Cultivation of Microorganisms:

Cultivation: is the process of propagating organisms by providing the proper environmental conditions. Growing microorganisms require elements present in their chemical composition. Nutrients must provide these elements in metabolically accessible form. In addition, organisms require metabolic energy to synthesize macromolecules and maintain essential chemical gradients across their membranes. Factors that must be controlled during growth include the nutrients, pH, temperature, aeration, salt concentration, and ionic strength of the medium.

Bacterial Nutrition :

Nutrients in growth media must contain all the elements necessary for the biologic synthesis of new organisms, nutrients are classified according to the elements they supply in to:

a-Carbon - building blocks of cell components

b-Nitrogen - production of proteins, nucleic acids

c-Hydrogen - occur in organic compounds

d-Oxygen - involved in the production of energy

e-Minerals, Trace Elements - required in small amount for enzyme function such as (Mg^{2+} , Fe^{2+} ,K^{+}, Ca^{2+},Zn^{2+} ,etc…)

Environmental Factors :

1-Temperature

Different microbial species vary widely in their optimal temperature ranges for growth :

a) **Psychrophilic** forms grow best at low temperatures (-5–15°C) and are usually found in such environments as the Arctic and Antarctic regions.

b) **psychrotrophs** have a temperature optimum between 20°C and 30°C but grow well at lower temperatures. They are an important cause of food spoilage.

c) **Mesophilic** forms grow best at 30–37°C.

d) **Thermophilic** forms grow best at 50–60°C.

e) Some organisms are **hyperthermophilic** and can grow at well above the temperature of boiling water, which exists under high pressure in the depths of the ocean. Most organisms are mesophilic; 30°C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.

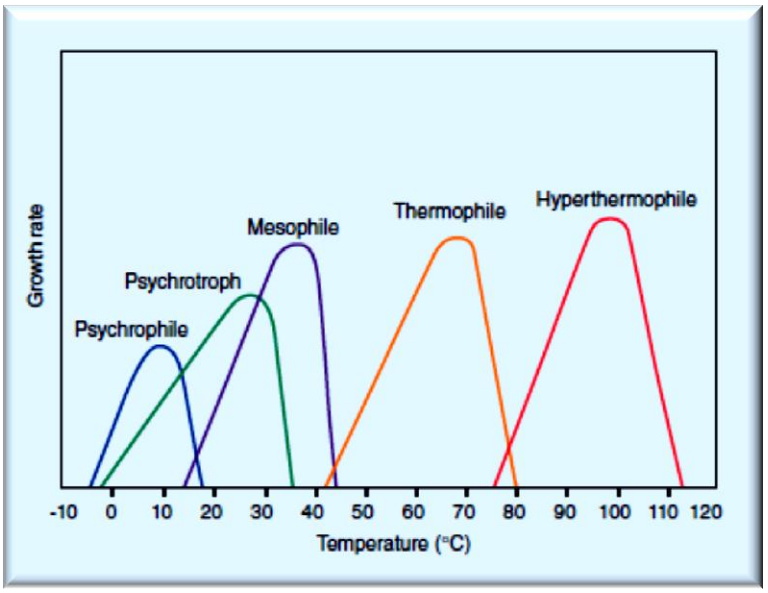


FIGURE 1: Temperature requirements for growth. Prokaryotes are commonly divided into five groups based on their optimum growth temperatures.

2-Hydrogen Ion Concentration (pH)

Most organisms have a fairly narrow optimal pH range. The optimal pH must be empirically determined for each species.

Most organisms **(neutralophiles)** grow best at a pH of 6.0– 8.0, although some forms **(acidophiles)** have optima as low as pH 3.0, and others **(alkaliphiles)** have optima as high as pH 10.5.

3- Aeration:

Many organisms are **obligate aerobes**, specifically requiring oxygen as hydrogen acceptor; some are **facultative anaerobes**, able to live aerobically or anaerobically; some are **obligate anaerobes** requiring a substance other than oxygen as hydrogen acceptor and are sensitive to oxygen inhibition; some are **microaerophiles,** which require small amounts of oxygen (2%–10%) for aerobic respiration (higher concentrations are inhibitory); and others are **aerotolerant anaerobes,** which are indifferent to oxygen.

Bacteria can be classified into the following type according to the basis of their ability to synthesize essential metabolism.

A- **Autotrophs**:-

These are bacteria which are able to synthesize their own organic food from inorganic substances. They use carbon dioxide for obtaining carbon and utilize hydrogen sulphide (H2S) or ammonia (NH3) or hydrogen (H2) as the source of hydrogen to reduce carbon

B- **Heterotrophs**:-

Microbes obtain their carbon from organic compound, such as sugar, protein and lipids. Hydrogen is usually obtained from water, and oxygen is obtained from atmosphere or from water where it is found in dissolved state.

Bactreial Growth:-

- It is an increase in all the cell components, which ends in multiplication of cell leading to an increase in population.
- It involves - an increase in the size of the cell & an increase in the number of individual cells.

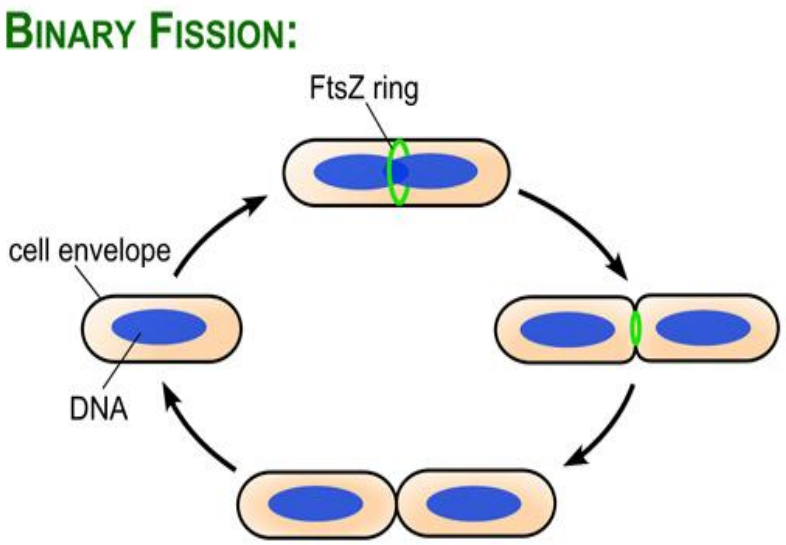
Generation Time:-

- Interval of time between two cell divisions
  OR
- The time required for a bacterium to give rise to 2 daughter cells under optimum conditions
- Also called population **doubling time**.

Bactreial Reproduction:

- **Binary Fission:-**

Most bacteria rely on binary fission for propagation. Conceptually this is a simple process; a cell just needs to grow to twice its starting size and then split in two. But, to remain viable and competitive , a bacterium must divide at the right time, in the right place, and must provide each offspring with a complete copy of its essential genetic material.



The Growth Curve In Batch Culture :-

If a fixed volume of liquid medium is inoculated with microbial cells taken from a culture that has previously been grown to saturation and the number of viable cells per milliliter is determined periodically and plotted, a curve of the type shown in Figure 3 is usually obtained

Growth is shown as *L* = log (numbers) where numbers is the number of colony forming units per ml, versus *T* (time.)

the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modelled with four different phases: **lag phase** (A), **log phase** or exponential phase (B), **stationary phase** (C), and **death phase**(D).

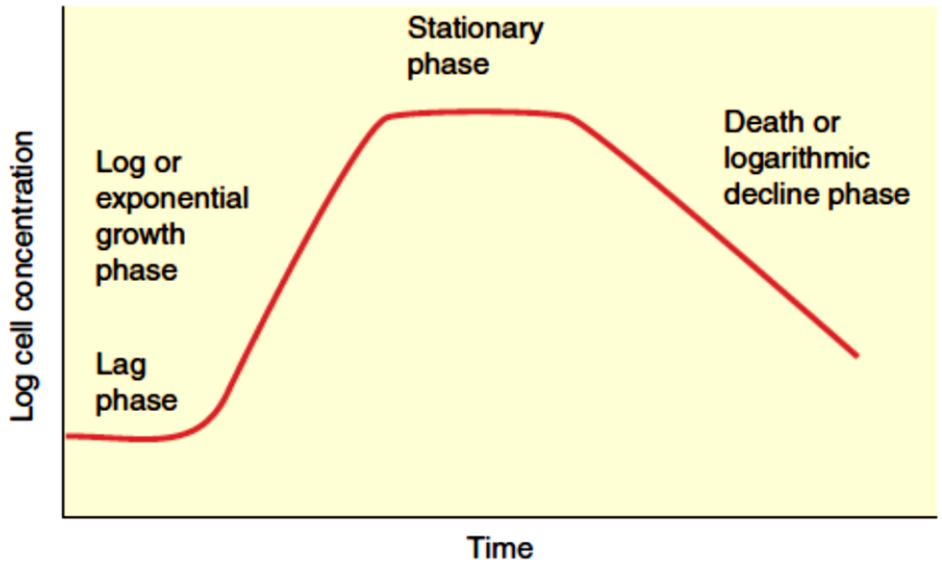


Figure 3: A bacterial Growth Curve.

1-Lag Phase :

1- The bacteria are adapted to the new environment and synthesis cellular components such as ribosome , enzymes and other proteins .

2- There is no increase in cell number .

3- During this phase bacteria are growing in size ,but they are not undergoing binary fission .

4- The bacteria have maximum cell size in the end of the lag phase

2- Exponential phase (log phase):

1- The exponential growth is expressed as the the bacteria generation time .

2- There is logarthmic increased in cell number .

3- During this phase ,the condition are optimal for growth and binary fission occurs.

4-In the log phase the cell are small and stain uniformly.

3-Stationary phase :

1- There is no increase or decrease in cell number in this stage ,in other word , cell growth (division) equals cell death.

2- The birth rate decrease due to limited nutreint , lack of space and the build up of secondary metabolic products (e.g. toxins).

3- The insufficient supply of nutrient is also causes some bacteria to form spore during this phase.

4-Cell frequently are gram varaible and show irregular staining due to the presence of intracellular storage granules .

4-The decline phase (Death phase):

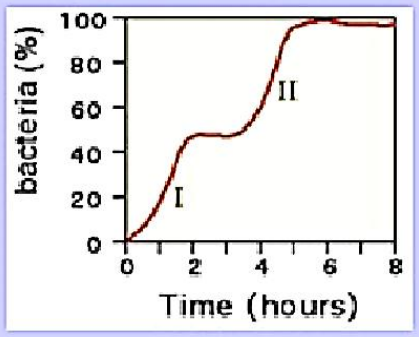
1- This phase is characterise by an exoponential death of cells.

2- When the media runs out of nutrients and there are too many toxin .Cell beigin to die as faster rate.

3- Involution form are common in decline phase.

BIPHASIC GROWTH CURVE

- A particular type of growth curve seen in cultured microorganisms in which they have two exponential growth stages separated by a plateau phase. This double-hump curve is produced when the microbes are cultured using two carbon sources, one of which must be used up before the second can be used.



Bacterial Genetics:

Genetics is the study of **genes** including the structure of genetic materials, what information is stored in the genes, how the genes are expressed and how the genetic information is transferred. Genetics is also the study of heredity and variation.

Genes are sequences of nucleotides within DNA that code for functional proteins. The genetic material of bacteria and plasmids is DNA. The two essential functions of genetic material are replication and expression. Most prokaryotic genes are carried on **bacterial chromosome** . while many bacteria contain additional genes on **plasmids**.

1-Bacterial chromosome:

most bacteria have single, covalently closed, circular chromosomes. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids.

2- Plasmids:

Plasmids are extra-chromosomal elements found inside a bacterium. These are not essential for the survival of the bacterium but they confer certain extra advantages to the cell.

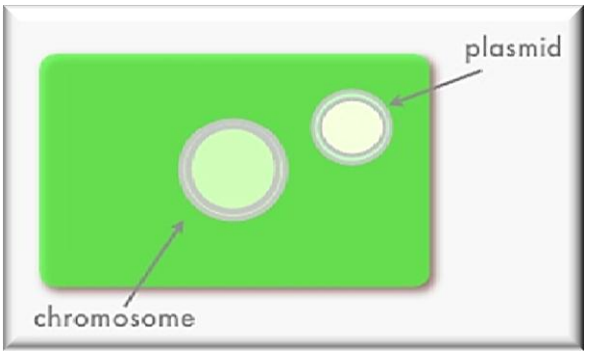
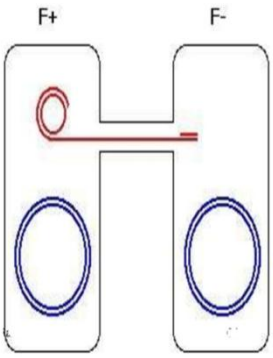


Figure 4: Bacterial genome

Types of plasmids:

- **F factor:** This is also known as **fertility factor** or **sex factor**. Most plasmids are unable to mediate their own transfer to other cells. F factor is a plasmid that codes for **sex Pilli** and its transfer to other cells. Those bacteria that possess transfer factor are called F+, such bacteria have sex pili on their surface. Those cells lacking this factor are designated F-. The F factor plasmid is transferred to other cells through conjugation. An F- cell will become F+ when it receives the fertility factor from another F+ cell.



- **R factor:** Those plasmids that code for the transmissible drug resistance are called R factor. These plasmids contain genes that code for resistance to many antibiotics. R factors may be transferred by conjugation and its transfer to other bacteria is independent of the F factor. Bacteria possessing such plasmids are resistant to many antibiotics and this drug resistance is transferred to closely related species.

❖ **Col Factor :** Responsible for colicin production.

Importance of plasmids:

1. Codes for resistance to several antibiotics.
2. Codes for the production of bacteriocines.
3. Codes for the production of toxins
4. Codes for resistance to heavy metals
5. Plasmids carry virulence determinant genes.
6. Codes resistance to uv light (DNA repair enzymes are coded in the plasmid).
7. Codes for colonization factors that is necessary for their attachment. Eg, as produced by the plasmids of *Yersinia enterocolitica*, *Shigella flexneri*, Enteroinvasive *Escherichia coli*.
8. Contains genes coding for enzymes that allow bacteria unique or unusual materials for carbon or energy sources. Some strains are used for clearing oil spillage.

Mutation :

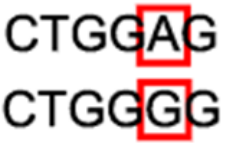
Mutation is defined as any change in base sequence of DNA. it occur in two form: **Transition** (purine replaced by purine or pyrimidine replaced by pyrimidine) or **Transversion (** purine replaced by pyrimidine or vice versa)

Mutations result from damage to DNA which is not repaired, errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements. Mutations play a part in both normal and abnormal biological processes including: evolution, cancer.

Types of Mutations:

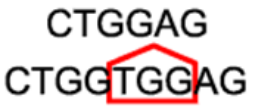
1- **Substitution**

- A substitution is a mutation that exchanges one base for another (i.e., a change in a single "chemical letter" such as switching an **A** to **G**).



2- **Insertion**:

- Insertions are mutations in which extra base pairs are inserted into a new place in the DNA.

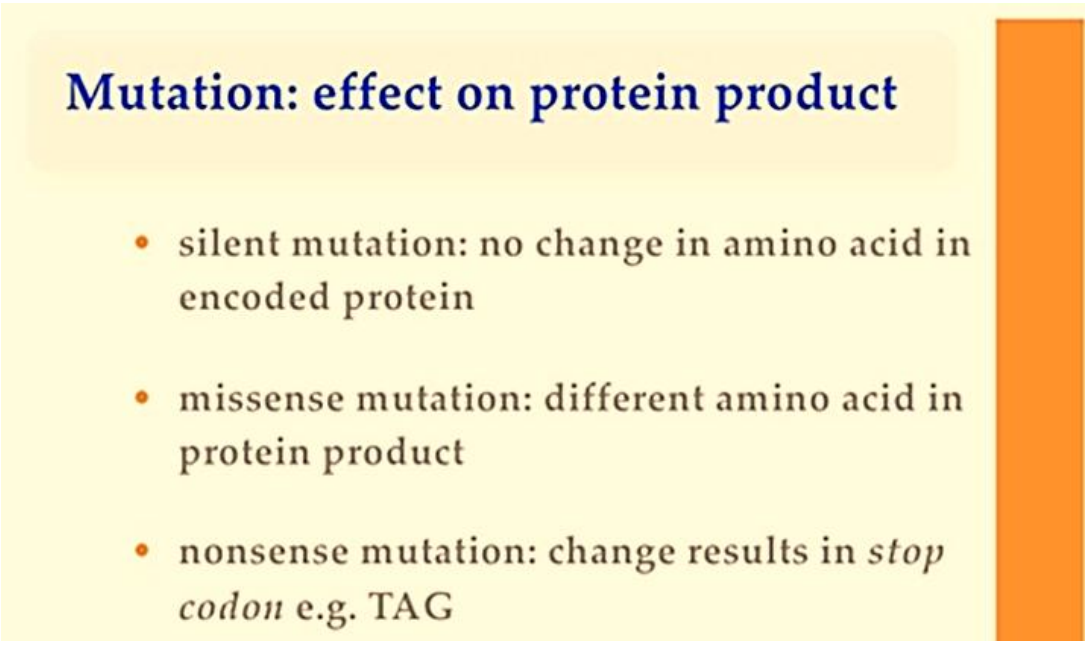


3- **Deletion**:

- Deletions are mutations in which a section of DNA is lost, or deleted.

Frame shift:

Since protein-coding DNA is divided into codons three bases long, insertions and deletions can alter a gene so that its message is no longer correctly parsed. These changes are called **frame-shifts**.



Exchange of Genetic Information:

1- Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. *Bacillus*, *Haemophilus*, *Neisseria*, *Pneumococcus*) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.

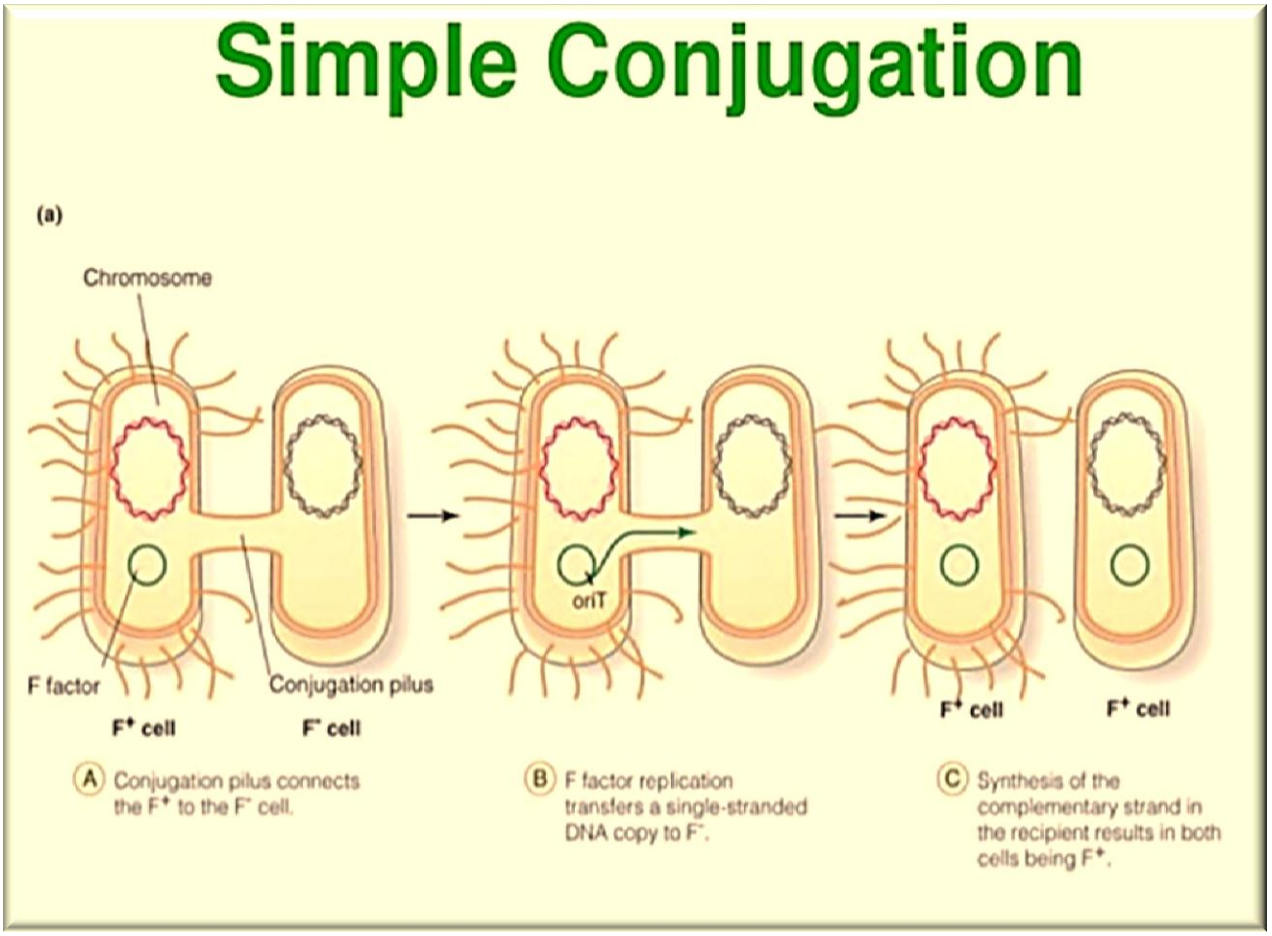
2- Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage.

3-Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient.

The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the **F factor or fertility factor or sex factor**. The F factor has genes on it that are needed for its replication and for its ability to transfer DNA to a recipient. One of the things the F factor codes for is the ability to produce a sex pilus (F pilus) on the surface of the bacterium. This pilli is important in the conjugation process.



Recombinant DNA Technology:

Recombinant DNA technology procedure by which DNA from different species can be isolated , cut and spliced together . The new recombinant molecules are then multiplied in quantity in population of rapidly dividing cells (e.g. bacteria ,yeast). Currently it is relatively easy to cut out a specific piece of DNA produce a large number of copies , determine its nucleotide sequence , slightly alter it and then as a final step transfer it back in to cell in .

Recombinant DNA technology is based on a number of important things :

1- Bacteria contain extra chromosomal molecules of DNA called plasmids which are circular

2- Bacteria also produce enzyme called restriction endonuclease that cut DNA molecules at specific places in to many smaller fragments called restriction

fragments. each enzyme cuts DNA at specific site defined by sequence of bases in the DNA called recognition site.

3- Restriction enzyme cuts only double helical segments that contain a particular sequence and it makes its incisions only within that sequence to give sticky end and blunt end.

4- Insertion DNA fragment in appropriate plasmid (vector) to generate recombinant molecule .

5- Introduce recombinant molecule in to new host.

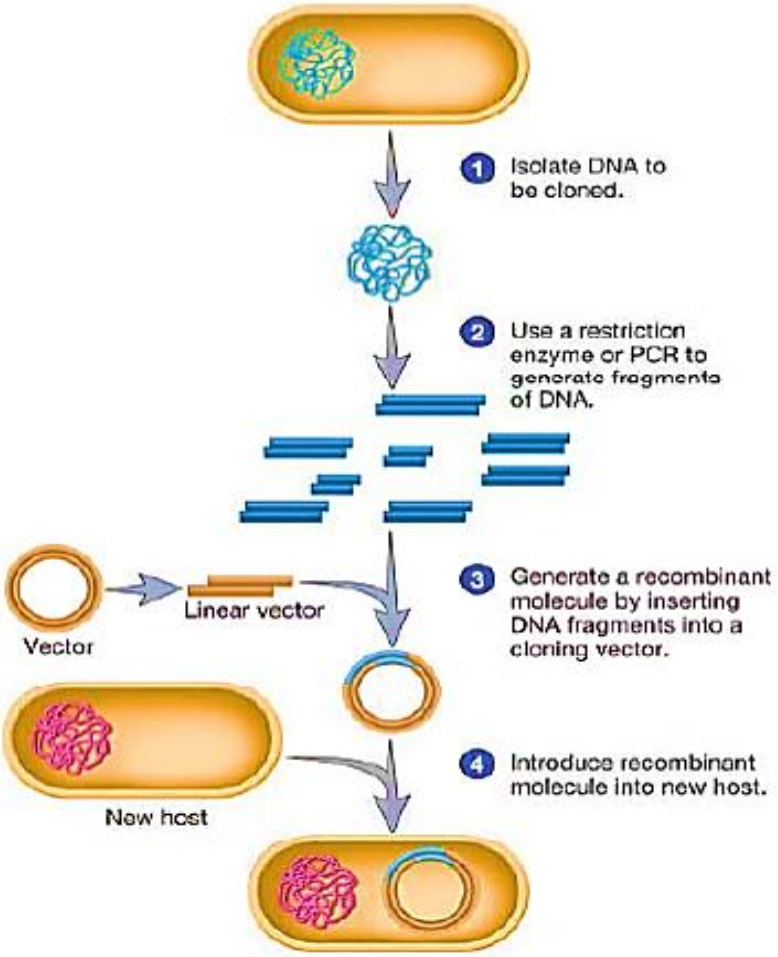


Figure : Steps in recombinant DNA techniques .

Restriction Enzymes :

They are proteins produced in a bacteria cell that cut DNA at a specific site. Also known as restriction endonucleases . This enzyme are primarily found in bacteria and given abbreviations based on genus and species of the bacteria . one of the first to be isolated was **Eco R1** it was isolated from *Escherichia coli* strain called RY13.

Some enzymes cut in a staggered fashion - "sticky ends"

EcoRI 5'…GAATTC…3'
3'…CTTAAG…5'

Some enzymes cut in a direct fashion – "blunt ends"

PvuII 5'…CAGCTG…3'
3'…GTCGAC…5'

H.W.// *Why don't bacteria destroy their own DNA with their restriction enzymes?*