# BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

- It is a technique using live organisms or their cellular components or enzymes to produce useful products and processes for man.
- According to **European Federation of Biotechnology (EFB),** "Biotechnology is the integration of natural science and organisms, cells, parts there of and molecular analogues for products and services."
- The term biotechnology coined by **Karl Ereky (1917).**
- Development of biotechnology can be studied under two phases.
  - **(i) Old or Traditional Biotechnology:** It is also known as conventional technology that has been used for many centuries. Curd, bread, wine, ghee, and other alcoholic beverages, idli, dosa, cheese, paneer have been produced using traditional biotechnology.
  - **(ii) Modern Biotechnology:** It is new branch developed during 1970. It involves development of highly new and useful traits in crop varieties and animal breeds by the use of genetic engineering **e.g. In vitro fertilization** leading to a **'Test tube baby'** synthesining a gene and using it, developing a DNA vaccine or correcting a defective gene are all parts of modern biotechnology.

**Principles of Biotechnology:**

- **(i) Genetic engineering:** In this Technique, chemistry of genetic material (DNA & RNA) is altered and introduced these into host organisms and thus alter the phenotype of the host organism is called **genetic engineering (Recombinant DNA technology)**.
- **(ii) Maintenance of Microbial contamination free (sterile) surrounding in chemical engineering:** It helps in the growth of only the desired micro-organism/eukaryotic in large quantities for the manufacture of biotechnological products such as antibiotics, vaccines, enzymes, medicines, hormones etc.

## Conceptual development of the Genetic Engineering Principles:

- Sexual reproduction promotes variations while asexual reproduction preserves genetic information. The former is more advance then latter.
- Traditional hybridisation procedures cause inclusion and multiplication of undesirable genes along with the desired genes.
- The technique of genetic engineering involves formation of **recombinant DNA (rDNA)**, use of gene cloning and gene transfer. It examines this limitation and permits to isolate only one or a set of desirable genes without introducing undesirable genes into the target organism.
- A piece of DNA is incorporated into the genetic material of the recipient where it may multiply and be inherited alongwith the host DNA. Thus alien DNA is linked with the **'Ori'** site or origin of replication (It is a site of initiation of replication) can replicate and multiply itself in the host organism that is called **cloning**. Thus **genetic engineering is alternately called recombinant DNA technology or gene cloning**. It is helpful in forming multiple identical copies of any template DNA.
- **Stanley Cohen and Herbert Boyer (1972)** firstly constructed **recombinant DNA**. They isolated piece of DNA from a plasmid carrying **antibiotic-resistance gene** of the bacterium ***Salmonella typhimurium*** and fused it to the **plasmid of *E. coli.*** The plasmid is used as a vector to carry an alien piece of DNA into the host organism.The linking of these two is performed by **DNA ligase** resulting recombinant DNA is created in vitro. Now it is transferred into *E.coli* where it can replicate in the presence of the new host's **DNA polymerase enzyme** and form multiple copies that represent gene cloning.

- **Genetically modified organism (GMO) or Transgenic organism** can be developed by the following three basic steps.
- Identification of DNA with desirable genes.
- Introduction of the identified DNA into the host.
- Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

**Tools of Recombinant DNA Technology:**

**(1) Enzymes** **(2) Cloning Vectors** **(3) Competent host**

**(1) Enzymes:** Variety of specific enzymes are employed in genetic engineering.

**(i) Restriction Enzymes:** They break DNA molecules. They are of three types.

**(a) Exonucleases:** They separate nucleotides from the terminal ends of DNA in one strand of duplex.
**(b) Endonucleases:** They produce cuts within the DNA.

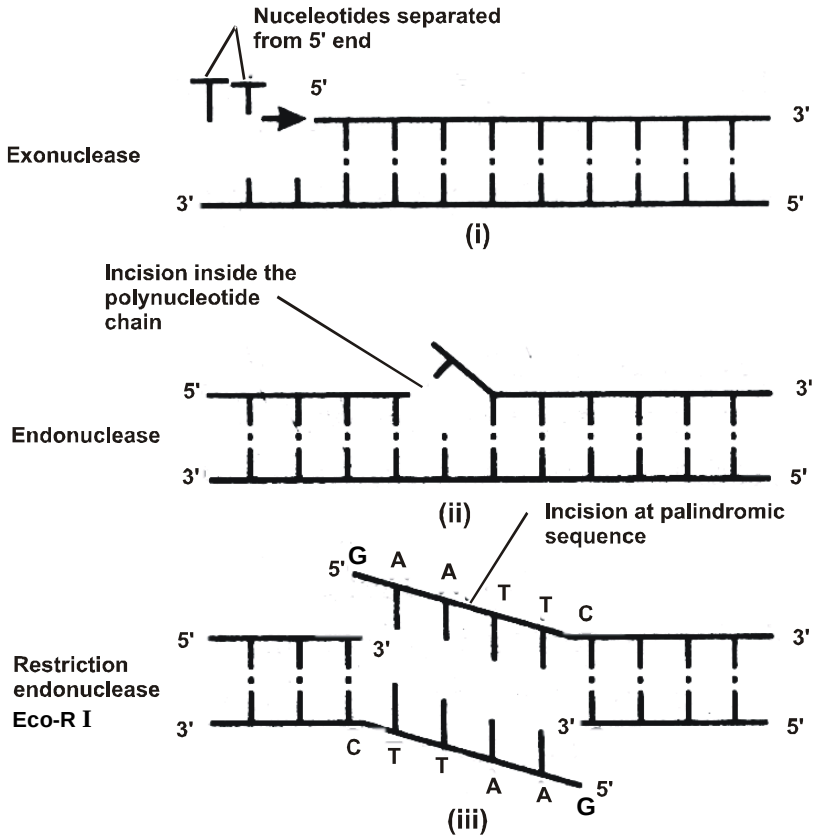



**Fig:- (i), Action of exonuclease (ii), Action of endo nuclease (iii), Action of restriction enzyme.**

**(c) Restriction endonucleases :**

- They cut the DNA strands at specific base sequence in palindrome site.
- They function as **'molecular scissors' or chemical scalpels**.
- **W. Arber, H. Smith and D. Nathans** discovered these enzymes.
- The first restriction endonuclease was **Hind II** isolated from ***Haemophilus influenzae Rd***. It was found that Hind II always cut DNA molecules at a particular point by recognising a sequence of Hind II. It produces blunt ends.

- The convention for naming these enzymes is the first letter of the name comes from the bacterium's genus name and the second two letters come from the species of the prokaryotic cell from which they were isolated, **e.g. 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**.
- The restriction endonuclease inspects the length of a DNA sequence. Once it recognises specific sequence, it binds to the DNA and cuts each of the two strands of the double helix at specific points in their sugar phosphate back bones. Special sequence in the DNA recognised by restriction endonuclease is called palindromic nucleotide sequence.
- The palindromes are groups of letters that form the same words when read in both directions forward and backward. **e.g.**

MALAYALAM

The palindromes in DNA are base pair sequences that are the same when read forward (left to right) or backward (right to left) from a central axis of symmetry. For **e.g.** the following sequences read the same on the two strands in 5'→3' direction. This is also true when we read in the 3' → 5' direction.

5' — G A A T T C — 3'
3' — C T T A A G — 5'

**Fig : Palindromic sequence**

- Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites but between the same two bases of the opposite strands. This leaves single stranded unpaired bases at cut ends. These ends with unpaired bases are called sticky ends or cohesive ends. The latter are named so because they form hydrogen bonds with their complementary cut counter parts. The sticky ends facilitate the action of the enzyme DNA ligase.

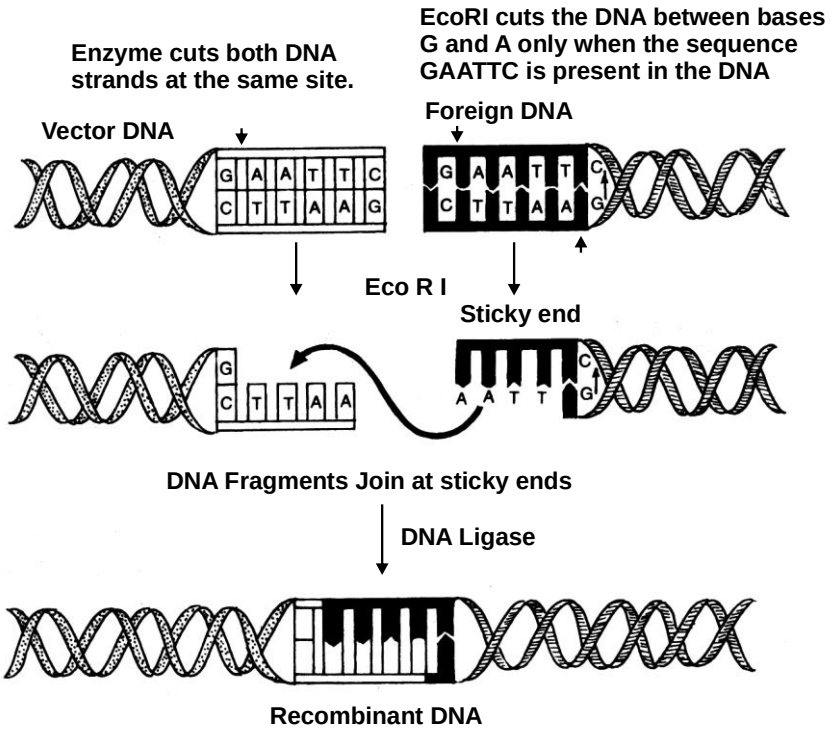



**Fig:- Formation of recombinant DNA by action of restriction endonuclease enzyme- Eco RI**

| Some restriction enzymes, type II, their source, recognition sequence and site of cleavage. | | | |
|---|---|---|---|
| **S.No.** | **Restriction Enzyme** | **Source** | **Recognition sequence and site of Cleavage** |
| 1. | Eco R I | *Escherichia coli RY* 13 | 5'-G↓A-A-T-T-C-3'<br>3'-C-T-T-A-A↑G-5 |
| 2. | Hin d II | *Haemophilus influenzae Rd* | 5'G-T-C↓G-A-C-3'<br>3'-C-A-G↑C-T-G-5' |
| 3. | Hin d III | *Haemophilus influenzae Rd* | 5'-A↓A-G-C-T-T3'<br>3'-T-T-C-G-A↑A-5' |
| 4. | Bam H I | *Bacillus amyloliquefaciens H* | 5'-G↓G-A-T-C-C-5'<br>3'-C-C-T-A-G↑G-5' |
| 5. | Sal I | *Streptomyces albus* | 5'-G↓T-C-G-A-C-3'<br>3'-C-A-G-C-T↑G-5' |
| 6. | Sma I | *Serratia marcescens* | 5'-C-C-C↓G-G-G-3'<br>3'-G-G-G↑C-C-C-5' |
| 7. | Alu I | *Arthrobacter luteus* | 5'-A-G↓C-T-3'<br>3'-T-C↑G-A-5' |
| 8. | Eco R II | *Escherichia Coli* R245 | 5'↓C-C-T-G-G-3'<br>3'-G-G-A-C-C↑5' |
| 9. | Hae III | *Haemophilus aegyptius* | 5'-G-G↓C-C-3'<br>3'-C-C↑G-G-5' |
| 10. | Sca I | *Streptomyces caespitosus* | 5'-A-G-T↓A-C-T-3'<br>3-T-C-A↑T-G-A-5' |

## Resonate the Concept

**(I) Type I Enzymes :**

Cleave at site remote from recognition site.

Requires both ATP and S-adenosyl methionine to function.

Multifunctional protein with both restriction and methylase activities.

**(II) Type II enzymes :**

Cleave within or at short specific distances from recognition site.

Most require magnesium.

Single functional enzymes independent of methylase.

**(III) Type III enzymes :**

Cleaves at sites a short distance from recogenition site.

Required ATP (but do not hydrolyse it)

S-adenosyl methionine is not required for the function of enzyme, it is only a part of a complex with a methylase.

**Why type II restriction endonuclease are used in genetic engineering?**

Type II restriction endonucleases always cleave at or near their recognition sites. They produce small, well-defined fragments of DNA that help to characterize genes and genomes and that produce recombinant DNAs.

**(ii) DNA Ligases (Molecular gum):** They form **phosphodiester bonds** between adjacent nucleosides and covalently link two individual fragments of double-stranded DNA. They help in sealing gaps in DNA fragments. Thus they act as a **molecular glue**. **$T_4$ DNA ligase** is mostly utilized in the **rDNA technology**.

**(iii) Alkaline Phosphatase:** It is helpful to remove the phosphate group from the 5' end of DNA molecule, leaving a free 5' hydroxyl group. It can be isolated from calf intestine or bacteria. It is used to prevent unwanted self ligation of vector DNA molecules in process of rDNA technology.

(**iv**) **Reverse Transcriptase**: it is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription.it is mainly associated wth retrovirus.

**(v) DNA Polymerase:** It is employed to polymerize replication of DNA on DNA template or complementary DNA (cDNA). It catalyses in 5'→3' and 3'→5' exonucleolytic degradation of DNA. DNA polymerase firstly investigated by **A. Kornberg in *E.coli*** and is now known as **DNA polymerase I**. other two enzymes are **DNA polymerase II and DNA polymerase III**. The latter is more active than Polymerase II & I. It produces a parallel strand in the presence of ATP on DNA template.

**(vi) Lysing enzymes:** These enzymes are used to isolate the DNA from the cell in genetic engineering. There are three types of lysing enzymes-

i. **Cellulase -** are the enzymes that hydrolyze β-1,4 glycosidic linkages in cellulose chains.
ii. **Chitinase -** chitin is an abundant biopolymer that is relatively resistant to degradation. Chitinase are hydrolytic enzymes that break down glycosidic bonds in chitin.
iii. **Lysozyme -** also called as glycosidase, is the enzyme responsible for cleaving the bond between N-acetyl muramic acid and N-acetyl glucosamine.

**(2) Cloning Vectors:**

- Vector DNA is able to carry a foreign DNA segment and replicate inside the host cell.

**Characteristics of a Cloning vector:** The following are the features that are required to facilitate cloning into a vector.

**(i) Origin of replication (ori):** It has specific sequence from where replication starts if any piece of foreign DNA is linked to this sequence.

**(ii) Selectable marker:** The vector also requires to identify and eliminate non-transformants and selectively permit the growth of the transformants. Genes encoding resistance to antibiotics such as Tetracycline, ampicillin, kanamycin or chloramphenicol are useful in selectable markers for *E.coli*.

**(iii) Recognition Sites (Cloning sites):**

- Vector bears one unique restriction endonuclease recognition site which enables foreign DNA to be inserted into the vector during the formation of recombinant DNA molecule.
- Most of the commonly used vectors contain unique recognition sites for several restriction enzymes in a small region of DNA which is referred to as a polylinker or multiple cloning site (MCS). A polylinker provides flexibility in the choice of restriction enzyme (s) that can be used for cloning.

**Plasmids, Bacteriophage, Cosmids, Phagemids, Yeast artificial chromosomes (YACs), Bacterial artificial chromosomes (BACs), Transposons etc**. are used as vectors in rDNA technology. Out of them plasmid and bacteriophage vectors are commonly used.

**(i) Plasmid:**

- It is extra chromosomal, self–replicating, usually circular, double -stranded DNA molecules, found in many bacteria and some yeast. They may occur as one or two copies or in multiple copies (500–700) inside the host organism. The ideal plasmid vector is pBR322.

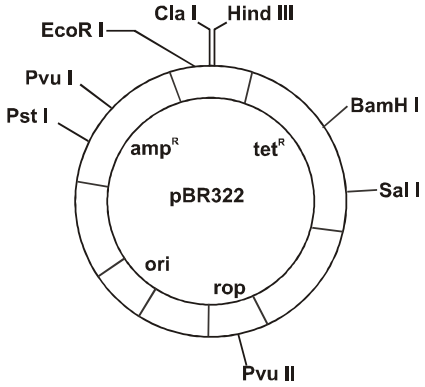
In pBR322
p- plasmid
BR- stands for Bolivar and Rodriguez (who constructed this plasmid)
322 - is a number given to distinguish this plasmid from other developed in the same laboratory.

**Structure of pBR 322:** Structurally it has following regions.

**(a) Origin of replication or (*Ori* ) :** It is a site of replication. It proceeds production of multiple copies per cell.

**(b) Antibiotic resistance genes:** amp$^r$ gene (ampicillin resistance gene) and tet$^r$ (tetracycline resistance gene) gene.

**(c) Unique recognition sites for restriction endonucleases:** It has a variety of unique recognition sites for restriction endonucleases. Two unique sites, Pst I and Pvu I are located within the amp$^r$ gene and Bam HI, sal I are within tet$^r$ gene. Some other unique restriction sites are Eco RI, Cla I, Hind III, Pvu II, rop codes for the proteins involved in the replication of the plasmid.




- Plasmid vector can clone only a small fragment of DNA (5 – 10 kb).

**Selection using Antibiotic resistance:** The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. For example, 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 but can still be selected out from non-recombinant ones by plating the transformant on ampicillin-containing medium.

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

**Disadvantage of pBR322:** Selection of recombinants due to inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics.

Overcoming the disadvantage of pBR322 by using another plasmid pUC8. **(p-plasmid, UC-University of California, 8 -** is a number given to distinguish this plasmid from other developed in the same laboratory.**)**

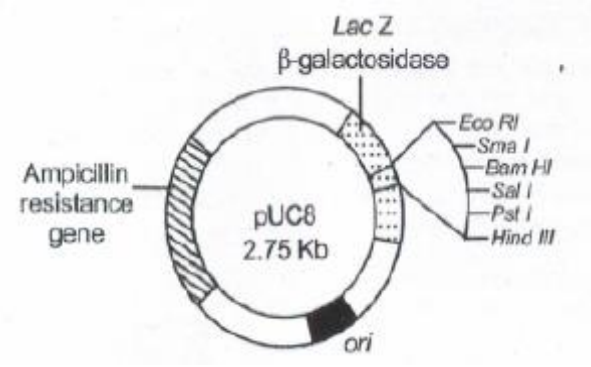



**Fig. : Structure of pUC8**

## Advantage :

**(1)** Higher copy number (500-700).

**(2)** Identification of recombinant cells achieved by a single step i.e., plating cells onto agar medium containing ampicillin and X-gal (chromogenic substrate for β-galactosidase enzyme encoded by lac Z).

**Colour Reaction (Blue/white selection) :** Alternative selectable maker in pUC8 plasmid differentiates recombinants from non-recombinants on the basis of their **ability to produce colour** in the presence of a chromogenic substrate **X-gal (5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside)**. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. This results into inactivation of the enzyme, which is referred to as insertional inactivation. The presence of a chromogenic substrate 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 and the colonies do not produce any colour, these are identified as recombinant colonies.

**(ii) Bacteriophage:** These viruses infect bacterial cells by injecting their DNA into these cells. The DNA of phage undergoes replication by the use of bacterial machinary and forms number of phages which burst out of the cell and reinfect neighbouring cells.

Several bacteriophages are being used as cloning vectors but most commonly used are

**(i) Lambda (λ) phage vector:** This vector allows cloning of DNA fraqments upto 23 kb length (1 kilobase = 1000 nucleotide long base sequence). The sequence that is cut to produce the cohesive, single stranded extensions located at the ends of the linear DNA molecules of certain phages is named as cos sites having 12 unpaired complementry bases.

**(ii) M13 phage vector:** It is filamentous phage which infects: *E.coli*. Foreign DNA can be inserted into it without disrupting any of essential genes.

**(iii) Cosmid:** It is constructed by combining certain features of plasmid and the 'cos' sites of phage lambda. It can be used to clone DNA fragments upto 45 kb in length.

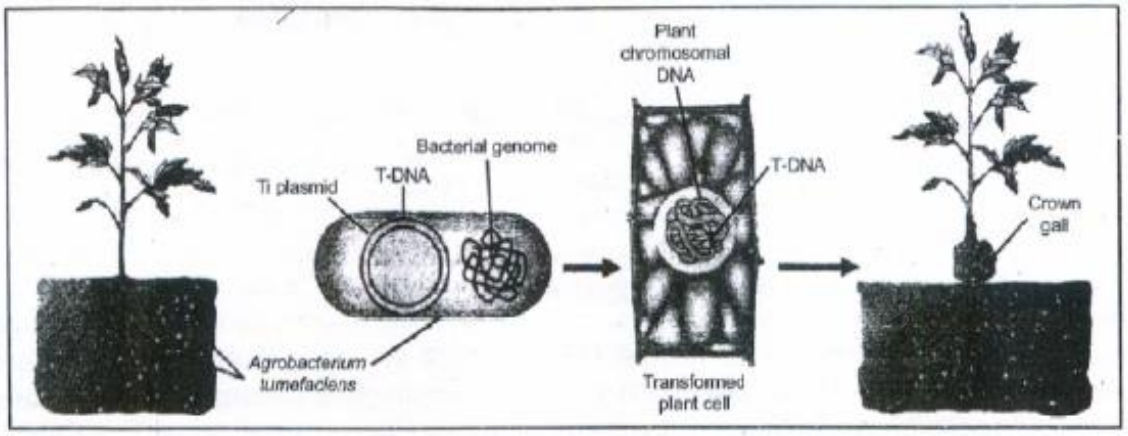
**(iii) Bacterial Artificial Chromosome (BAC):** They can accommodate upto 300–350 kb of foreign DNA and are also being used in genome sequencing projects. The basis of these vectors is natural, or extra-chromosomal plasmid of *E.coli* – the Fertility or F-Plasmid.

**(iv) Yeast Artificial Chromosome (YAC):** These vectors are employed to clone DNA fragements of more than 1 Mb in size.

## Vectors for cloning genes in plants:

**Ti plasmid of *Agrobacterium tumefaciens* :** *Agrobacterium tumefaciens*, a pathogen of several **dicot plants** is a soil-borne,rod shaped, motile, gram negative bacterium.It is able to deliver a piece of DNA known as '**T-DNA**' to transform normal plant cells lnto a tumor and direct these tumor cells to produce the chemicals required by the pathogen. This bacterium invades plants at the site of wound, transforming them and nearby cells to form a tumor called **crown gall**.

The **wounded dicot plant cells** release certain chemicals, phenolic compound named as **Acetosyringone** and sugars which are recognized as signal by *Agrobacterium*.When the bacterium contacts a damaged plant cell, it delivers a T-DNA (transferred DNA) fragment of Ti plasmid into host cell that integrates at a random position in plant cells chromosome. The tumor inducing (Ti) plasmid of A. tumefaciens has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanism to deliver genes of our interest into a variety of plants as shown in figure below.




## Vectors (virus) used to clone genes in animals:

**(a) Retroviruses:** These viruses in animals have the ability to transform normal cells into cancerous cells. 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). e.g. *Bovine leukemia virus*, *Lentivirus*.

**(b) Shuttle Vector:** These vector can **replicate in both eukaryotic cell and** prokaryotic cell i.e., these vectors contain two types of origin of replication and selectable marker genes, one for eukaryotic cell and another for prokaryotic cell. e.g. YEP ( Yeast Episomal Plasmid) and Modified Ti plasmid.

**(3) Competent host (For Transformation with Recombinant DNA):**

- It is essential for transformation with recombinant DNA.
- Since DNA is a hydrophilic molecule, it can not pass through membranes, so the bacterial cells must be made capable 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 (rDNA) can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42ºC (heat shock), and then putting them back on ice, This enables the bacteria to take up the recombinant DNA.

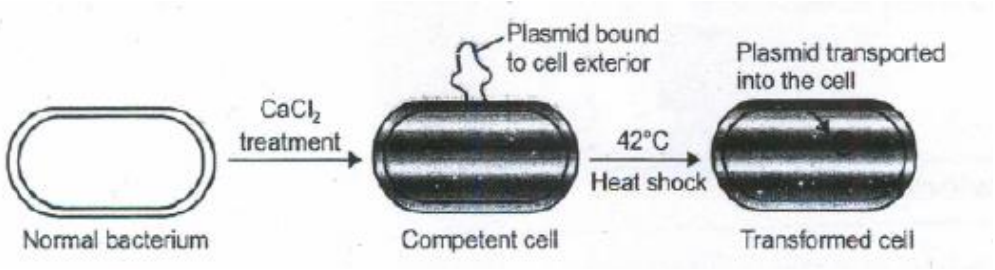



**Fig.** The binding and uptake of DNA by a competent bacterial cell through transformation

### Direct gene Transfer:

It involves alternative methods to introduce the recombinant DNA into recipient cells of animals without carrier molecules. Some alternative methods are as follow.

**(i) Microinjection:** Foreign DNA is directly injected into the nucleus of animal cell or plant cell by using micro needles or micro pipettes. It is used in oocytes, eggs and embryo. Jeffey **S. chamberlain et al (1993)** of Human genome Centre, Michigan University U.S.A have cured mice that inherited a neuromuscular disease which is like muscular dystrophy of humans.

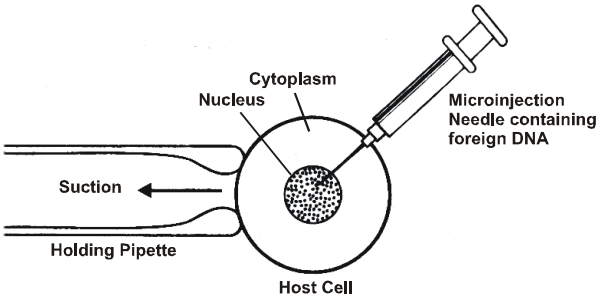



**Fig:- Introduction of foreign DNA in a host cell with a microinjection Needle.**

**(ii) Gene gun or Particle gun or Biolistic:** In this process, DNA coated onto **microscopic pellets of gold or tungsten** is literally shot with high velocity into target cells. Although it is developed for plants yet this technique is also used to insert genes into animal that promote tissue repair into cells (Particulary cancer of mouth) near wounds leading to reduction of healing time.

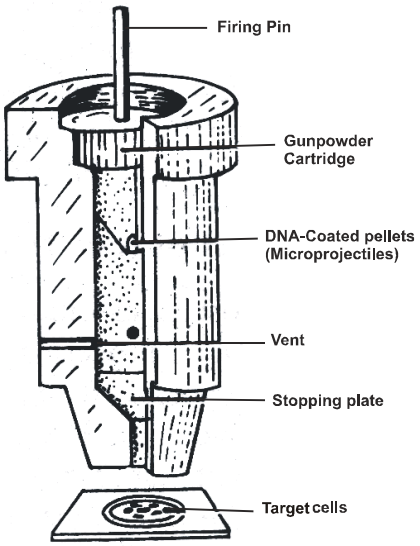



**Fig :- Gene gun.**

**(iii) Direct DNA Injection:** Direct injection of DNA into skeletal muscle led to the possibility of using Gene as vaccines. Due to low level of expression therapeutic benefits for the treatment of genetic disorder could not be derived. This method gave birth to the concept of DNA vaccine or genetic immunization.

**(iv) Electroporation:** In this method the electrical impulses induce transient (temporary) pores in the plant cell membrane through which the DNA molecules are incorporated into the plant cells.

### Test your Resonance with concept

**1.** The most extensively used bacteria in genetic engineering is
(1) *Bacillus* (2) *Clostridium* (3) *Escherichia* (4) *Salmonella*

**2.** Restriction endonucleases are called so as they
(1) Synthesize DNA (2) Restrict nuclear activity
(3) Cleave DNA into fragments (4) Break DNA randomly

**3.** Which type of restriction enzymes are used in recombinant DNA technology?
(1) Type-I (2) Type-II (3) Type-III (4) All of the above

**4.** Each restriction endonuclease recognises a ..... sequences in the DNA.
(1) Nucleotide sequence (2) Nucleoside sequence
(3) Palindromic nucleotide sequence (4) Both 1 and 2

**5.** Restriction endonucleases are enzymes which
(1) recognize a specific nucleotide sequence for binding of DNA ligase
(2) restrict the action of the enzyme DNA polymerase
(3) remove nucleotides from the ends of the DNA molecule
(4) Make cuts at specific positions within the DNA molecule

**Answers**

**1.** (3) **2.** (3) **3.** (2) **4.** (3) **5.** (4)

## Process of Recombinant DNA Technology:

The main steps of rDNA technology are as follow

(1) Isolation of the genetic material (DNA)
(2) Fragmentation of DNA at specific locations by restriction endonuclease
(3) Separation and Isolation of DNA fragments
(4) Gene amplification by PCR
(5) Insertion of recombinant DNA into the host cell / Organism
(6) Culturing the host cells in a nutrient medium at a large scale for obtaining the foreign gene product
(7) Extraction of the desired product utilises downstream processing

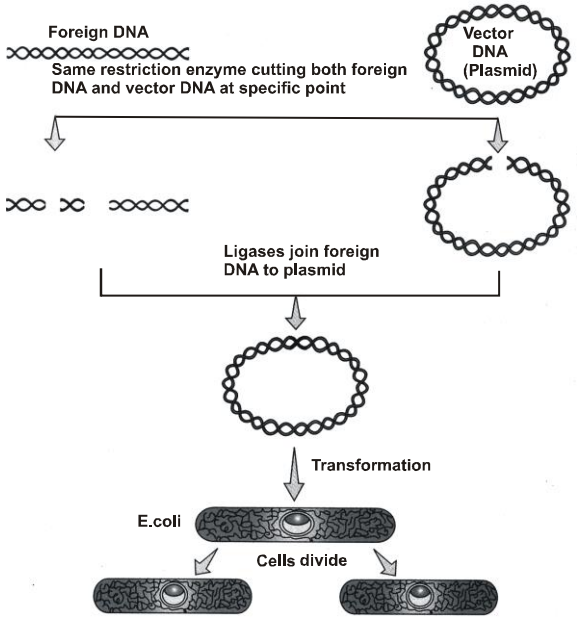



**Fig : Diagrammatic representation of recombinant DNA technology**

**(1) Isolation of the Genetic Material (DNA):**

- The procedure for total DNA preparation from a culture of bacterial cells can be divided into four stages (shown in figure bellow) :
  1. A culture of bacteria is grown and then harvested.
  2. The cells are broken open to release their contents according to cell type

| | | |
|---|---|---|
| **Bacterial** | **-** | **Lysozyme** |
| **Fungal** | **-** | **Chitinase** |
| **Plant cell** | **-** | **Cellulase** |

  3. This cell extract is treated to remove all components except the 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)
  4. The resulting DNA solution is concentrated.

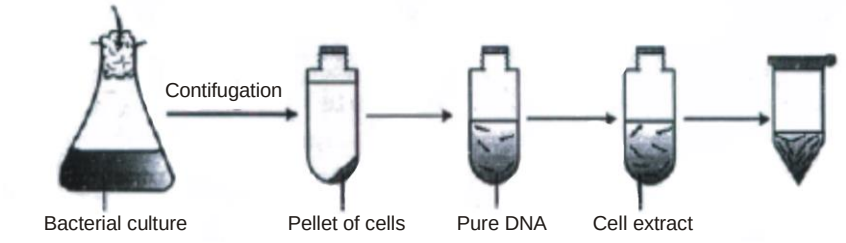



**Fig. : The basic steps in preparation of total cell DNA from a culture of bacteria**

**(2) Fragmentation of DNA at Specific Locations by RE**

Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme. Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. Both vector and insert should be digested with the same restriction enzyme.

**(3) Separation and Isolation of DNA Fragments**

**A Separation of DNA fragments by 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 positive electrode anode under an electric field through a medium/matrix,
- Nowadays, 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.
- Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion. This process is repeated with the vector DNA also.
- The separated DNA fragments can be visualised only after staining the DNA with a compound such as Ethidium bromide or Acridine orange, 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.

**B Isolation of desired DNA fragment**

- 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.

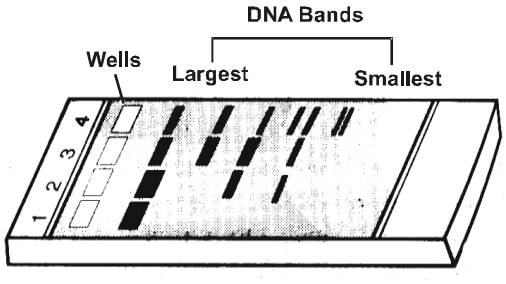



**Fig:- A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested lanes of DNA fragments (lanes 2 to 4)**

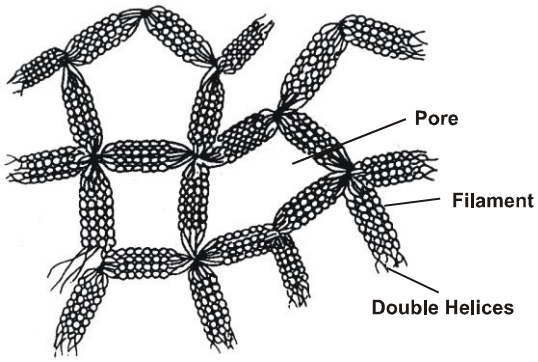



**Fig :- Formation of pores in the agarose gel.**

**(4) Gene amplification by PCR:**

- It was discovered by **Kary Mullis in 1985**.
  PCR is DNA replication on a grander scale. The polymerase chain reaction relies on the use of several essential chemical ingredients, including the following:
- DNA polymerase: A major limitation of early PCR method was that fresh DNA polymerase had to be added during every cycle. '**Taq polymerase**', a heat-resistant enzyme isolated from the bacterium ***Thermus aquaticus*****,** eliminated the need to add fresh polymerase during life cycle. *Thermus aquaticus* is a thermophilic bacterium that can survive temperatures up to 95°C. In fact, its natural habitat is the hot spring ecosystem of Yellowstone National Park. This innovation greatly improved the quantity and quality of PCR products.
- **Pfu DNA polymerase** is also an enzyme found in the hyperthermophilic archaeon ***Pyrococcus furiosus***
- A small amount of DNA to serve as the initial template (nanograms).
- The four deoxyribonucleotides to serve as the substrates for the DNA polymerase and the raw ingredients of the new DNA molecules.
- Few necessary ions and salts.
- A pair of primers (small chemically synthesized oligonucleotides made up of DNA that are complementary to the regions of DNA) with exposed 3'-OH groups that will bind to the particular sequence of interest in the DNA template.

The DNA polymerases can only add new nucleotides to the 3'-OH end of a growing strand. They, therefore, require the presence of a primer to get started, because they cannot begin synthesis de-novo. In fact, two primers are required - one to initiate replication of each of the two DNA strands.

**PCR or Polymerase chain reaction** involves synthesis of multiple copies of the gene of interest in vitro.

**(i) Denaturation:** In this process, the target DNA is heated to a high temperature (usually **94º to 96ºC**), resulting in the separation of the two strands. Each single strand of the target DNA then acts as a template for DNA synthesis.

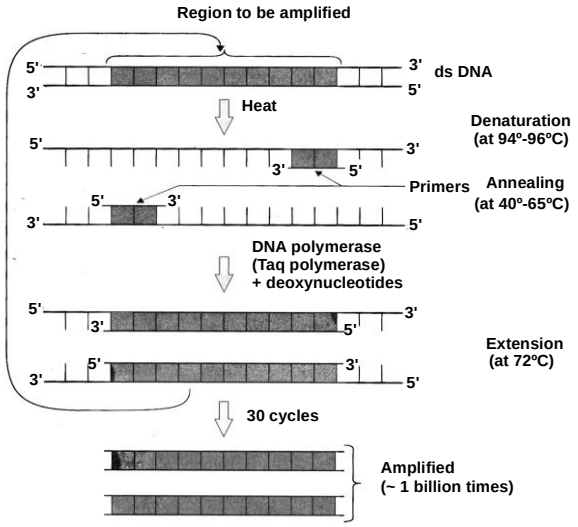



**Fig:- Schematic representation of polymerase chain reaction (PCR) (AIPMT Mains. 2012)**

**(ii) Primer annealing:** The two sets of primers (small chemical synthesized oligonucleotides that are complementary to the regions of DNA) undergo biochemical process of annealing at an optimum temperature of **40-65ºC.**

**(iii) Extension:** The final step is extension, where in **Taq polymerase (thermophile bacterium *Thermus aquaticus*)** synthesizes the DNA region between the primers, using dNTPs (deoxynucleoside triphosphates) and $Mg^{2+}$. It means the primers are extended towards each other so that the DNA segment lying between the two primers is copied. The optimum temperature for this polymerization step is **72ºC.** The extension product of one cycle can serve as a template for subsequent cycles as a result, from a single template essentially is possible to generate $2^n$ molecules after n number of cycles.

## Resonate the Concept

- **RAPD (Random Amplified polymorphic DNA) :** are DNA fragments amplified by PCR using short synthetic fragment (generally 10 bp) of random sequence. These oligonucleotides serve as both forward and reserve primer, and are usually able to amplify fragments from 1-10 genomic sites simultaneously.
- **RT-PCR (Reverse transcription polymerase chain reaction)-** is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement through the use of reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR.

## Resonate the Concept

**Application of PCR:**

- **Diagnosis of Pathogens:** Pathologists used techniques based on detecting specific enzymes or antibodies against disease-related proteins. But these techniques cannot be used for detecting infectious agents that are difficult to culture or that persist and very low levels in infected cells. To overcome these problems, PCR-based assays have been developed that detect the presence of gene sequences of the infectious agents.
- **Diagnosis of Specific Mutation:** PCR is used to detect the presence of a specific mutation.

**(5) 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. So, if a recombinant DNA bearing gene for resistance to an antibiotic (e.g., ampicillin) is transferred into E. coli cells, the host cells become transformed into ampicillin-resistant cells. If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow, untransformed 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.

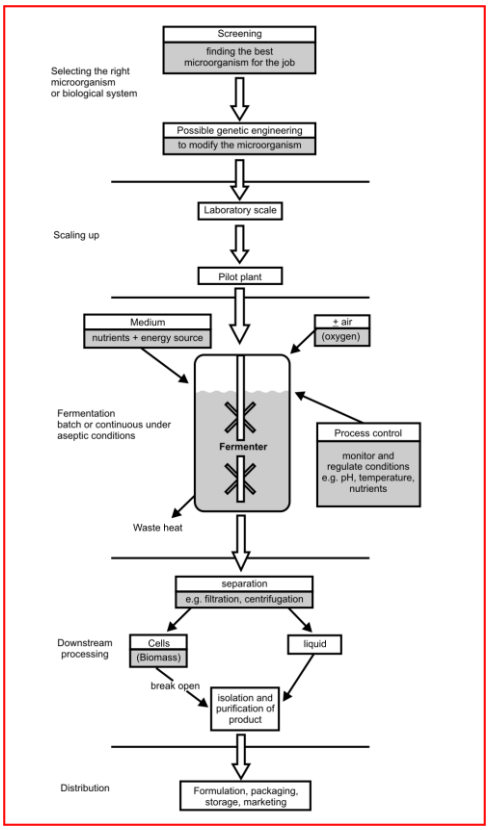



**Fig. : Overview of a biotechnological process**

**(6) Insertion of Recombinant DNA into the Host Cell/Organism**

After initial investigations using ordinary laboratory apparatus it is usual to make a 'pilot plant' that involves use of a small 'fermenter' such as large shake flasks in laboratories. Fermenter is the tank or vessel in which the process will be carried out. Optimum nutrient and physical condltions for maximum yield must be determined.

New factors come into play when the process has to scaled up from pilot production to full-scale (100-1000 *l*). Some of the important factors are as follows:

- Maintaining aseptic conditions. It is easy to contaminate both inputs and outputs to the main fermenter.
- Physical factors, such as mixing and aerating the media and getting rid of waste heat, create the biggest problems in moving from one scale to another.
- To supply enough oxygen in large-scale cultures, air must be forced through the medium because the simple agitation used at the laboratory scale is inadequate. Small bubbles are more effective than large bubbles, so a sparger is used (a tube with small holes). The mixture may also be stirred.
- Anti-foaming agents are required to reduce the foaming caused by stirring and aeration.

- Heat is produced by the activity of microorganisms and large-scale production. Cooling water must be circulated around the fermenter.
- To keep conditions constant, such as supply of nutrients, pH and oxygen concentration, throughout the medium on a large scale. Sophisticated monitoring devices and control processes are needed.

**Fermenters / bioreactors:**

These are chambers in which microorganisms are cultured in a liquid/solid medium. To produce the desired product in large quantities, bioreactors were developed where large volumes (100-1000 L) of culture can be processed.

Bioreactors are 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).

**Fermenter design and use:** The most commonly used bioreactors are of stirring type, which are shown in figure given below.

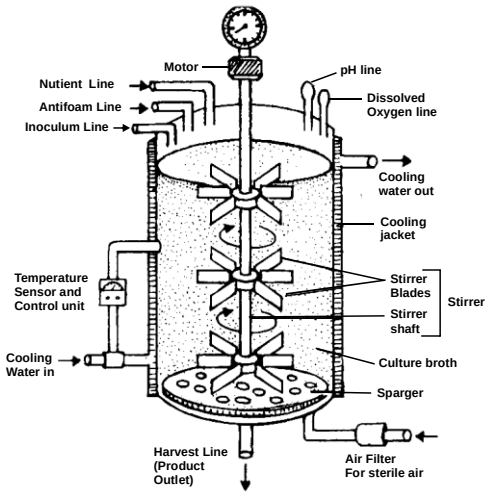



**Fig :- Simple stirred tank bioreactor for continuous culture.**

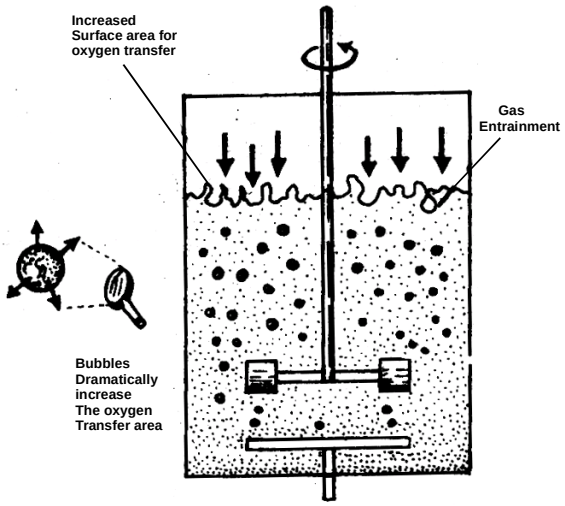



**Fig:- Sparged- stirred tank bioreactor through which sterile (free from any germs) air bubbles are sparged.**

A **stirred-tank reactor** is usually cy!indrical 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.

The processes which take place in fermenters are referred to as fermentations. The term fermentation originally applied only to anaerobic processes but is now used more broadly to include all the processes, whether aerobic or anaerobic. The product is either the cells themselves (biomass) or some useful cell product.

**All operations must be carried out under sterile conditions to avoid contamination of the culture.**

**Types of Fermentation:**

(i) Batch fermentation (closed system)

(ii) Continuous culture (open system)

In **batch fermentation** the conditions are set up and not changed from outside once the fermentation starts; for example, no more nutrients are added. This is why the process is described as a closed system. The process is stopped once sufficient product has been formed. The contents of the fermenter are then removed, the product isolated, the microorganism discarded and the fermenter is cleaned and set up for a fresh batch.

**In continuous culture system**, the used medium is drained out from one side, while fresh medium is added from other to maintain the cells in their physiologically most active log/exponential phase. This type of culture method produces a larger biomass leading to higher yields of desired protein.
Small volume cultures (in shake flasks) cannot yield appreciable quantities of products.
Continuous culture involves continuous, long-term operation over many weeks, during which nutrient medium is added as fast as it is used, and the overflow is harvested.

**(7) Extraction of the Desired Product Utilises Downstream Processing**

**Downstream processing is the name given to the stage after fermentation when the desired product is recovered and purified**.

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. These downstream processes include separation and purification of the desired product.
Usually the contents of the fermenter are first separated in a liquid component and a solid component which contains the cells. This is usually done by filtration or centrifugation.The liquid may contain the desired product in solution or it may be the cells or some product inside the cells that is needed.
A whole range of biochemical separation and purification techniques is available, such as drying, chromatography, solvent extraction and distillation. As an indication of the importance of downstream processing, it involves over 90% of the 200 staff employed by Eli Lilly in their human insulin plant.
After purification the product has to formulate with suitable preservative. 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.

## Test your Resonance with concept

**1.** Polymerase chain reaction involves use of a DNA polymerase enzyme which is
(1) A terminal transferase (2) Thermolabile
(3) Thermostable (4) Modular enzyme

**2.** Transfer of DNA bands from an agrose gel to a nitrocellulose or nylonmembrane is referred to as
(1) western transfer (2) Northern transfer (3) eastern transfer (4) gene transfer

**3.** During “gene cloning” which is called as “gene taxi”
(1) Vaccine (2) Plasmid (3) Bacterium (4) Protozoa

**4.** Restriction endonucleases were used by Cohen and Boyer’s DNA experiments for
(1) Isolation of cloned bacterial plasmids (2) Cleaving the bacterial plasmid
(3) isolation of human insulin (4) both 1 and 2

**5.** Identify the correct sequence of performing PCR.
(a) Annealing of primers
(b) Extensions of primers using the nucleotides provided in the reaction and the genomic DNA as template.
(c) Denaturation of the double stranded DNA.
(d) A segment of DNA is amplified approximately a billion times.
(1) abdc (2) cabd (3) dcab (4) badc

### Answers

**1.** (3) **2.** (4) **3.** (2) **4.** (2) **5.** (2)

**Resonate the Concept**

**For AIIMS Only**

1. **RFLP (Restriction Fragment length polymorphism) :** These are produced by single base alterations in the recognition sequence as the result of which the pattern of cut has been changed in DNA and segments of variable length are produced. RFLP are used as marker.

**Uses:**

**(i)** To determine relationship between different strains & species.
**(ii)** To utilize in DNA Fingerprinting.
**(iii)** To select desired organisms containing suitable features.
**(iv)** To utilize as a marker for the identification of varieties.

2. **Reporter gene:** Reporter or marker genes present in cells produce a specific phenotype by which they can be easily detected. In Genetic engineering, regenerated plants (Transgenic plants) can be tested for the transfer of gene, with the help of one of the several screenable markers (also called reporter genes) gene which is also transferred along with the desirable gene. **E.g. npt II (Neomycin phosphotransferase II gene)**.

# BIOTECHNOLOGY AND ITS APPLICATIONS

- **Biotechnology is mainly employed for industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals**. The application of biotechnology include therapeutics, diagnostics and genetically modified crops for agriculture, processed food, bioremediation, waste treatment and energy production.
- Three research areas of biotechnology are as follow.
  **(i)** Providing the best catalyst in the form of improved organism; generally a microbe or pure enzyme.
  **(ii)** Creating optimal conditions through engineering for a catalyst to act.
  **(iii)** Downstream processing technologies to purify the protein / organic compound.

## BIOTECHNOLOGICAL APPLICATION IN AGRICULTURE:

Three options can increase the food production.

**(i)** Agrochemical based agriculture.
**(ii)** Organic agriculture.
**(iii)** Genetically engineered crop-based agriculture.

### Green Revolution

In 20$^{th}$ century significant increase in agricultural productivity of grains (particularly wheat and rice) was observed, resulting from

**(i)** Introduction of improved crop varieties i.e high-yielding varieties.
**(ii)** Better management practices (irrigation, mechanisation and soil conservation technique).
**(iii)** Use of agrochemicals (fertilisers or pesticides) its early dramatic success was in Mexico and the Indian subcontinent.

The green revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population.

**Difficulties:** Green Revolution was not a complete success in developing world due to various factors.

- For farmers in devolping world, agrochemicals are often too expensive.
- Further increases in yield with existing varieties are not possible using conventional breeding.
- The new varieties required large amounts of fertilizers, and pesticides to produce high yields, raising concern about cost and potentially harmful effects.
- minimising the use of fertilizers and chemicals so that their harmful effects on the environment are reduced.

**For overcoming the above stated problems use of genetically modified crops is a possible solution**

Gene cloning provides a new dimension to crop breeding by enabling directed changes to be made to the genotype of a plant, eliminating the random processes inherent in conventional breeding.

Two general strategies have been used:

**(a) Gene addition**: in which cloning is used to alter the characteristics of a plant by providing it with one or more new genes.

**(b) Gene subtraction**: in which genetic engineering techniques are used to inactivate one or more of the plants, existing genes.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called genetically modified organism (GMO) or transgenic organisms.

## TRANSGENIC PLANTS

## Genetic modified crops or GM Crops:

They represent crops that have one or more useful foreign genes or transgenes.

GM crops has two advantages.

**(a)** Any gene of any organisms or a synthetic gene can be incorporated.

**(b)** Genotypic change is precisely control.

This technique is superior to breeding programmes because in breeding only the already present genes are reshuffled and that changes would occur in all traits for which the parents are different.

**Application of DNA Technology in the production of Transgenic Plants:**

GM plants have been useful in many ways:

**(i)** Made crops more tolerant to abiotic stresses (Cold, drought, salt heat).

**(ii)** Reduced reliance on chemical pesticides (pest-resistant crops).

**(iii)** Helped to reduce post harvest losses.

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

**(v)** Enhanced nutritional value of food, e.g. Vitamin 'A' enriched rice.

In addition to these uses, genetic engineering has been used to create tailor- made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

## Gene Addition

- Recombinant DNA techniques are being used to develop transgenic plants.
- Plasmid of ***Agrobacterium tumefaciens*** is widely used as **vector** to introduce new genes into dicot plants. The former is called **Ti plasmid (Tumor inducing plasmid)**, so called because in nature, it induces tumors in broad leaf plants such as tomato, tobacco and soyabean. It does not infect cereals.
- *Agrobacterium* initiates formation of cancerous growth called a **crown gall tumor**. For genetic engineering purposes, its strains are developed in which tumor-forming genes are eliminated. These transformed bacteria can still infect plant cells.

- The part of Ti plasmid transferred into plant cell DNA, is called the **T-DNA**. This T-DNA with desired DNA spliced into it, is inserted into the chromosomes of the host plant where it produces copies of itself.
- Such plant cells are then cultured, induced to multiply and differentiate to form plantlets. Transferred into soil, the plantlets grow into mature plant, carrying the foreign gene, expressed throughout the new plant.

  ***Agrobacterium tumefaciens* is called natural genetic engineer** because it integrates its plasmid's gene into plant genome and these genes carried by its plasmid produce effect in several parts of the plant.

### (i) Insect Resistant transgenic plant:

**Bt Cotton –**

- ***Bacillus thuringiensis* bacterium form crystal protein (Cry protein)** contain a toxic insecticidal protein. That kill certain insects like **Lepidopterans (tobacco budworm, armyworm), coleopteran (beetles) and dipterans (flies, mosquitoes).**

  The Bt toxin proteins exist as inactive **protoxins** but once an insect ingests the inactive toxin it is converted into an active form of toxin due to the alkaline pH of the alimentary canal that solublizes the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores which cause cell swelling and lysis and finally cause death of the insect.
- **Bt toxin** genes were isolated from ***Bacillus thuringiensis*** and incorporated into the several crop plants like **cotton**. The toxin is coded by a gene named ***cry***.
- ***cry* I Ab** gene has been incorporated in **Bt corn** to protect the same from **corn borer**.
- Two ***cry* genes IAc and *cry* II Ab** have been introduced in cotton. **The genetically modified crop** is called **Bt cotton** that shows **resistance** against **cotton bollworms**.

### The endotoxins of *Bacillus thuringiensis*

Insects not only eat plants; bacteria also form occasional part of their diet. In response, several types of Bacteria have evolved defense machanisms against insect predation, an example being *B. thuringiensis* which, during sporulation, forms-intracellular crystalline bodies that contain an insecticidal protein called the endotoxin.This toxin is encoded by a gene named ***cry***. The endotoxin that accumulates in the bacterium is an inactive precursor. After ingestion by the insect, this protoxin is cleaved by proteases (alkaline conditions in gut), resulting in shorter versions of the protein that display the toxic activity, by binding to the inside of the insects mid gut and damaging the surface epithelium by creating pores that cause swelling and lysis. So, that insect is unable to feed and consequently starves to death. The choice of gene depends on the crop and the targeted pest, as most Bt toxins are insect group specific.

This toxin called Bt toxin as produced by *Bacillus thuringiensis* has been cloned in bacteria and been expressed in plants to provide resistance to insects without the need for insecticides in effect created a bio-pesticide. Examples are Bt cotton, corn, rice, tomato, potato and soya bean etc.

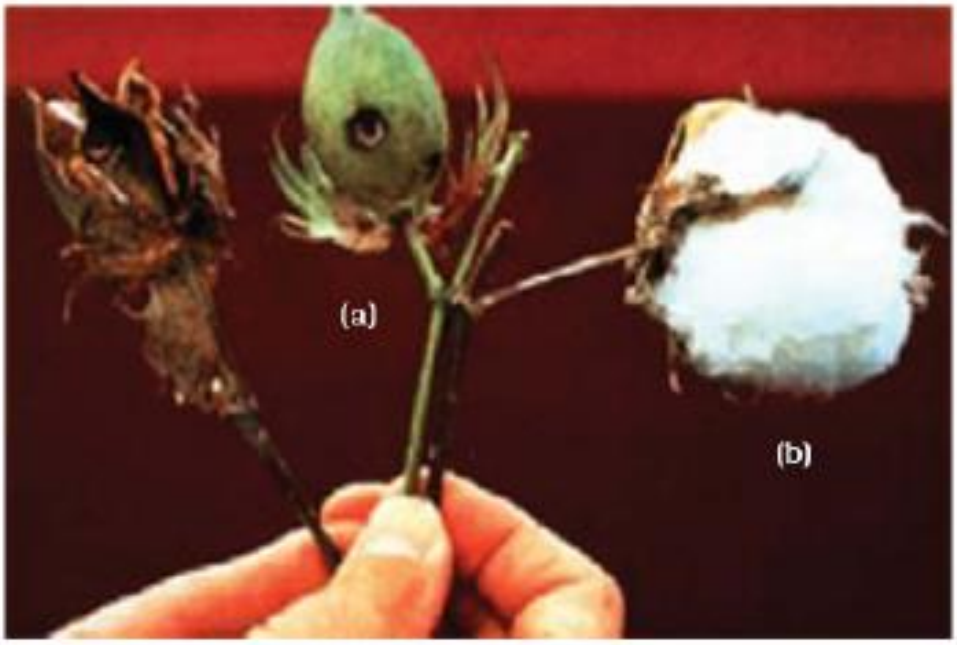



**Figure - Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll**

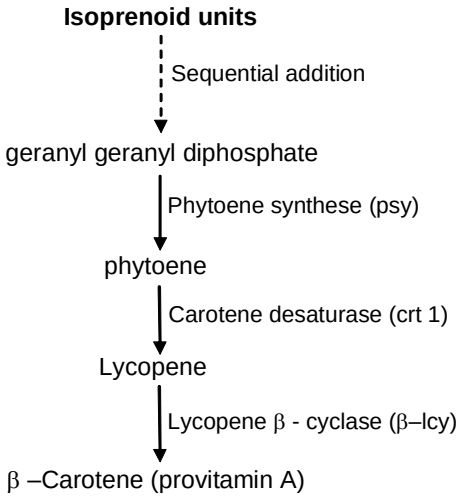
**(ii) Golden Rice:**

Golden rice was developed by **Ingo Potrykus** and **Peter Beyer** to combat vitamin A and Iron deficiency as this could accumulate more β-carotene.

Golden rice is a transgenic variety of rice *(Oryza sativa)* which contains good quantities of β-carotene **(provitamin A -** inactive state of vitamin A). It is required by all individuals as it is present in retina of eyes. Deficiency of vitamin A causes night blindness and skin disorders. β-carotene is a principal source of vitamin A. Due to β-carotene the grains (seeds) of this GM rice are pale yellow instead of pearly white in colour, thus the rice is commonly called **golden rice**.

It is a transgenic variety of rice with genes for synthesis of β-carotene taken from **Daffodil** *(Narcissus pseudonarcissus)* and inserted into the genome of a temperate strain of rice using *Agrobacterium tumefaciens,* as the vector to effect the transfer.

Golden rice required the introduction of three genes encoding the enzymes phytoene synthase, Carotene desaturase and lycopene β-cyclase.



**(iii) Production of hirudin from transgenic *Brassica napus* seeds**

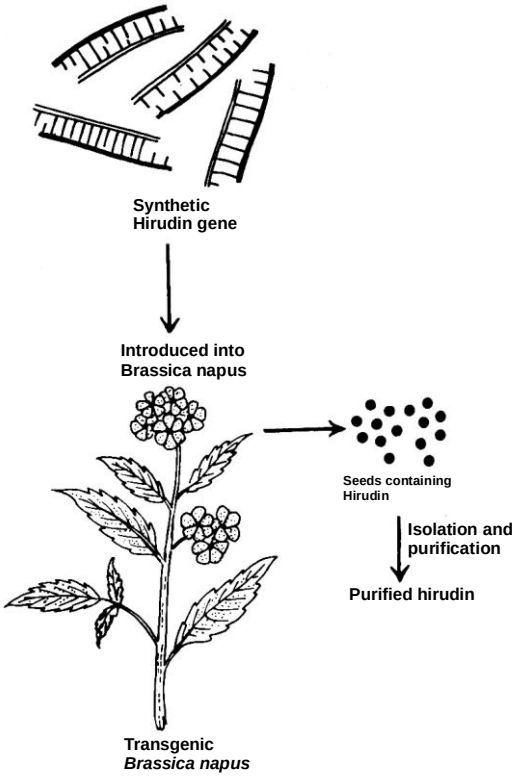



**Fig :- Diagrammatic representation of the production of hirudin from transgenic *Brassica napus* seeds.**

Hirudin is a naturally occurring, anticoagulant produced and secreted in the salivary glands of the medicinal leech, *Hirudo medicinalis*, with a number of desirable properties. Hirudin has been made in variety of microorganisms including yeast and *E. coli* but these entail significant costs associated with fermentation. Therefore, the gene-encoding hirudin was chemically synthesized and introduced into *Brassica napus* using *Agrobacterium*-mediated transformation. The resulting transgenic plant yielded seeds in which hirudin accumulates. The hirudin is purified and used as medicine.

**(iv) Herbicide resistant transgenic plants**

The herbicide **glyphosate** [**Roundup** (Trade name)] inhibits photosynthesis. It blocks the activity of **enolpyruvylshikimate phosphate synthase (EPSPS)**, a key enzyme involved in the biosynthesis of phenyl alanine, tyrosine and tryptophane. The genes epsps/aroA confer resistance to transgenic plants which can be selected. This gene obtained from mutant strain of *Agrobacterium*. Thus Roundup ready transgenic plants has been produce and commercialised. Herbicide tolerence has been developed in Maize, Cotton**,** Soyabean and Tobacco etc.

**Gene Subtraction**

This is another method of changing the genotype of a plant by either inactivation of the gene or sometimes **knockout of the gene**. There are several possible strategies for inactivating a chosen gene in a living plant, the most successful so far in practical terms being the use of anti-sense RNA.

**The strategy adopted to prevent this infestation is based on the process of RNA interference (RNAi):** RNAi was first discovered in 1998 by Andrew Fire and Craig Mello in the nematode worm ***Caenorhabditis elegans*** and later found in a wide variety of other organisms, including mammals.The importance of this discovery is reflected by the fact that 2006 Nobel prize for medicine was awarded to Fire & Mello.

RNAi is a naturally occurring mechanism that leads to the "silencing" of genes. In consequence, the respective protein is no longer synthesized. In nature this mechanism is used for the regulation of specific genes and is also applied as a defence against viruses. This technique has been used for **loss of function** studies where a gene (responsible for parasitism) is specifically silenced. It takes place in all eukaryotic organisms as a method of cellular defense.This method involves silencing of a specific mRNA due to formation of dsRNA molecule formed by binding of complementary RNA (anti-sense RNA) molecule to original mRNA, thereby preventing translation of the original mRNA (silencing). The source of this complementary RNA could be from infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

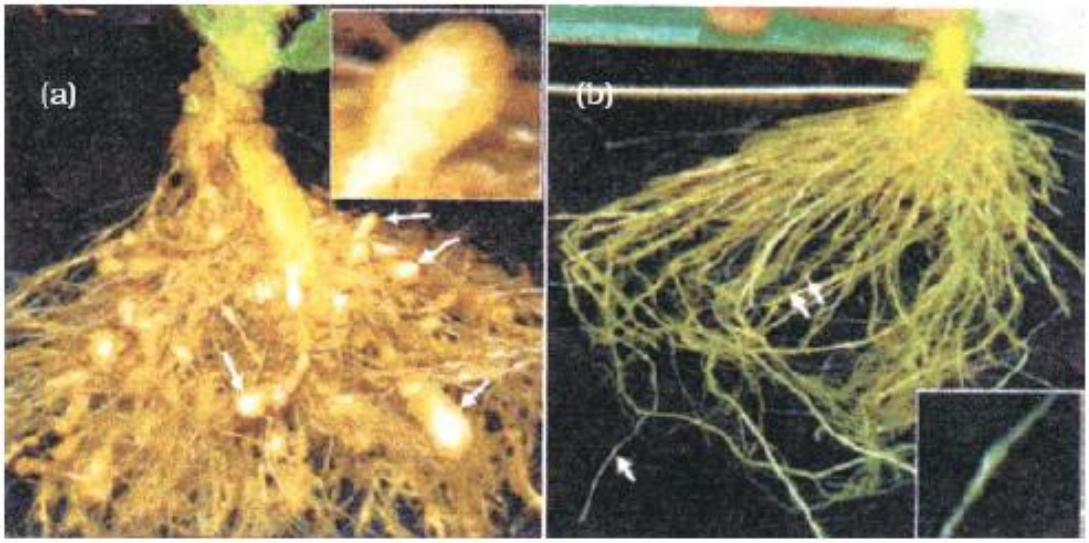



**Fig.** Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

**Use of anti-sense RNA in creating pest resistant plants:**

Several nematodes parasite have wide variety of host plants and animals including human beings. They attack nearly every food and fibre crop grown by invading the plants roots. It feeds on the roots cells causing roots to grow into large galls/knots, damaging the crop and reducing its yield. Hence, called root-knot nematode.

A nematode *Meloidegyne incognita* infects the roots of tobacco plants causing a great reduction in yield. The most cost effective tactic for preventing such damage was to bioengineer resistant plants that prevent the nematode from feeding on the roots.

Using *Agrobacterium* vectors, nematode specific genes (responsible for parasitism) 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 forming dsRNA. These two RNA's being complementary to each other formed a dsRNA that was taken up by the parasitic nematode and initiated RNAi, thus silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.

Different steps involved in making tobacco plant resistant to nematode are briefly described below:

1. Double-stranded RNAs are processed into approximately 21-23 nucleotide RNAs with two nucleotides. An RNase enzyme called **Dicer** cuts the dsRNA moelcules (from a virus, transposon, or through transformation) into small interfering RNAs (siRNAs).

2. Each siRNA complexes with ribonucleases (distinct from Dicer) to form an RNA-induced silencing complex (RISC).
3. The siRNA unwinds and RISC is activated.
4. The activated RISC targets complementary mRNA molecules. The siRNA strands act as guides where the RISCs cut the transcripts in an area where the siRNA binds to the mRNA. This destroys the mRNA.
5. When mRNA of the parasite is destroyed no protein was synthesized. It resulted the death of the parasite (nematode) in the transgenic host. Thus the transgenic plant got itself protected from the parasite.

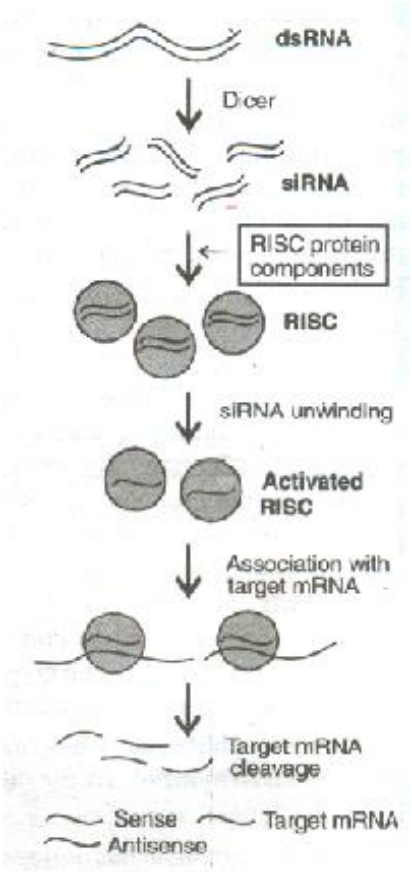



**Fig. The steps in RNA interference (RNAi)**

**Flavr Savr-tamato (post harvest losses and delayed fruit ripening):**

The tomato variety Flavr Savr developed by Calgene, presents an example where an expression of a natural tomato gene has been blocked.

In 'Flavr Savr' transgenic tomato, expression of a native tomato gene for **polygalacturonase** has been blocked. This gene produces enzyme **polygalacturonase** which promotes softening of fruit. The production of this enzyme was reduced in the Flavr Savr transgenic tomato. The non-availability of this enzyme prevents over-ripening because the enzyme is essential for degradation of cell walls.

Transformation was carried out by introducing recombinant plasmid into ***Agrobacterium tumefaciens*** and then allowing the bacteria to infect tomato stem segments. The transformed fruits, although undergoing a gradual softening could be stored for a prolonged period before beginning to spoil.

**Advantages-**

- longer shelf life of fruit
- superior taste
- Good flavour
- higher quantity of total soluble solids.

| S.No. | Transgenic plants | Useful application |
|---|---|---|
| 1 | Bt Cotton | Pest resistance, herbicide tolerance and high yield. It is resistant to boll worm infestation. |
| 2 | Wheat | Resistant against the herbicide. |
| 3 | *Brassica napus* | A gene encoding hirudin (a protein that prevents blood clotting) is synthesized chemically and then transferred into *Brassica napus*, This gene is activated in the plant and starts synthesizing hirudin which acccumulates in seeds. The hirudin is then extra |
| 4 | Tobacco | CPTI (Cow pea trypsin inhibitor) gene has been introduced in tobacco to show resistance against pests. |
| 5 | Flavr Savr Tomato | Increased shelf-life (delayed ripening) and better nutrient quality. |
| 6 | Golden Rice | Vitamin A-rich |
| 7 | Potato | Higher protein content |
| 8 | Corn, Brinjal | Insect resistance |
| 9 | Soyabean, Maize | Herbicide resistance |

## Test your Resonance with concept

**1.** Flavr Savr variety of Tomato is
(1) High yielding hybrid variety (2) High yielding new variety
(3) Transgenic vaiety (4) Polyploid

**2.** Golden rice was created by transforming rice with two beta-carotene biosynthesis genes, namely
(1) Psy and Crt 1 genes (2) LCY-e (3) CHY -1 (4) CHY - 2

**3.** GM brinjal in India has been developed for resistance against
(1) Virus (2) Bacteria (3) Fungi (4) Insects

**4.** Cry II Ab & Cry I Ab produce toxins that control.
(1) Cotton bollworm & corn borer respectively
(2) Corn borer & Cotton bollworm respectively
(3) Tobacco budworms & nematodes respectively
(4) Nematodes & tobacco budworms respectively

**5.** Golden rice is:
(1) A variety of rice grown along the yellow river in china
(2) Long stored rice having yellow colour tint
(3) A transgenic rice having gene for $\beta$ carotene
(4) Wild veriety of rice with yellow coloured grains.

### Answers

**1.** (3) **2.** (1) **3.** (4) **4.** (1) **5.** (3)

## Advantage of Transgenic Plants:

Due to genetic modification GM plants have been useful in many ways:

1. **Pest Resistance Crops:** Growing GM crops can help to reduce the use of chemical pesticides, e.g. Bt Cotton.
2. **Tolerance:** GM crops have made more tolerance to abiotic stresses (cold, drought, salt, heat, etc.)
3. **Reduction in Post-harvest Losses**: They have helped to reduce post harvest losses, e.g. Flavr Sarv transgenic tomato.
4. **Prevention of Early Exhaustion of Fertility of Soil:** Increased efficiency of mineral usage by plants prevents early exhaustion of fertility of soil.
5. **Increasing Nutritional Value of Food:** GM plants enhance nutritional value of food, e.g. golden rice is rich in vitamin A.
6. **Herbicide Resistance:** Herbicides (weed killers) do not harm the GM crops.
7. **Alternative Resources to Industries:** GM plants have been used to create alternative resources to industries in the form of starches, fuels and pharmaceuticals. Researchers are working to develop edible vaccines, edible antibodies and edible interferons.
8. **Disease Resistance:** Many viruses, bacteria and fungi cause plant diseases. Scientists are working to create genetically engineered plants having resistance to these diseases.
9. **Phytoremediation:** Plants such as popular trees have been genetically engineered to clean up heavy metal pollution from contaminated soil.

## Disadvantages of Transgenic Plants:

1. **Environmental hazards**
   - **(i) Unintended harm to other organisms**: Pollen from Bt corn caused high mortality rates in monarch butterfly caterpillars. Monarch caterpillars consume milkweed plants, not corn, but the fear is that if
     pollen from Bt corn is blown by the wind on to milkweed plants in neighbouring fields, the caterpillars could eat the pollen and perish.
   - **(ii) Reduced effectiveness of pesticides:** Just as some populations of mosquitoes developed resistance to the now-banned pesticide DDT, many people are concerned that insects will become resistant to Bt or other crops that have been genetically modified to produce their own pesticides.
   - **(iii) Gene transfer to non-target species**: Another concern is that crop plants engineered for herbicide tolerance and weeds will cross-breed, resulting in the transfer of the herbicide resistance genes from the crops into the weeds. These “superweeds” would then be herbicide tolerant as well. Other introduced genes may cross over into non-modified crops planted next to GM crops.

2. **Human health risks:**
   - **(i) Allergies**: The transgenic food may cause toxicity and or produce allergies. The enzyme produced by the antibiotic resistance gene can cause allergies, because it is a foreign protein.
   - **(ii) Effect on Bacteria of Alimentary canal:** The bacteria present in the human alimentary canal can take up the antibiotic resistance gene that is present in the GM food. These bacteria can become resistant to the concerned antibiotic and will be difficult to manage.
3. **Economic concerns:** Bringing a GM food to market is a lengthy and costly process and of course agro-biotech companies wish to ensure a profitable return on their investment.

## BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

The recombinant DNA technologies processes have made immense impact in the area of healthcare
Advantages of recombinant therapeutics:

1. Enables mass production
2. Safe and more effective drugs
3. 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 globally and **12 of these** are presently being **marketed in India**.

| Important Recombinant Proteins and Their Therapeutic Uses. | | |
|---|---|---|
| **S.No.** | **Recombinant Proteins** | **Therapeutic Uses** |
| 1 | OKT-3 | Used for reversal of acute kidney Transplantation rejection. OKT-3 therapeutic antibody. |
| 2 | ReoPro | Prevention of blood clots. |
| 3 | Tissue Plasminogen activator (T-PA) | Used for acute myocardial infarction. |
| 4 | Asparaginase | Treatment of some types of cancer. |
| 5 | DNase | Treatment of cystic fibrosis |
| 6 | Human Insulin (Humulin) | Treatment of diabetes mellitus |
| 7 | Blood Clotting Factor VIII | Treatment of Haemophilia A |
| 8 | Blood Clotting Factor IX | Treatment of Haemophilia B |
| 9 | Hepatitis B Vaccine | Prevention of Hepatitis B |
| 10 | Platelet derived growth factor | Approved for diabetic/skin ulcers. It also stimulates wound healing |
| 11 | Interferon alpha (INF- alpha) | Used as vaccine for hepatitis C |
| 12 | Hirudin | Used as an anticoagulant |

## (i) Genetically engineered Insulin:

- Insulin is secreted by the **Beta cells of the islets of Langerhans of the pancreas**. In 1921 **Banting and Best** demonstrated that administration of insulin could cure diabetes in human beings.
- In human, **insulin is composed of 51 amino acids** arranged in two polypeptide chains, **A having 21 amino acids and B with 30 amino acids. The two polypeptide chains are interconnected by two disulphide bridges or S-S linkages.**
- The hormone develops from a storage product called **pro-insulin**. The latter contains **three chains A, B and C**. out of them C-chain with 33 amino acids is removed prior to insulin formation.
- 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 prohormone (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.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 disulfide bonds to form human insulin.

- Efforts were made to synthesize human insulin through rDNA technology. Two DNA sequences were prepared by Eli Lilly for the two chains, A and B of insulin by reverse transcription of their mRNA.
- Plasmids of ***Escherichia coli*** **(e.g. pUC-18)** and insulin gene are treated with the same restriction endonuclease. It produces sticky ends in both. The two are joined together by DNAs ligase. It produces recombinant DNA in the form of plasmids carrying the insulin genes are attached in the vector plasmids adjacent to Z-gene encoding β- galactosidase.
- A culture of plasmid free *Escherichia coli* is now inoculated with recombinant plasmids in the presence of calcium chloride or other chemical that increases the ability of bacterial cells in obtaining plasmids from outside.
- The genetically engineered bacteria are tested for the formation of fusion polypeptide consisting one insulin subunit and β-galactosidase sequence.

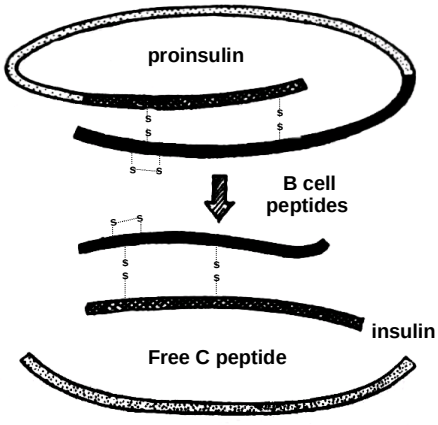



**Fig:- Maturation of pro-insulin into insulin after removal of C-peptide**

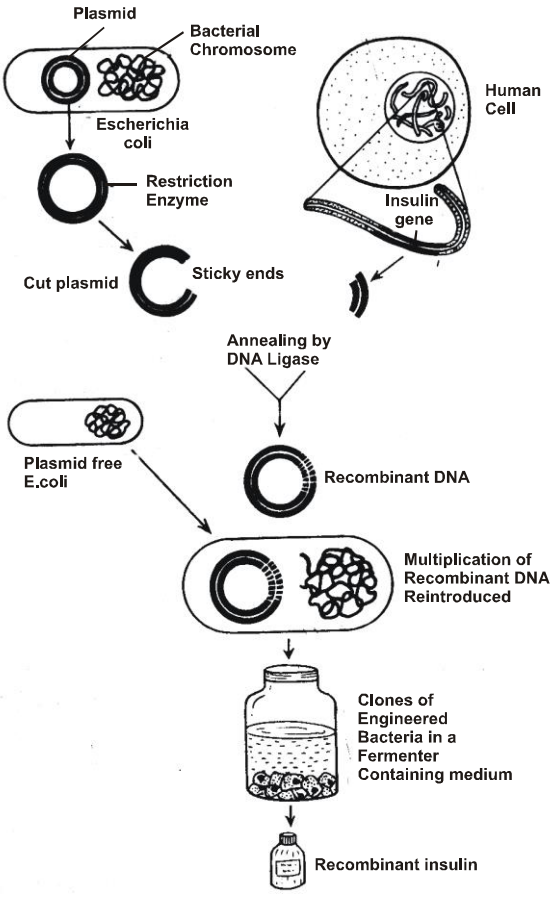



**Fig :- Indirect gene transfer for the production of human insulin.**

- Insulin is extracted from the harvested bacteria and purifying the fusion polypeptides, treating them with Cyanogen bromide for separating polypeptide of β-galactosidase and purifying insulin subunits. The latter are then mixed. Insulin is formed spontaneously. Since it is exactly similar to human insulin, the same is also called **humulin**.

**(ii) Human Growth Hormone (hGH) – Somatotropin:** hGH is secreted by the antierior lobe of pituitary gland. Secretion of hGH is regulated by two other hormones secreted by hypothalamus. These hormones are:

**(a) Somatotropin-releasing hormone** which stimulates the anterior lobe of pituitary gland to release somatotropin or growth hormone.

**(b) Somatostatin or growth-inhibiting hormone** which inhibits the secretion of growth hormone from the anterior lobe of pituitary gland.

hGH is essential to remove pituitary dwarfism that occurs due to non secretion of hormone from anterior pituitary. Biosynthesis of somatotropin was achieved through gene cloning procedures.

**(iii) Gene Therapy (Gene addition) :** It is advanced technique of genetic engineering by which a faulty gene can be replace by normal healthy functional gene **e.g. Adenosine Deaminase (ADA) Deficiency.**

## Gene therapy

Gene therapy is a collection of methods that allows correction of a gene defect to cure an inherited disease by providing the patient with a correct copy of that defective gene 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 and compensate for the non-functional gene.Gene therapy has now been extended to include attempts to cure any disease by introduction of a cloned gene into the patient.

The first clinical gene therapy was given in 1990 to a four-year old girl with **adenosine deaminase** (ADA deficiency).Gene for enzyme ADA is situated on chromosome number 20. Mutation in this gene causes **severe combined immune deficiency disorder (SCID)**. Because of absence, defect or deficeincy of adenosine deaminase enzyme body become unable to break down a sunstance **Deoxyadenosine** that get build up and destroy infection fighting immune cells called T and B lymphocyte.

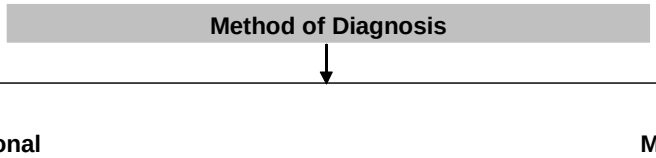
This enzyme is crucial for the immune system to function because in its absence lymphocyte proliferation is inhibited. Without T and B cells, ADA-deficient children are wide open to the attacks of viruses and bacteria.

In some children, ADA deficiency can be treated by **bone marrow transplantation** and in others it can be treated by **Enzyme Replacement Therapy (ERT)**. In ERT patient is given an intravenous injection of ADA or enzyme lacking in the content. But the problem with both 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.

**(iv) Molecular Diagnosis:**

For effective treatment of a disease, early diagnosis and understanding the pathophysiology is very important. Presence of a pathogen (bacteria viruses) is normally suspected only when the pathogen has produced a disease symptoms. By this time, the concentration of pathogen is already very high in the body. Methods of detection fall under two categories:




**Traditional**

1. Conventional method
2. Known biology or association of single analysts (glucose cholestrol ets.)
3. Early detection is not possible
4. Involves serum and urine analysis

**Modern**

1. Recombinant DNA technology
2. Polymerase chain reaction
3. Enzyme Linked Immuno Sorbent Assay (ELISA)
4. Serve purpose of early diagnosis

## Modern methods of diagnosis:

**1. PCR**

PCR is widely used in the multiplication of genetic materials of those bacteria or virus that are less in number and the symptoms of the disease are not yet visible. The former is used to detect HIV in suspected AIDS patients. It is also used to detect mutations in gene in suspected cancer patients and also many other genetic disorders.

- It helps to detect very low concentration of bacteria or virus at the time when the symptoms of the disease are not visible by amplification of their nucleic acid.
- PCR is now routinely used to detect HIV in suspected AIDS patient.
- It is being used to detect mutations in genes in suspected cancer patients too.
- It is a powerful technique to identify many other genetic disorders.

**2. ELISA**

Based on the principle of antigen-antibody interaction.

Infection by pathogen can be detected by the presence of antigens such as proteins, glycoproteins, etc or by detecting the antibodies synthesised against the pathogen.

**3.** Many techniques used for early diagnosis based on RDT include RFLP, VNTR, DNA sequencing, etc.

**4. Autoradiography**

It is a method allowing the detection/localisation of radioactive isotope within a biological sample.

**Purpose:**

To demonstrate the distribution of a radiolabled compound within an organism or a cell or to localise the site of incorporation of this compound into the cells or tissues.

Some molecular diagnostic techniques such as RFLP make use of hybridisation probes to detect mutated genes causing disease.

A single stranded DNA or RNA tagged with a radioactive molecules called probe allowed to hybridise to its complementary DNA in a clone of cells followed by detection causing autoradiography. The clone having themutated gene will not appear on the photographic film, because the probe will not have complementarity with the mutated gene.

To detect hybridisation of the probe to its target sequence, the probe is tagged (or labelled) with a molecular marker of either radioactive molecule or fluorescent molecule (more recently). Commonly used marker are $P^{32}$ incorporated into the phosphodiester bond in the probe DNA.

## TRANSGENIC MICROORGANISMS

Various microorganisms, particularly bacteria have been modified through the techniques of genetic engineering to meet specific needs.

1. **Crop Production and Protection:** Several bacteria have been modified by introduction of foreign genes to control, (i) insects by production of endotoxins, (ii) fungal disease by production of chitinases, which suppress fungal flora in the soil and (iii) by production of antibiotic which will degrade the toxin produced by pathogen. There are also positive measures where the $N_2$ fixing efficiency of bacteria *Rhizobia* can be increased by transfer of useful nif genes. (nif means nitrogen fixation)
2. **Biodegradation of Xenobiotics and Toxic Wastes:** Bacteria can be modified genetically for degradation of xenobiotics (Wastes from nonbiological systems) and other waste material. Bacterial genes for this purpose are isolated from bacteria found at waste sites. For example bacteria *Pseudomonas* are not very efficient degraders but multiple genes may sometimes be needed for efficient biodegradation. Therefore, for efficient biodegradation, efficient degraders have to be prepared through genetic engineering.
3. **Production of Chemicals and Fuels:** Genetic engineering also has an important impact on microbial production of chemicals and fuels. Examples: (i) genetically engineered strains of *Bacillus amyloliquefaciens* and *Lactobacillus casei* have been prepared for production of amino acids on a large scale (ii) *E. coli* and *Klebsiella planticola* carrying genes from *Z. mobilis* could utilize glucose and xylose to give maximum yield of ethanol.
4. **Living Factory for the Production of Proteins:** In bacteria, genetic engineering turns the bacterium into a living factory for the production of proteins. Examples: The transfer of genes for human insulin, human growth hormone (hGH) and bovine growth hormone.

## TRANSGENIC ANIMALS

Recombinant DNA technology is used to introduce foreign genes in to the genomes of the animal. Animals that have had their DNA manipulated to possess and express an extra (foreign) gene are known as transgenic animals. The genome of their animals has been changed and they can carry genes from other species. Examples of transgenic animals include rats, rabbits, pigs, sheep, cow, monkey and fish although over **95% transgenic animals are mice**.

Why are these transgenic animals being produced?

The two most common reasons are:

Some transgenic animals are produced for specific economic trait.

Other transgenic animals are produced as disease models (animals genetically manipulated to exhibit disease symptoms so that effective treatment can be studied).

| S. No. | Transgenic Animals | Useful application |
|---|---|---|
| 1 | Cow, Sheep, Goat | Therapeutic : human proteins in their milk |
| 2 | Pig | Organ transplantation without risk of rejection |
| 3 | Rabbits | Molecular farming or gene farming. |
| 4 | Mouse | Contains a human gene that causes breast cancer. This enables the researchers to study the very early development of cancer. |
| 5 | Fish (Common Carp, Catfish, salmon, gold fish, Zebra fish, Rainbow trout) | They contain human growth hormone (HGH) They attain a size twice of that shown by nontransgenic fish |
| 6 | Chicken | It contains good quality of food. |

**Polly and Molly** (Born 1997), two **ewes,** were the **mammals** to have been successfully cloned from an adult somatic cell and to be **transgenic** animals at the same time.

**Dolly the sheep** was the first animal (mammal) to be successfully cloned from an adult somatic cell where there was no genetic modification carried out on the adult donor nucleus. Polly and Molly, like Dolly the sheep, were cloned at the **Roslin Institute** in **Edinburgh, Scotland**.
The first transgenic sheep to produce **α-1-antitrypsin** was **Tracy**.

**ANDi** (Transgenic Monkey) was the first genetically modified monkey. The **GFP (green fluorescent protein)** gene was inserted into the monkey's chromosome.

## Application of transgenic animals:

**1. Medicine**

**(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.

**(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.

**2. Agriculture**

**(i) Breeding**: Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g. increased milk production, high growth rate). Traditional breeding is a time-consuming, difficult task. When technology using molecular biology was developed, it became possible to develop traits-in animals in a shorter time and with more precision. In addition, it offers the farmer an easy way to increase yields.

**(ii) Quality**: Transgenic cows exist that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool. In the past, farmers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product.

**(iii) Disease resistance**: Scientists are attempting to produce disease-resistant animals, such as influenza-resistant pigs, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals.

**3. Industrial Applications:**

In 2001, two scientists at Nexia Biotechnologies in Canada introduced spider genes into the cells of lactating goats. The goats began to manufacture silk along with their milk and secrete tiny silk strands from their body. By extracting polymer strands from the milk and weaving them into thread, the scientists can create a light, tough, flexible material that could be used in such applications as military uniforms, medical microsutures, and tennis racket strings.

## Application of Recombinant DNA Technology/Genetic engineering

1. **Molecular Analysis of Diseases:** DNA research has helped in understanding the molecular basis of diseases like sickle cell anaemia, thalassemias, etc.
2. **Production of Proteins in Abundance:** Using recombinant DNA technique several proteins have been produced in abundance for curing the diseases. These are insulin, growth hormone, interferons, vaccines, erythroprotein and blood clotting factors.
3. **Laboratory Diagnostic Application:** rDNA technology makes the diagnosis of many diseases (e.g. AIDS) simple and quick.
4. **Gene Therapy:** The genetic diseases like sickle cell anaemia can be cured through gene therapy.
5. **Prenatal Diagnosis of Diseases:** DNA collected from the amniotic fluid surrounding the foetus can be used for predicting the genetic diseases.
6. **Application of forensic Medicine:** rDNA technology has greatly helped to identify criminals by DNA fingerprinting and settle the disputes of parenthood of children.
7. **Agricultural Application:** rDNA technology is used for developing transgenic plants which resist drought and diseases and increase their productivity. It imporves quality of food.
8. **Industrial Application:** Enzymes synthesized by rDNA technology are used to produce sugars, cheese and detergents.
9. **Application to Animals:** It is used for developing test tube babies to overcome infertility and production of transgenic animals.
10. **Evolution:** rDNA technique is of great use in joining several missing links in the evolution. This is done by amplifying the DNA of extinct animals.

**Other Applications of Genetic engineering**

1. **To improve renewable fuel production:**
   - Enhanced or engineered microorganisms for fermentation of ethanol, other fuels.
   - Engineered microorganisms or plants to manufacture enzymes used in fuel production.
   - Improved algal strains for biofuel production.
   - Selected or engineered plant species with favourable traits for use as improved biofuel feedstock.
2. **Bioremediation:**
   - Bioremediation is the use of **microoraganism** metabolism to remove pollutants.
     ***In situ*** bioremediation involves treating the contaminated material at the site, while ***ex situ*** involves the removal of the contaminated material to be treated elsewhere.
   - ***Pseudomonas putida*** or **super bugs** was developed by A.M. Chakarvorty and is used for **clearing oil spills**.

## Biopatent :

- A patent is the right granted by a government of an inventor to prevent others from commercial use of his invention. When patents are granted for biolgical entities and for products derived from them, these patents are called **biopatent**.
- Biopatents are granted for following:
  (i) Strains of microorganisms
  (ii) Cell lines
  (iii) Genetically modified strains of plants and animals
  (iv) DNA sequences
  (v) The proteins encoded by DNA sequences
  (vi) Various biotechnological procedures
  (vii) Production processes
  (viii) Products
  (ix) Product applications.

- There is an opposition from social groups to the biopatents. These objections are mainly ethical and political. Some biopatents are very broad in their coverage. For example, one patent cover **"all transgenic plants of Brassica family".**

**Advantages of biopatents:**
Direct incentive for genetic engineering.
Iincreased economic growth.

**Disadvantages:**
allows private, monopoly rights over cells, genes, animals and plants. It means that people will not share vital research information because they are afraid that it will be patented by someone else. The people will not research in areas that are dominated by patents. It will lead to research programmes dominated by patentability and profitability rather than need.

## Biopiracy:

- Multinational companies and some organisations exploit and / or patent biological resources or bioresources of other nations without proper authorisation from the countries concerned, this is called **biopiracy**.
- Institutions and companies of industrialised nations are poor in biodiversity and traditional knowledge related to the utilisation of the resources but are rich in technology and financial resources whereas developing countries are rich in biodiversity and traditional knowledge related to bioresources but poor in technology and financial resources.
- All those organisms that can be used to derive commercial benefits represent biological resources. Traditional knowledge related to bioresources in the knowledge developed by various communities over long periods of history, regarding the utilisation of the bioresources.
- ***Pentadiplandra brazzeana*** is a West African plant. It produces **brazzein protein** that is approximately **2000 times as sweet as sugar**. Local people have been using the super-sweet berries of this plant for centuries. But the protein brazzein was patented in U.S.A. The gene encoding brazzein was also isolated, sequenced and patented in U.S.A. It is proposed to transfer the brazzein gene into maize and express it in maize kernels. These Kernels will then be used for the extraction of brazzein. This development could have serious implications for countries exporting large quantities of sugar.

- Institution and companies of industrialised nations are collecting and exploiting the bioresources, as follows
  - **(i)** They are collecting and patenting the genetic resources themselves. For example, a patent granted in U.S.A. covers the entire **'basmati' rice** germplasm indigenous to our country.
  - **(ii)** The bioresources are being analysed for identification of valuable biomolecules. A biomolecule is a compound produced by a living organism. The biomolecules are then patented and used for commercial activities.
  - **(iii)** Useful genes are isolated from the bioresources and patented. These genes are then used to generate commercial products.
  - **(iv)** The traditional knowledge related to bioresources is utilised to achieve the above objectives. In some cases, the traditional knowledge itself may be the subject of a patent.

## Bioethics:

- Ethics involves rules of conduct by which a community regulates its behaviour and decides as to which activity is legitimate and which is not. **Bioethics** includes rules of conduct that may be used to regulate our activites in relation to the biological world.
- The main bioethical concerns pertaining to biotechnology are briefly mentioned as follows
  - **(i)** Introduction of a transgene from one species into another species violates the integrity of species.
  - **(ii)** Biotechnology may pose unforeseen risks to the environment, including risk to biodiversity. Thus it could disturb the existing ecological balance.
  - **(iii)** Transfer of human genes into animals (and vice-versa) dilutes the concept of 'humanness'.
  - **(iv)** When animals are used for production of pharmaceutical proteins, they are virtually reduced to the status of a factory.
  - **(v)** Use of animals in biotechnology causes great suffering to them.
  - **(vi)** It is disrespectful to living beings, and only exploits them for the benefit of human beings.
  - **(vii)** Scientists can not rule out the possibility of other biological damage. It can accidentally create new infectious agents.
- 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 common persons of a particular region / country. There are numerous 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. In 1997, and American company got patent rights one 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 equivalent, 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 immediatedly counter these patent applications, other countries / individuals may encash on our rich legacy and we may not be able to do anything about it.
- 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 introducting GM-organisms for public services.

## Test your Resonance with concept

**1.** Which of the following are transgenic plant:-
(1) Flavr savr & golden rice (2) Bt cotton & Bt brinjal
(3) Both (1) and (2) (4) None of these

**2.** Use of bio-resources by multinational companies & other organization without proper authorization from the countries & people concerned without compensatory payment is known as
(1) Biopiracy (2) Biopatent (3) Bioweapon (4) Bioethics

**3.** During the processing of the prohormone "Proinsulin" into the mature "insulin".
(1) C - peptide is added to proinsulin
(2) C - peptide is removed from proinsulin
(3) B - peptide is added to proinsulin
(4) B - peptide is removed from proinsulin

**4.** An example of gene therapy is
(1) Production of injectable hepatitis B vaccine
(2) Production of vaccines in food crop
(3) Introduction of adenosine deaminase gene in infants affected with SCID
(4) Production of test tube babies through artificial insemination and implantation

**5.** **Assertion :** The antibiotics produced by *Streptomyces* species have found greatest commercial application.
**Reason :** Some of the important life saving antibiotics such as pencillin and polymixin–B are produced by *Streptomyces.*
(1) (2) (3) (4)

### Answers

**1.** (3) **2.** (1) **3.** (2) **4.** (3) **5.** (3)

### Resonate the Concept

1. **Cytoplast:** A protoplast devoid of nucleus prepared by centrifugation or other approaches.
2. **Southern blotting:** In this technique, DNA segments are diagnosed by hybridising them with radioactively labelled DNA or RNA probes.
3. **Northern blotting:** RNA can be identified by labelled DNA or RNA probe.
4. **Western blotting:** It is useful to identify protein with the help of labelled antibody.
5. **Chimeric Gene:** In recombinant DNA technology; a gene constructed by combining coding sequence from one gene with the regulatory sequences of the other gene (usually of a different organism).
6. **Probe:** 15-30 bases long oligonucleotides (RNA, DNA) labelled with 32P nonradioactive label, used to detect complementary nucleotide sequences in recombinant DNA technology, diseases, diagnosis, etc. constitute a probe.
7. **In vitro clonal propagation in plants is characterized by PCR and RAPD.**