PRE-MEDICAL

ZOOLOGY

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STUDY MATERIAL

Biotechnology : Principles & Processes and Applications

ENGLISH MEDIUM

Copyright Statement

HERBERT BOYER (1936)

Herbert Boyer was born in 1936 and brought up in a corner of western Pennsylvania where railroads and mines were the destiny of most young men. He completed graduate work at the University of Pittsburgh, in 1963, followed by three years of post-graduate studies at Yale.

In 1966, Boyer took over assistant professorship at the University of California at San Francisco. By 1969, he performed studies on a couple of restriction enzymes of the E. coli bacterium with especially useful properties. Boyer observed that these enzymes have the capability of cutting DNA strands in a particular fashion, which left what has became known as 'sticky ends' on the strands. These clipped ends made pasting together pieces of DNA a precise exercise.

This discovery, in turn, led to a rich and rewarding conversation in Hawaii with a Stanford scientist named Stanley Cohen. Cohen had been studying small ringlets of DNA called plasmids and which float about freely in the cytoplasm of certain bacterial cells and replicate independently from the coding strand of DNA. Cohen had developed a method of removing these plasmids from the cell and then reinserting them in other cells. Combining this process with that of DNA splicing enabled Boyer and Cohen to recombine segments of DNA in desired configurations and insert the DNA in bacterial cells, which could then act as manufacturing plants for specific proteins. This breakthrough was the basis upon which the discipline of biotechnology was founded.

BIOTECHNOLOGY - PRINCIPLES & PROCESSES

01. INTRODUCTION

- **Introduction**
- **Principles of Biotechnology**
- **Tools of Recombinant DNA Technology**
- **Processes of Recombinant DNA Technology**

Biotechnology term given by **Karl Ereky**.

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

Types of Biotechnology :- Two types –

(1) OLD/TRADITIONAL

- Old biotechnology are based on the natural capabilities of micro organisms.
  e.g. formation of Citric acid, production of penicillin by *Penicillium notatum*, making curd, bread or wine, which are all microbe-mediated processes, a form of biotechnology.

(2) NEW/MODERN

- Based on Recombinant DNA technology.
  e.g. Human gene producing Insulin has been transferred and expressed in bacteria like *E.coli.*
- The European Federation of Biotechnology (EFB) has given a definition of biotechnology that encompasses both traditional view and modern molecular biotechnology. The definition given by EFB is as follows: **'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.**
- **Paul berg (Father of genetic engineering)**. He transferred gene of SV-40 virus (simian virus) in to *E.coli* with the help of λ – phage. (Nobel prize - 1980)
- **Stanley Cohen and Herbert Boyer :** First made recombinant DNA by linking an antibiotic resistance gene with a native plasmid of ***Salmonella typhimurium. (1972)***

02. PRINCIPLES OF BIOTECHNOLOGY

Among many, the two core techniques that enabled birth of modern biotechnology are :

(1) GENETIC ENGINEERING/RECOMBINANT DNA TECHNOLOGY

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

(2) BIOPROCESS ENGINEERING

Maintenance of sterile (microbial contamination-free) ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

The concept of genetic engineering was the outcome of two very significant discoveries made in bacterial research. These were–

- Presence of extrachromosomal DNA fragments called **plasmids** in the bacterial cell, which replicate independent of chromosomal DNA of the bacterium.
- Presence of enzymes **restriction endonucleases** which cut DNA at specific sites.
- These enzymes are, therefore, called **'molecular scissors'**.

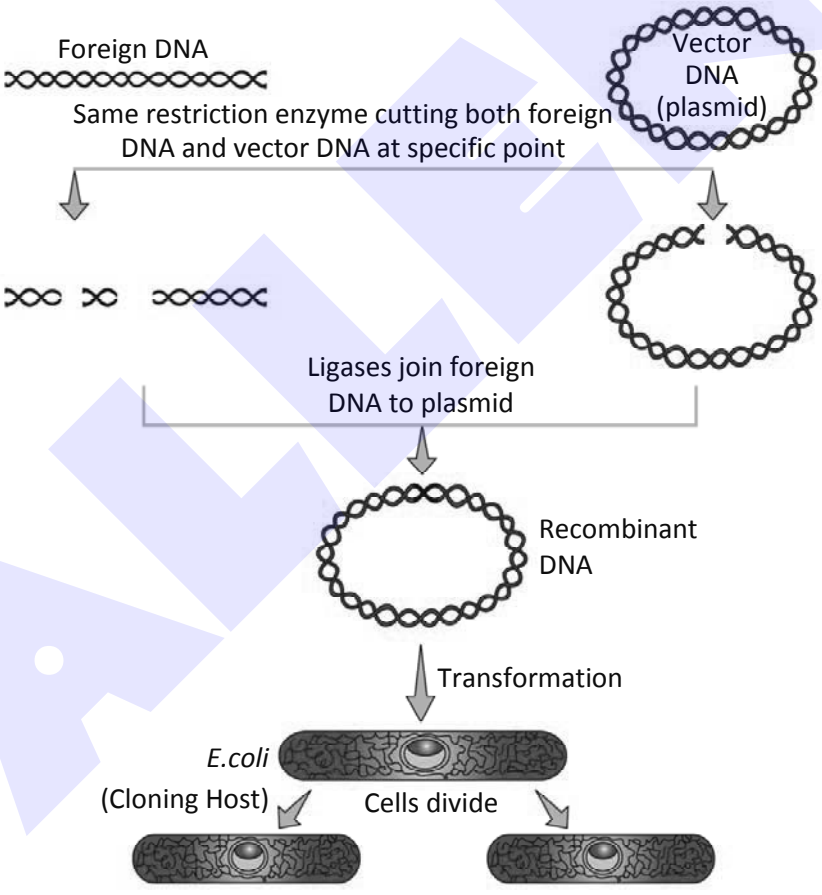
The main basis of Recombinant DNA Technology is DNA cloning :- It is making multiple identical copies of any template DNA.

Three basic steps in genetically modifying an organism (of DNA cloning) -

(a) identification of DNA with desirable genes;

(b) introduction of the identified DNA into the host;

(c) maintenance of introduced DNA in the host and transfer of the DNA to its progeny.



Diagrammatic representation of recombinant DNA technology

NCERT Page No. 197, Figure No. 11.2

03. TOOLS OF RECOMBINANT DNA TECHNOLOGY

- Genetic engineering involves cutting of desired segments of DNA and joining of this desired segments in a vector to produce a recombinant DNA (rDNA).
- The 'biological tools' used in the synthesis of recombinant DNA include **enzymes, vehicle or vector DNA, desired DNA** and **host cells**.

(1) ENZYMES

- A number of specific kinds of enzymes are employed in genetic engineering.
- These include lysing enzymes, cleaving enzymes, synthesising enzymes and joining enzymes.

(A) Lysing enzymes - These enzymes are used for opening the cells to get DNA for genetic experiment.

- Bacterial cell : is commonly digested with the help of **lysozyme**.
- Plant cell : is commonly digested with the help of **cellulase** and **pectinase**.
- Fungal cell : is commonly digested with the help of **chitinase**.

(B) Cleaving enzymes – These enzymes are used for cleave DNA molecules. Cleaving enzymes are of two types; exonuclease and endonuclease.

(i) Exonuclease remove nucleotides from the ends of the DNA.

(ii) Endonuclease make cuts at specific positions within the DNA.

eg. Restriction endonuclease

Restriction Endonuclease Enzymes (Molecular scissor or molecular knife) :-

- Restriction enzymes belong to a larger class of enzymes called endonuclease.
- Restriction enzymes are used in recombinant DNA technology because they can be used in vitro to recognise and cleave within specific DNA sequence (typically consisting of 4 to 8 nucleotides).
- This specific 4 to 8 nucleotide sequence is called **restriction site** and is usually **palindromic,** this means that the DNA sequence is the same when read in a 5'-3' direction on both DNA strand

$\longleftarrow\!\!\!\!\!\!\!\!\longrightarrow$

AND MADAM DNA

- Restriction enzymes are obtained from bacteria.

What is function of restiction enzymes in bacteria?

- They are useful to bacteria because the enzyme bring about fragmentation of viral DNA without affecting the bacterial genome. This is an adaptation against bacteriophages.
- These enzymes exist in many bacteria.
- Modification in the form of methylation, by methylation the bacterial DNA modifies and therefore protects it's own chromosomal DNA from cleavage by these restriction enzymes.
- The first restriction endonuclease–**Hind II**, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later. It was found that Hind II 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 for Hind II.
- Restriction enzyme (EcoRI) was discovered by Arber, Smith & Nathans.
- Besides Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

Nomenclature of enzyme –

- **The first letter** : indicates bacterial genus (In italic)
- **Second** and **third letter** : indicate speceis of bacteria (In italic)
- **Fourth letter** : indicates strain of bacteria (optional)
- **Roman numerical** : signifying the order in which the enzymes were isolated from that strain of bacteria.

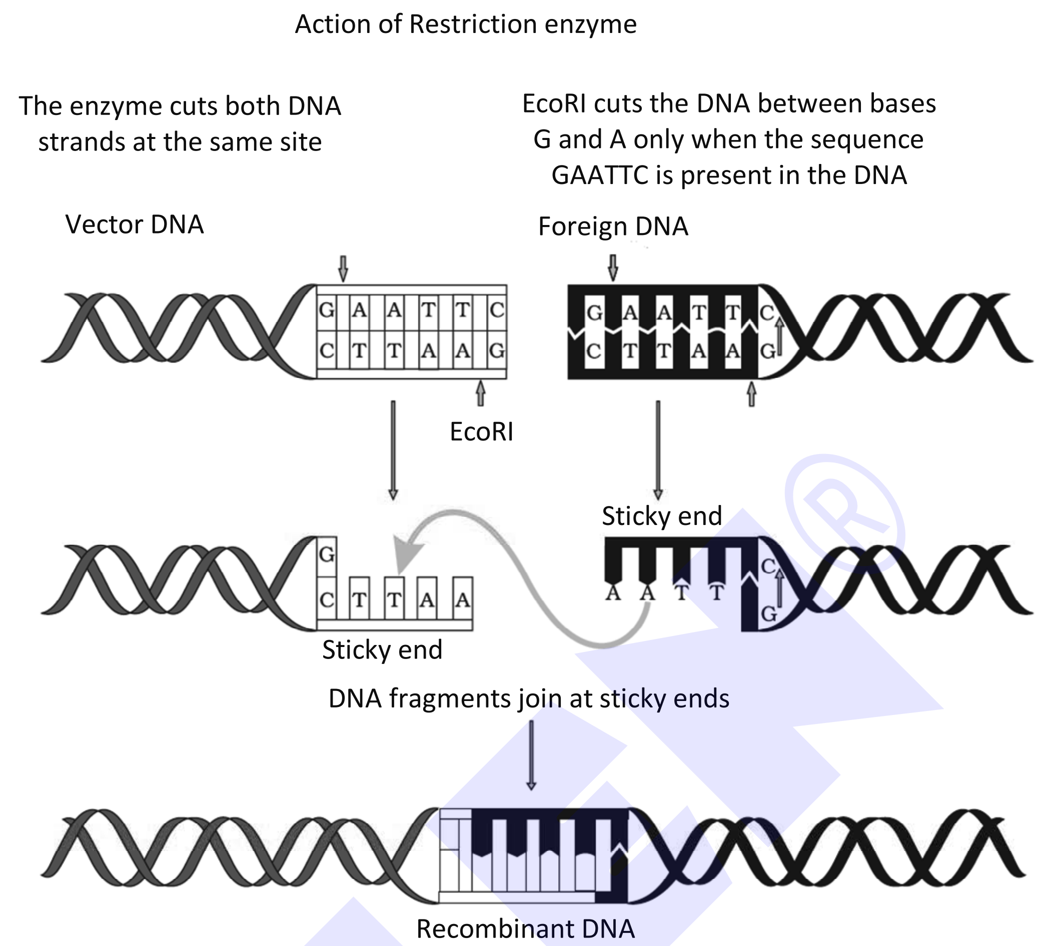
- EcoRI comes from *Escherichia* coli RY 13. In EcoRI, the letter 'R' is derived from the name of strain. Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

Restriction enzymes forms two types of ends on the basis of mode of cutting.

(a) Sticky end (Free end) :

- Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands.
- This leaves single stranded portions at the ends. There are overhanging stretches called sticky ends on each strand. These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

  eg. EcoRI, Hind III, Bam HI, Sal I



Steps in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

NCERT XII Page No. 196, Figure No. 11.1

(b) Blunt end (non sticky end) :

- Some enzymes cleave both strand of DNA at the same nucleotide position, typically in the centre of the recognition sequence resulting in blunt end or flush end.

  eg. Sma I, EcoRV, Hae III, Hind II

Sma I (*Serratia marcescens*)

$$\begin{array}{ccccc} \text{5' C C C}\downarrow\text{G G G 3'} & & \text{5' C C C 3'} & + & \text{5' G G G 3'} \\ & \Rightarrow & & & \\ \text{3' G G G}\uparrow\text{C C C 5'} & & \text{3' G G G 5'} & + & \text{3' C C C 3'} \end{array}$$

EXAMPLES OF RESTRICTION ENZYME

Recognition sequences of some restriction endonucleases

Name	Recognition sequence	End after cleavage		Source
Eco RI	– G A A T T C – – C T T A A G –	– G – C T T A A	A A T T C – G –	*Escherichia coli*
Hind III	– A A G C T T – – T T C G A A –	– A – T T C G A	A G C T T – A –	*Haemophilus influenzae*
Bam HI	– G G A T C C – – C C T A G G –	– G – C C T A G	G A T C C – G –	*Bacillus amyloliquefaciens*
Hae III	– G G C C – – C C G G –	– G G – C C	C C – G G –	*Haemophilus aegyptius*
EcoRV	– G A T A T C – – C T A T A G –	– G A T – C T A	A T C – T A G –	*Escherichia coli*
Hind II	– G T C G A C – – C A G C T G –	– G T C – C A G	G A C – C T G –	*Haemophilus influenzae*

(C) Synthesizing enzymes :

- These enzymes are used to synthesize new strands of DNA, complementary to existing DNA or RNA template. They are of two types; reverse transcriptases and DNA polymerases.
  - **(i)** **Reverse transcriptases** help in the synthesis of complementary DNA strands on RNA templates;
  - **(ii)** ***DNA polymerases*** help in the synthesis of complementary DNA strands on DNA templates.

(D) Joining enzymes :

- These enzymes help in joining the DNA fragments. For example **DNA ligase** is used to join DNA fragments. Joining enzymes are, therefore, called molecular glues.

(2) VEHICLE DNA OR VECTOR DNA

- The DNA used as a carrier for transferring a fragment of foreign DNA into a suitable host is called **vehicle or vector DNA.**
- Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

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

(A) Origin of Replication (ori) :

- This is a sequence from where replication starts and any piece of DNA when linked to the vector having this sequence (ori) 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.

(B) Selectable Marker :

- In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.
- Normally, the genes encoding resistance to antibiotics such as ampicilin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for *E. coli*.
- Enzyme forming gene also act as selectable marker gene. eg. Lac Z gene

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

(C) Restriction Sites/Cloning Sites :

- In order to link the alien DNA, the vector needs, **recognition sites** for the commonly used restriction enzymes. The ligation of alien DNA is carried out at a restriction site present in one of the two **antibiotic resistance** genes.
- Vector should have restriction site for many enzymes but only one restriction site for each enzyme, Otherwise vector will get fragmented.

Selection of vector depends on :-

(a) Size of desired gene

(b) Type of host

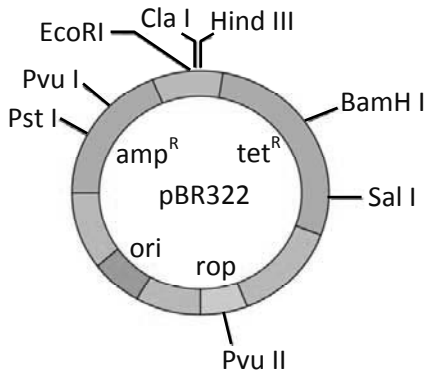
Some examples of vectors :-

(a) Plasmids

- They are double stranded, circular and extra chromosomal DNA segments found in bacteria which can replicate independently.
- Plasmids can be taken out of bacteria and made to combine with desired DNA segments by means of restriction enzymes and DNA ligase.
- A plasmid carrying DNA of another organism integrated with it, is known as **recombinant plasmid** or **hybrid plasmid** or **Chimeric plasmid**.

eg. **pBR 322**, **PUC 18** (used for gene transfer in bacteria)

Ti plasmid, **Ri** Plasmid (used for gene transfer in dicot plant)



E. coli cloning vector pBR322 showing restriction sites (Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes (amp^R and tet^R). Rop codes for the proteins involved in the replication of the plasmid.

NCERT XII Page No. 199, Figure No. 11.4

(b) Viruses

- The DNA of certain viruses is also suitable for use as a vehicle DNA.
- Bacteriophage DNA is also used as a vector DNA for gene transfer.

e.g. λ-Bacteriophage, **M-13**-Bacteriophage

Retro virus – useful for gene transfer in animal cell

	Vector type	**Insert size kb**
(1)	Plasmid	0.5 – 8
(2)	Bacteriophage lambda	9 – 23
(3)	Cosmid	30 – 45
(4)	BAC	50 – 300
(5)	YAC	1000 – 2500

(3) DESIRED DNA / ALIEN DNA / FOREIGN DNA / PASSENGER DNA

- It is the DNA which is transferred from one organism into another by combining it with the vehicle DNA. The passenger DNA can be complementary, synthetic.

(A) Complementary DNA (cDNA)-

- It is synthesized on *mRNA* template with the help of reverse transcriptase and necessary nucleotides.
- cDNA formed through reverse transcription is shorter than the actual or *in vivo* gene because of the absence of introns or non-coding regions.

(B) Synthetic DNA (sDNA)-

- It is artificially synthesized with the help of DNA polymerase.
- **Kornberg** (1961) synthesized first synthetic DNA.
- **Khorana** (1968) synthesized first artificial gene (DNA) without a template.

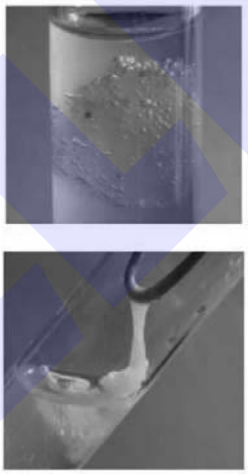
(4) HOST CELL

- This cell is used for DNA cloning.
- Host cell may be plant cell, animal cell, bacterial cell *(E.coli)*, fungal cell (Yeast).

04. PROCESSES OF RECOMBINANT DNA TECHNOLOGY

(1) ISOLATION OF DNA

- The DNAs which are to be used as passenger DNA and the vehicle DNA are extracted out of their cells by lysing the cells with the suitable enzyme.
- The DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus).
- You know that 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.
- DNA that separates out can be removed by spooling method.



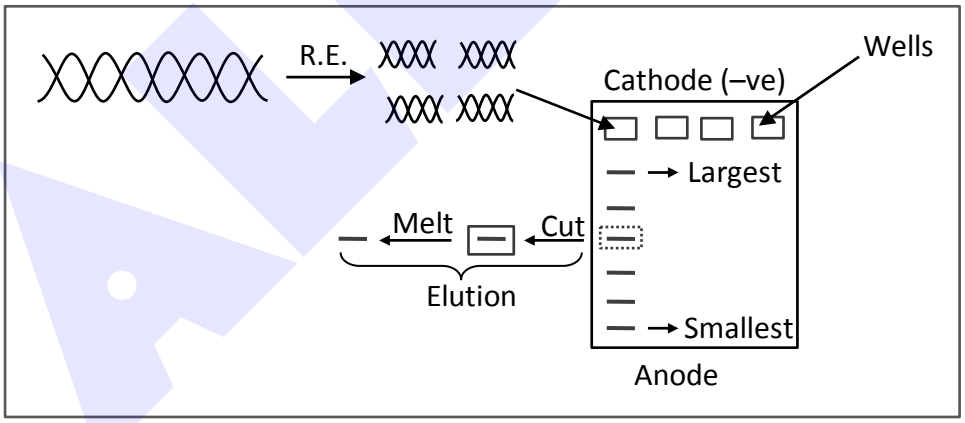
DNA that separates out can be removed by spooling
NCERT XII Page No. 201, Figure No. 11.5

(2) FRAGMENTATION OF DNA BY RESTRICTION ENDONUCLEASES

- 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.
- Both the passenger and vehicle DNAs are then, cleaved by using the same restriction endonuclease so that they have complementary sticky ends.

GEL ELECTROPHORESIS

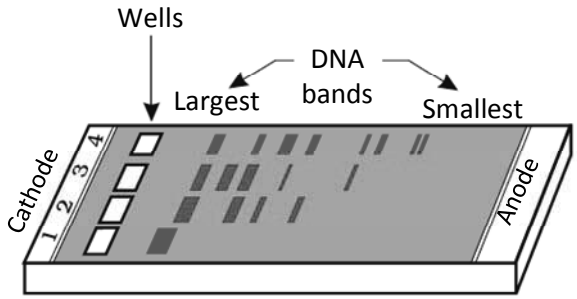
- Gel electrophoresis is a method that separates macromolecules either nucleic acid (DNA or RNA) or proteins-on the basis of size (Sieving effect).
- In this technique a gel (matrix) is required.
- Nowadays the most commonly used gel is agarose.
- Agarose is a natural polymer extracted from sea weeds.
- DNA is loaded on wells or cathode (negative electrode)
- Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode (positive electrode) under an electric field through a medium/matrix.
- 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** (you cannot see pure DNA fragments in the visible light and without staining).
- You can see **bright orange coloured bands** of DNA in a ethidium bromide stained gel exposed to UV light.



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

Application of gel electrophoresis:-

1. Separation of DNA Fragments
2. Checking of the progression of restriction digestion.



A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

NCERT XII Page No. 198, Figure No. 11.3

(3) AMPLIFICATION OF GENE OF INTEREST BY USING PCR

PCR (Polymerase Chain Reaction) :-

- Also known as Thermal Cycle
- Technique was invented by Kary Mullis in 1983.
- Use :- Amplification of DNA
- Based on :- in vitro DNA replication / in vitro DNA synthesis.

Requirements :-

(1) Eppendorf tube

(2) Sample DNA

(3) Two sets of DNA primer

(Small chemical synthesized oligonucleotides)

(4) All types of deoxyribonucleotides

(dATP, dGTP, dCTP, dTTP)

(5) PCR machine

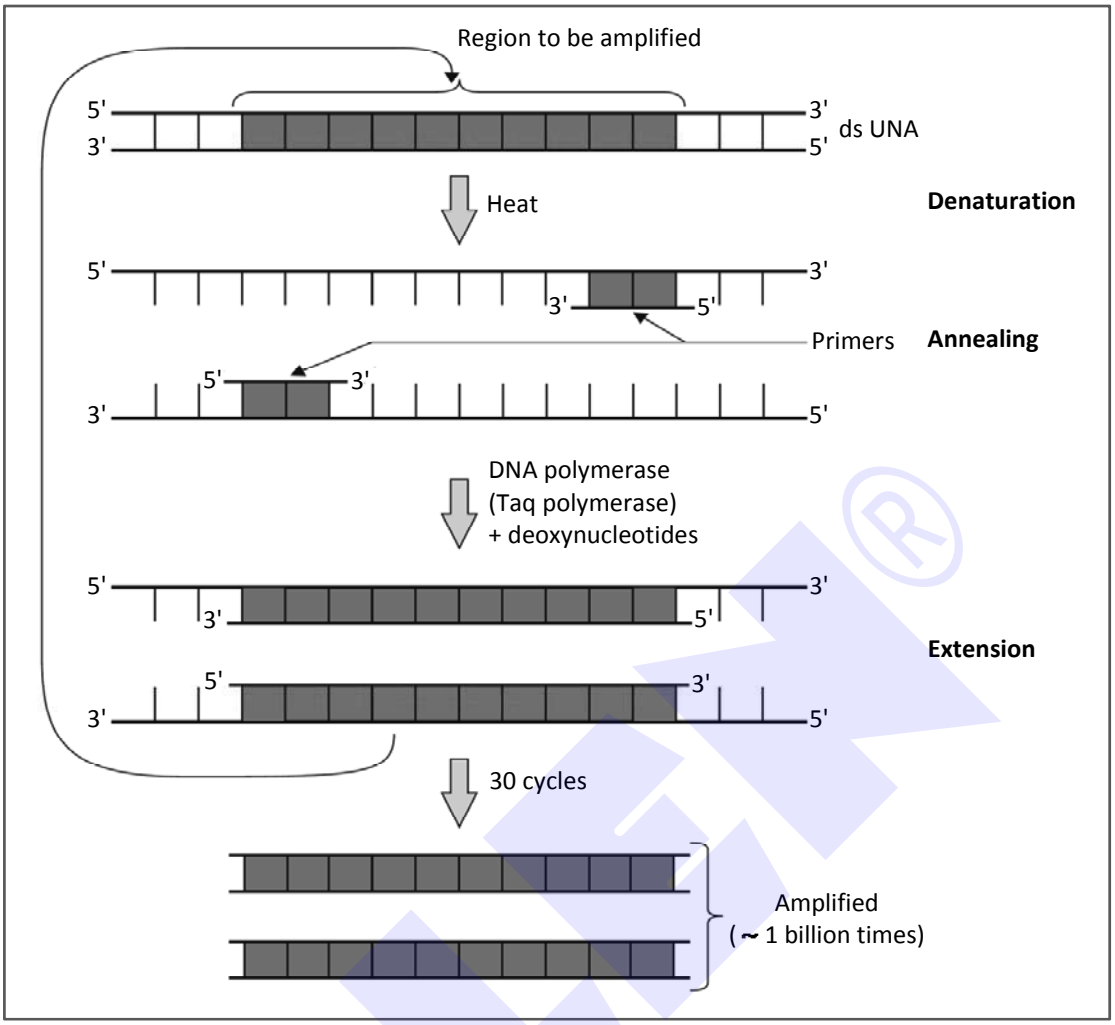
(6) A special type of DNA polymerase enzyme which is stable at high temperature (Thermo Stable Enzyme).

e.g.

I. Taq Polymerase : Isolated from *Thermus aquaticus* bacterium.

II. Pflu Polymerase : Isolated from *Pyrococus furiosus* bacterium.

III. Vent Polymerase: Isolated from *Thermococcus litoralis* bacterium.



3 Steps of PCR :-

(1) Denaturation (94°) :-

In this step a double stranded DNA becomes single stranded.

(2) Annealing/Cooling (54°) :-

In this step two DNA primers are attached at 3' end of single stranded DNA.

(3) Extension (72°) :-

In this process Taq polymerase enzyme synthesize new DNA strand.

The taq Polymerase 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.

After n cycle of PCR total number of DNA is = 2^n (n :- no. of Cycles)

(4) LIGATION OF THE DNA FRAGMENT INTO A VECTOR

The complementary sticky ends of the passenger and vehicle DNAs are joined with ligase enzyme. This gives rise to a recombinant DNA.

(5) TRANSFERRING THE RECOMBINANT DNA INTO THE HOST CELL (GENE TRANSFER)

Transfer of desired genes from one organism into another is an important aspect of genetic engineering.

Gene transfer is achieved by two kinds of transfer methods:

(A) Indirect method through vectors or carriers

(B) Direct or vector less transfer method.

(A) Indirect Method :-

(i) Gene Transfer in bacterial cell :

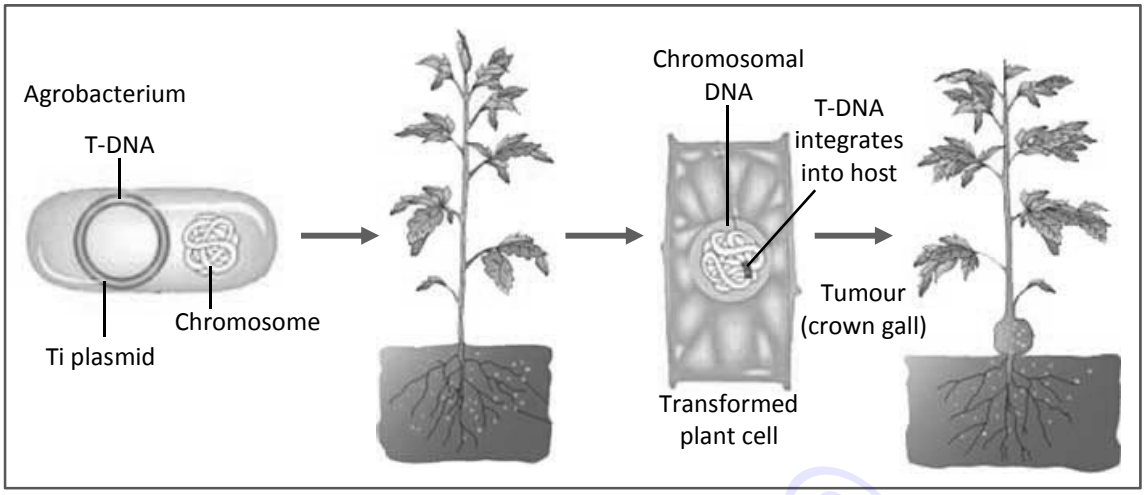
- Since DNA is a hydrophilic molecule, it cannot pass through cell membranes.
- In order to force bacteria to take up the plasmid, the bacterial cells must first be made '**competent**' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium, 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.

Note -

- The bacteria to be used as host cell should be without plasmids.
- The host cells are treated with calcium chloride.

(ii) Gene Transfer in plant cell :

- A plant pathogenic *bacterium-**Agrobacterium tumefaciens*** produces crown galls or plant tumours in almost all dicotyledonous plants.
- Tumour formation is induced by it's plasmid which is therefore called Ti plasmid (Ti = Tumour inducing)
- *Agrobacterium tumefaciens* naturally transfers some part of Ti-plasmid in to host plant DNA without any human effort so it is called **natural genetic engineer** of plant.
- In the transformation process two essential component in Ti-plasmid –
  - T-DNA – (Transfer DNA)
  - Vir-region – (Virulence region)
- Inside the host plant cell, T-DNA is separated from Ti-plasmid, and integrated into host plant DNA that causes crown gall tumour.
- Vir-region contains genes which are essential for T-DNA transfer and integration to host plant DNA.
- When we use Ti plasmid as a vector, first we remove the tumour causing gene (ti gene) from T-DNA region. Then desired gene is inserted in place of it. Now, this plasmid is called **disarmed plasmid**.
- Same as Ri plasmid of ***A.rhizogenes*** (causing hairy root disease) are also used as vector for gene transfer into plant cell.



(iii) Gene transfer in animal :

- Retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.

(B) Direct Method

Foreign genes can also be transferred directly by the following methods:

(i) Electroporation- It creates transients (temporary pores) in the plasma membrane to facilitate entry of foreign DNA.

(ii) Chemical mediated genetic transformation- It involves certain chemicals such as polyethylene glycol (PEG), that help in the uptake of foreign DNA into host cells.

(iii) Microinjection- It is the introduction of foreign genes mainly into animal cells using micropipettes or glass needles.

(iv) Particle gun/Biolistic method / Gene gun- It is a technique in which tungsten or gold particles coated with foreign DNA are bombarded into target cells to facilitate entry of the foreign genes.

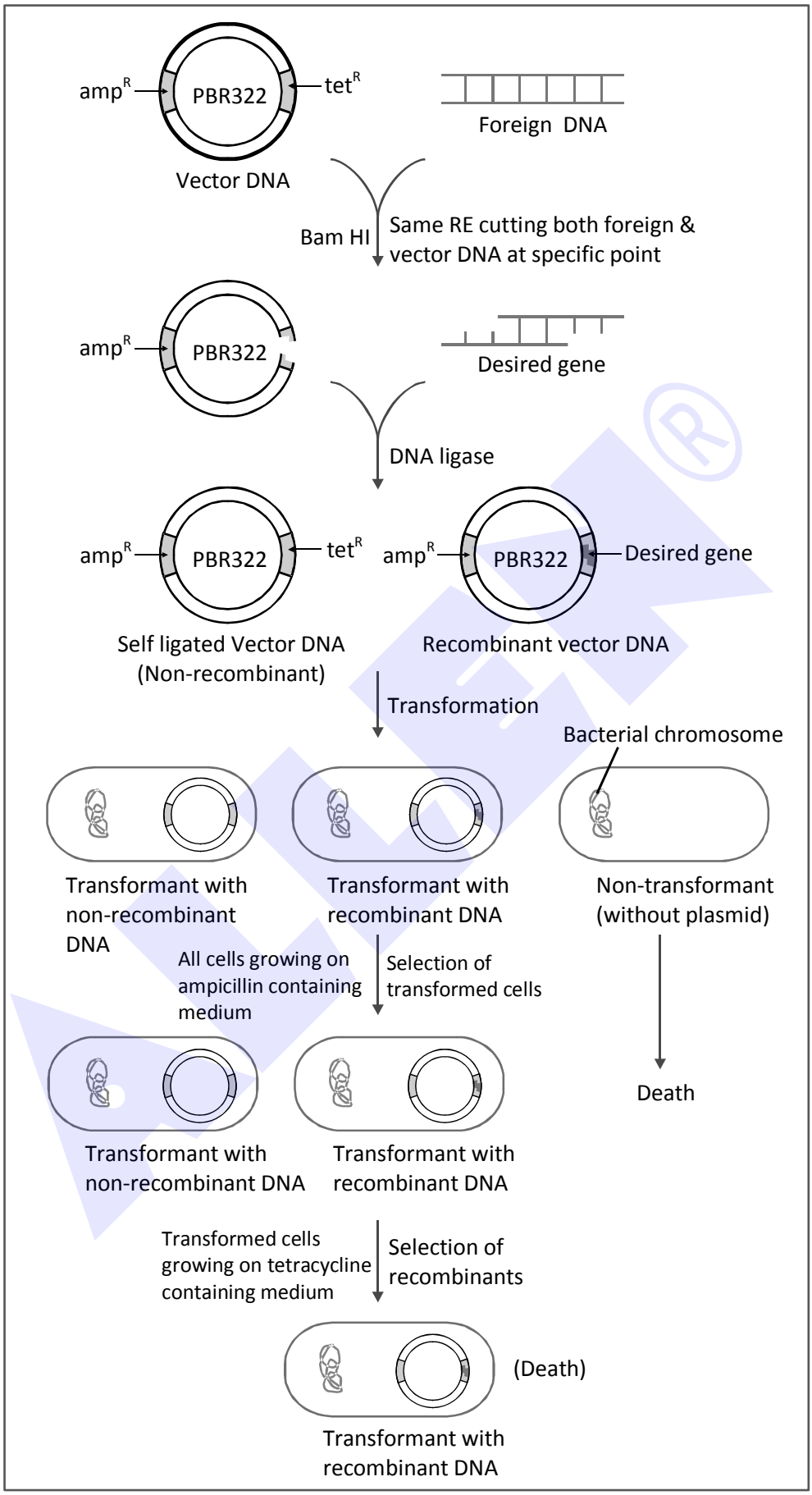
(v) Liposome mediated gene transfer- In this method, DNA is enclosed within lipid bags. These lipid bags are fused with protoplast.

(6) SELECTION OF TRANSFORMANT WITH RECOMBINANT DNA

(A) Selection by Two Antibiotic Resistance Gene

- You can ligate a foreign DNA at the BamH I site of tetracycline resistance gene in the vector pBR322. The recombinant plasmids will lose tetracycline resistance due to insertion of foreign DNA (insertional inactivation) now, it can be selected out from non-recombinant ones by following bases.
- The transformants (plasmid transfer) growing on ampicillin containing medium are then transferred on a medium containing tetracycline.
- 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.

Insertional inactivation : Due to insertion of desired gene within selectable marker gene of vector, selectable marker gene become inactive or loose their function. This is called Insertional inactivation.


amp^R
PBR322
tet^R
Foreign DNA
Vector DNA
Bam HI
Same RE cutting both foreign & vector DNA at specific point
amp^R
PBR322
Desired gene
DNA ligase
amp^R
PBR322
tet^R
amp^R
PBR322
Desired gene
Self ligated Vector DNA (Non-recombinant)
Recombinant vector DNA
Transformation
Bacterial chromosome
Transformant with non-recombinant DNA
Transformant with recombinant DNA
Non-transformant (without plasmid)
All cells growing on ampicillin containing medium
Selection of transformed cells
Death
Transformant with non-recombinant DNA
Transformant with recombinant DNA
Transformed cells growing on tetracycline containing medium
Selection of recombinants
(Death)
Transformant with recombinant DNA

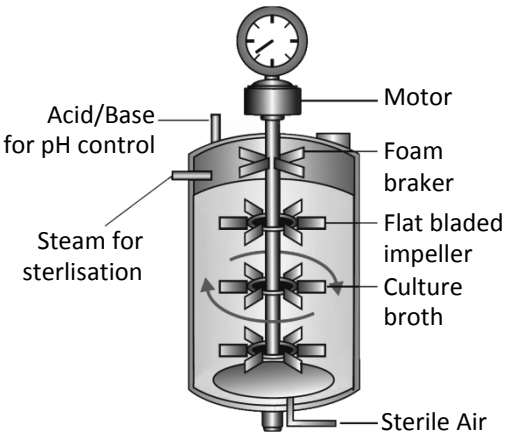
(B) Selection by one Lac Z gene and one antibiotic resistance gene

- Selection of recombinants due to inactivation of antibiotic resistance gene is a cumbersome (troublesome) procedure because it requires simultaneous plating on two plates having different antibiotics.
- Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate.
- In this, desired gene is inserted within the coding sequence of an enzyme, which is referred to as **insertional inactivation.** The presence of a chromogenic substrate X–gal gives blue coloured colonies if the plasmid in the bacteria does not have an insert. Presence of insert results into insertional inactivation of the β-galactosidase (reporter enzyme) and the colonies do not produce any colour, these are identified as recombinant colonies.

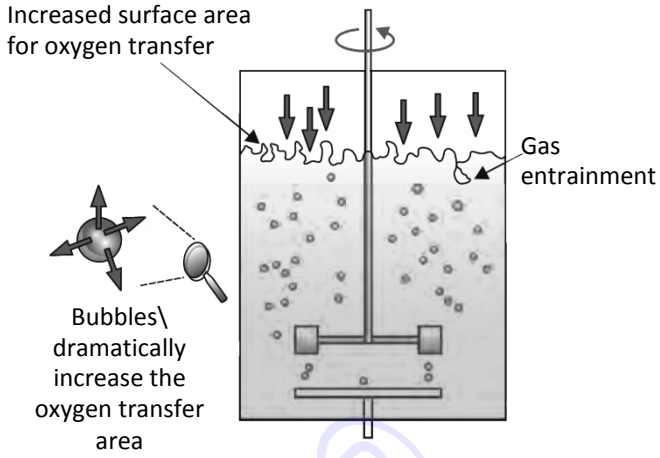
(7) OBTAINING THE FOREIGN GENE PRODUCT

- 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.
- After having cloned 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.
- The cells can also be multiplied in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain 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.
- Small volume cultures cannot yield appreciable quantities of products. To produce in large quantities, the development of **bioreactors,** where large volumes (100-1000 litres) of culture can be processed, was required. Thus, bioreactors can be thought of as **vessels in which 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).

The most commonly used bioreactors are of stirred type :-



(a) Simple stirred – tank bioreactor



(b) Sparged stirred – tank bioreactor

NCERT XII Page No. 204, Figure No. 11.7

- 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.
- **The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.**

(8) DOWNSTREAM PROCESSING

- After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product.
- The processes include **separation and purification**, which are collectively referred to as downstream processing.
- The product has to be formulated with suitable preservatives. Such formulation has to undergo through 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.

BEGINNER'S BOX — PRINCIPLE TO PROCESS OF RECOMBINANT DNA THECHNOLOGY

1. Transfer of any gene into a completely different organism can be done through
(1) Genetic engineering (2) Tissue culture
(3) Transformation (4) RNA interference

2. DNA probe is used in :-
(1) Gel electrophoresis (2) Northern blotting (3) DNA finger printing (4) Interferon synthesis

3. First artificial gene synthesized by khorana was a gene of
(1) Arginine (2) Lysine
(3) Alanine t-RNA of yeast (4) Valine t-RNA

4. pBR 322 is an artificial gene vector which does not have
(1) Ampicillin marker gene (2) Cos site
(3) Restriction site for cla I enzyme (4) Ori

5. The thermostable enzyme Taq & Pfu isolated from thermophilic bacteria are :-
(1) RNA polymerase (2) DNA primers (3) DNA polymerases (4) DNA ligase

- The two very significant discoveries made Biotechnology successful are :

  (i) Plasmid

  (ii) Restriction Endonuclease

Note : First discovered R.E. – Hind II

- During nomenclature of R.E., Roman numerical signifies the order in which the enzyme was isolated from the strain of bacteria.

Tools of Recombinant DNA Technology

- Enzyme
  - Lysing
    - Lysozyme
    - Cellulase & Pectinase
    - Chitinase
  - Cleaving
    - Exonuclease
    - Endonuclease
  - Synthesizing
    - Reverse transcriptase
    - DNA polymerase
  - Joining
    - DNA ligase
- Vechicle or Vector DNA
  - Plasmid
  - Bacteriophage
- Desired DNA
  - c-DNA Complementary DNA
  - s-DNA Synthetic DNA
- Host
  - Plant
  - Animal
  - Bacteria *(E.coli)*
  - Fungi (Yeast)

Gene Transfer Method

- Indirect
  - Vector or carrier mediated
    - (i) *Agrobacterium tumefaciens*
    - (ii) *Agrobacterium rhizogenes*

    } For plant
  - (iii) *Retro virus-for animal cell*
- Direct
  - Vector less
    - Electroporation
    - Chemical mediated
      - Polyethylene glycol (PEG)
    - Microinjection
      - Best for animal cell
    - Particle gun / Biolistic
      - For plant cell
    - Liposome mediated

BIOTECHNOLOGY AND ITS APPLICATION

01. INTRODUCTION

- **Introduction**
- **Biotechnological Applications in Agriculture**
- **Biotechnological Applications in Medicine**
- **Transgenic Animals**
- **Ethical Issues**

- Biotechnology, essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.
- The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production.
- Three critical research areas of biotechnology are:
  - Providing the best catalyst in the form of improved organism usually a microbe or pure enzyme.
  - Creating optimal conditions through engineering for a catalyst to act, and
  - Downstream processing technologies to purify the protein/organic compound.

02. BIOTECHNOLOGICAL APPLICATION IN AGRICULTURE

- Let us take a look at the three options that can be thought for increasing food production
  - agro-chemical based agriculture;
  - organic agriculture; and
  - genetically engineered crop-based agriculture.
- The **Green Revolution** succeeded in tripling the food supply but yet it was not enough to feed the growing human population.
- Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilisers and pesticides).
- However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding.
- Is there any alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields? Is there a way to minimise the use of fertilisers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution.
- Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms **(GMO)**.
- GM has been used to create **tailor-made plants** to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Use of genetically modified (GM) plant :-

(a) To enhance nutritional quality of food.

eg. Golden rice : Vitamin A enriched rice. (In this rice, gene of β-carotene is transferred)

(b) Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).

(c) Helped to reduce post harvest losses.

eg. Flavr Savr Tomato : Transgenic variety of **Tomato** –Pectin degrading enzyme polygalacturonase is inhibited in **Flavr Savr Tomato** So that tomato variety remains fresh and retain flavour much longer. Flavr Savr Tomato was developed by anti-sense technology.

(d) Increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

(e) To produce biopharmaceutical products.

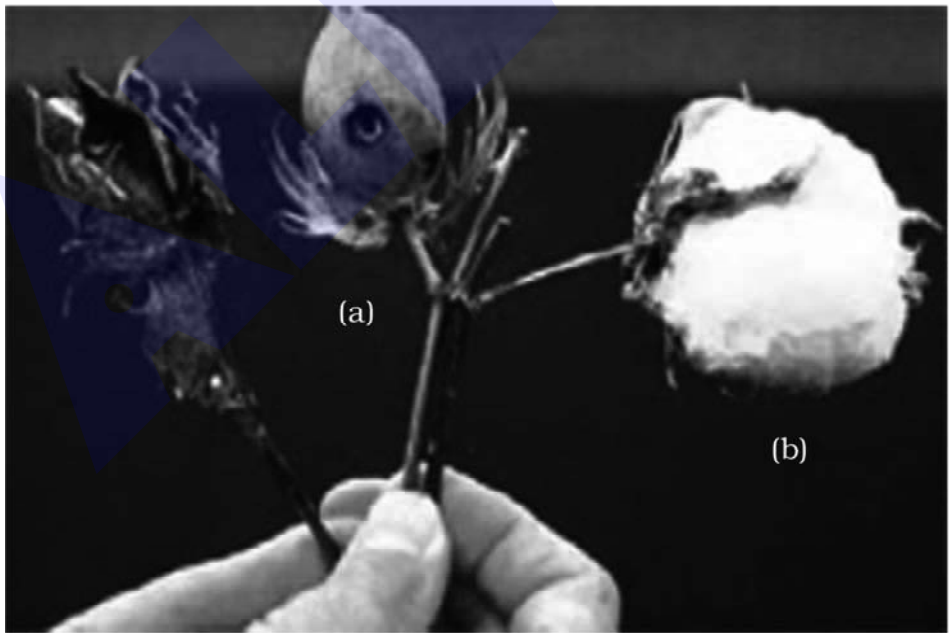
eg. Production of Hirudin : Hirudin is a protein that prevents blood clotting. The gene encoding hirudin was chemically synthesized and transferred into ***Brassica napus***, where hirudin accumulates in seeds. The hirudin is purified and used as medicine.

(f) To produce herbicide resistant plant.

eg. **First transgenic plant was tobacco.** It contains resistant gene against herbicide (Glyphosate).

(g) **Pest-resistant crops** : Reduces reliance on chemical pesticides.

(i) Insect resistant plant



Cotton boll : (a) destroyed by bollworms;
(b) a fully mature cotton boll

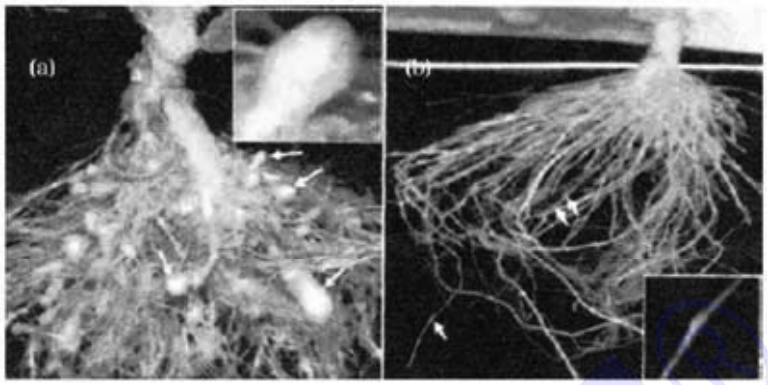
NCERT XII Page No. 209, Figure No. 12.1

- **Bt cotton** : Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as :
  (i) Lepidopterans (tobacco budworm, armyworm)
  (ii) Coleopterans (beetles)
  (iii) Dipterans (flies, mosquitoes)
- *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic **insecticidal protein** .
- The Bt toxin protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals.
- The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of the insect.
- ***Bacillus thuringiensis***, produces **crystal** [Cry] **protein**. This Cry protein is toxic to larvae of certain insects. Each Cry protein is toxic to a different group of insects.
- The gene encoding Cry protein is called "**cry gene**". This cry gene is isolated and transferred into several crops. A crop expressing a cry gene is usually resistant to the group of insects for which the concerned Cry protein is toxic.
- The choice of cry gene depends upon the :-
  (i) crop (ii) targeted pest
  *Most Bt toxins are insect – group specific.
- There are a number of them, for example, the **proteins encoded by the genes cryIAc and cryIIAb control the cotton bollworms**.
- **cryIAb controls corn borer.**

(ii) Nematode resistant plant :

- Several nematodes parasitise a wide variety of plants and animals including human beings.
- A nematode ***Meloidegyne incognitia*** infects the roots of tobacco plants and causes a great reduction in yield.
- A novel strategy was adopted to prevent this infestation which was based on the process of **RNA interference** (RNAi).
- RNAi takes place in all eukaryotic organisms as a method of cellular defense.
- This method involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing).
- The source of this complementary RNA could be from :-
  (i) an infection by viruses having RNA genomes or
  (ii) mobile genetic elements (transposons) that replicate via an RNA intermediate.
- Using Agrobacterium vectors, nematode-specific genes were introduced into the host plant.
- The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells.
- These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode.

- The consequence was that the parasite could not survive in a transgenic host expressing specific interferring RNA.
- The transgenic plant therefore got itself protected from the parasite.



Host plant-generated dsRNA triggers protection against nematode infestation : (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

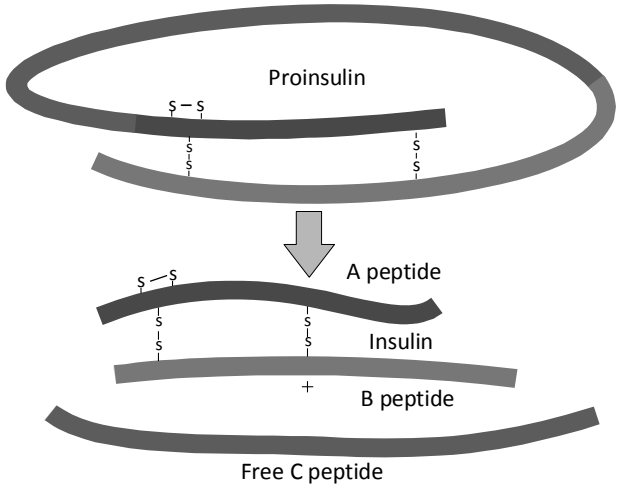
NCERT XII Page No. 210, Figure No. 12.2

03. BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

- The recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. Further, the recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.
- At present, about 30 recombinant therapeutics have been approved for human-use the world over.
- In India, 12 of these are presently being marketed.

(A) Genetically engineered insulin

- It is a proteinaceous hormone having 51 Amino acids arranged in two polypeptides A and B having 21 and 30 Amino Acids, respectively and joined by S-S disulphide bridges.
- **Sir Edward Albert Sharpey Schafer** (1916) was the first to note that diabetes of some persons was because of failure of some islands of pancreas to produce a substance which he called insulin (Derived from the latin word : insula meaning island).



Maturation of pro-insulin into insulin (simplified)

NCERT XII Page No. 211, Figure No. 12.3

- Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs.
- Insulin from an animal source, though caused some patients to develop allergy or other types of reactions to the foreign protein.
- Insulin consists of two short polypeptide chains: chain A and chain B, that are linked together by disulphide bridges.
- In mammals, including humans, insulin is synthesised as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the **C peptide**.
- This C peptide is not present in the mature insulin and is removed during maturation into insulin.

Insuline formed by r-DNA technique :-

- The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form. In 1983, Eli Lilly an American company prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains.
- Chains A and B were produced separately, extracted and combined by creating disulphide bonds to form human insulin.
- This insulin has been given the trade name **humulin** and has been approved for clinical use.

(B) Gene therapy :

- A new system of medicine gene therapy, may be used to treat some hereditary diseases such as SCID, haemophilia etc.
- Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo.
- Here genes are inserted into a person's cells and tissues to treat a disease.
- Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.
- **The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency.**
- This enzyme is crucial for the immune system to function. The disorder is caused due to the deletion of the gene for adenosine deaminase.
- In some children ADA deficiency can be cured by bone marrow transplantation; in others it can be treated by enzyme replacement therapy, in which functional ADA is given to the patient by injection. But the problem with both of these approaches is that they are not completely curative.
- As a first step towards gene therapy, lymphocytes from the blood of the patient are grown in a culture outside the body.
- A functional ADA cDNA (using a retroviral vector) is then introduced into these lymphocytes, which are subsequently returned to the patient.

- However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.
- However, if the gene isolated from marrow cells producing ADA is introduced into cells at early embryonic stages, it could be a permanent cure.

(C) Medical diagnosis of disease (Molecular diagnosis)

- You know that for effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible.
- Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

(i) PCR :-

- Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR.
- PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too.
- It is a powerful techqnique to identify many other genetic disorders.

(ii) Hybridisation using probe :-

- A single stranded DNA or RNA, tagged with a radioactive molecule (probe) is allowed to hybridise to its complementary DNA in a clone of cells followed by detection using autoradiography.
- The clone having the **mutated gene will hence not appear on the photographic** film, because the probe will not have complementarity with the mutated gene.

(iii) ELISA :-

- ELISA is based on the principle of antigen-antibody interaction.
- Infection by pathogen can be detected by the presence of antigens (proteins, glycoproteins, etc.) or by detecting the antibodies synthesised against the pathogen.

04. TRANSGENIC ANIMALS

- Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as **transgenic animals.**
- Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over **95 percent of all existing transgenic animals are mice.**

(i) Normal physiology and development :

- Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as **insulin-like growth factor**.
- By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(ii) Study of disease :

- Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease.
- These are specially made to serve as models for human diseases so that investigation of new treatments for diseases is made possible.
- Today transgenic models exist for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.

(iii) Biological products :

- Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make.
- Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as **human protein (α-1-antitrypsin) used to treat emphysema.** Similar attempts are being made for treatment of phenylketonuria (PKU) and cystic fibrosis.
- **In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.**

(iv) Vaccine safety :

- Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans.
- Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(v) Chemical safety testing :

- This is known as toxicity/safety testing. The procedure is the same as that used for testing toxicity of drugs.
- Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied.
- Toxicity testing in such animals will allow us to obtain results in less time.

Applications of Recombinant DNA products

Medically useful recombinant products	Applications
Human insulin	Treatment of insulin - dependent diabetes
Human growth hormone	Replacement of missing hormone in short stature people.
Calcitonin	Treatment of rickets.
Chorionic gonadotropin	Treatment of infertility.
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilla A/B.
Tissue Plasminogen Activator (TPA)	Dissolving of blood clots after heart attacks and strokes.
Erythropoetin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during dialysis or side effects of AIDS patients treated by drugs.
Platelet derived growth factor	Stimulation of wound healing
Interferon	Treatment of pathogenic viral infections, cancer
Interleukin	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertusis, meningitis, etc.

Application of Genetically Engineered Microbes

Microbes	Applications
Escherichia coli (gut bacterium)	Production of human insulin, human growth factor interferons, interleukin and so on.
Bacillus thuringiensis (soil bacterium)	Productions of endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide for plant protection.
Rhizobium meliloti (bacterium)	Nitrogen fixation by incorporating "nif" gene in cereal crops.
Pseudomonas putida (bacterium)	Scavenging of oil spills by digesting hydrocarbons of crude oil.
Bacterial strains capable of accumulating heavy metal	Bioremediation (cleaning of pollutants in the enviroment).
Trichoderma (fungus)	Production of enzyme chitinases for biocontrol of fungal diseases in plants.

Transgenics and their potential applications

Transgenic organism	Useful appications
Bt Cotton	Pest resistance and high yield.
Flavr Savr Tomato	Increased shelf-life (delayed ripening) and better nutrient quality
Golden Rice	Vitamin A and Fe – rich
Cattles (cow, sheep, goat)	Therapeutic human proteins in their milk
Pig	Organ transplantation without risk of rejection

05. ETHICAL ISSUES

- The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.
- Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have unpredicatable results when such organisms are introduced into the ecosystem.
- Therefore, **the Indian Government has set up organisations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.**
- The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.

06. BIO-PATENT

- A patent is a right granted by a government to an **inventor** to prevent others from commercial use of his invention. A patent is granted for –
  - (a) An invention [including product]
  - (b) An improvement in an earlier invention
  - (c) The process of generating products and
  - (d) A concept or design.
- There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.
- Rice is an important food grain, the presence of which goes back thousands of years in Asia's agricultural history. There are an estimated **200,000 varieties of rice** in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and **27 documented varieties of Basmati** are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries.
- **In 1997, an American company got patent rights on Basmati rice** through the **US Patent and Trademark Office**. This allowed the company to sell a 'new' variety of Basmati, in the US and abroad. This 'new' variety of Basmati had actually been derived from Indian farmer's varieties. **Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty.** The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent.
- Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, e.g., turmeric, neem. If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

07. BIO-PIRACY

- **Bio-piracy** is the term used to refer to the use of bio-resources by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.
- Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialisation.
- There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.
- The Indian Parliament has recently cleared the **second** amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.

BEGINNER'S BOX — BIOTECHNOLOGY APPLICATION TO BIO-PIRACY

1. Bt toxin kills the insect by

(1) Blocking the nerve conduction (2) Damaging the surface of trachea

(3) By creating pores in the tracheal system (4) By creating pores in the mid gut

2. Which is not an application of modern biotechnology?

(1) Production of humulin (2) Developing a DNA vaccine

(3) Gene therapy (4) Production of cheese and butter

3. Transgenic *Brassica napus* has been used for the synthesis of :-

(1) Hirudin (2) Heparin (3) Polgalacturonase (4) Cry protein

4. Transgenic tobacco plant was developed by the process of RNA interference, was resistant against the infection of :-

(1) Algae : *Scenedesmus* (2) Fungi : *Fusarium*

(3) Bacteria : *Bacillus thuringienesis* (4) Nematode : *Meloidegyne incognitia*

5. The first clinical gene therapy was given for treating

(1) Rheumatoid arthritis (2) Adenosine deaminase deficiency

(3) Diabetes (4) Chicken pox

Golden Key Points

- First transgenic animal was mouse containing gene for growth hormone. This enlarged mouse was known as **Supermouse**.
- First introduced transgenic crop in India (2002) is **Bt-cotton**.
- **Charles Weismann** of university of Zurich, obtained **interferon** through recombinant *E.coli* (1980)
- Microbes have been engineered to produce Human Growth Hormone (**HGH**) for curing dwarfism.
- **Vaccines** which are produced by genetic engineering e.g. for Hepatitis-B and Herpes virus.
- Nitrogen fixation genes may be transferred from bacteria to the major food crops to boost food production without using expensive fertilizers.
- **Bioremediation** : In pollution control, microbes have been engineered to break up the crude oil spills. **Dr. Ananda Mohan Chakrabarty** introduced plasmids from different strains into a single cell of ***Pseudomonas putida*.** The result was new genetically engineered bacterium which would clean the oil spills called **"Superbug"** (Oil eating bug). He transferred four types of genes/plasmids in this bacteria. These are OCT, XYL, CAM & NAH.
- **Genetic modified food** – The food prepared from genetically modified crop[transgenic] is called genetically modified food or **G.M. Food**.

BEGINNER'S BOX — ANSWERS KEY

PRINCIPLE TO PROCESS OF RECOMBINANT DNA THECHNOLOGY

Que.	1	2	3	4	5
Ans.	**1**	**3**	**3**	**2**	**3**

BIOTECHNOLOGY APPLICATION TO BIO-PIRACY

Que.	1	2	3	4	5
Ans.	**4**	**4**	**1**	**4**	**2**

Applications of Biotechnology

- **Pest Resistant Plants**
  - Insect resistance plant
    - CryIAc and CryIIAb control cotton bollworms
    - CryIAb Control corn borer
  - Nematode resistance plant
    - RNA interference (RNAi)
    - (i) Source → Two copy of a gene
    - (ii) Vector → Agrobacterium
- **Medical**
  - Genetically Engineered insulin e.g. Humulin
  - Gene Therapy e.g. ADA gene (c DNA) introduced into lymphocyte using retrovirus
  - Molecular diagnosis e.g. PCR, ELISA, RDT
- **GM Crop**
  - Enhance nutritional quality of food e.g. Golden Rice
  - Reduce post harvest losses e.g. Flavr-Savr Tomato
  - Produce biopharmaceutical products e.g. Hirudin
  - Herbicide Resistance plant
    e.g. Resistance gene against glyphosate in tobacco

Transgenic Animals

Study of Diseases
- Cancer
- Cystic fibrosis
- Rheumatoid arthritis
- Alzheimer

Vaccine Safety
- Transgenic mice are being developed for use in testing the safety of vaccines (such as-polio vaccine)

Biological Product
- α-1 antitrypsin used to treat Emphysema
- Rosie, produced human protein (alpha-lactalbumin) enriched milk