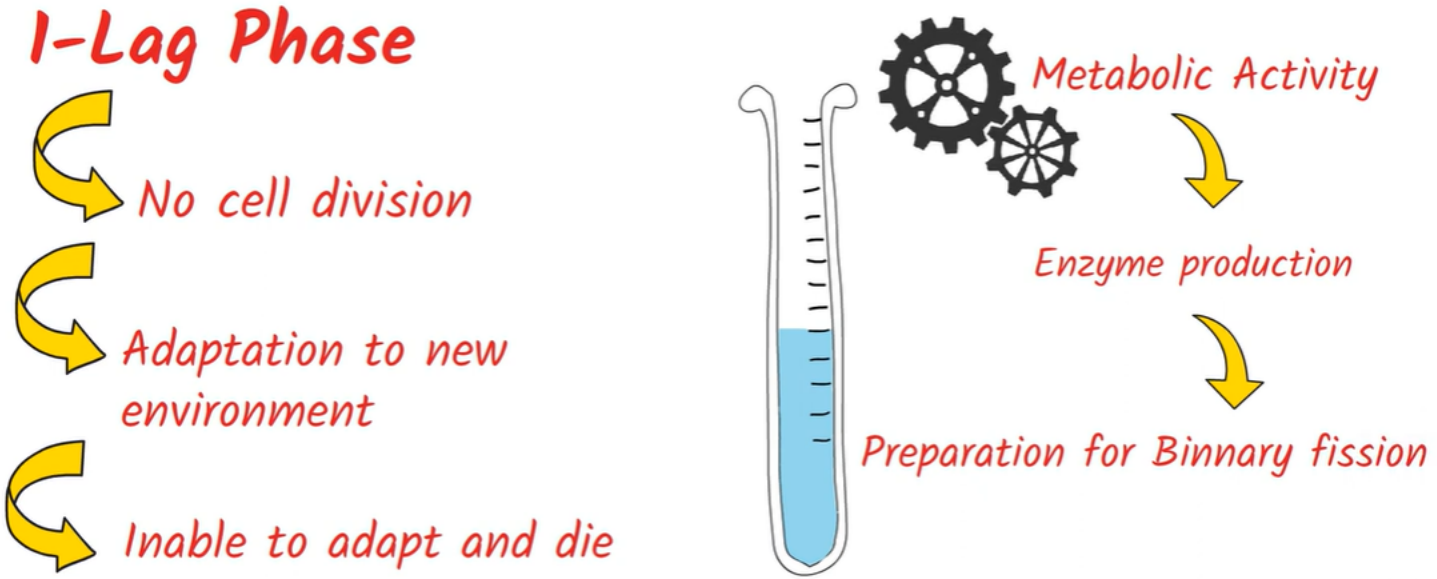
- Doubling phase
- Metabolically active
- Accumulation of toxic products starting.
- Change in Ph

Stationary phase

- Death Rate = Birth rate
- Depletion of nutrients
- Accumulation of Toxic substance increasing
- Sporulation occur in this phase

Decline phase

- Rate of death is increase
- Full of toxic products in this phase
- After some time due to Continuously death, culture become sterile.



Bacterial Growth Curve in HINDI AND URDU

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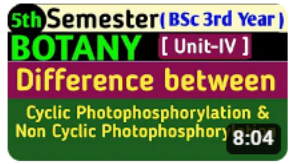

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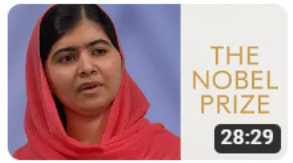

Malala Yousafzai: Nobel Peace Prize Lecture 2014
Nobel Prize
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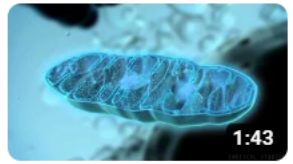

How Mitochondria Produce Energy
CorticalStudios
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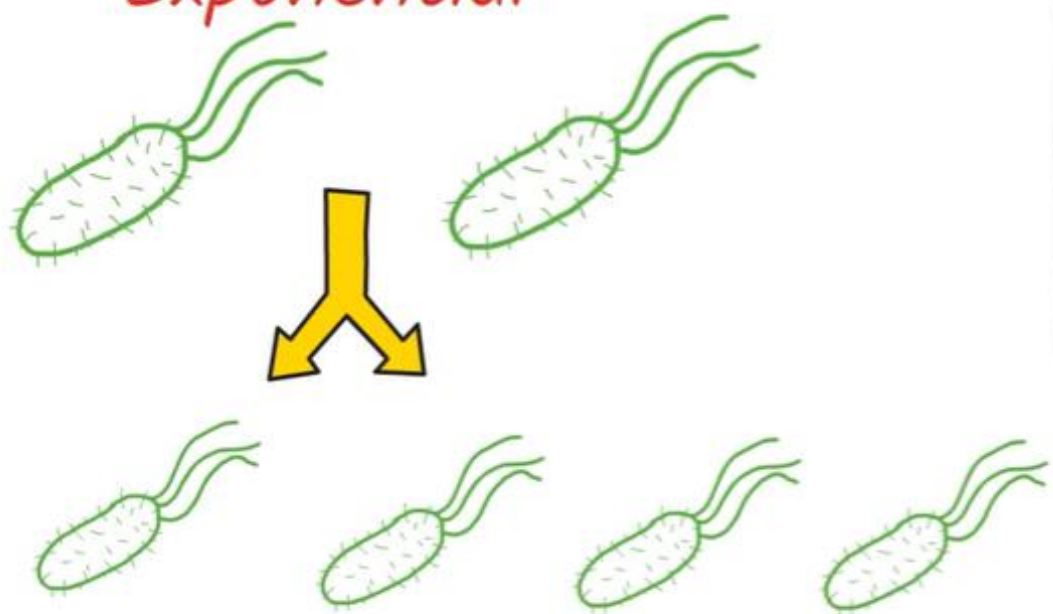
What if Everything was Free? + more videos | #aumsum #kids...
It's AumSum Time

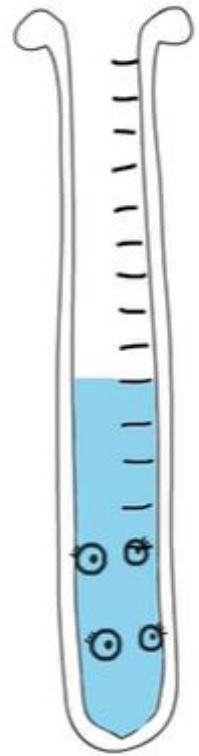
2-Log Phase

Cell Grow in number

↓

Exponential





Generation Time

↓

Duration of cell division

↓

Species
Environmental Factors

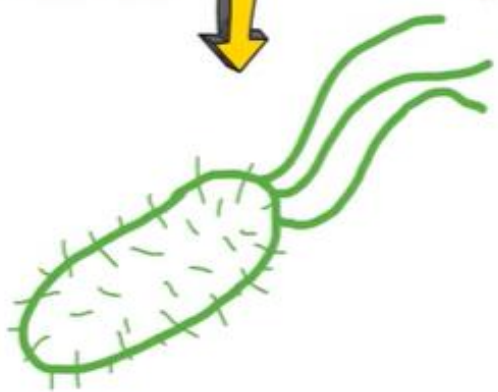
3-Stationary Phase

Reproductive and death rate equalize

Limited Nutrients

Oxygen

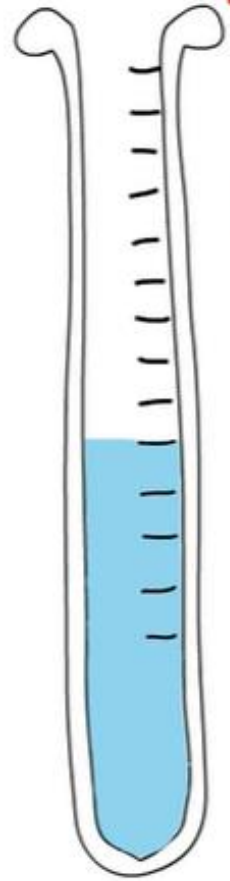
Dead



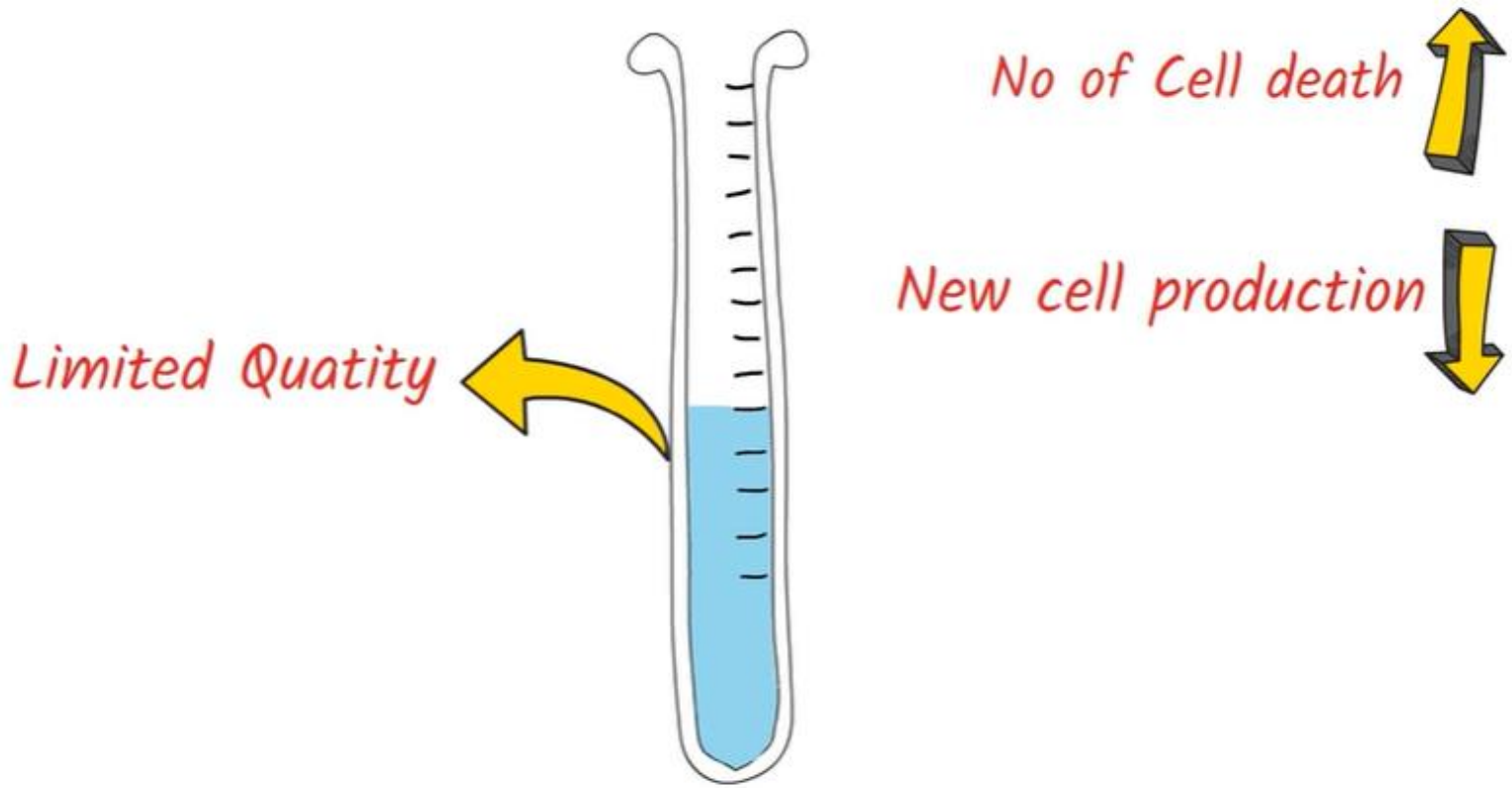
Waste materials accumulation

Competative cell

Utilize Nutrients



4-Decline or Death Phase



The Generation time is calculated using the following formula

$$G = \frac{t}{n}$$

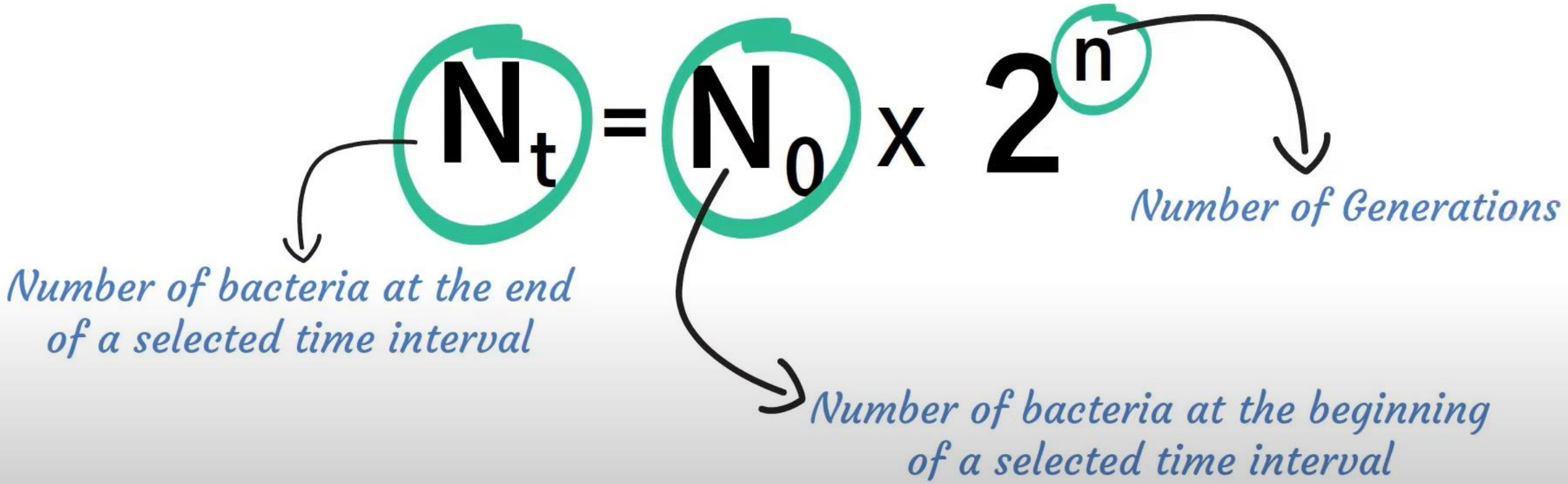
Where

G = Generation time

t = Time interval

n = Number of generations

To know the 'n' value, the following formula will be used



Let's find the value of 'n'

$$N_t = N_0 \times 2^n$$

Let us apply 'log' on both sides of the equation

$$\text{Log } N_t = \log (N_0 \times 2^n)$$

This is in the form of log (ab)
Hence, can be written as log a + log b

$$\text{Log } N_t = \log N_0 + \log 2^n$$

This is in the form of $\log a^b$ and can be written as b log a

$$\log N_t = \log N_0 + n \log 2$$

If a is equal to b, b is also equal to a
So, let us invert the equation

$$\log N_0 + n \log 2 = \log N_t$$

If we take the $\log N_0$ to the right side of the equation

$$n \log 2 = \log N_t - \log N_0$$

$$\Rightarrow n = \frac{\log N_t - \log N_0}{\log 2}$$

As per the logarithm table, log 2 value is equal to 0.301

$$n = \frac{\log N_t - \log N_0}{0.301}$$

$$\Rightarrow n = \frac{1}{0.301} (\log N_t - \log N_0)$$

As this is in the form of log a- log b,
it can be written as log a/b

$$n = \frac{1}{0.301} (\log N_t / N_0)$$

$$\Rightarrow n = 3.3 \log N_t / N_0$$

$$\Rightarrow n = 3.3 \log N_t / N_0$$

Let's put the value of 'n' in the G= t/n formula

$$G = \frac{t}{3.3 \log N_t / N_0}$$

Factor affecting bacterial growth

- Ph (7)
- Temperature (37)
- Oxygen requirement (Aerobic, Anaerobic, Microerophilic, F.Anaeobes)
- Light (photosynthetic)
- Salt Concentration (Halophiles)
- Osmotic pressure
- Water
- Nutrient availability

- **Radiations**
- **Metal Ions**

Types of culture in industry

Batch culture

Fixed amount of nutrient added
at the time of batch start.

Growth curve can be seen such as
Lag,Log,Stationary even sometime decline.

Products are formed for short time,
& reuse machine for next culture

continuous flow culture

Continuous nutrient can be added
During machine in running mode

Growth curve is maintained in
Log phase all the time

Products are formed for long periods of time,
in continuous manner

THANK YOU