Biotechnology: Principles and Processes

Biotechnology: Principles and Processes

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

Biotechnological products and processes are:

- *In vitro* fertilisation for ‘test-tube’ babies.
- Synthesising a gene and using it
- Developing a DNA vaccine
- Correcting a defective gene.

B. John Ebenezer

Biotechnology: Principles and Processes

The definition of Biotechnology given by EFB (European Federation of Biotechnology)

The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services.

Molecular analogues (*molecular structure closely similar to that of another*)

Genetic engineering is a technique to alter the chemistry of genetic material (DNA and RNA) to introduce these into host organisms and thus change the phenotype of the host organism.

B. John Ebenezer

Biotechnology: Principles and Processes

Asexual reproduction **preserves** the genetic information.

Sexual reproduction permits **variations** which may be beneficial to the organism as well as the population.

Sexual reproduction leads to **inclusion** and **multiplication of undesirable genes** *along with the desired genes.*

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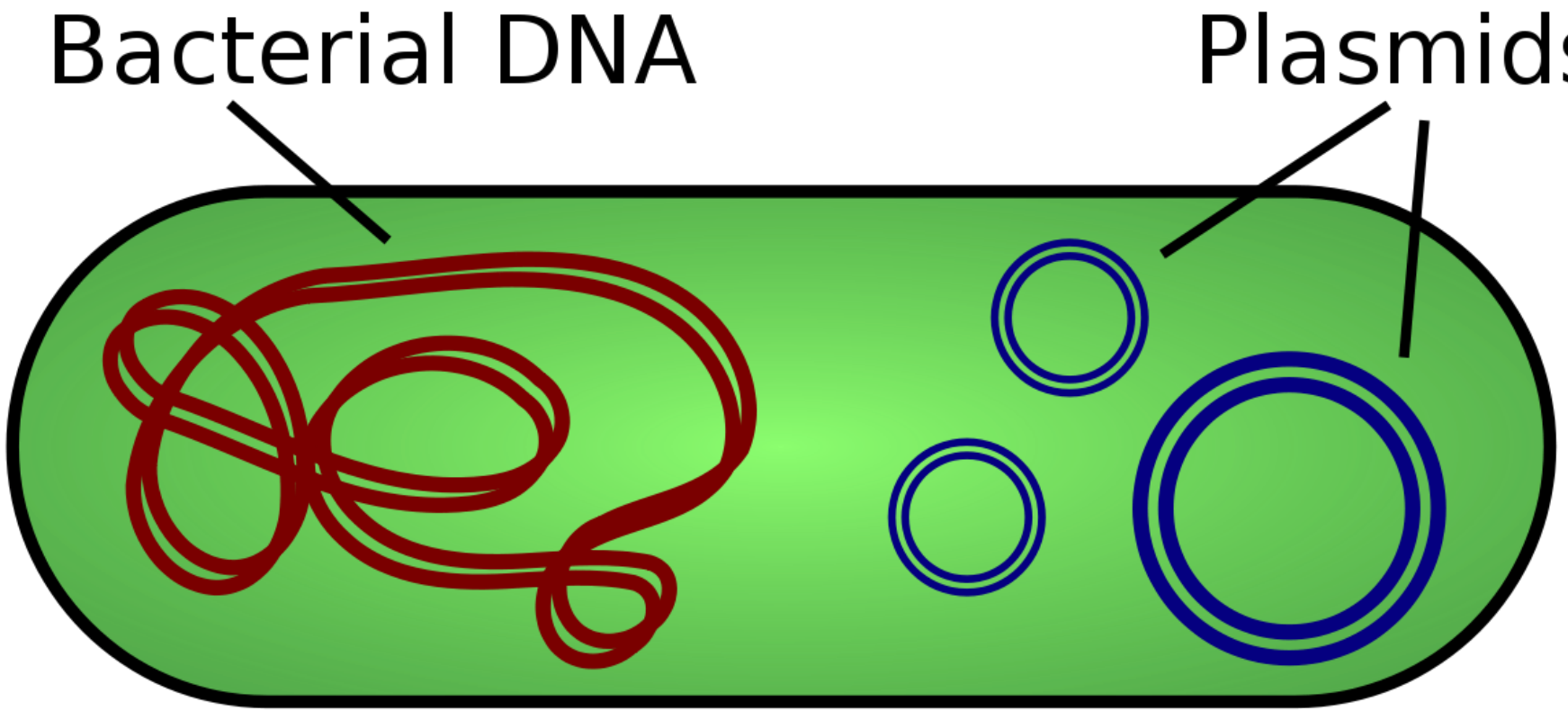
Biotechnology: Principles and Processes

The **linking of alien DNA** with the **origin of replication** in order to replicate and multiply itself in the host organism is known as cloning.

This can also be called as making **multiple identical copies** of template DNA.

The techniques of genetic engineering which include creation of **recombinant DNA,** use of **gene cloning** and **gene transfer, overcome inclusion and multiplication of undesirable genes** *along with the desired genes.*

B. John Ebenezer


Bacterial DNA
Plasmids

Biotechnology: Principles and Processes

Two core techniques that enabled birth of modern biotechnology are :

(i) Genetic engineering:

Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.

(ii) Maintenance of sterile (microbial contamination free) ambience:

It enables the growth of only the **desired microbe**/eukaryotic cell in **large quantities** for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

B. John Ebenezer

Biotechnology: Principles and Processes

Plasmid:

The autonomously replicating, circular, extra-chromosomal DNA is known as plasmid.

Vectors:

The plasmid DNAs which deliver the alien pieces of DNA into the host organisms are called as vectors.

Restriction enzymes:

The enzymes used for **cutting DNA** at specific locations, are called restriction enzymes or molecular scissors.

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Biotechnology: Principles and Processes

The piece of DNA transferred into an alien organism **would not be able to multiply** itself in the progeny cells of the organism.

But, when it gets **integrated into the genome of the recipient, it multiplies** and be inherited along with the host DNA.

This is because the alien piece of DNA has **become part of a chromosome,** which has the ability to replicate.

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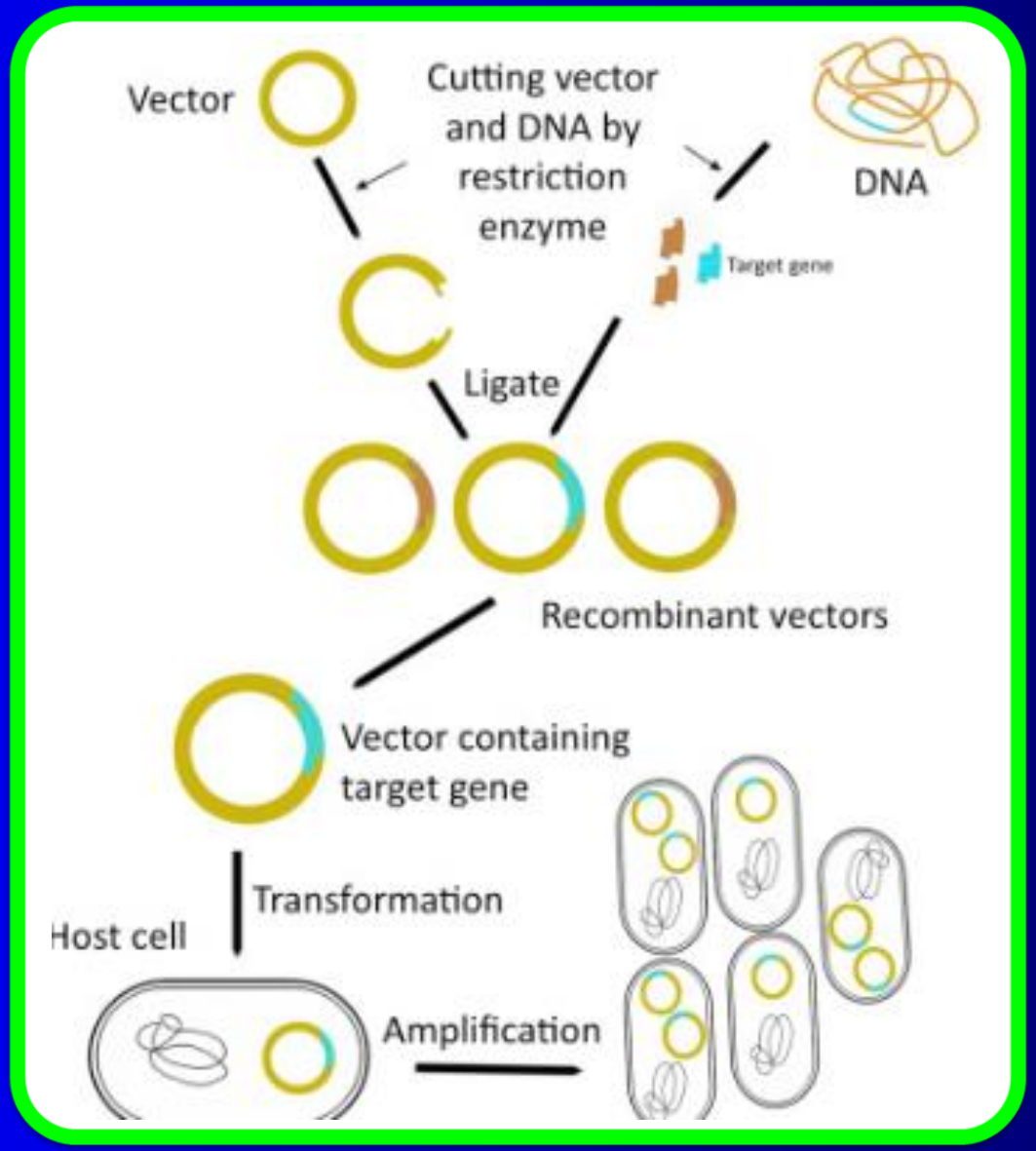
Biotechnology: Principles and Processes



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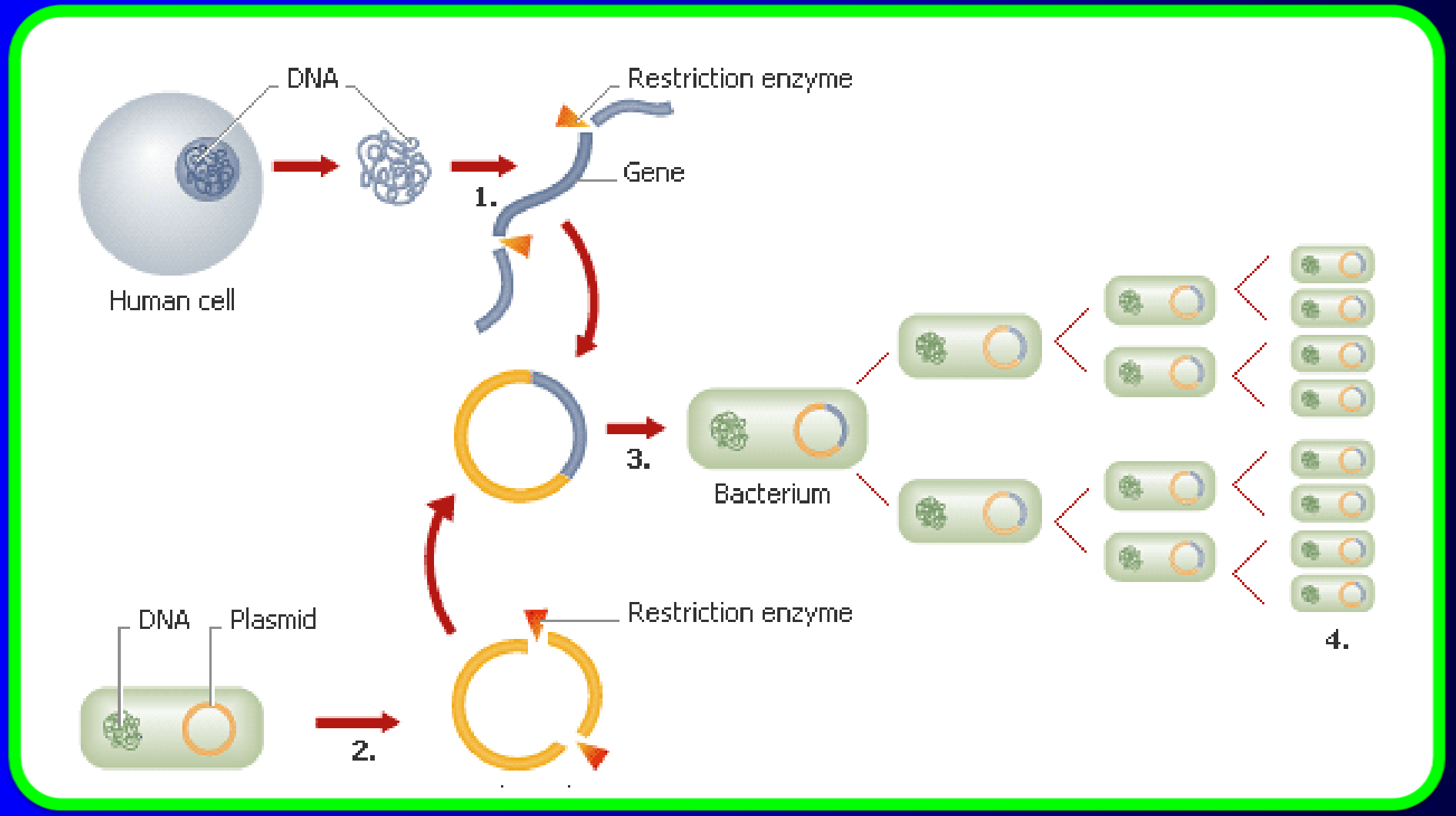
Biotechnology: Principles and Processes



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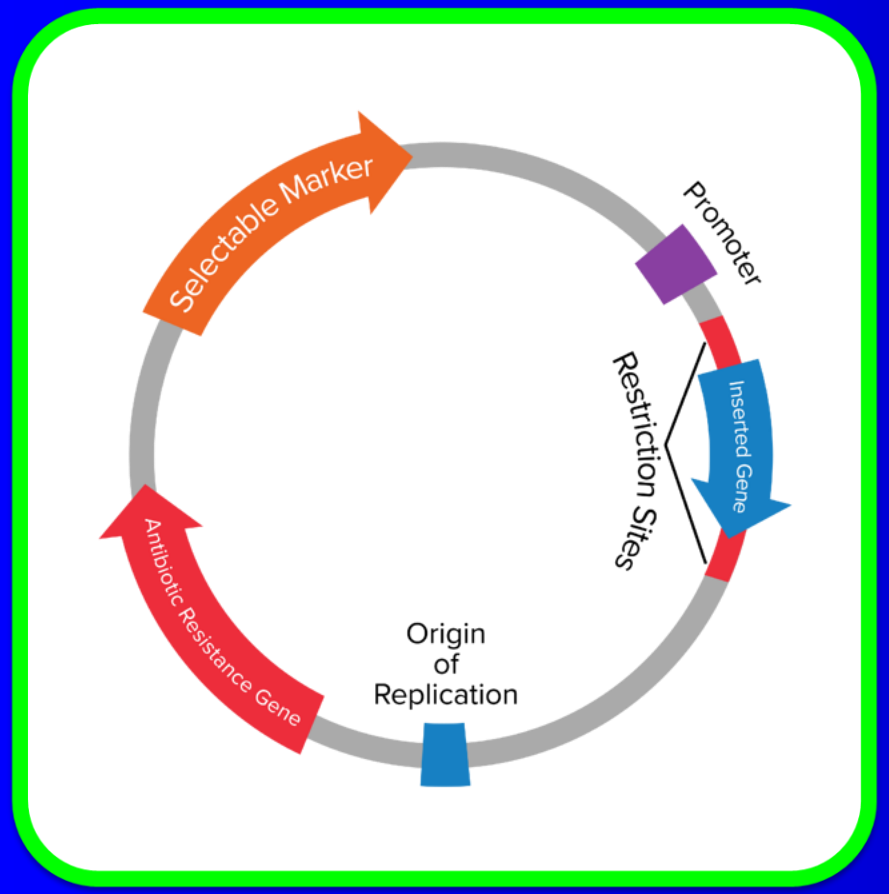
Origin of Replication



In a chromosome there is a specific DNA sequence called the **origin of replication.**

It is responsible for initiating replication.

OR

Origin of replication is **a sequence** from where replication starts.

Any piece of DNA which is linked to this sequence can be made to replicate within the host cells.

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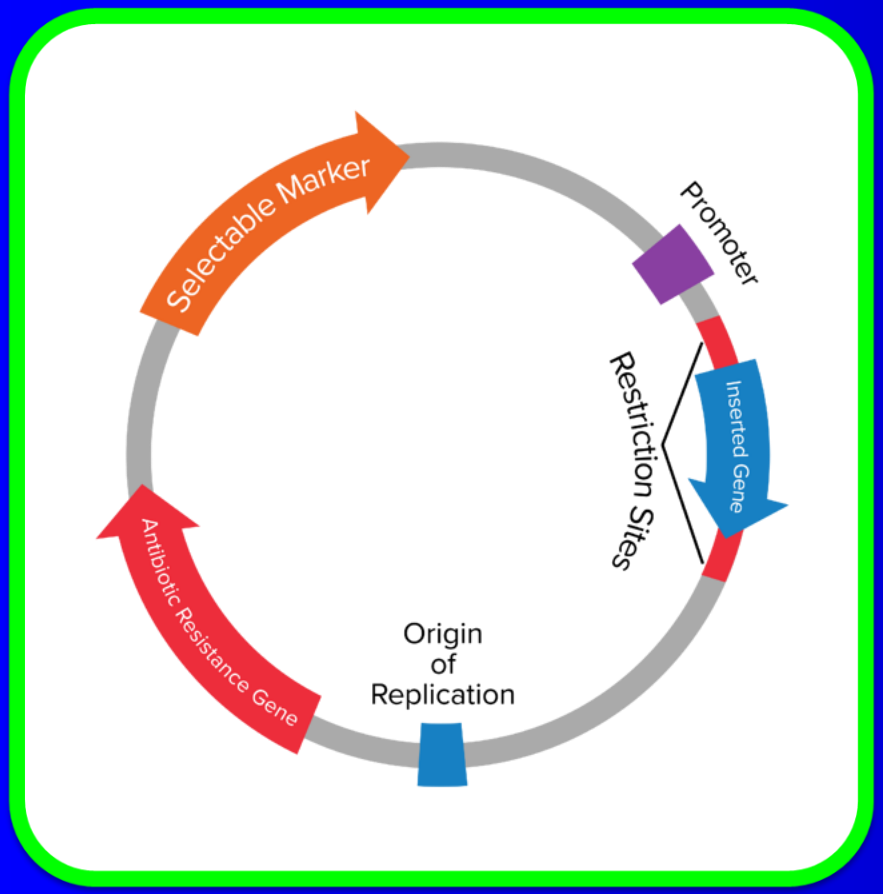
Origin of Replication



This sequence is also responsible for **controlling the copy number** of the linked DNA.

So, if we want to recover many copies of the target DNA it should be cloned in a vector whose origin **support high copy number.**

B. John Ebenezer

Construction of Artificial Recombinant DNA Molecule

The first recombinant DNA emerged by linking a gene encoding **antibiotic resistance** with a native **plasmid** of *Salmonella typhimurium*.

(Plasmid is an autonomously replicating circular extra-chromosomal DNA)

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Artificial Recombinant DNA Molecule

Stanley Cohen and **Herbert Boyer** made Artificial Recombinant DNA in 1972.

They isolated antibiotic resistance gene by **cutting out a piece of DNA from a plasmid** (*Salmonella typhimurium*) which confers antibiotic resistance.

The cutting of DNA at specific locations became possible with the discovery of the so-called 'molecular scissors'– **restriction enzymes**.

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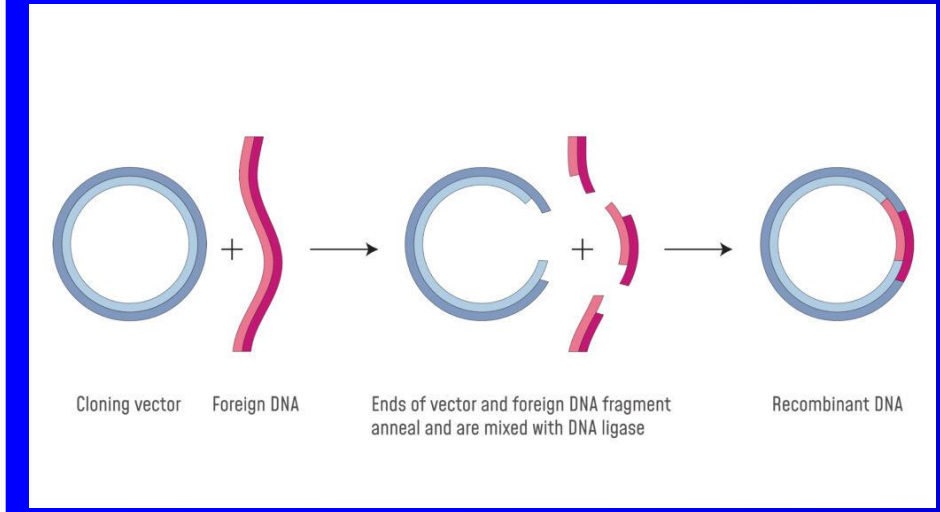
Artificial Recombinant DNA Molecule



The cut piece of DNA was then **linked** with the **plasmid DNA** of *Escherichia coli*.

These plasmid DNA act as **vectors** to transfer the piece of DNA attached to it into the host organism.

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Artificial Recombinant DNA Molecule

When this DNA was transferred into ***Escherichia coli***, a bacterium closely related to ***Salmonella,*** it could replicate using the new host's **DNA polymerase enzyme** and make **multiple copies.**

The **ability to multiply copies** of **antibiotic resistance gene** in *E. coli* is called **cloning** of antibiotic resistance gene in *E. coli*.

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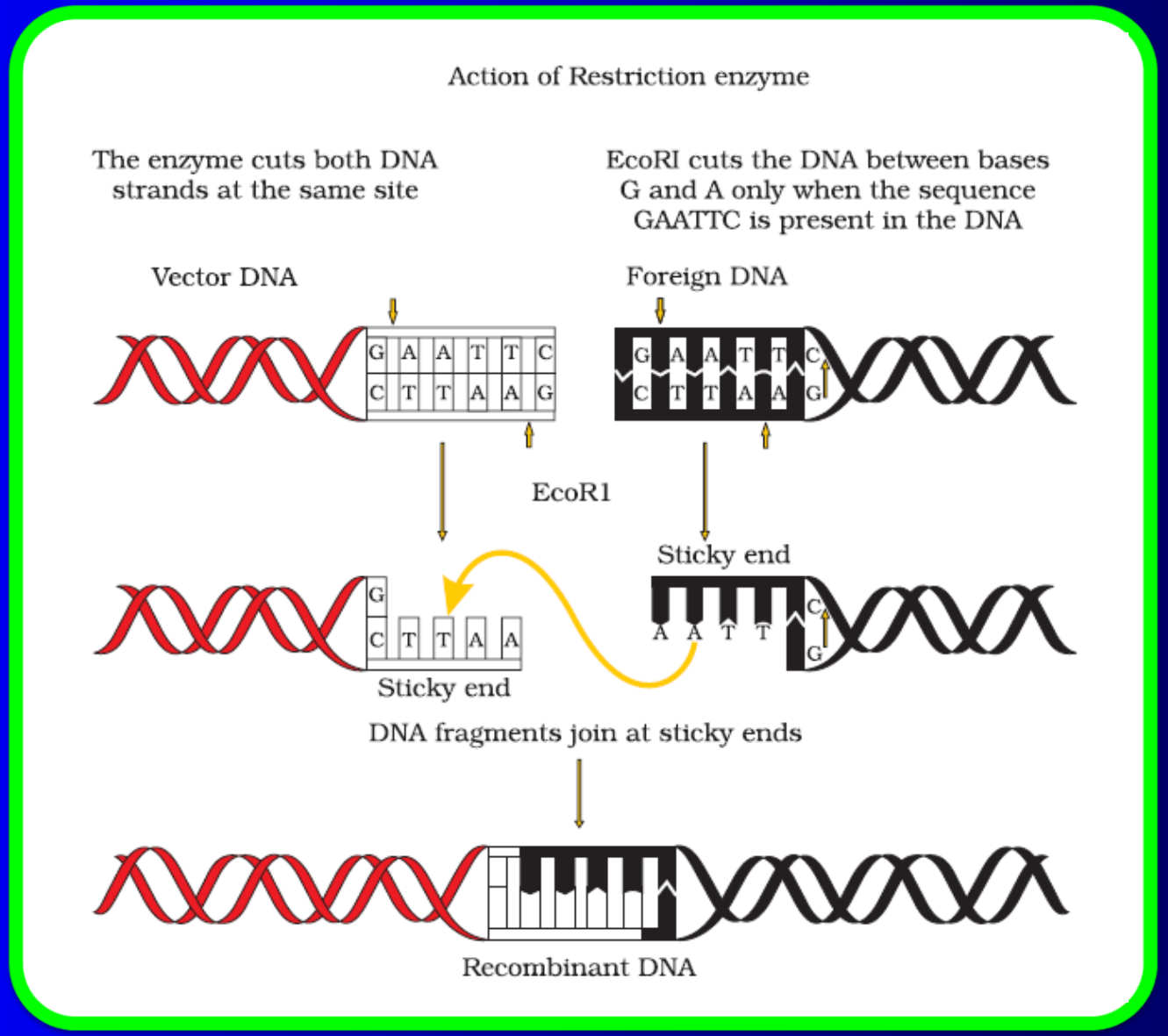
Construction of Artificial Recombinant DNA Molecule



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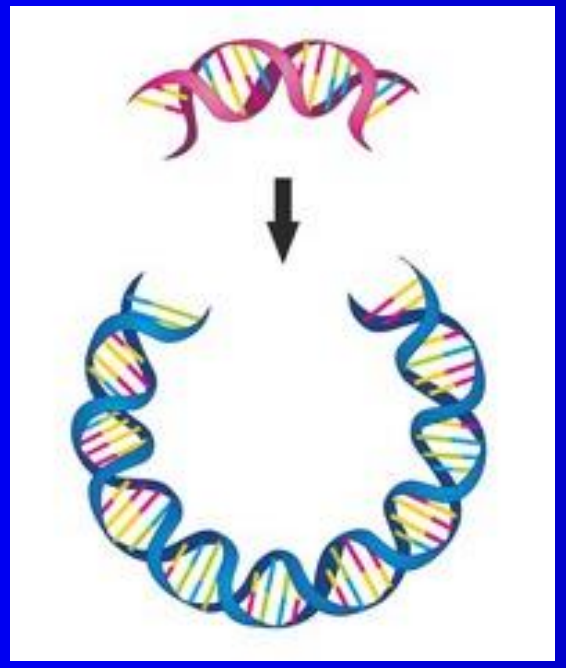
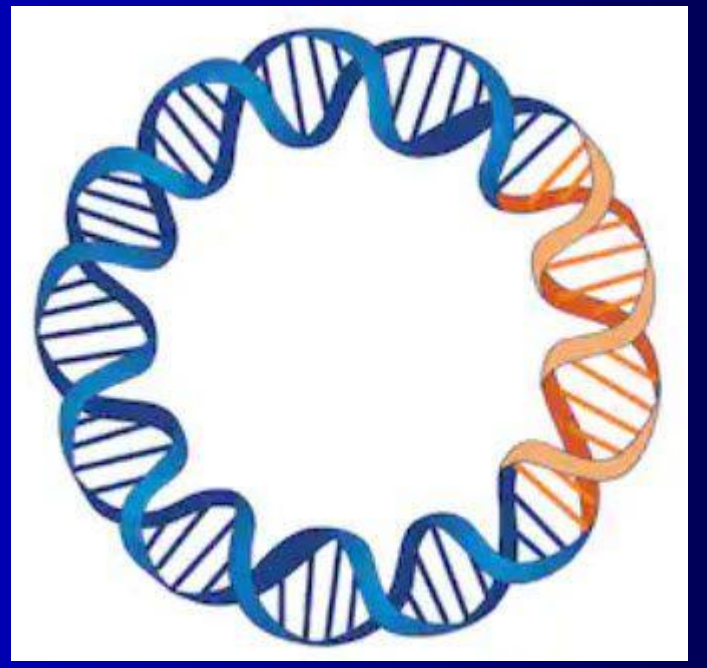
Construction of Artificial Recombinant DNA Molecule





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Construction of Artificial Recombinant DNA Molecule

The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme **DNA ligase,** which acts on cut DNA molecules and **joins their ends.**

This makes a new combination of circular autonomously replicating DNA created *in vitro* and is known as recombinant DNA.

B. John Ebenezer

Genetically Modified Organisms

The three basic steps involved in genetically modifying an organism:

Identification of DNA with desirable genes.

Introduction of the identified DNA into the host.

Maintenance of DNA introduced in the host and transfer of the DNA to its progeny.

B. John Ebenezer

Restriction enzymes

The enzymes used for cutting DNA at specific locations are called **restriction enzymes or molecular scissors.**

More than **900 restriction enzymes** have been isolated from **over 230 strains of bacteria.**

Each recognizes different **recognition sequences.**

The first restriction endonuclease is **Hind-II**, (Haemophilus influenzae)

The specific DNA nucleotide sequence was isolated and characterised five years later.

Restriction enzymes belong to a larger class of enzymes called **nucleases.**

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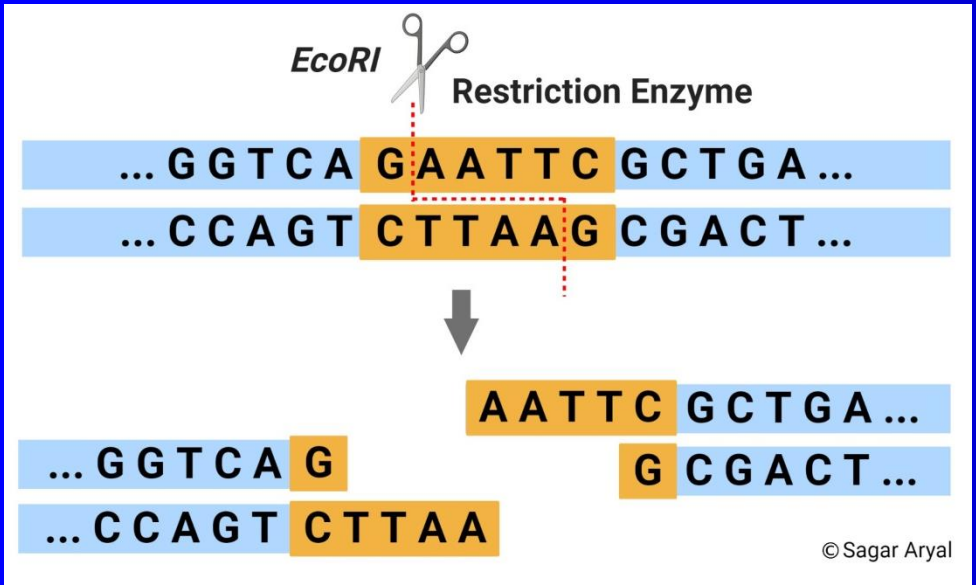
Palindrome



The groups of letters that form the same words when read both forward and backward, e.g. MALAYALAM.

The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

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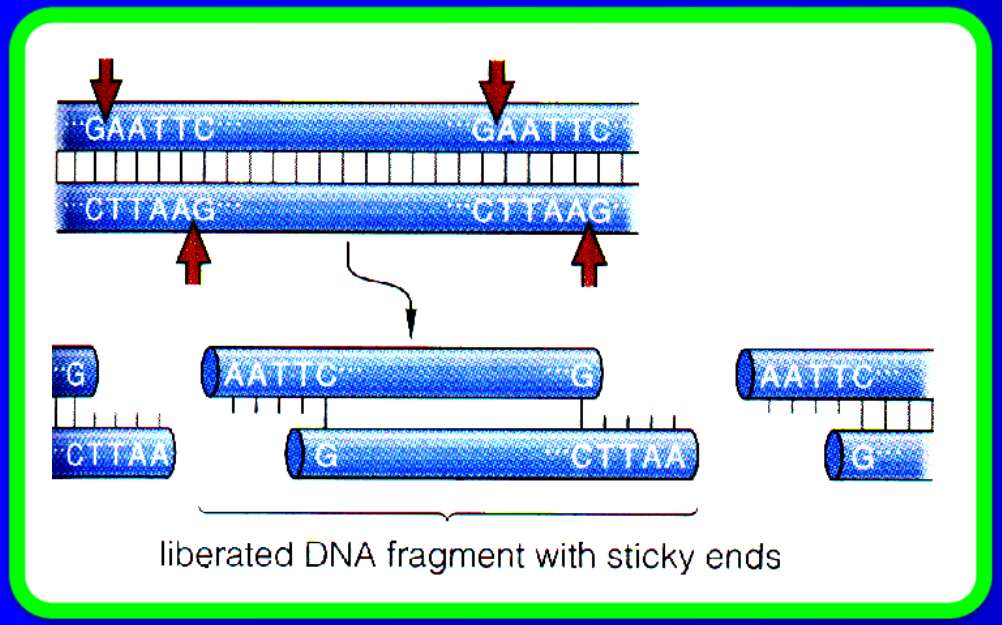
Recognition Sequence



Restriction enzymes always cut DNA molecules at a particular point by ***recognising a specific sequence of six base pairs***.

This specific base sequence is known as the **recognition sequence.**

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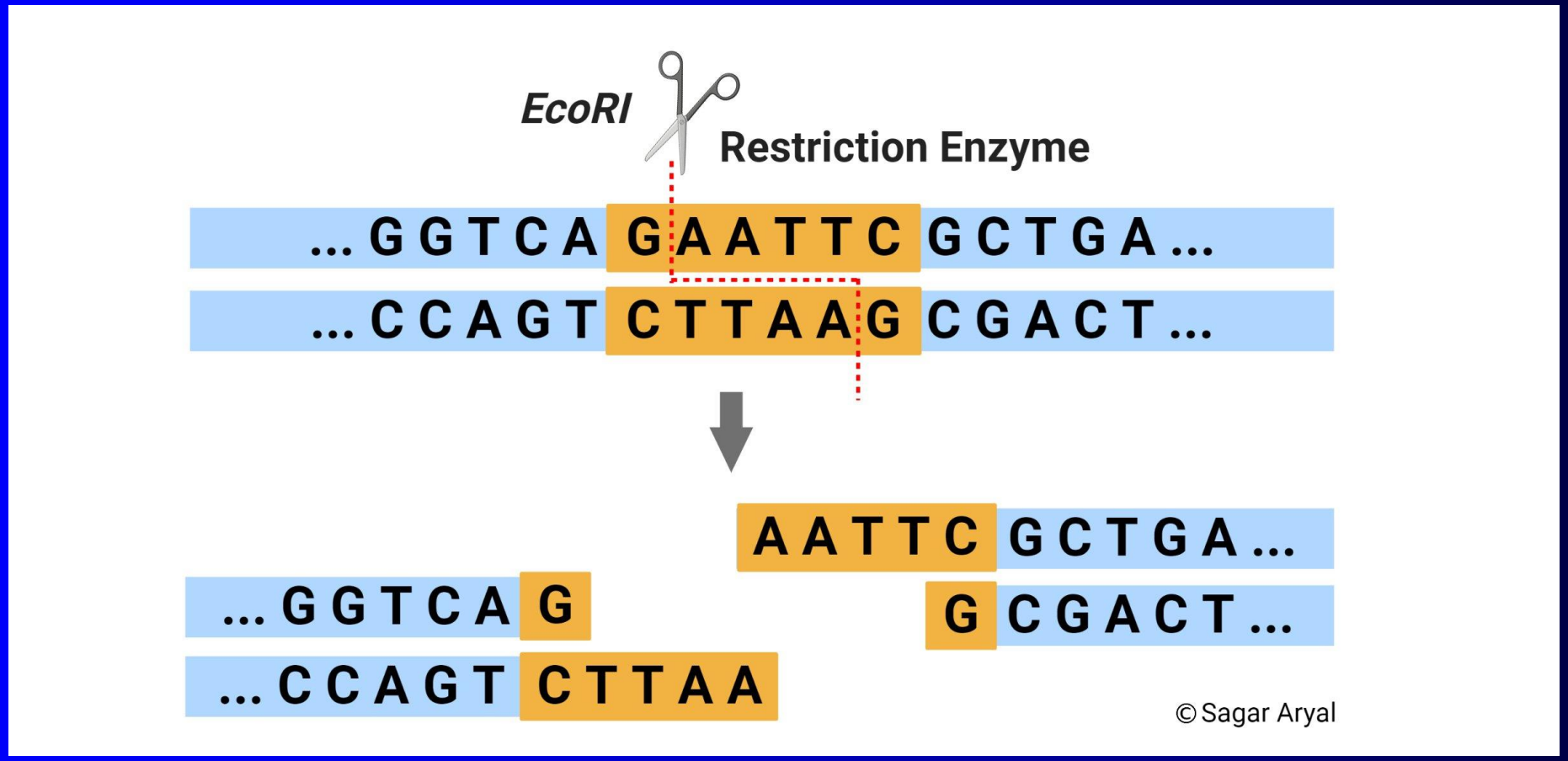
Recognition Sequence



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Naming of Enzymes

Naming of Enzymes

The **first letter** of the name comes from the genus.

The **second two letters** come from the species of the prokaryotic cell from which they were isolated.

e.g., **EcoRI** comes from ***E****scherichia* ***co****li* **R**Y13.

In EcoRI, the letter ***R*** is derived from the name of the **strain.**

Roman numbers following the names indicate **the order** in which the enzymes were isolated from that strain of bacteria.

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Naming of Enzymes

BamHI	**B**acillus **am**yloliquefaciens (H is a strain)
PstI	**P**rovidencia **st**uartii
PvuI	**P**roteus **vu**lgaris
HindII	**H**aemophilus **in**fluenzae
Sal I	**S**treptomyces **al**bus

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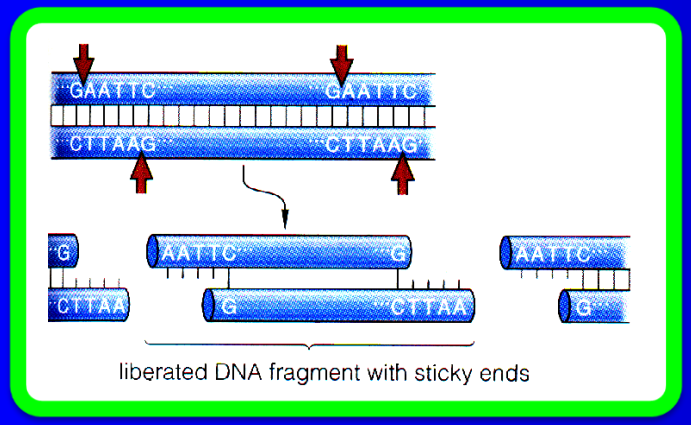
Nucleases

Endonucleases

Cut the DNA at a specific site



liberated DNA fragment with sticky ends

Exonucleases

Remove nucleotides from the cut ends of DNA

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Nucleases

The two kinds of nucleases are **exonucleases** and **endonucleases**.

Exonucleases **remove nucleotides** from the ends of the DNA whereas; endonucleases make cuts at specific positions within the DNA.

Each restriction endonuclease functions by inspecting the length of a DNA sequence.

Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbones

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Cloning Vectors

Cloning Vectors

Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

So they are used as cloning vectors.

Bacteriophages are used because of their **very high copy numbers per cell**.

Some plasmids have **only one or two copies per cell**

The others have **15-100 copies per cell**.

The copy numbers can be still higher.

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Cloning Vectors

If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage.

Vectors used at present are engineered in such a way that they help;

- **Easy linking** of foreign DNA and
- **Selection of recombinants** from non-recombinants.

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Features required to facilitate cloning into a vector

The following features are required to facilitate cloning into a vector.

1. Origin of replication (ori)

This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells.

This sequence is also responsible for controlling the copy number of the linked DNA.

So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.

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2. Selectable Markers

Selectable markers help in **identifying and eliminating non-transformants** and selectively permitting the growth of the transformants.

The **genes encoding resistance to antibiotics** such as **ampicillin, chloramphenicol, tetracycline or kanamycin** are considered useful selectable markers for *E. coli*

The normal *E. coli* cells do not carry resistance against any of these antibiotics.

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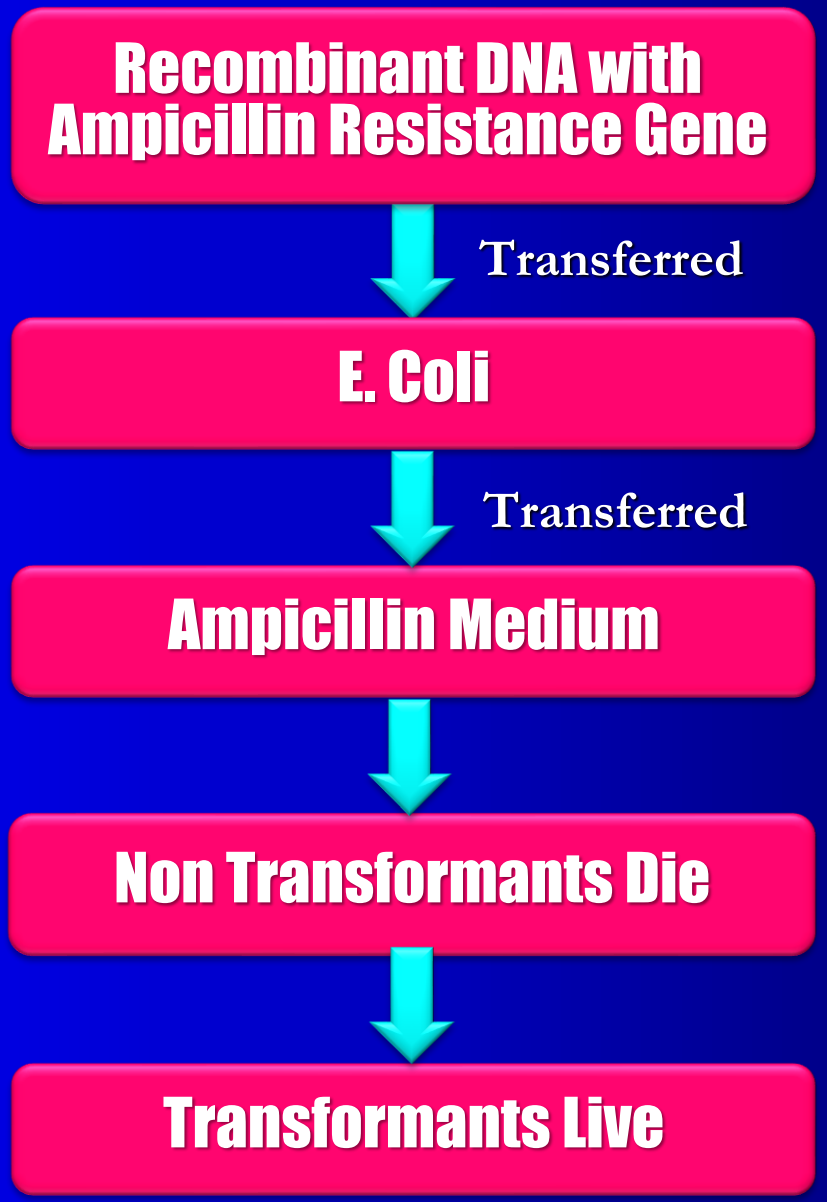
Ampicillin Resistance gene as Selectable Marker



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Selectable Markers

Recombinant DNA with Ampicillin Resistance Gene

Transferred

E. Coli

Transferred

Ampicillin Medium

Non Transformants Die

Transformants Live

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Selectable Markers

Insertion of Recombinant DNA into the Host Cell/Organism

Recipient cells after making them **competent** to receive, take up DNA present in its surrounding.

↓

Recombinant DNA bearing **gene for resistance** to an antibiotic (e.g., ampicillin)

↓

Transferred into *E. coli* cells

↓

The host cells become **transformed** into ampicillin-resistant cells.

↓

Spread the transformed cells on agar plates containing ampicillin

↓

Only **transformants will grow**, **non-transformed recipient cells will die.**

Since, due to ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a **selectable marker.**

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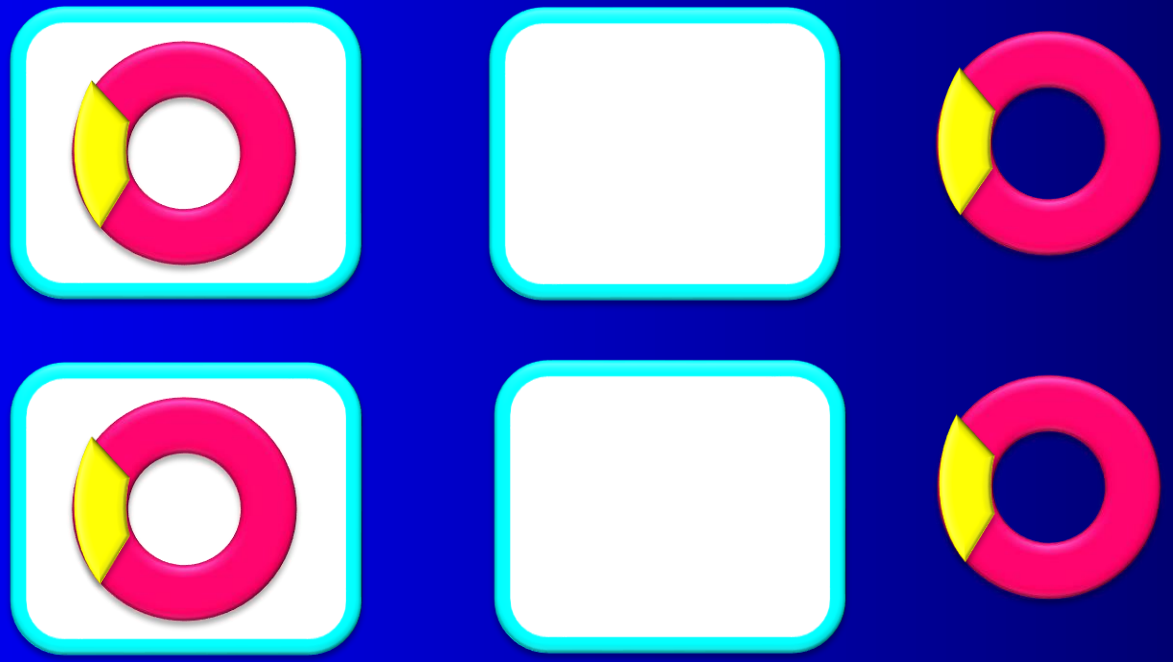
Transformants and Non-Transformants



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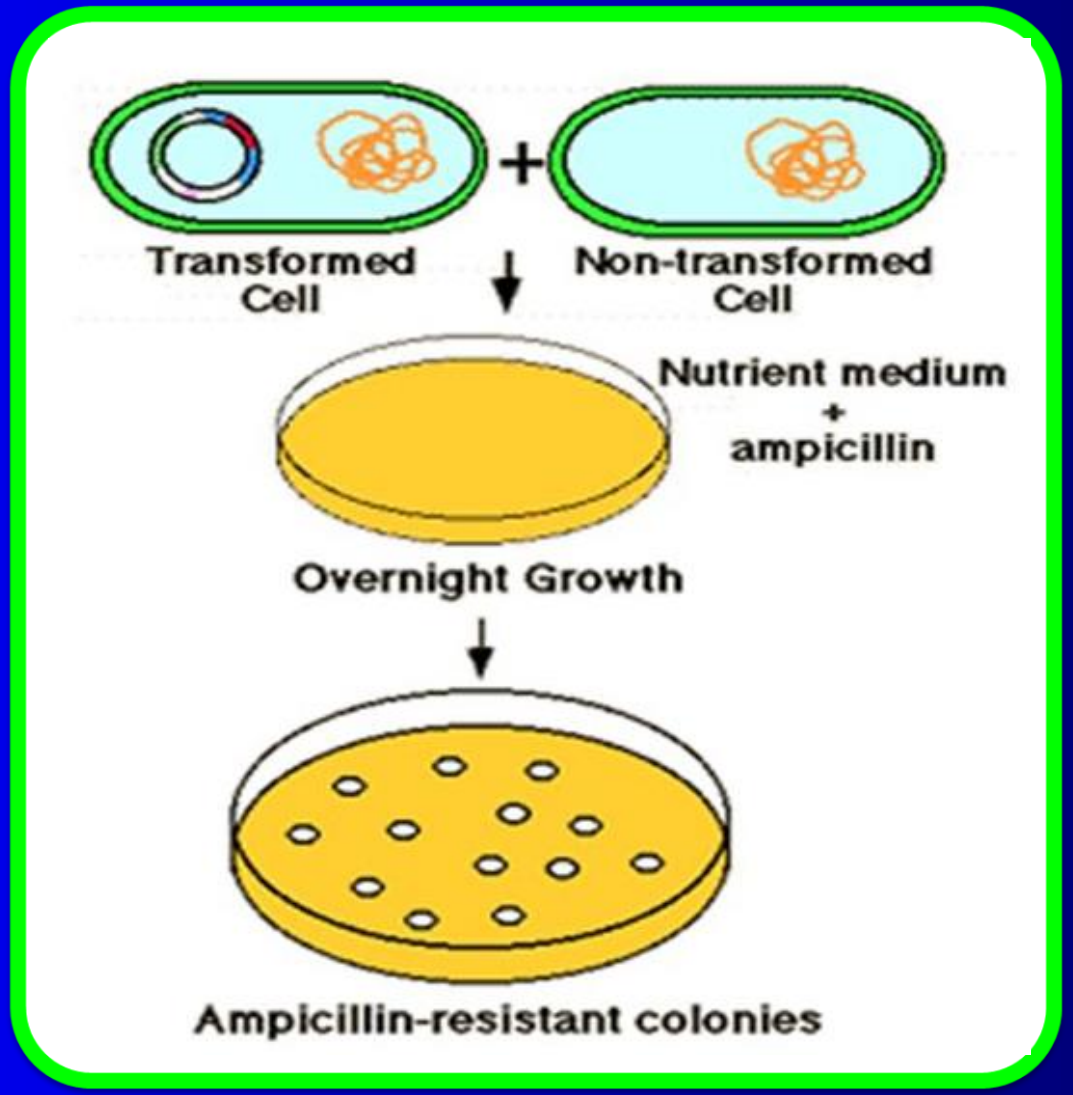
Selectable Markers



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3. Cloning Sites

In order to link the alien DNA, the vector needs to have very few, preferably single, **recognition sites** for the commonly used restriction enzymes.

Presence of **more than one recognition sites** within the vector will make several fragments, which will complicate the gene cloning.

The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance genes.**

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Cloning Sites

A recombinant DNA is inserted within the coding sequence of an enzyme **alpha-galactosidase.**

This results into inactivation of the enzyme, which is referred to as **insertional inactivation.**

If the plasmid in the bacteria **does not have an insert,** the colonies give **blue colour** in the presence of a **chromogenic substrate**

If the plasmid in the bacteria **has an insert,** it results into insertional inactivation of the a-galactosidase and the colonies do not produce any colour, these are identified as recombinant colonies.

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Recombinant DNA

Inserted at the

Coding Sequence of alpha galactosidase enzyme

Insertional Inactivation

Inactivation of enzyme alpha galactosidase

Plasmid in bacteria with insert

In the presence of Chromogenic Substrate

Colour is not produced

No colour

Recombinants

Plasmid in bacteria without insert

In the presence of Chromogenic Substrate

Colour Produced

Blue colour

Non-Recombinants

5. Vectors for cloning genes in plants and animals

Process of selecting recombinants from non-recombinants using alternative selectable markers.

Alternative selectable markers differentiate recombinants from non-recombinants on the basis of their ability to **produce colour in the presence of a chromogenic substrate.**

Recombinant DNA.

Inserted

In the coding sequence of a- galactosidase enzyme

Insertional inactivation

Inactivation of the enzyme a-galactosidase

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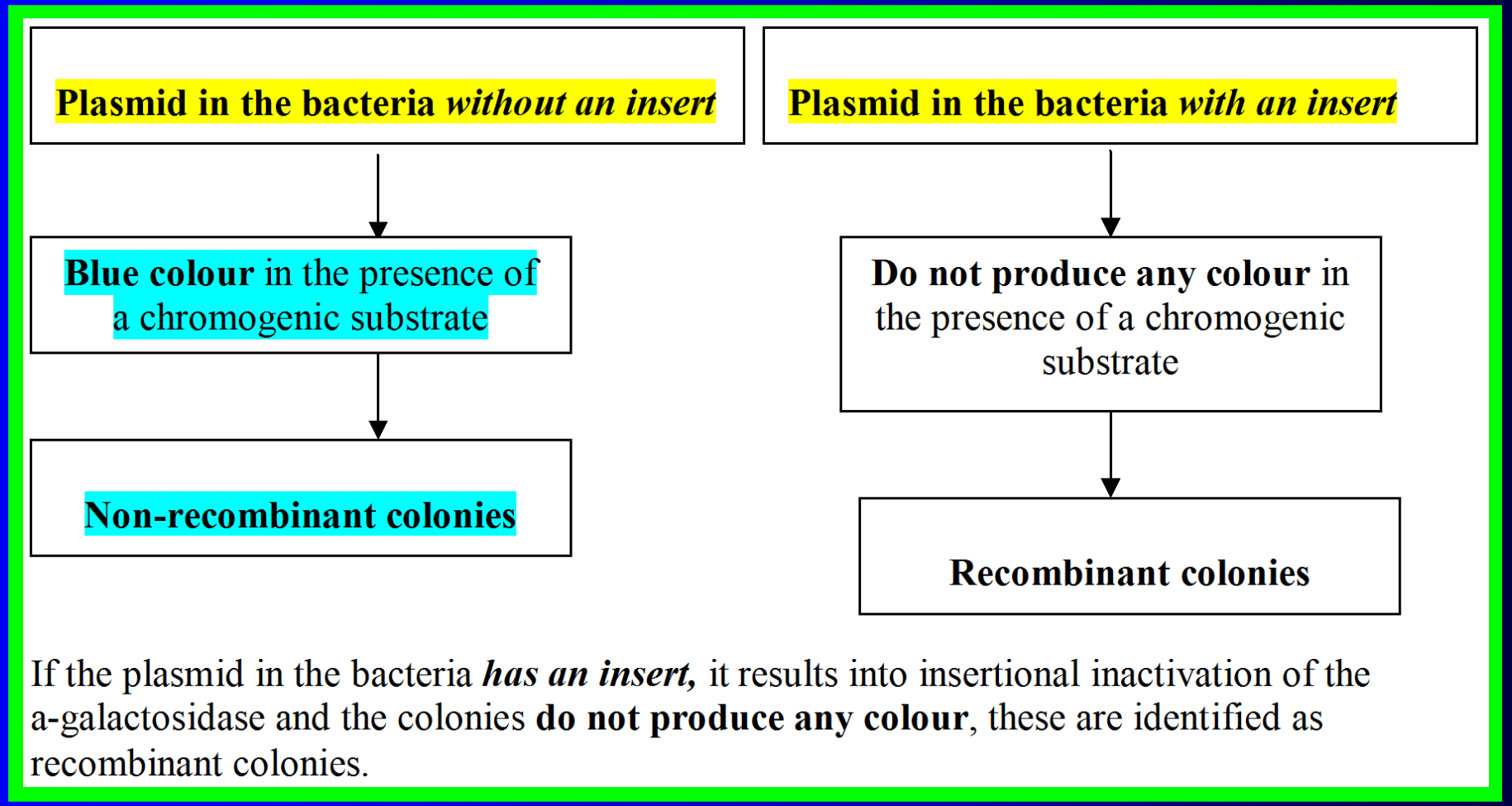
5. Vectors for cloning genes in plants and animals



If the plasmid in the bacteria ***has an insert,*** it results into insertional inactivation of the a-galactosidase and the colonies **do not produce any colour**, these are identified as recombinant colonies.

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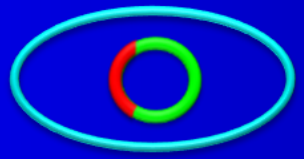
Alternative Selectable Marker Insertional Inactivation of Alpha Galactosidase

Desirable Gene

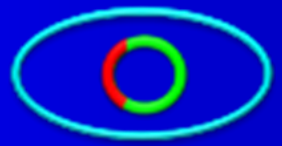
Plasmid

Recombinant DNA

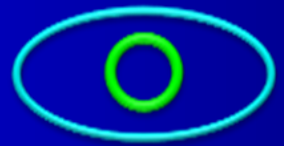


Bacterial cell with insert

Bacterial cell without insert



Recombinants



Non Recombinants

Colourless colony

Blue Colour colony

A recombinant DNA is inserted within the coding sequence of an enzyme **alpha-galactosidase.**

Plasmid in the bacteria **with an insert** results into insertional inactivation of a-galactosidase and produces colourless colonies in the presence of **chromogenic substrate**

Plasmid in the bacteria **without an insert produce blue colour colonies** in the presence of **chromogenic substrate.**

pBR322 Vector

P=plasmid BR= Boliver and Rodriguez (researchers) 322= is the no given to distinguish this from the other vectors.

If a foreign DNA is ligated at the Bam HI site of **tetracycline resistance gene** in the vector pBR322.

The recombinant plasmids will **lose tetracycline resistance** due to insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformants on ampicillin containing medium.

The transformants growing on ampicillin containing medium are then transferred on a medium containing tetracycline.

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pBR322 Vector

The recombinants will grow in ampicillin containing medium but not on that containing tetracycline.

But, non-recombinants will grow on the medium containing both the antibiotics.

In this case, one antibiotic resistance gene helps in selecting the transformants.

The other antibiotic resistance gene gets inactivated due to insertion of alien DNA, and helps in selection of recombinants.

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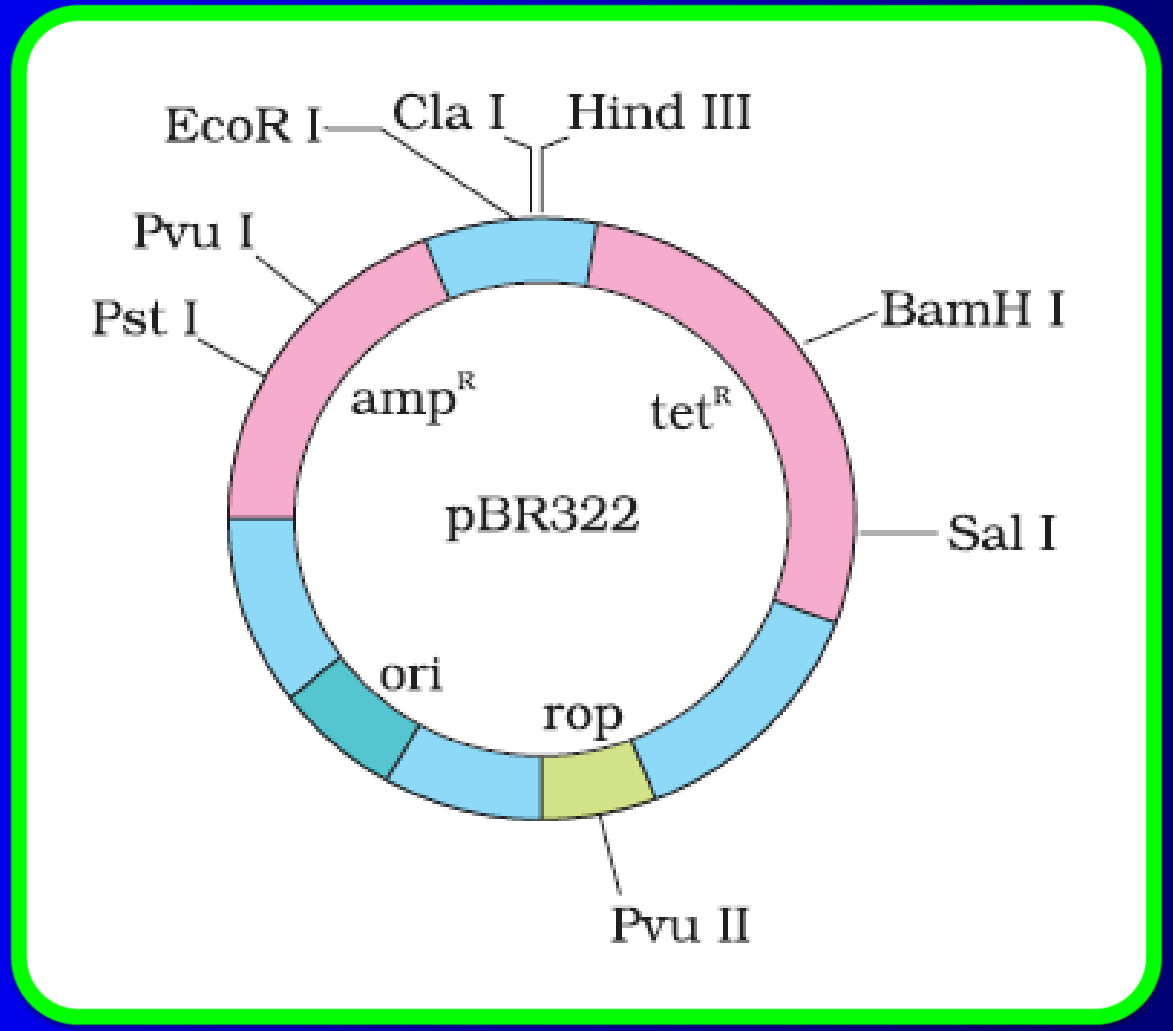
Cloning Sites



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Cloning Sites

BamHI	Bacillus amyloliquefaciens (H is a strain)
PstI	Providencia stuartii
PvuI	Proteus vulgaris
HindII	Haemophilus influenzae
Sal I	Streptomyces albus

Rop is a gene and also known as **repressor of primer** which codes for a small protein responsible for keeping the copy number.

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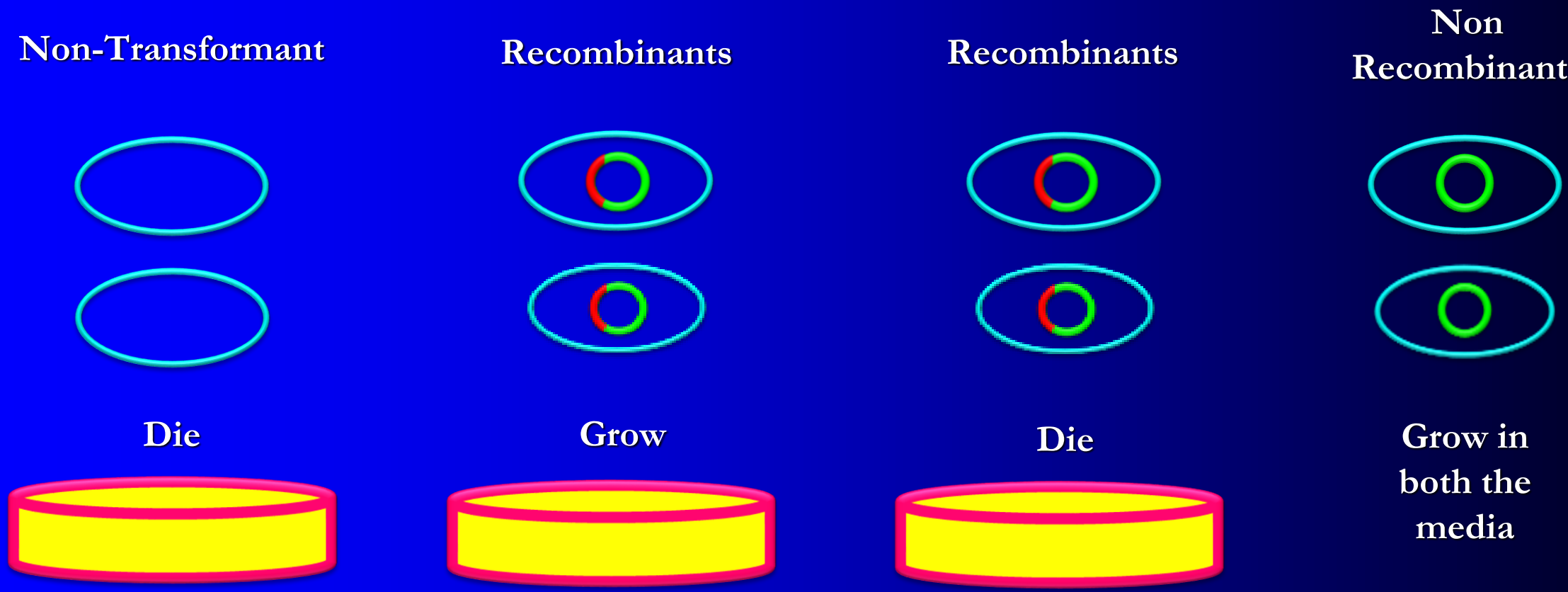
Cloning in pBR322 Vector



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Vectors for cloning genes in plants and animals

Agrobacterium tumifaciens, a pathogen of several dicot plants is able to deliver a piece of DNA known as '**T-DNA to transform normal plant cells into a tumor** and **direct these tumor cells to produce the chemicals** required by the pathogen.

Similarly, **retroviruses** in animals have the ability to **transform normal cells into cancerous** cells.

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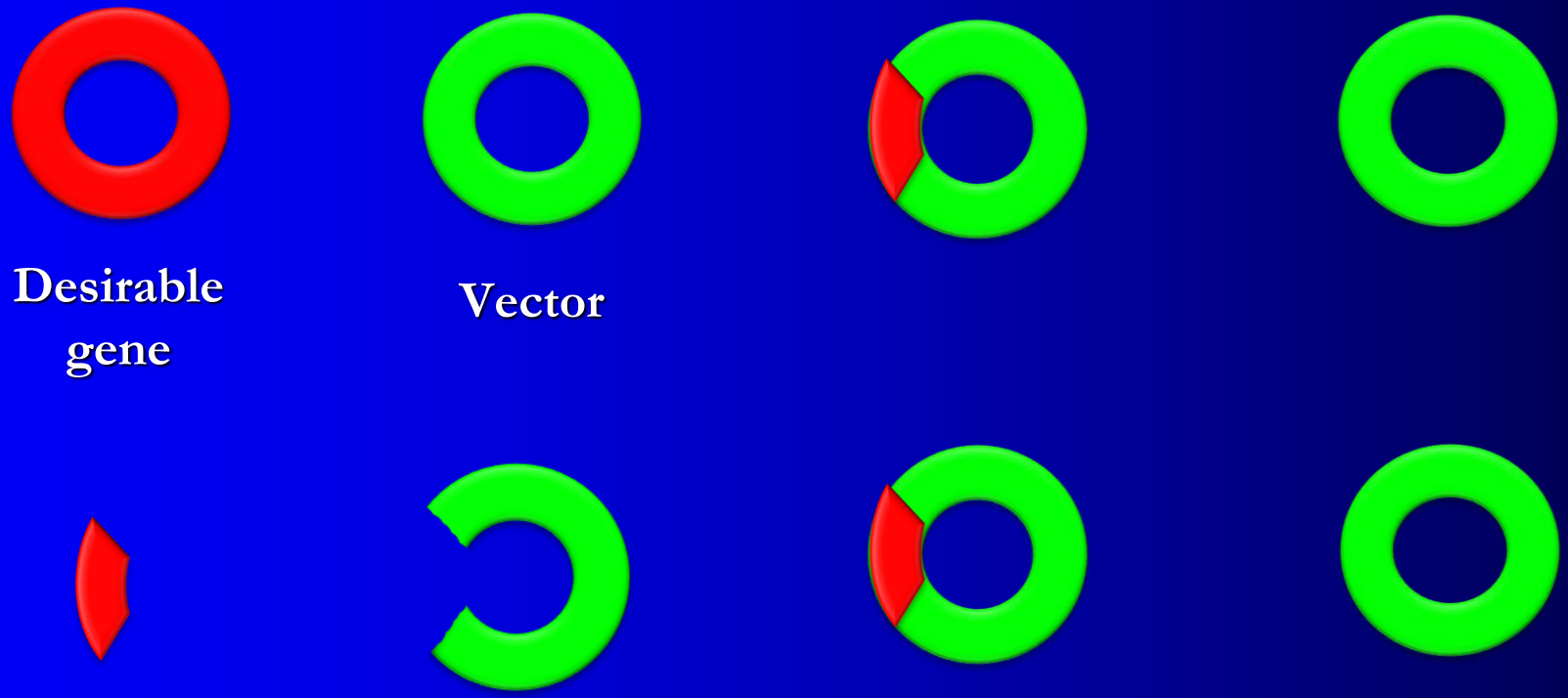
Recombinants and Non-Recombinants



Desirable gene

Vector

Recombinant genes

Non Recombinant genes

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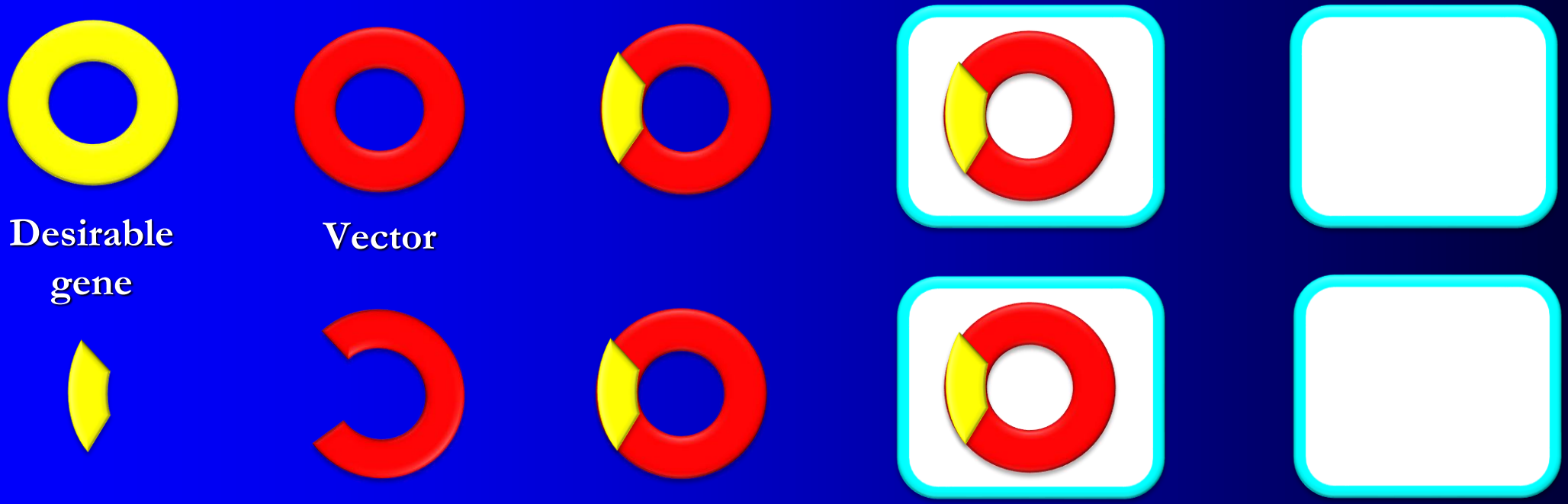
Transformants and Non-Transformants



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Vectors for cloning genes in plants and animals

The tumor inducing (Ti) plasmid of ***Agrobacterium tumifaciens*** has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants.

Similarly, **retroviruses** have also been disarmed and are now used to deliver desirable genes into animal cells.

So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).

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Competent Host (For Transformation with Recombinant (DNA)

Since DNA is a hydrophilic molecule, it cannot pass through cell membranes.

In order to force bacteria to take up the plasmid, the bacterial cells must be made competent to take up DNA.

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Heat Shock Method

Making the bacterial cell competent to take up DNA

Incubating the Bacterial cells + Recombinant DNA on ice

↓

Placing them briefly at 42°C (heat shock)

↓

Placing them back on ice

Enables the bacteria to take up the recombinant

Heat Shock Method

The bacterial cells are made competent to take up DNA by treating them with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock), and then putting them back on ice.

This enables the bacteria to take up the recombinant DNA. This is not the only way to introduce alien DNA into host cells.

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Micro-injection

In a method known as **micro-injection,** recombinant DNA is directly injected into the nucleus of an animal cell.

Biolistics or gene gun

The **cells are bombarded** with high velocity micro-particles of **gold or tungsten coated with DNA.** This method is known as biolistics or gene gun.

Disarmed pathogen vectors

This method uses disarmed pathogen vectors, which when allowed infecting the cell, transferring the recombinant DNA into the host.

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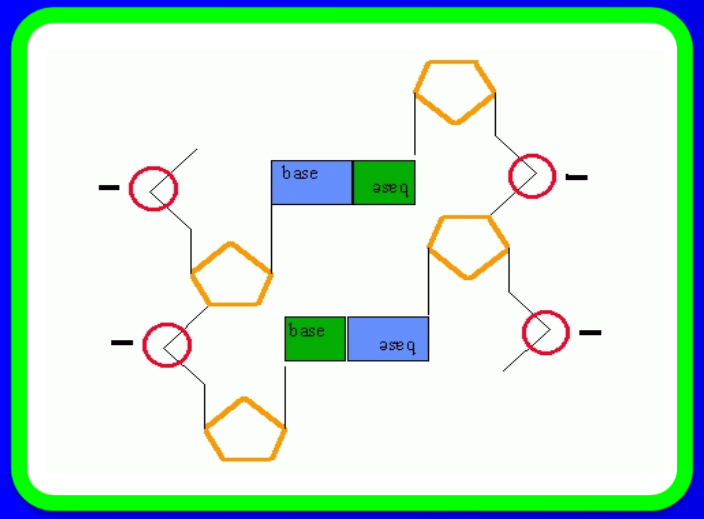
Gel Electrophoresis



The cutting of DNA by restriction endonucleases results in the fragments of DNA.

These fragments can be separated by a technique known as **gel electrophoresis.**

Since DNA fragments are **negatively charged molecules** they can be separated by forcing them to move towards the **anode** under an electric field through a medium/matrix.

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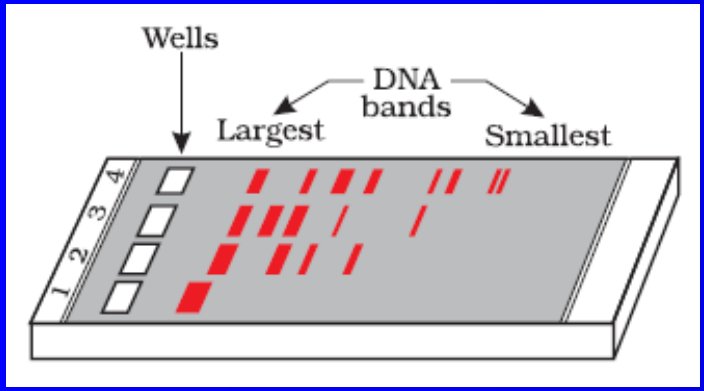
Gel Electrophoresis



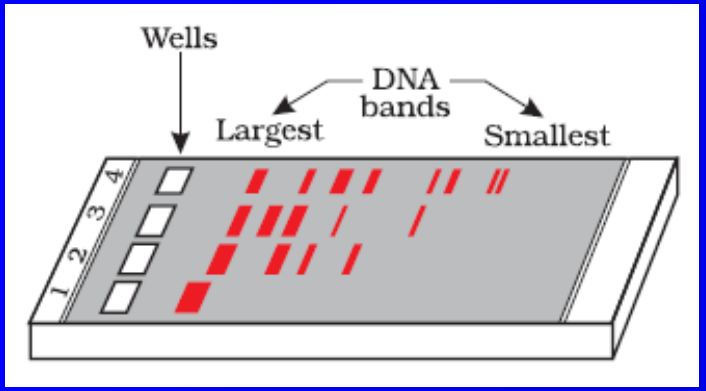
The most commonly used matrix is **agarose** which is a natural polymer extracted from sea weeds.

The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.

The separated DNA fragments can be visualised only after staining the DNA with a compound known as **ethidium bromide** followed by **exposure to UV radiation.**

B. John Ebenezer

Gel Electrophoresis



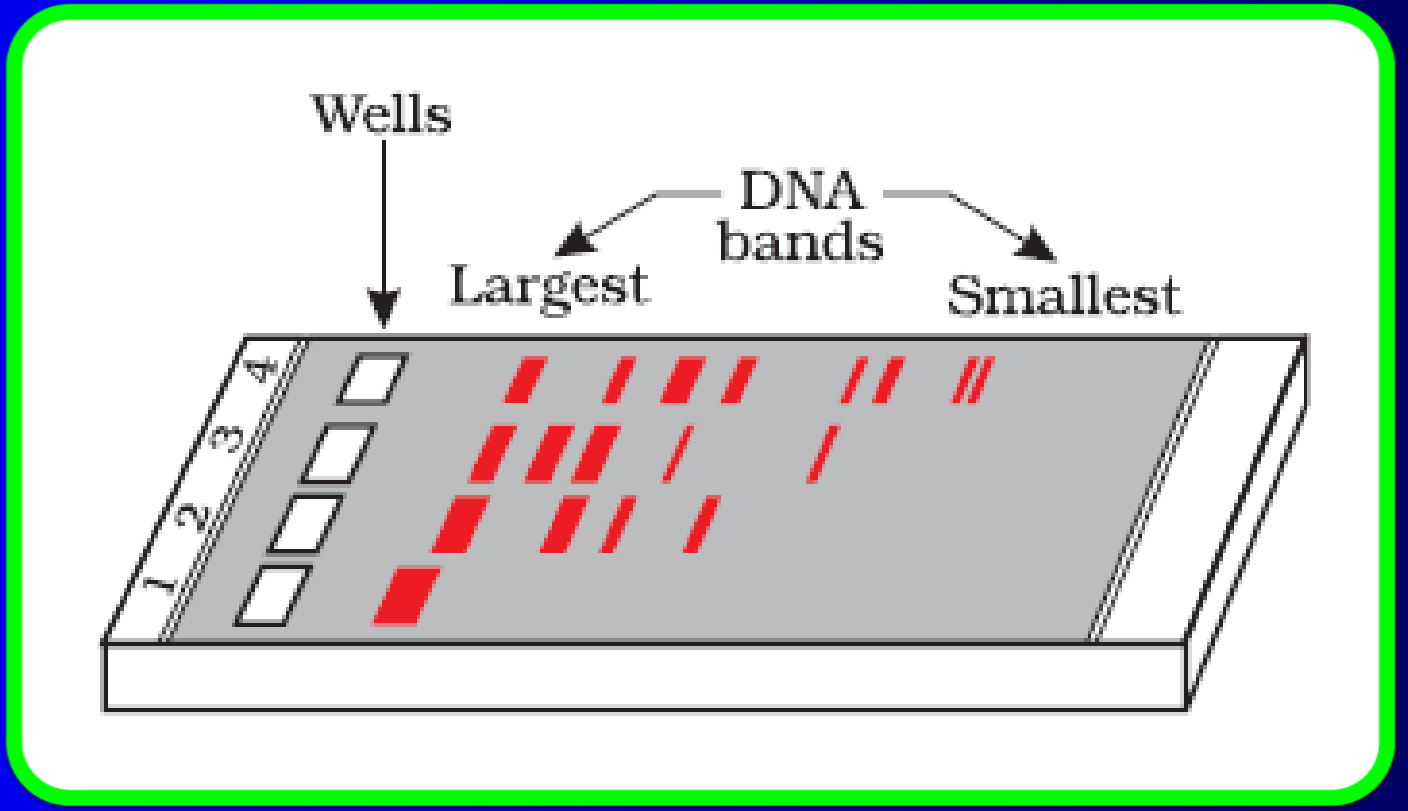
A bright orange coloured bands of DNA is seen in an **ethidium bromide** stained gel exposed to **UV light**

The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution.

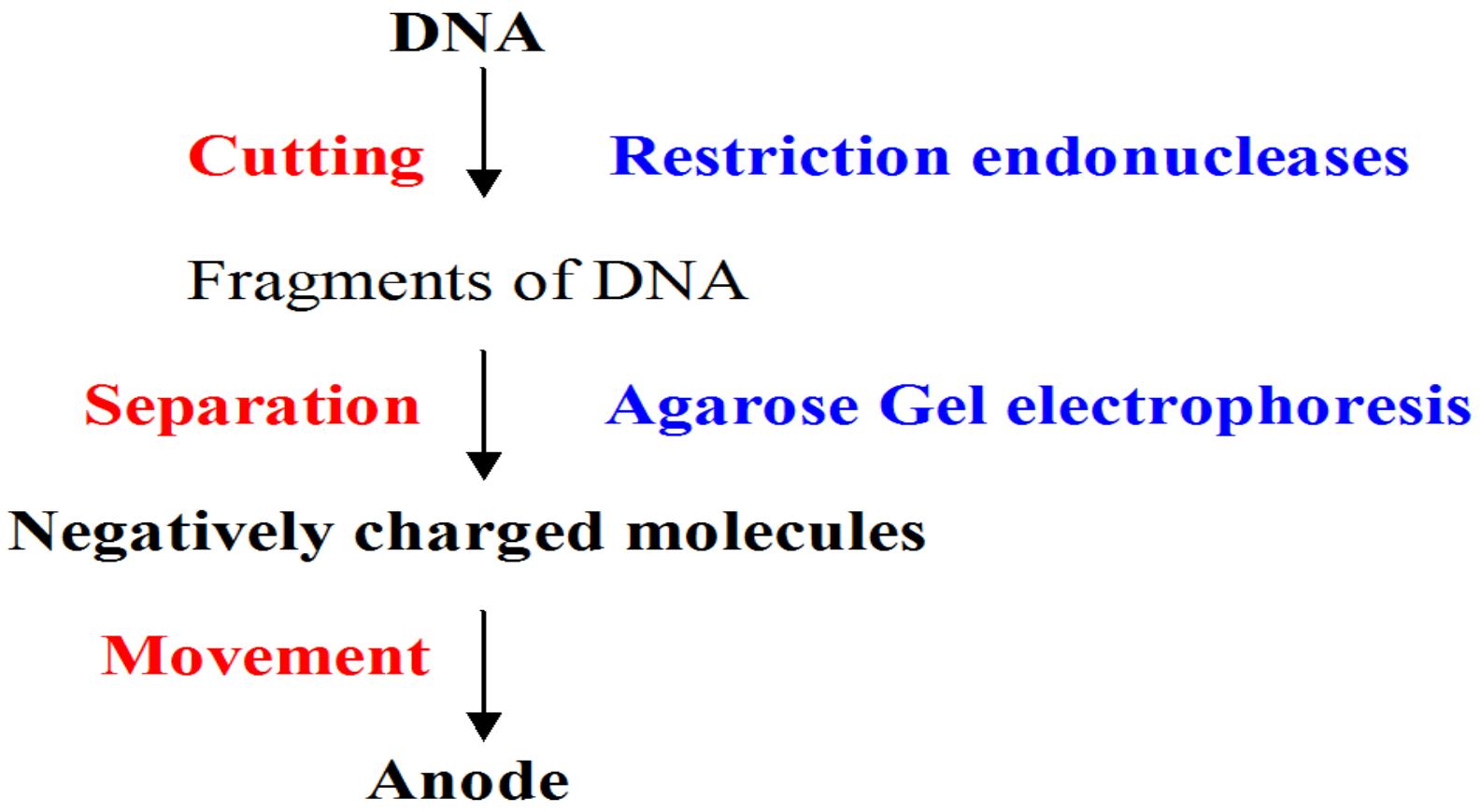
The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

B. John Ebenezer

Gel Electrophoresis



The process of isolation of DNA fragments



VISUALIZATION

DNA Fragments

Staining ↓

Ethidium bromide

Exposure ↓

UV radiation

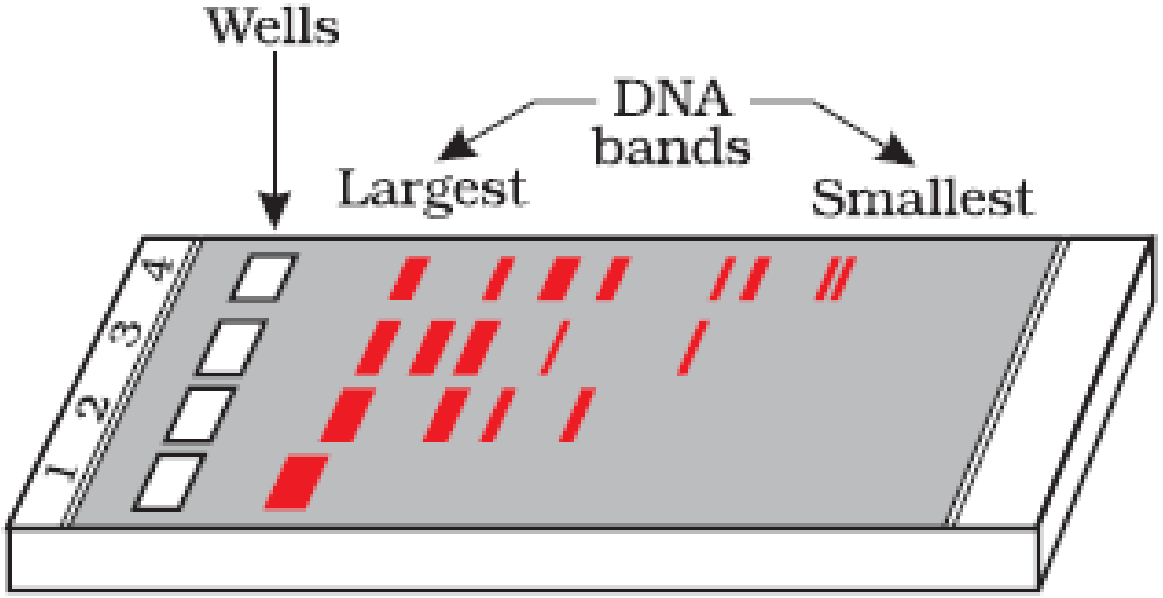
↓

Bright orange coloured bands of DNA is seen

Elution ↓ **Extraction from the gel piece**

Pure DNA fragments

Gel Electrophoresis



Processes of Recombinant DNA Technology

Steps of Recombinant DNA technology

Recombinant DNA technology involves several steps in specific sequence such as

- Isolation of DNA
- Fragmentation of DNA by restriction endonucleases
- Isolation of a desired DNA fragment
- Ligation of the DNA fragment into a vector
- Transferring the recombinant DNA into the host
- Culturing the host cells in a medium at large scale and extraction of the desired product

B. John Ebenezer

Isolation of the Genetic Material (DNA)

In order to cut the DNA with restriction enzymes, it needs to be in pure form, free from other macro-molecules.

Since the DNA is enclosed within the membranes, we have to break open the cell to release DNA along with other macromolecules such as **RNA, proteins, polysaccharides and also lipids.**

This can be achieved by treating the bacterial cells with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus).

B. John Ebenezer

Isolation of the Genetic Material (DNA)

Genes are located on long molecules of DNA interwined with proteins such as histones.

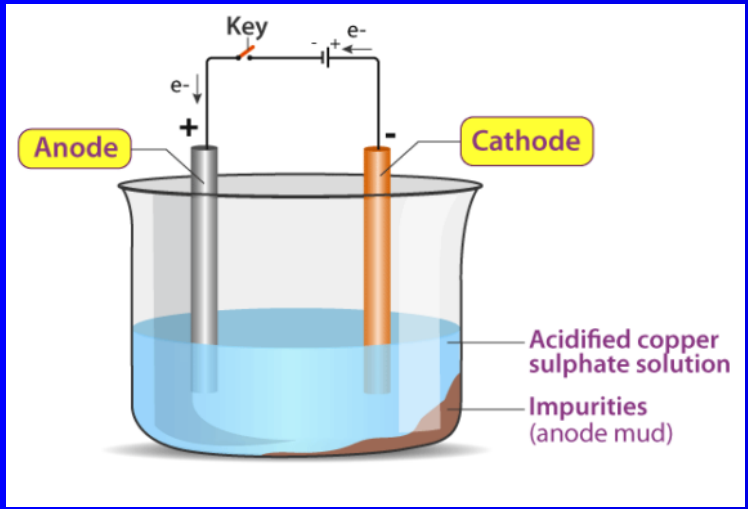
The **RNA** can be removed by treatment with **ribonuclease** whereas **proteins** can be removed by treatment with **protease**.

Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol.

This can be seen as collection of fine threads in the suspension.

B. John Ebenezer

Cutting of DNA at Specific Locations



Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme.

Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.

DNA is a negatively charged molecule, hence it moves towards the positive electrode (anode).

The process is repeated with the vector DNA also.

B. John Ebenezer

Cutting of DNA at Specific Locations

The joining of DNA involves several processes.

After cutting the source DNA as well as the vector DNA with a specific restriction enzyme, the cut out ‘gene of interest’ from the source DNA and the cut vector with space are mixed and ligase is added.

This results in the preparation of recombinant DNA.

B. John Ebenezer

Amplification of Gene of Interest using PCR

PCR stands for **Polymerase Chain Reaction**.

In PCR multiple copies of the gene of interest is synthesised *in vitro* using **two sets of primers** (small chemically synthesised **oligonucleotides** that are complementary to the regions of DNA) and the enzyme **DNA polymerase**.

The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as template.

If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made.

B. John Ebenezer

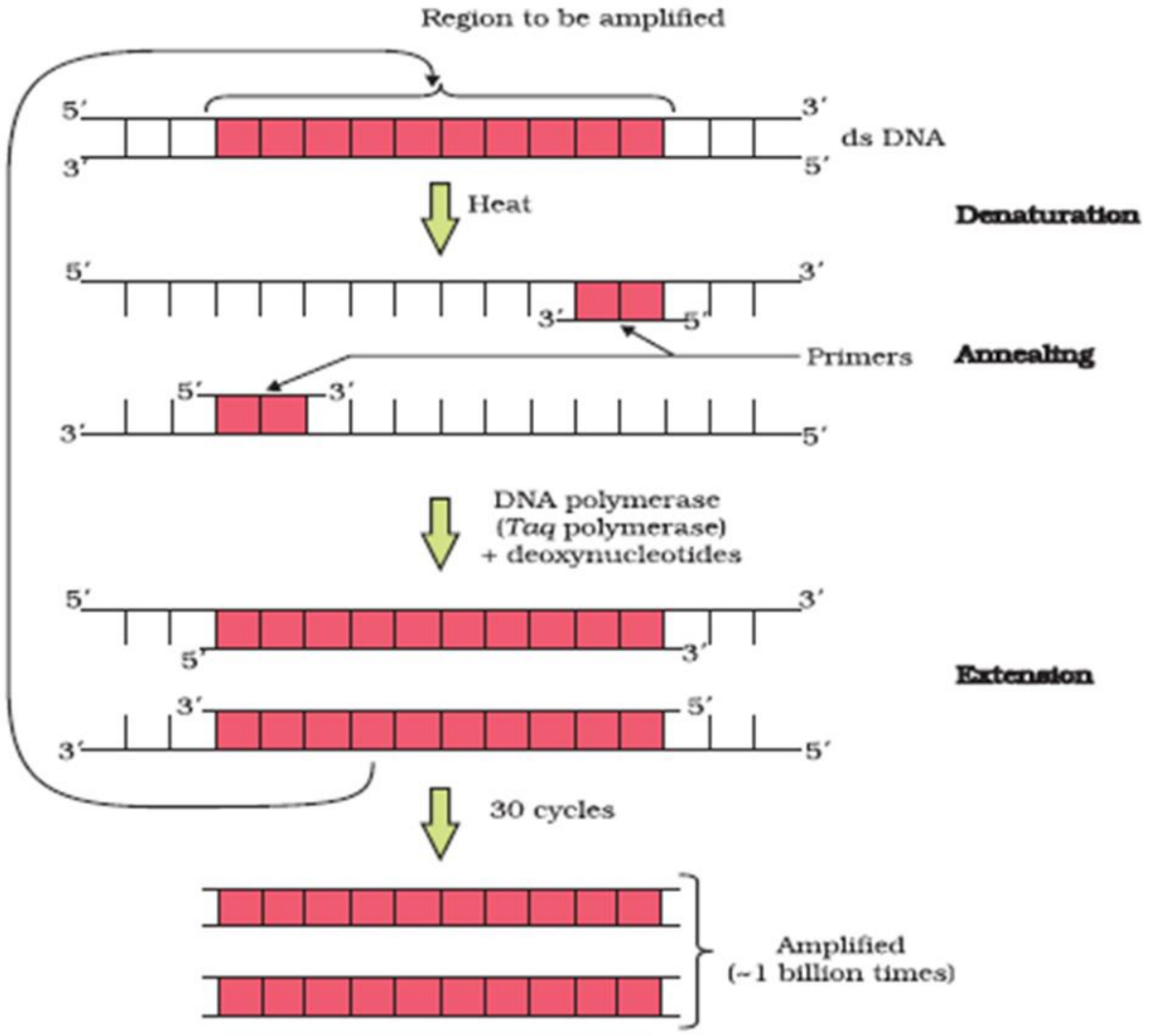
Amplification of Gene of Interest using PCR

Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, ***Thermus aquaticus***), which remain active during the high temperature induced denaturation of double stranded DNA.

The amplified fragment if desired can now be used to ligate with a vector for further cloning

B. John Ebenezer

Polymerase Chain Reaction



B. John Ebenezer

Insertion of Recombinant DNA into the Host Cell/Organism

There are several methods of introducing the ligated DNA into recipient cells.

Recipient cells after making them ‘competent’ to receive, take up DNA present in its surrounding.

Ampicillin resistance gene is the most commonly used selection marker.

If a recombinant DNA bearing ampicillin resistance gene is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cells.

B. John Ebenezer

Insertion of Recombinant DNA into the Host Cell/Organism

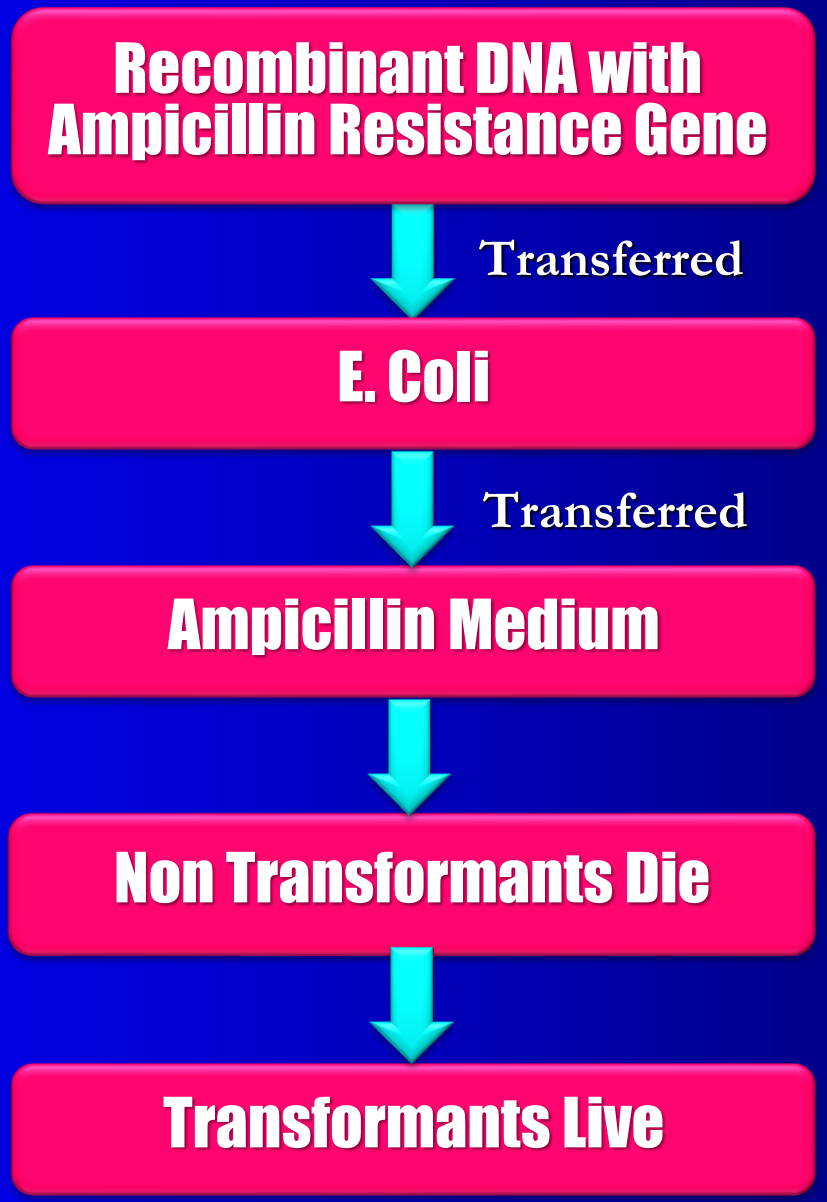
If we spread the transformed cells on agar plates containing ampicillin, only transformants grow, untransformed recipient cells die.

So, we can select transformed cells from non-transformed cells in the presence of ampicillin.

The ampicillin resistance gene in this case is used as a selectable marker.

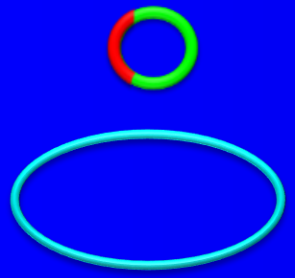
B. John Ebenezer

Ampicillin Resistance gene as Selectable Marker

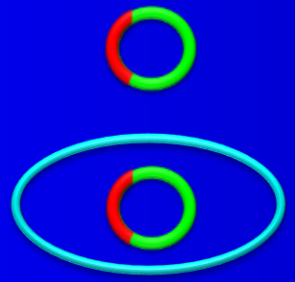


B. John Ebenezer

Ampicillin Resistance gene as Selectable Markers

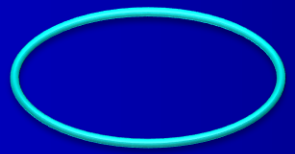


Non Transformant

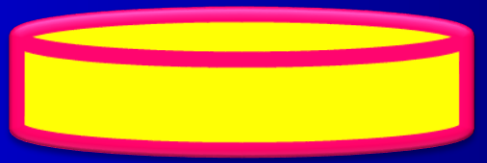


Transformant

Non Transformants



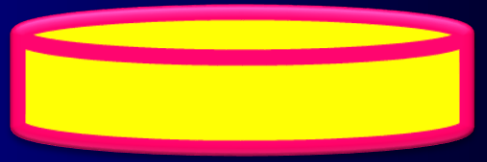
Die



Ampicillin Medium

Transformants

Grow



Ampicillin Medium

B. John Ebenezer

Obtaining the Foreign Gene Product

When you insert a piece of alien DNA into a cloning vector and transfer it into a bacterial, plant or animal cell, the alien DNA gets multiplied.

In almost all recombinant technologies, the ultimate aim is to produce a desirable protein.

Hence, there is a need for the recombinant DNA to be expressed.

The foreign gene gets expressed under appropriate conditions.

The expression of foreign genes in host cells involve understanding many technical details.

B. John Ebenezer

Obtaining the Foreign Gene Product

After cloning the **gene of interest** and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale.

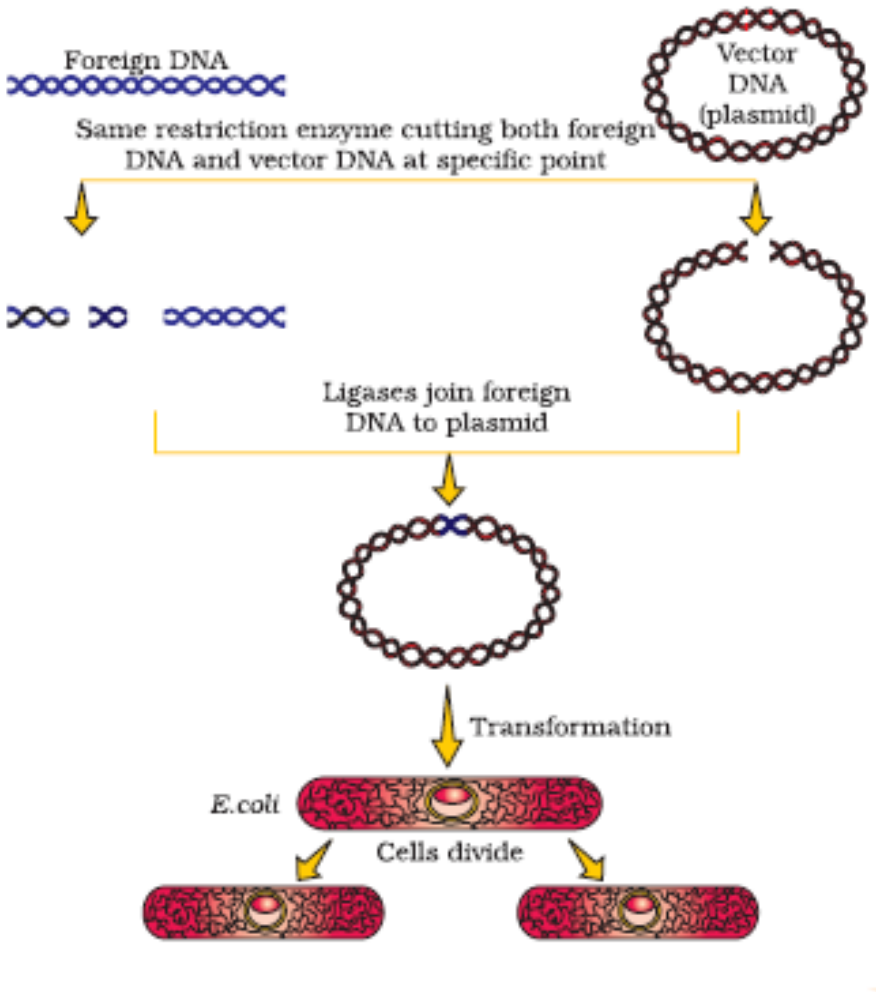
If any protein encoding gene is expressed in a heterologous host, is called a **recombinant protein**.

The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory.

The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

B. John Ebenezer

Recombinant DNA Technology



B. John Ebenezer

Obtaining the Foreign Gene Product

The cells can also be multiplied in a continuous culture system.

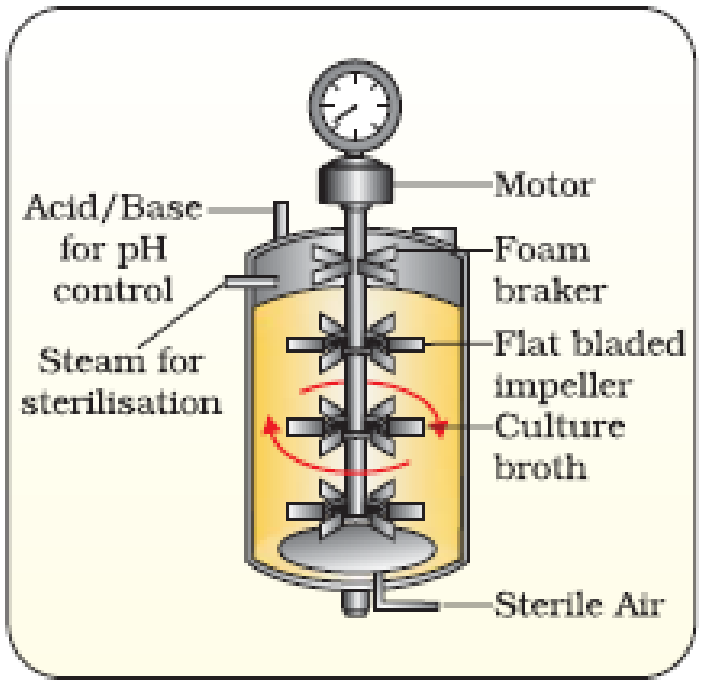
Here the used medium is drained out from one side while fresh medium is added from the other side.

This maintains the cells in their physiologically most active log/exponential phase.

This type of culturing method produces a larger biomass leading to higher yields of desired protein.

B. John Ebenezer

Bioreactors



Bioreactors are vessels in which large volumes (100-1000 litres) of **raw materials** are biologically converted into **specific products,** individual **enzymes,** etc., using microbial plant, animal or human cells.

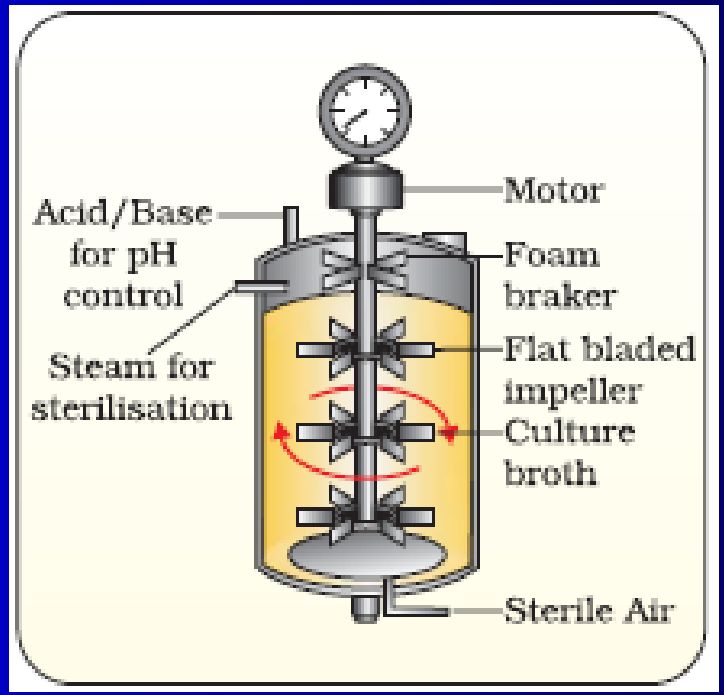
A bioreactor provides the **optimal conditions** for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

B. John Ebenezer

Bioreactors

The bioreactor has

- Agitator system
- Oxygen delivery system
- Foam control system
- Temperature control system
- pH control system
- Sampling ports so that small volumes of the culture can be withdrawn periodically.



B. John Ebenezer

Bioreactors

The most commonly used bioreactors are of stirring type.

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents.

The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor.

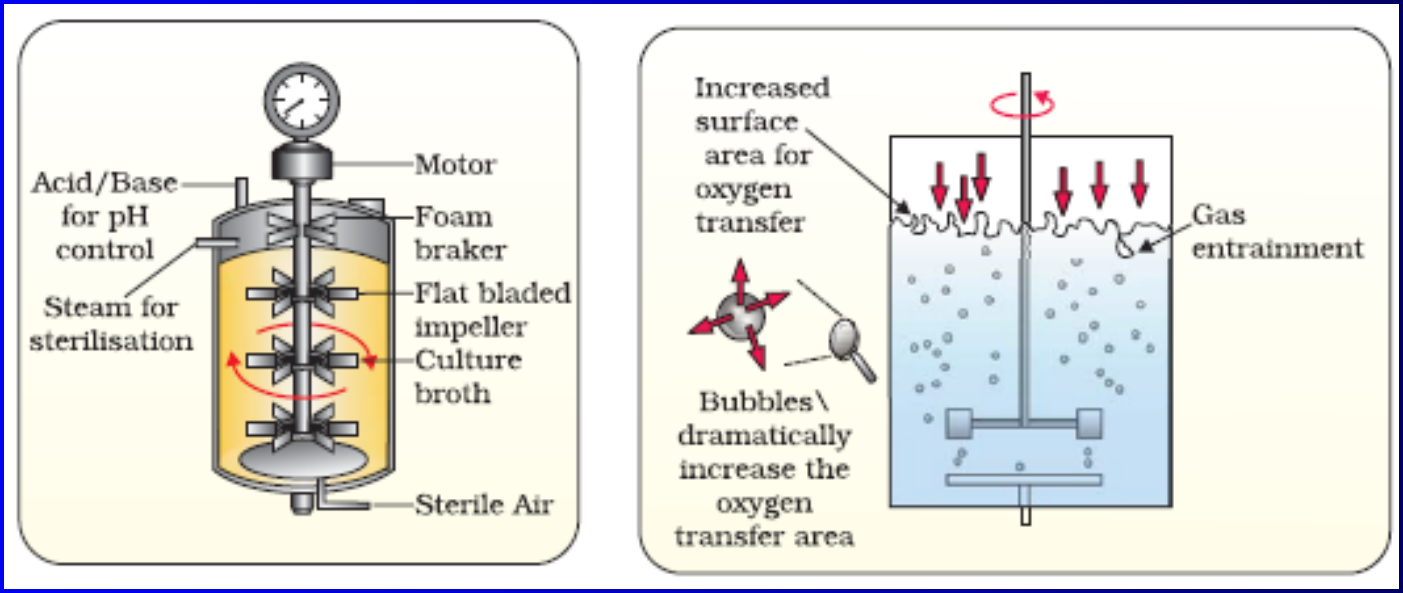
B. John Ebenezer

Bioreactors

The two types of bioreactors are

(a) Simple stirred-tank bioreactor;

(b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged.



B. John Ebenezer

Bioreactors

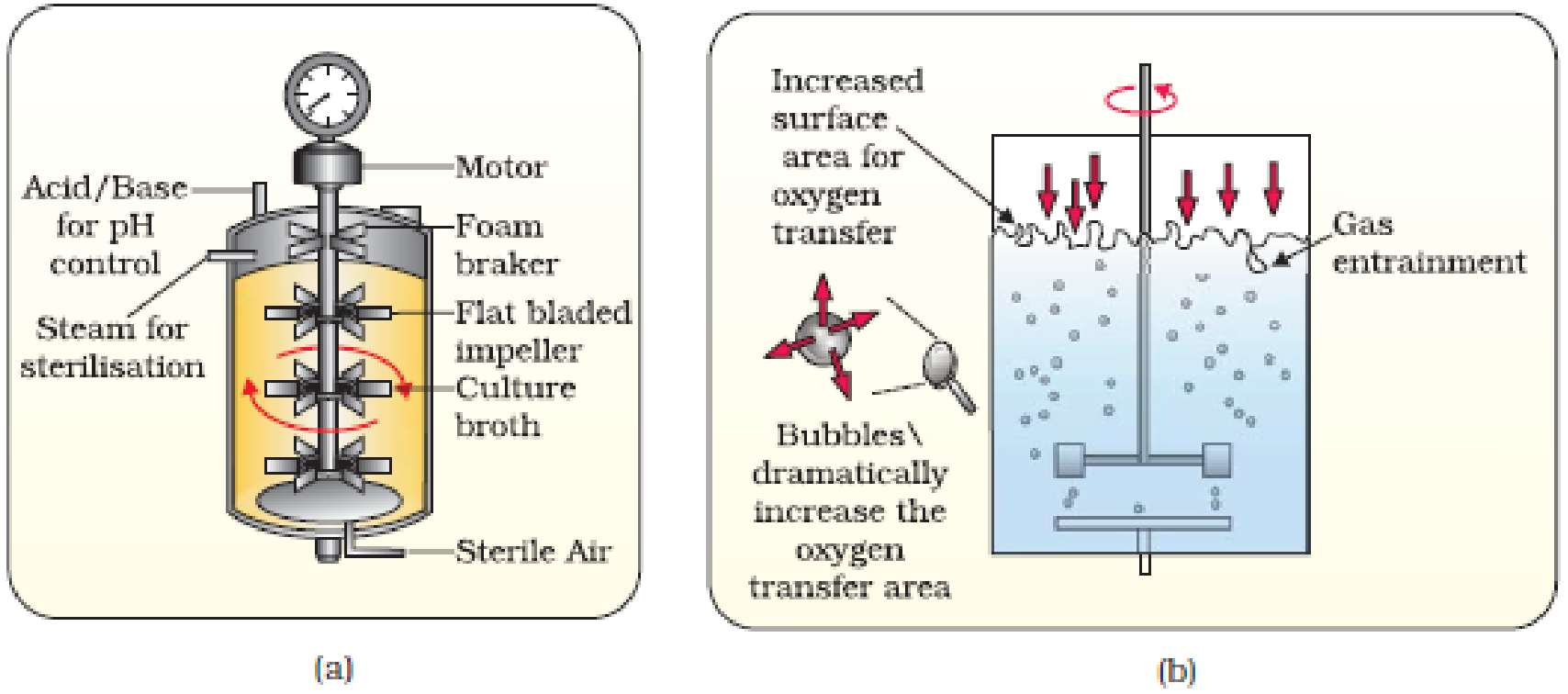
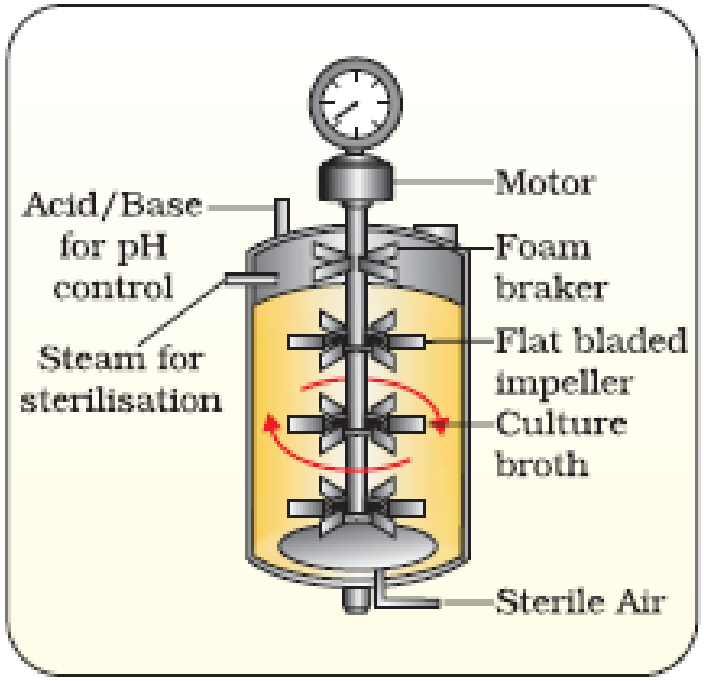


Figure 11.7 (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

B. John Ebenezer

Bioreactors



B. John Ebenezer

Downstream Processing

The processes of **separation** and **purification** of bio-product, are collectively referred to as downstream processing.

The product is **formulated** with suitable **preservatives.**

Such formulation has to undergo thorough **clinical trials** as in case of drugs.

Strict **quality control testing** for each product is also required.

The **downstream processing** and **quality control testing** vary from product to product.

B. John Ebenezer


God Bless You!