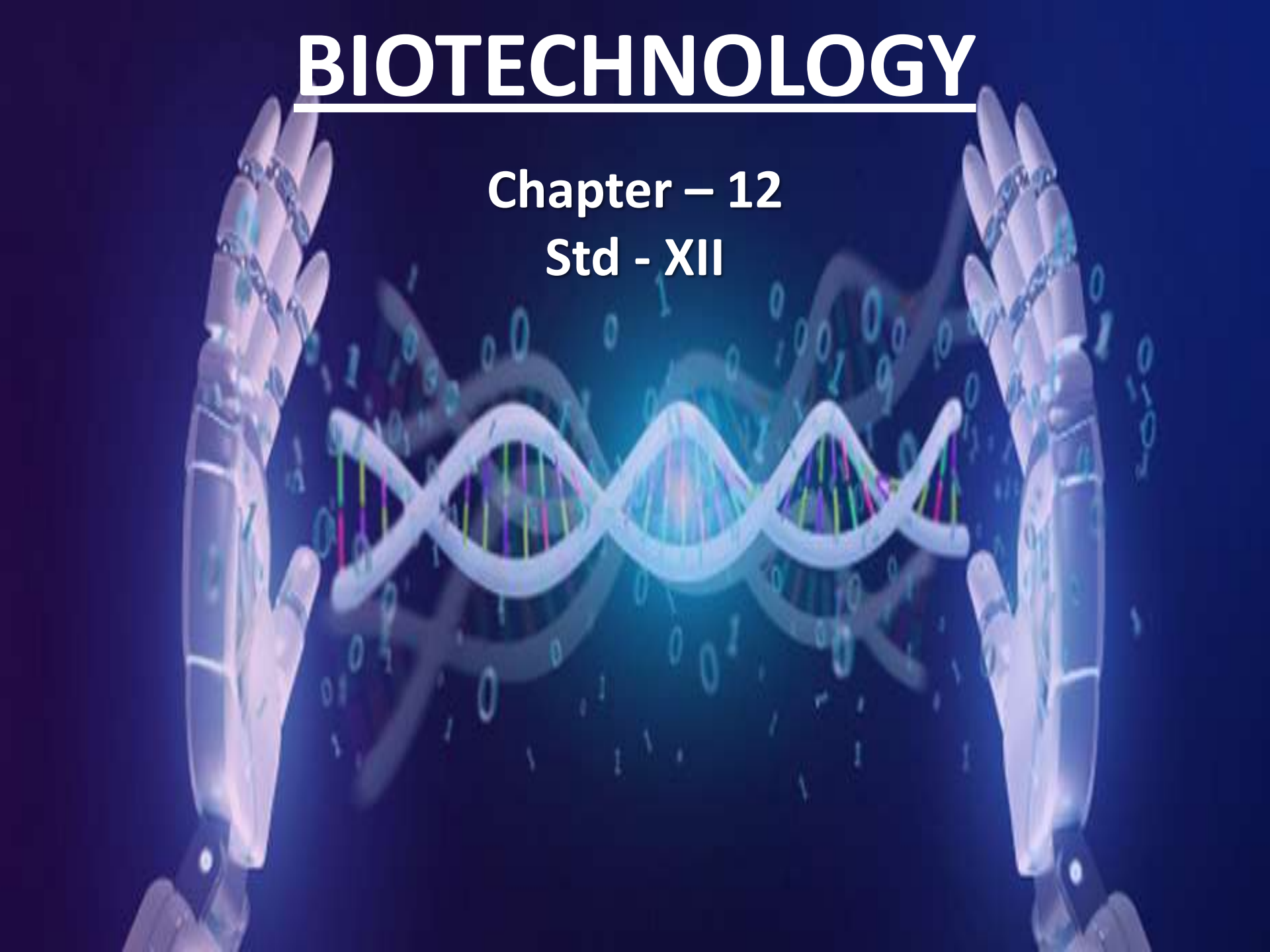
BIOTECHNOLOGY



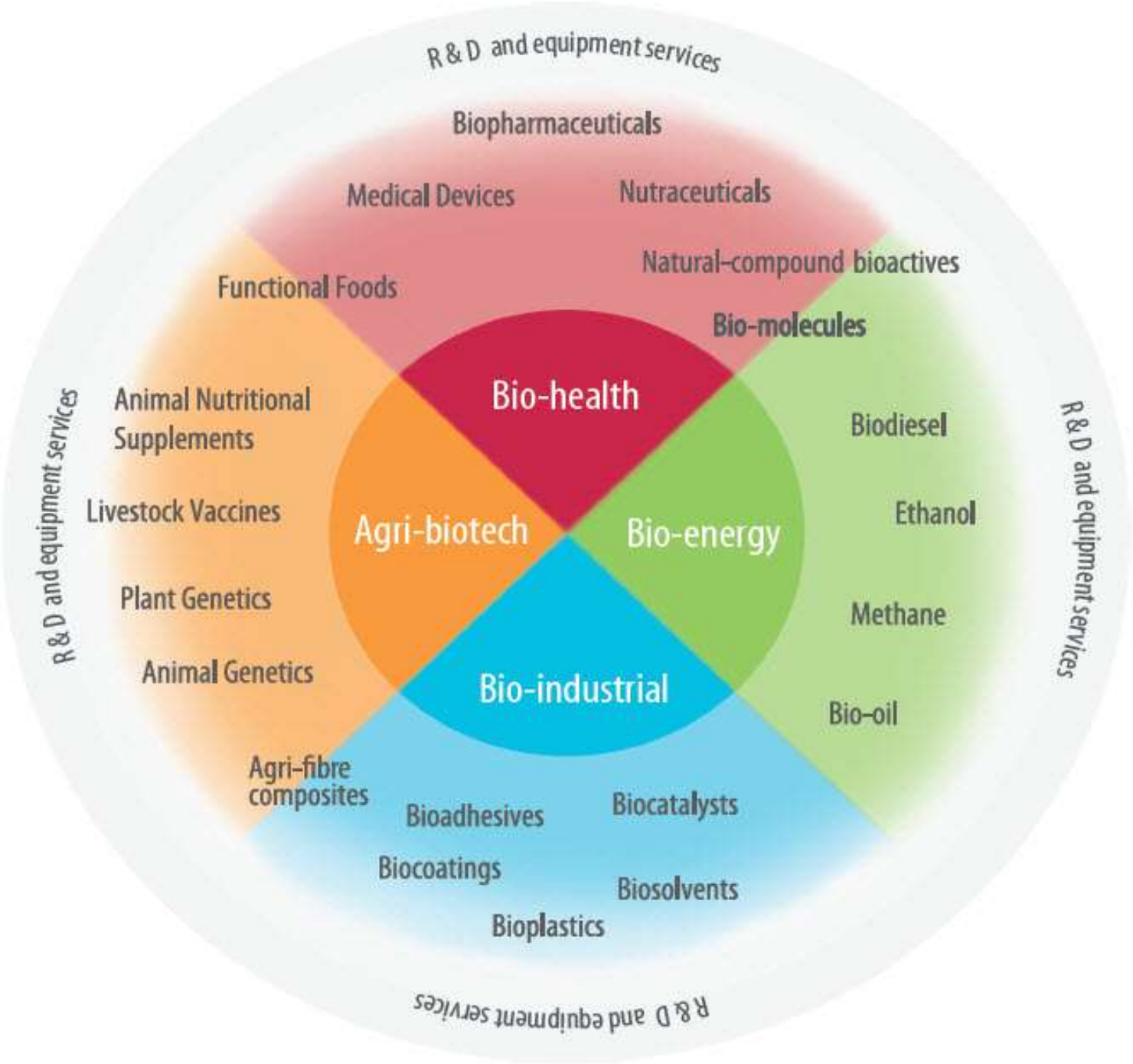
Chapter – 12
Std - XII

BIOTECHNOLOGY

- *It is an applied branch of biology.*
- *The term biotechnology was first used by Karl Ereky in 1919 to describe a process for large scale production of pigs.*

It has been broadly defined as 'the development and utilization of biological forms, products or processes for obtaining maximum benefits to man and other forms of life'.

According to OECD (Organization for Economic Cooperation and Development, 1981)- 'It is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service to the human welfare'.


R & D and equipment services
Biopharmaceuticals
Medical Devices
Nutraceuticals
Natural-compound bioactives
Functional Foods
Bio-molecules
Bio-health
Animal Nutritional Supplements
Biodiesel
Livestock Vaccines
Agri-biotech
Bio-energy
Ethanol
Plant Genetics
Methane
Animal Genetics
Bio-industrial
Bio-oil
Agri-fibre composites
Bioadhesives
Biocatalysts
Biocoatings
Biosolvents
Bioplastics
R & D and equipment services
R & D and equipment services
R & D and equipment services

- *It uses scientific principles of microbiology, genetics, biochemistry, chemical engineering, mathematics, statistics, computers, industrial processes, etc.*
- *Biological agent means plants & animal cells, micro-organisms, enzymes or their products.*
- *History of origin of biotechnology is as old as human civilization.*
- ***Development of biotechnology in terms of its growth, occurred in two phases viz,***
  - ***Traditional biotechnology and***
  - ***Modern biotechnology.***

Traditional biotechnology
(old biotechnology)

- ***It was primarily based on fermentation technology using microorganisms as in the preparation of curd, ghee, soma, vinegar, yogurt, cheese making, wine making, etc.***
- ***It became an art of kitchen in indian houses. It was more an art than science.***
- ***People were not aware as to how exactly the process occurs and the organisms causing this process.***
- ***The contributions made by several chemists, biochemists and microbiologist, over the time, could explain the mechanism of these process and also the nature of microorganisms causing the process.***

Modern biotechnology

- *During 1970 a new technique of 'recombinant DNA technology was developed and then established by Stanley Cohen and Herbert Boyer in 1973.*
- *The technique permits to change/ modify genetic (heritable) material for getting new specific products.*
- *The combination of biology and production technology based on genetic engineering evolved into modern biotechnology (new biotechnology*).

- ***There are two major features of technology that differentiate modern biotechnology from classical or old biotechnology :***

I. ***Capablity of science to change the genetic material for getting new specific products through rDNA technology, polymerase chain reaction (PCR), microarrays, cell culture and fusion, and bioprocessing.***

II. ***Ownership of technology and its socio-political impact.***

- ***Now the conventional industries, pharmaceutical industries, agro industries, food industries, etc. are also focussing attention to produce biotechnology- based products.***

PRINCIPLES AND PROCESSES OF BIOTECHNOLOGY

Modern biotechnology is based on two core techniques :

1. ***Genetic engineering and***
2. ***Chemical engineering.***

- ***Genetic engineering deals with alteration of genetic material (DNA and RNA).***
- ***Chemical engineering deals with maintaining sterile environment for manufacturing variety of useful products including vaccines, antibodies, enzymes, organic acids, vitamins, therapeutics, etc.***

- ***Genetic engineering is defined as the manipulation of genetic material towards a desired end and in a directed and predetermined way, using in vitro process.***

Manipulation of genes involves :

- ***Repairing of the defective genes or replacing of defective genes by healthy genes or normal genes.***
- ***Artificially synthesizing of a totally new gene.***
- ***Transfer of genes into a new location or into a new organism.***
- ***Introducing an altogether new gene.***
- ***Manipulation of genes for improvement of living organisms***
- ***Combining of genes from two organisms.***
- ***Altering the genotype, Gene cloning etc.***

- ***Genetic engineering is alternatively called recombinant DNA technology or gene cloning.***

What genetic engineering mean?

Genetic engineering is the process of manually adding new DNA to an organism. The goal is to add one or more new traits that are not already found in that organism. Examples of genetically engineered (transgenic) organisms currently on the market include plants with resistance to some insects, plants that can tolerate herbicides, and crops with modified oil content .

Examples of Genetic Modification

- OncoMouse
  - Modified Lab Mouse Used for Cancer Research
- Xenotransplantation
  - Transplant of tissues from one species to another.
  - Modified for human acceptance
- GMO's
  - Genetically Modified Organisms
  - Both animals and crops modified to be less susceptible to disease, have higher yields, etc.
  - Biofuel Creation

Technique of gene cloning and rDNA Technology

- *In gene cloning, a gene of known function can be transferred from its normal location into a different cell via a suitable vector.*
- *The transferred gene is replicated normally and is handed over to the next progeny.*

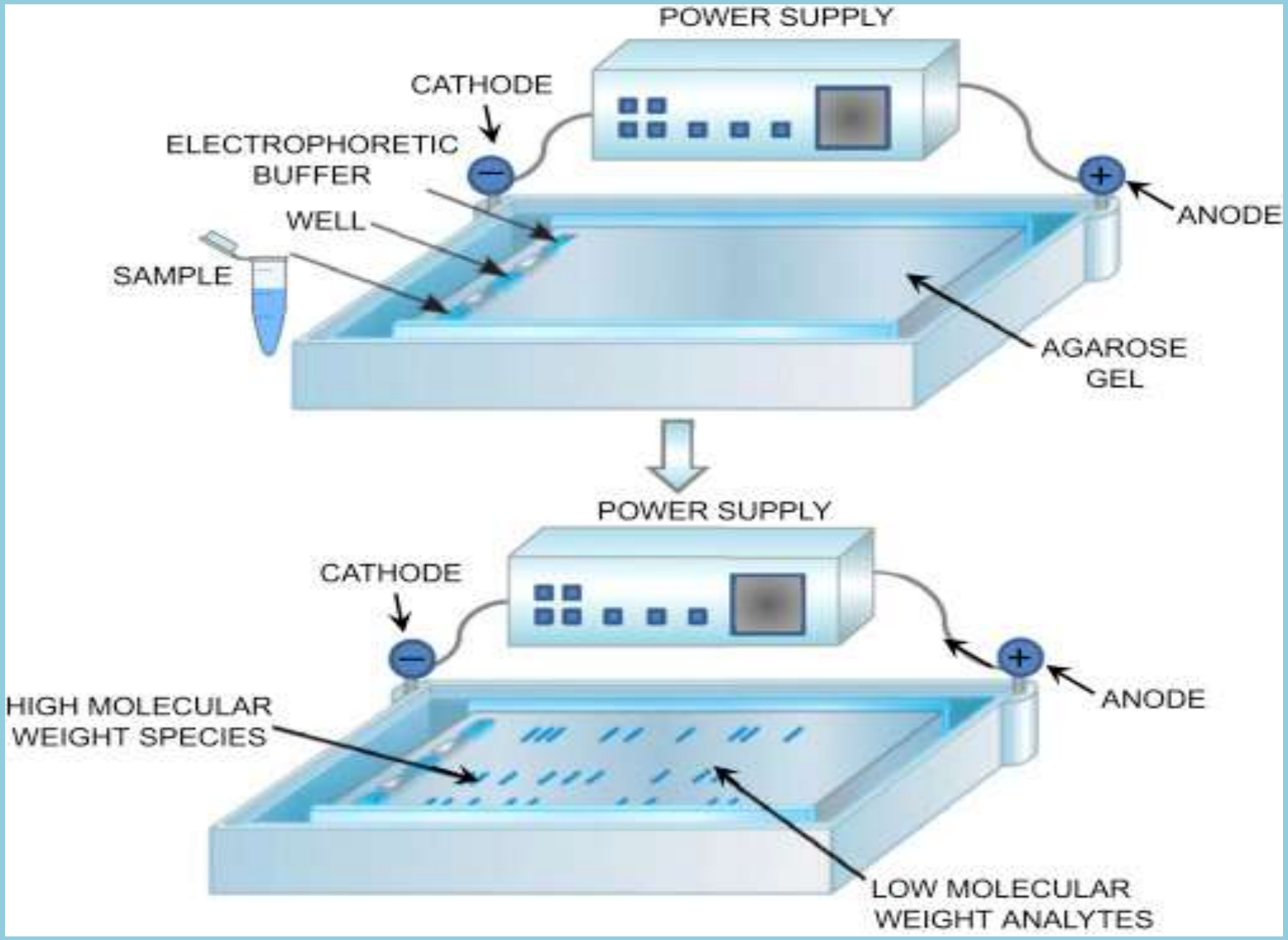
A. Tools and techniques for gene cloning / rDNA technology

The basic requirements for the technique are :

I. DIFFERENT INSTRUMENTS (DEVICES) :

- ***Macromolecule such as DNA, RNA, proteins are synthesized in the living cells which vary in their molecular weight, solubility, presence of charges, absorbance of light, etc.***
- ***Several techniques are used to isolate and characterize the macromolecules.***
- ***The size of different types of molecules varies and therefore their molecular weights also vary.***
- ***The techniques used on the basis of molecular weight, are gel permeation, osmotic pressure, ion exchange chromatography, spectroscopy, mass spectrometry, electrophoresis, etc.***

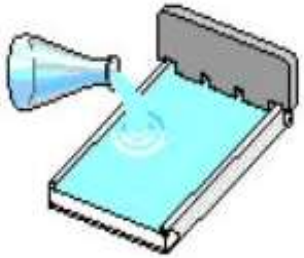
- *Electrophoresis is the separation of charged molecules, by applying an electric field.*
- *It is applied for the separation of DNA, RNA and proteins.*
- *DNA being negatively charged, migrates to anode.*
- *Small fragments of DNA molecules, move faster and thus separate faster.*
- *Use of Agar-rose gel electrophoresis, PAGE, SDA PAGE are the different methods of electrophoresis.*


POWER SUPPLY
CATHODE
ELECTROPHORETIC BUFFER
WELL
SAMPLE
ANODE
AGAROSE GEL
POWER SUPPLY
CATHODE
ANODE
HIGH MOLECULAR WEIGHT SPECIES
LOW MOLECULAR WEIGHT ANALYTES

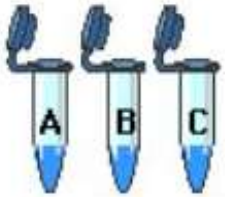
Gel Electrophoresis

- **Conclusions**

1. Make gel.



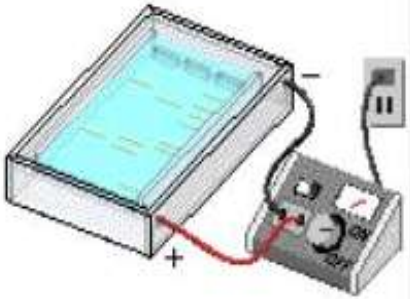
2. Obtain prepared DNA samples.



3. Load samples into gel.

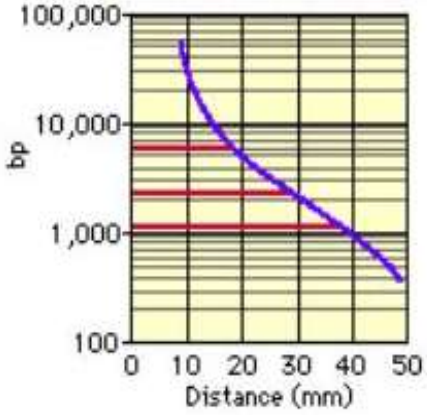
DNA = negatively charged

4. Separate fragments by electrophoresis.

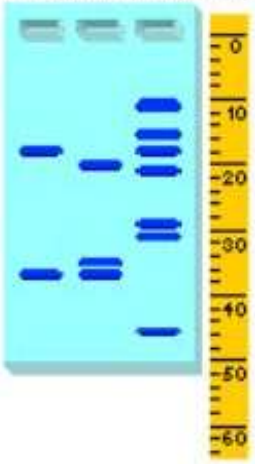


correlate distance to size

6. Prepare a standard curve. Determine fragment sizes.



5. Stain DNA fragments and measure distances.



smaller fragments travel faster & therefore farther

Polymerase chain reaction (PCR)

- *Polymerase chain reaction (PCR) is a device used for gene cloning or gene multiplication in vitro.*
- *Amplification of gene of interest, through PCR.*
- *Discovered by Kary Mullis in 1985.*
- *PCR can generate a billion copies of the desired segment of DNA or RNA, with high accuracy and specificity, in a matter of few hours.*
- *The process of PCR is completely automated and involves automatic thermal cycles for denaturation and re-naturation of double stranded DNA.*

- ***The device required for PCR is called thermal cycler.***
- ***PCR is in-vitro amplification of a desired DNA segment.***

It requires :

- ***DNA containing the desired segment to be amplified.***
- ***Several molecules of four deoxy-ribonuclueoside triphosphates (dNTPs).***
- ***Excess of two primer molecules.***
- ***Heat stable DNA polymerase and***
- ***Appropriate quantities of Mg++ ions.***


Applied Biosystems
GeneAmp® PCR System 9700
Copyright © 1996
Version 2.11
F1
F2
F3
F4
F5
POWER
STOP
1
2
3
4
5
6
7
8
9
ENTER
0
CE
GeneAmp®
PCR System 9700
Applied Biosystems

Mechanism of PCR

- ***At the start of PCR, the DNA segment, and excess of two primer molecules, four deoxyribo-nucleosides triphosphates and the thermostable DNA polymerase are mixed together in 'eppendorf tube' and the following operations are performed sequentially :***

Step i : ***The reaction mixture is heated to a temperature (90–98^{o} C) to separate two strands of desired DNA.***
This is called denaturation.

Step ii : ***The mixture is allowed to cool (40–60° C) that permits pairing of the primer to the complementary sequences in DNA. This step is called annealing.***

Step iii : ***The temperature (70–75° C) allows thermostable Taq DNA polymerase to use single-stranded DNA as template and adds nucleotides. This is called primer extension.***

It takes arround two minutes duration.

- ***One cycle takes arround 3 to 4 minutes.***
- ***To begin second cycle, DNA is again heated to convert double stranded DNA into single strands.***

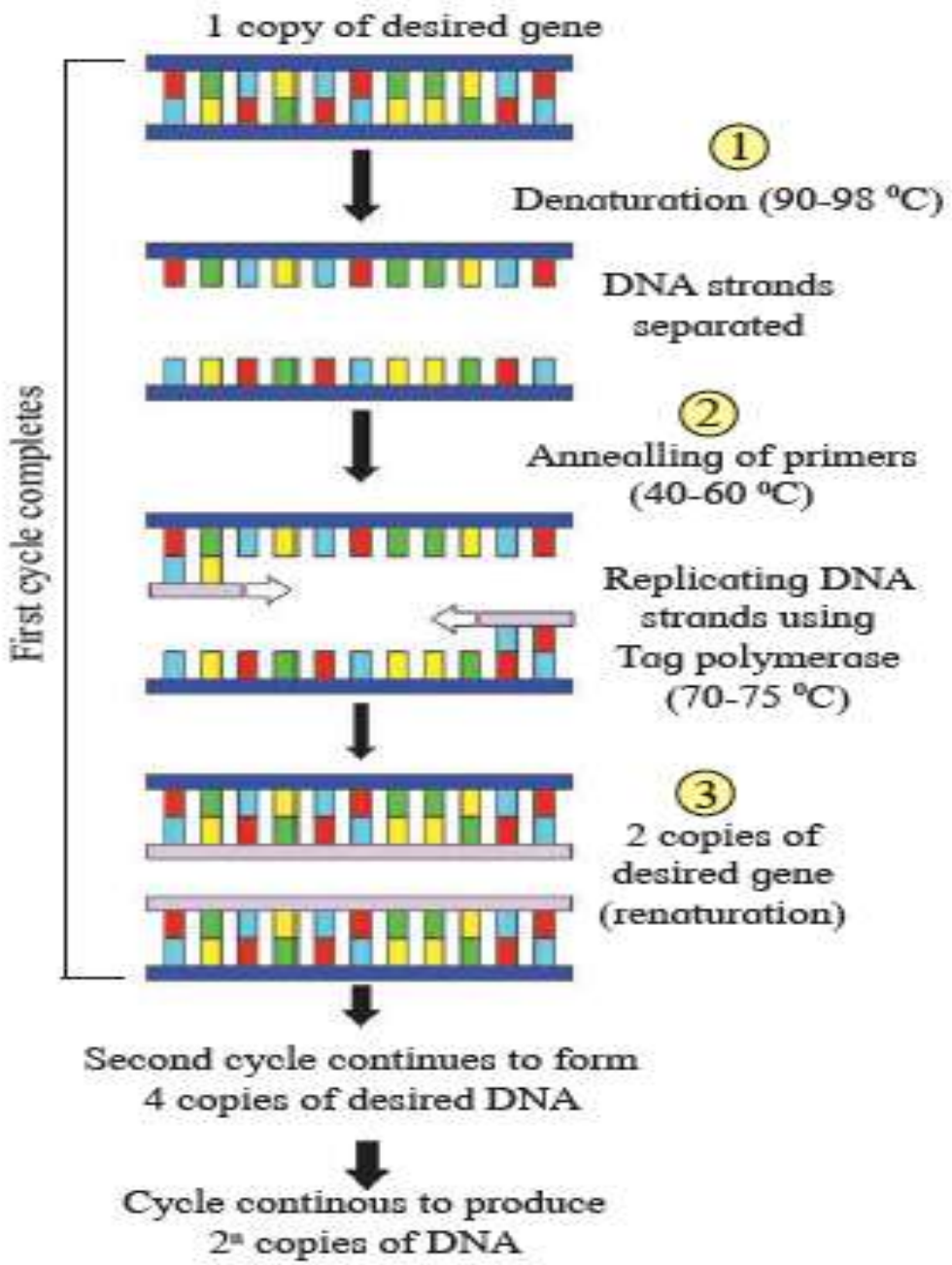
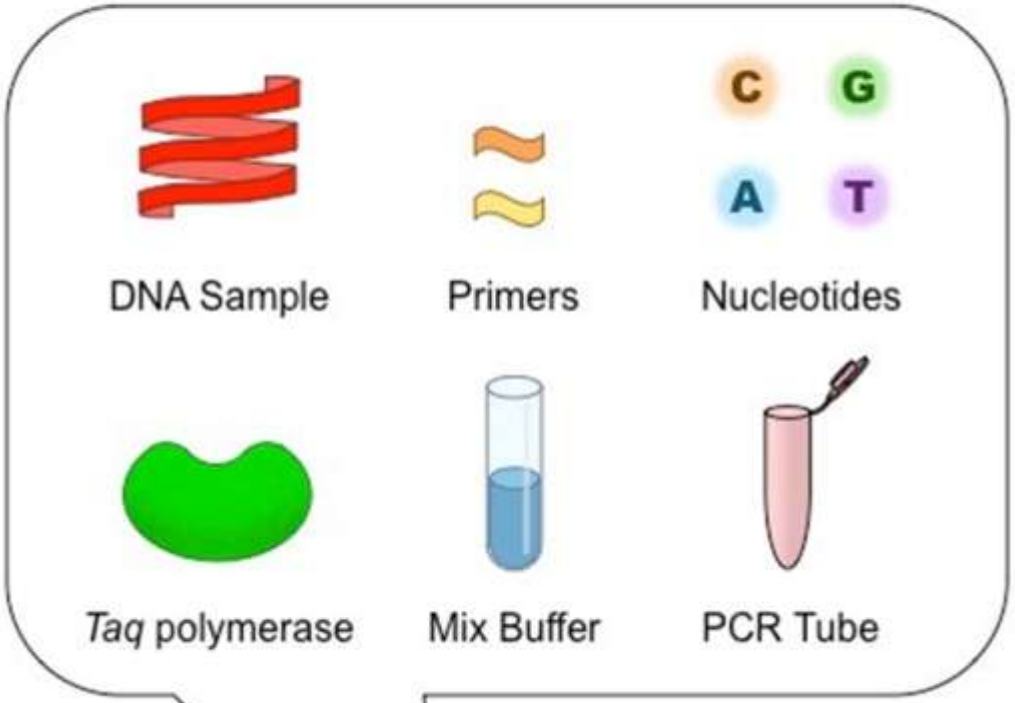


Fig. 12.1 : DNA replication through polymerase chain reaction.

T. aquaticus is a bacterium that lives in hot springs and hydrothermal vents, and Taq polymerase was identified as an enzyme able to withstand the protein-denaturing conditions (high temperature) required during PCR. Therefore, it replaced the DNA polymerase from E. coli originally used in PCR.

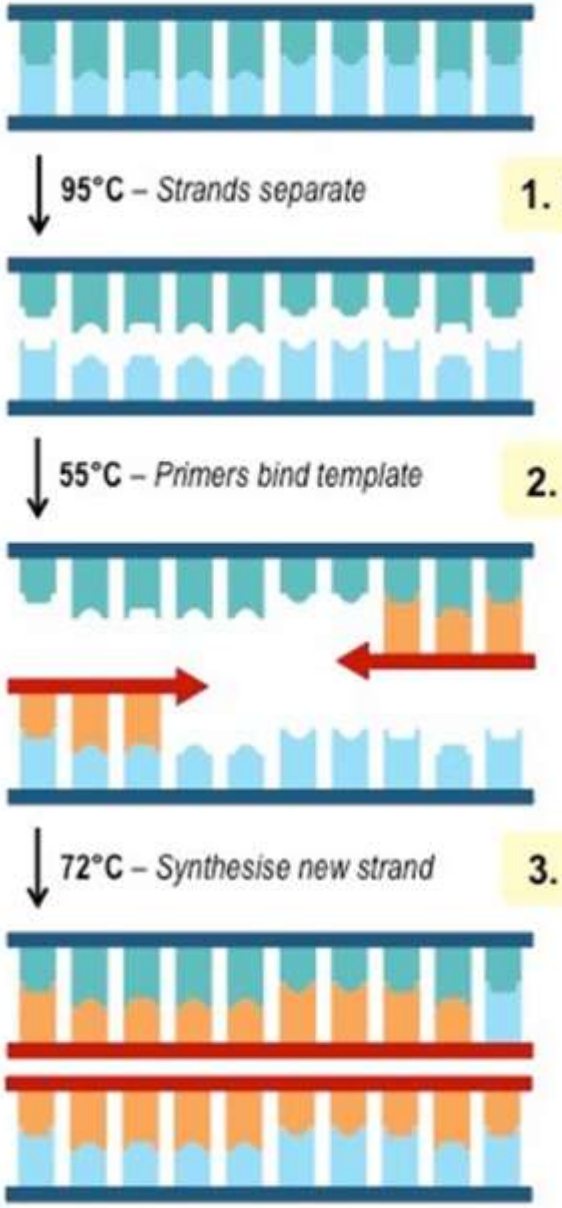
PCR Components



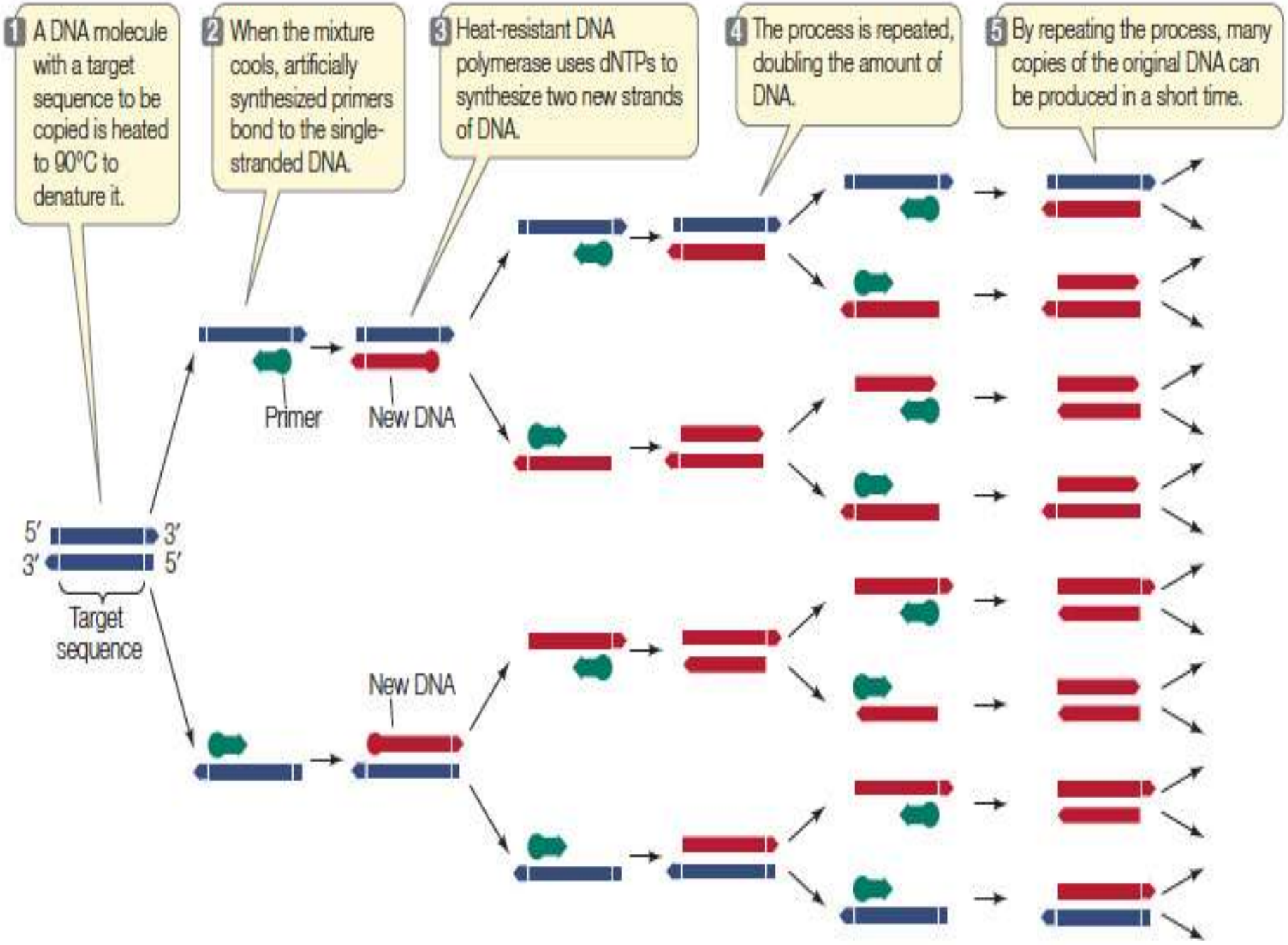
Thermal Cycler

PCR Cycle

PCR Process (ONE Cycle)



- *In an automatic thermal cycler, the given three steps are automatically repeated 20-30 times.*
- *Thus, at the end of 'n' cycles 2n copies of DNA segments, are produced.*
- *The machine performs the entire operations automatically and precisely.*
- *Once the desired number of cycles is completed, the amplified DNA segment is purified by gel electrophoresis.*
- *After its sequencing, the amplified DNA segment can be inserted into a cloning vector.*
- *Desired gene can also be obtained from gene library.*


1 A DNA molecule with a target sequence to be copied is heated to 90°C to denature it.
2 When the mixture cools, artificially synthesized primers bond to the single-stranded DNA.
3 Heat-resistant DNA polymerase uses dNTPs to synthesize two new strands of DNA.
4 The process is repeated, doubling the amount of DNA.
5 By repeating the process, many copies of the original DNA can be produced in a short time.
Primer
New DNA
New DNA
5′
3′
3′
5′
Target sequence

II. BIOLOGICAL TOOLS

Three types of biological tools are used viz,

- Enzymes

- Cloning vectors (vehicle DNA) and

- Competent host (cloning organisms)

A. Enzymes : ***Different enzymes include Lysozymes, Nucleases such as exonucleases, endonucleases, restriction endonucleases, DNA ligases, DNA polymerases, alkaline phosphatases, reverse transcriptases, etc.***

i. Restriction enzymes :

- ***Enzymes that cut the phosphodiester bonds of polynucleotide chains are called nuclease.***
- ***There of two types : exonuclease and endonuclease.***

- *Exonucleases cut nucleotides from the ends of DNA strands. Endonuclease cut DNA from within.*
- *During the 1970s, it was found that bacteria contain nucleases that would recognize short nucleotide sequence with duplex DNA and cut.*
- *The phospho-di-ester back bone at highly specific sites on both strands of duplex, is cut by these enzymes, called restriction endonucleases or simply restriction enzymes.*
- *They were given this name because they are used by the bacteria to destroy various viral DNAs that might enter the cell, thereby restricting the potential growth of the virus.*

- ***Thus, restriction enzymes serve as defence mechanism.***
- ***The bacteria protects its own DNA from nucleolytic attack by methylating the bases at susceptible sites, a chemical modification that blocks the action of the enzyme.***
- ***The restriction enzymes are thus the MOLECULAR SCISSORS that are used to recognize and cut DNA at specific sequences.***
- ***The sites recognized by them, are called recognition sequences or recognition sites.***
- ***Different restriction enzymes found in different organisms recognize different nucleotide sequences and therefore cut DNA at different sites.***

Table 12.2 : Source and recognition sequences (indicated by arrow) of various restriction enzymes:

Restriction Enzyme	Source (Organism and strain)	Recognition sequence	Product	End products produced
Alu I	*Arthobacter luteus*	5' ---A- G-↓-C -T--- 3' 3' ---T- C-↑-G-A--- 5'	5'---A-G 3'---T-C C-T---3' G-A---5'	Blunt ends
Bam HI	*Bacillus amyloliquefaciens H*	5'---G-↓-G-A-T-C-C---3' 3'---C-C-T-A-G-↑-G---5'	5'---G G-A-T-C-C---3' 3'---C-G-T-A-G G---5'	Sticky ends
EcoRI	*Escherichia coli* Ry13	5' ---G-↓-A-A-T-T-C---3' 3' ---C-T-T-A-A-↑-G--- 5'	5'---G A-A-T-T-C---3' 3'---C-T-T-A-A G---5'	Sticky ends
Hind II	*H. influenzae* Rd	5'--G-T-C-↓-G-A-C--3' 3'--C-A-G-↑-C-T-G--5'	5'--G-T-C 3'--C-A-G G-A-C--3' C-T-G--5'	Blunt ends

List of some restriction endonucleases and the site at which they cleave DNA

ii. Recognition sequences :

- ***The sequences recognized by restriction enzymes are 4 to 8 nucleotides long and characterized by a particular type of internal symmetry.***
- ***Consider the particular sequence recognized by the enzyme EcoRI .***

3'------ C T T A A G-----5'
5'------ G A A T T C-----3'

- ***When one reads the sequence in opposite direction (3' to 5' or 5' to 3') it is identical/ same.***
- ***A sequence with this type of symmetry is called a PALINDROME.***

- ***When the enzyme EcoRI attacks this palindrome, it breaks each strand at the same site in the sequence, which is indicated by the arrow between the A and G residues.***

3’ ---- C T T A A G -------5’

5’ ---- G A A T T C -------3’

- ***Restriction enzymes either cut straight across the DNA in the region of palindrome to give blunt ends or cuts producing short, single stranded projections at each end of DNA to produce, cohesive or sticky ends or staggered ends.***

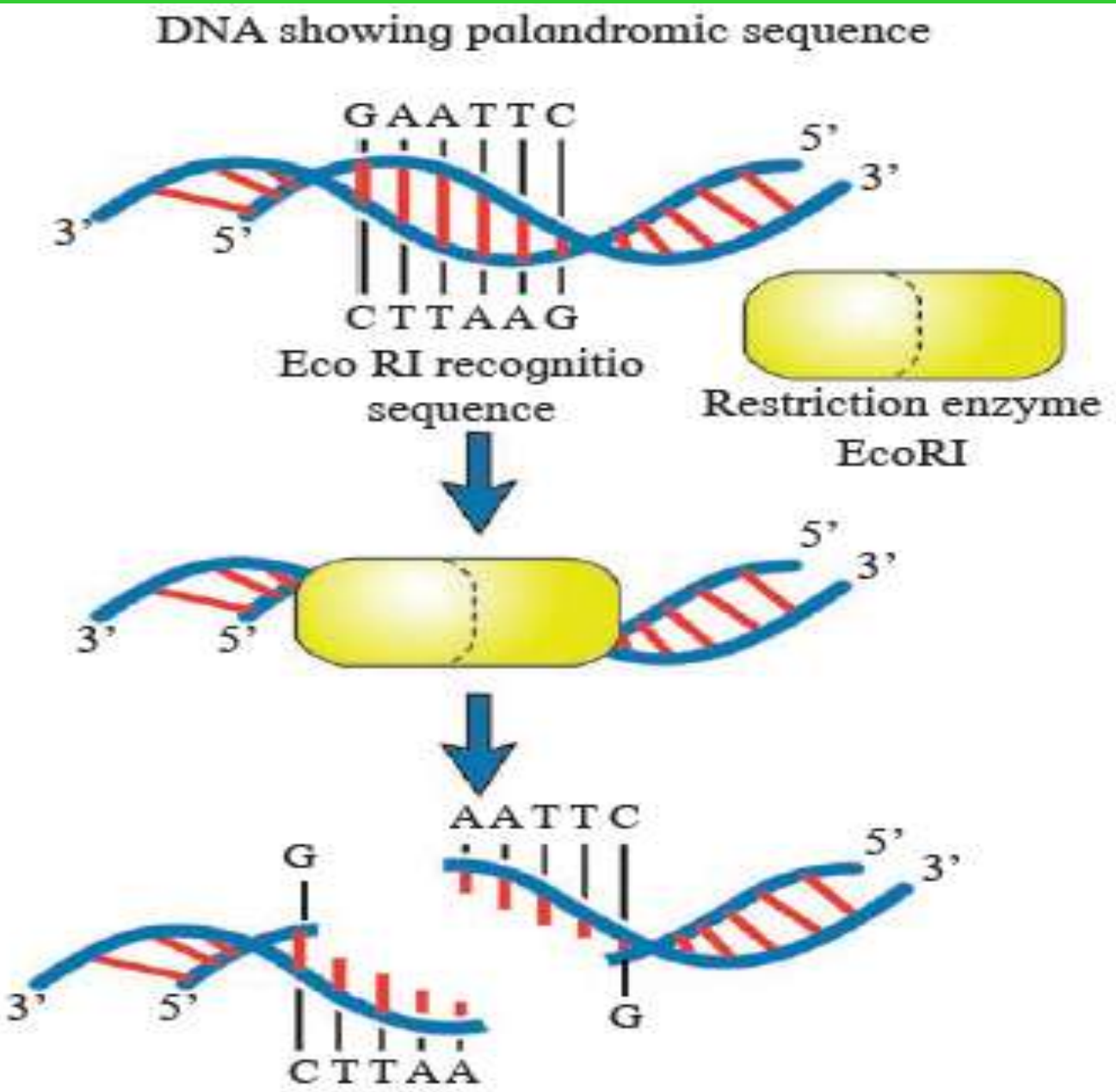


Fig. 12.3 : Mode of action of EcoRI to cleave DNA

- *Smith, Nathan and Arber achieved the discovery of restriction enzymes and for this they were awarded Nobel Prize for physiology and medicine in 1978.*

- *There are three types of restriction enzyme viz,*

Type I *- Which function simultaneously as endonuclease and methylase e.g. E co K.*

Type II *- Which have separate activities for cleaving and methylation. They are more stable and are used in rDNA technology e.g. E co RI, BgII. These enzymes cut DNA at specific sites within the pallindrome.*

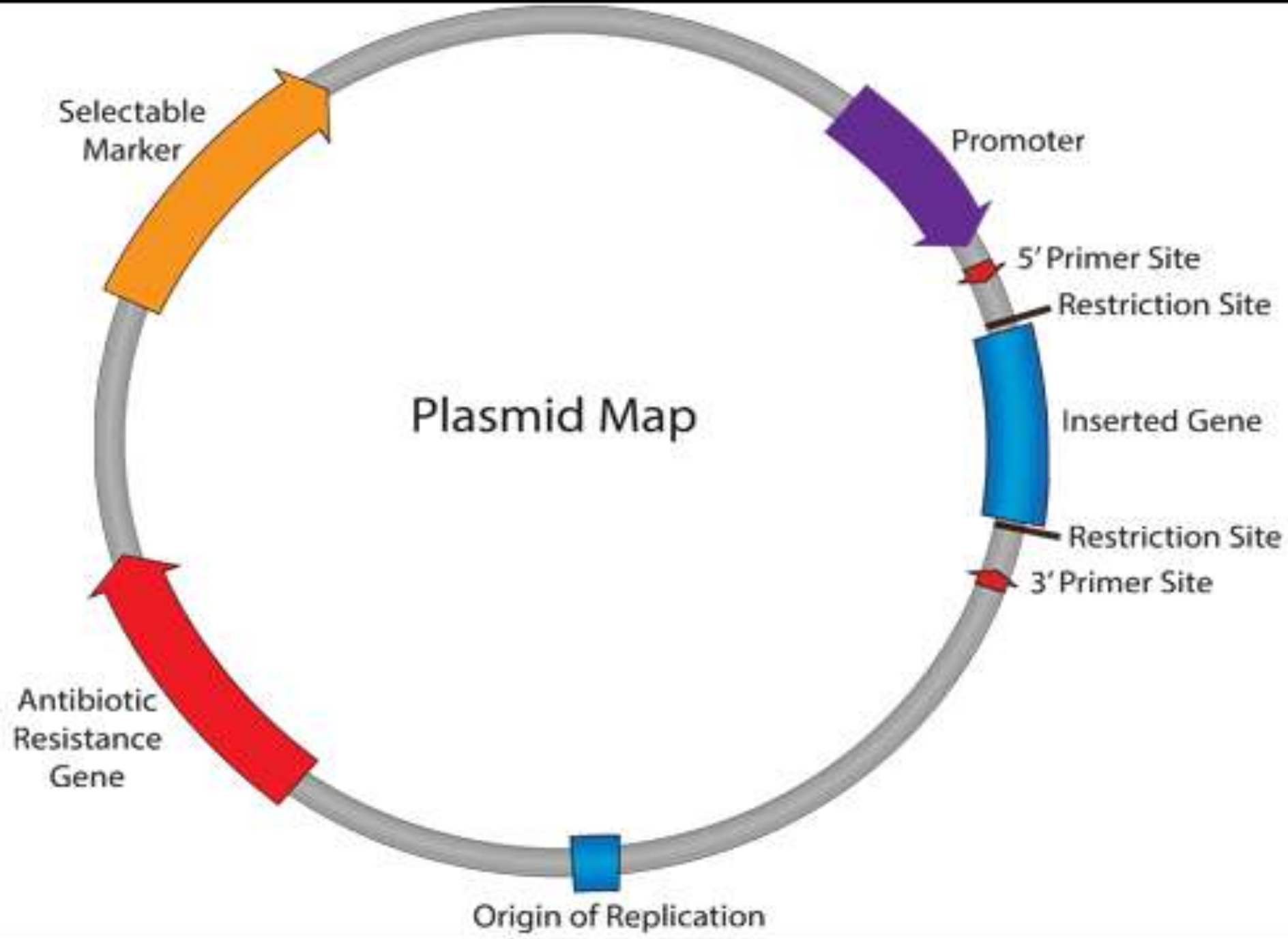
Type III *- Which cut DNA at specific non-pallindromic sequences e.g. HpaI, MboII.*

- *Thousands of type II R. E. enzymes are recognized/discovered.*

B. Cloning vectors (vehicle DNA)

- *Vectors are DNA molecules that carry a foreign DNA segment and replicate inside the host cell. Vectors may be :*
  - *Plasmids*
  - *Bacteriophages (M13, lambda virus)*
  - *Cosmid*
  - *Phagemids.*
  - *BAC (bacterial artificial chromosome)*
  - *YAC (yeast artificial chromosome)*
  - *Transposons*
  - *Baculoviruses and*
  - *Mammalian artificial chromosomes (MACs).*
- *Most commonly used vectors are plasmid vectors (pBR 322, pUC, Ti plasmid) and bacteriophages (lamda phase, M13 phage).*
- *Plasmids and bacteriophages are most commonly used as vectors.*

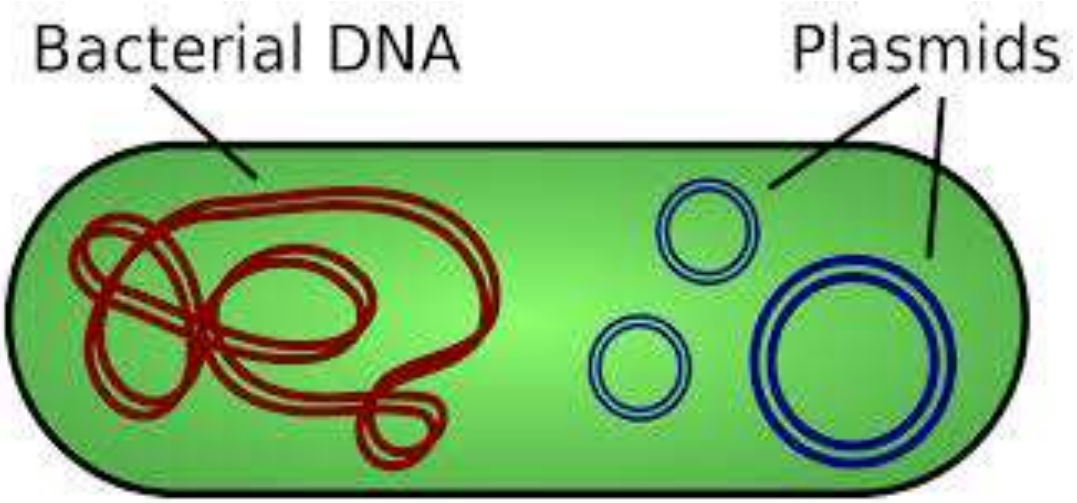
- *A good vector should have the ability of independent replication so that as the vector replicates (through ori gene) large number of copies of the DNA insert will be formed.*
- *Vector should be able to easily introduce into host cells.*
- *It should have marker genes for antibiotic resistance, must contain unique cleavage site in one of the marker genes for restriction enzyme.*
- *It should have at least suitable control elements like promoter, operator, ribosomal binding sites, etc.*
- *The plasmids obtained naturally do not posses all the characteristics. Hence, they are constructed by inserting gene for antibiotic resistance. e.g. pBR 322, pBR 320, pACYC 177 are the constructed plasmids.*
- *pBR 322 is mostly used in rDNA technology in plants.*


Selectable Marker
Promoter
5' Primer Site
Restriction Site
Plasmid Map
Inserted Gene
Restriction Site
3' Primer Site
Antibiotic Resistance Gene
Origin of Replication

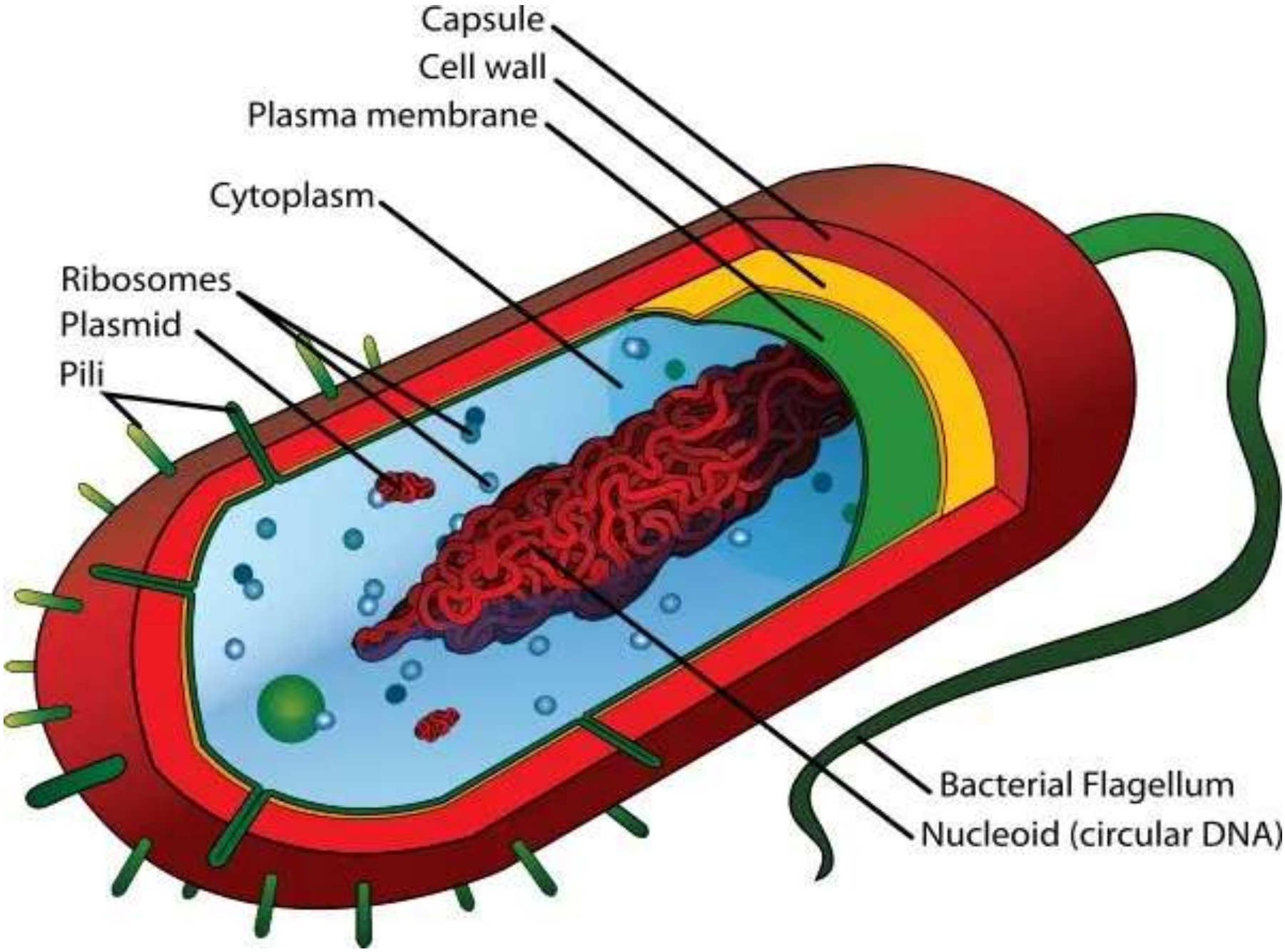
i. PLASMID

*(A **plasmid** is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria, however, **plasmids** are sometimes present in archaea and eukaryotic organisms.)*

- ***The plasmids most commonly used in recombinant DNA technology are those that replicate in E. coli.***



- ***Investigators have engineered these plasmids to optimize their use as vectors in DNA cloning.***


Capsule
Cell wall
Plasma membrane
Cytoplasm
Ribosomes
Plasmid
Pili
Bacterial Flagellum
Nucleoid (circular DNA)

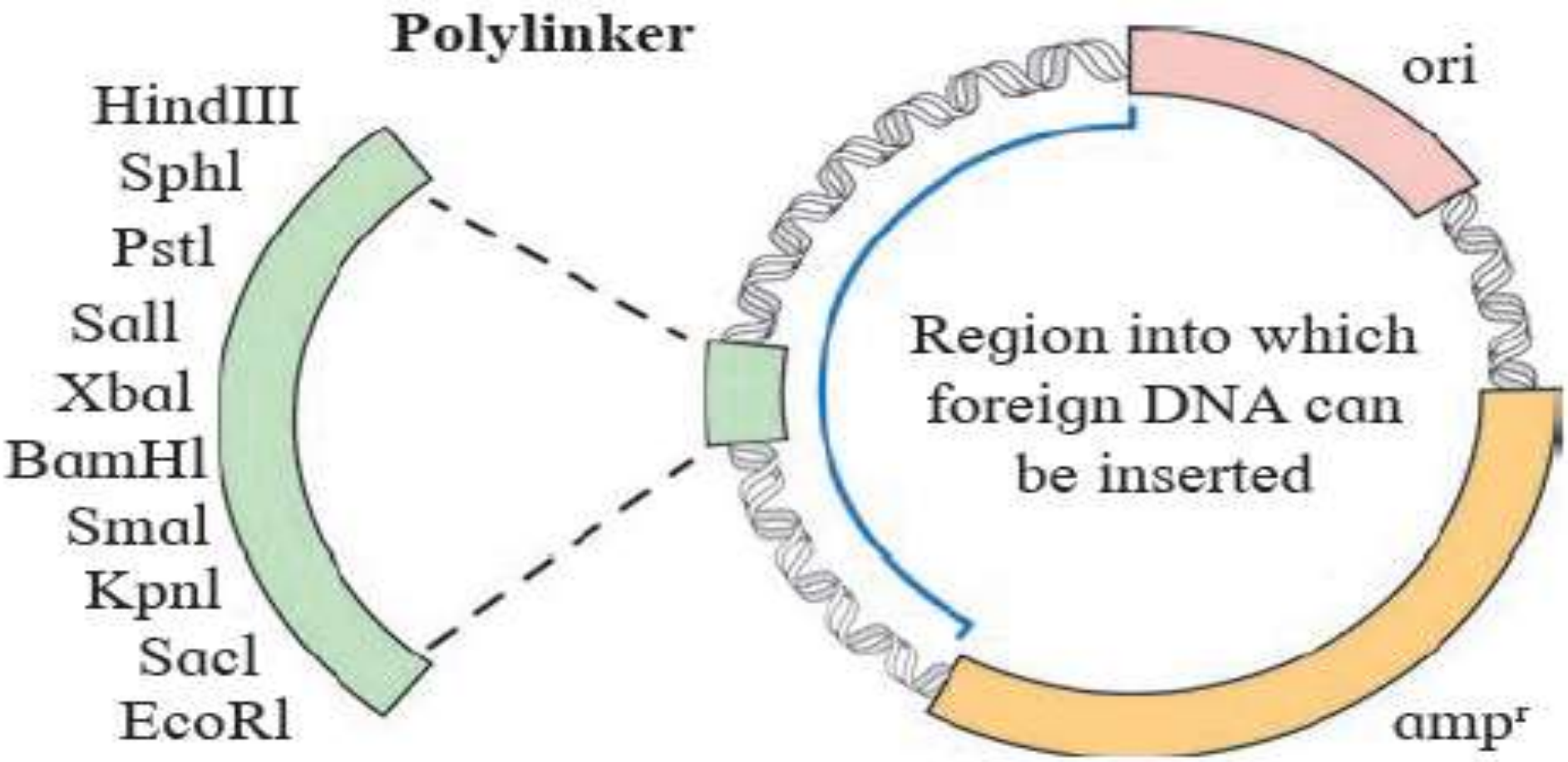
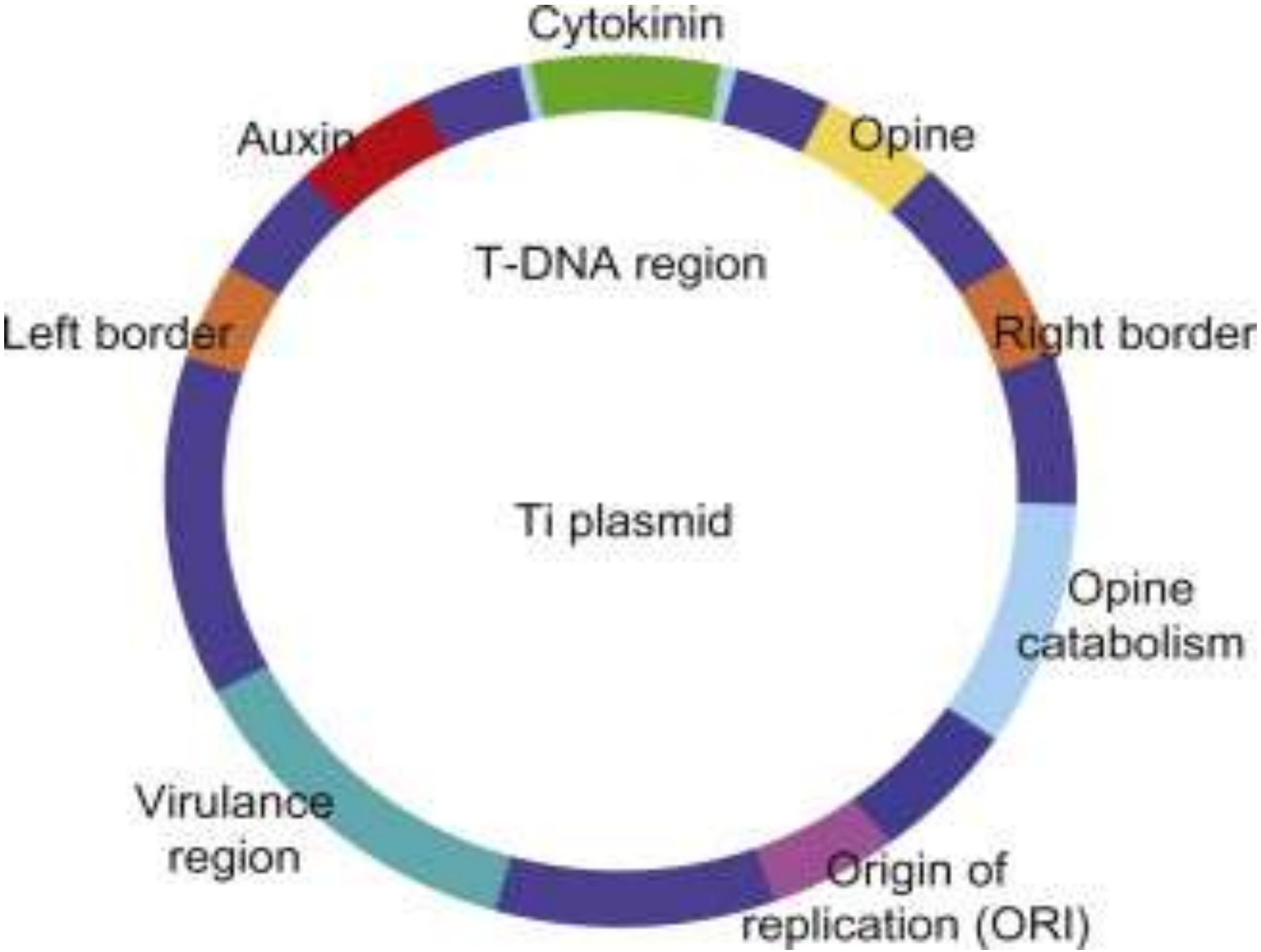


Fig. 12.4 : Plasmid cloning vector showing a replication origin (ori), a drug resistance gene (amp^r) and a region in which foreign DNA can be inserted.

ii. PLASMID VECTORS FOR PLANTS

- *An important vector for carrying new DNA into many types of plants is a plasmid that is found in Agrobacterium tumefaciens.*
- *This bacterium lives in the soil and causes a plant disease called crown gall, which is characterized by the presence of over- growths, or tumors, in the plant.*
- *A. tumefaciens contains a plasmid called Ti (for tumor-inducing).*
- *The Ti plasmid contains a transposon, called T DNA, which inserts copies of itself into the chromosomes of infected plant cells.*
- *The transposon, with the new DNA, can still be inserted into the host cell's chromosomes.*
- *A plant cell containing this DNA, can then be grown in culture or induced to form a new, transgenic plant.*


Cytokinin
Auxin
Opine
T-DNA region
Left border
Right border
Ti plasmid
Opine
catabolism
Virulance
region
Origin of
replication (ORI)

C. Competent host

(cloning organism)

Cloning organisms used are usually the bacteria like :

- ***Bacillus haemophilus***
- ***Helicobacter pyroli and***
- ***E.coli.***

Mostly E. coli is used for the transformation with recombinant DNA

METHODOLOGY FOR rDNA TECHNOLOGY

The steps involved in gene cloning are as follows :

a. Isolation of DNA (gene) from the donor organism

i. The desired gene to be cloned has to be obtained from the source organism (donor).

- *Initially the cells of the donor organism are sheared with the blender and treated with suitable detergent.*
- *Genetic material from the donor is removed, isolated and purified by using several techniques.*
- *Isolated DNA can be spooled on to a glass rod.*

ii. Isolated purified DNA is then cleaved by using restriction enzymes particularly Restriction Endonucleases (RE).

- ***These enzymes cleave DNA at specific sites, called restriction sites and break the DNA into fragments.***
- ***There are several types of restriction endonucleases.***
- ***Cleaved DNA fragments have cohesive, sticky, staggered ends or blunt ends.***

- ***From cleaved DNA fragments, a fragment containing desired gene is isolated and selected for cloning.***
- ***This is now called foreign DNA or passenger DNA.***
- ***A desired gene can also be obtained directly from genomic library or cDNA library.***

- *Gene library is a collection of different DNA sequences from an organism where each sequence has been cloned into a vector for ease of purification, storage and analysis.*

There are two types of gene libraries on the basis of the source of DNA used.

A. Genomic library :

- *It is a collection of clones that represent the complete genome of an organism.*
- *The genomic library of prokaryotes can be constructed by using plasmid vector.*
- *It is because prokaryotic genome does not contain repetitive DNA.*

B. cDNA library :

- ***It represents the library of eukaryotic organisms only.***
- ***DNA is produced from isolated mRNA by reverse transcription.***
- ***The DNA so made is called complementary DNA (cDNA).***
- ***The library is called cDNA library.***
- ***Eukaryotic DNA genome contains introns, regulatory genes and repetitive DNA.***
- ***Hence, the establishment of genomic library in eukaryotes is not meaningful.***

b. Insertion of desired foreign gene into a cloning vector (vehicle DNA)

- *The foreign DNA or passenger DNA is now inserted into a cloning vector or vehicle DNA.*
- *The most commonly used cloning vectors are plasmids of bacteria (pBR 322) and the bacteriophage viruses like lamda phage and M13.*
- *Plasmids are isolated from the vector organisms i.e. bacterium.*
- *By using same RE (which is used in the isolation of the desired gene from the donor), plasmid i.e. vector DNA is cleaved.*
- *Now by using enzyme DNA ligase, foreign DNA is inserted/ integrated into the vector DNA.*
- *The combination of vector DNA and foreign DNA is now called Recombinant DNA or Chimeric DNA and the technology is referred to as rDNA technology.*

c. Transfer of rDNA into suitable competent host or cloning organism

- *Finally the recombinant DNA is now introduced i.e. transferred for expression into a competent host cell of the suitable cloning organism which is usually a bacterium.*
- *Host cell takes up naked rDNA by process of 'transformation' and incorporates into its own chromosomal DNA which finally expresses the trait controlled by passenger DNA.*
- *The transfer of rDNA into a bacterial cell is assisted by divalent Ca++.*

- ***The cloning organisms used in plant biotechnology are E.coli and Agrobacterium tumifaciens.***
- ***The host/competent cell which has taken up rDNA is now called transformed cell.***
- ***Foreign DNA can also be transferred directly into the naked cell or protoplast of the competent host cell, without using vector.***
- ***This is done by using techniques like electroporation, microinjection, lipofection, shot gun, ultrasonication, biolistic method, etc.***
- ***But in plant biotechnology the transformation is through Ti plasmids of A. tumifaciens.***

Electroporation or electropermeabilization : ***is a microbiology technique in which an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell (also called electrotransfer).***

Microinjection : ***is the use of a glass micropipette to inject a liquid substance at a microscopic or borderline macroscopic level. The target is often a living cell but may also include intercellular space.***

Lipofection (or liposome transfection) : ***is a technique used to inject genetic material into a cell by means of liposomes, which are vesicles that can easily merge with the cell membrane since they are both made of a phospholipid bilayer.***

A shot gun /gene gun or biolistic particle delivery system : is a device used to deliver exogenous DNA (transgenes), RNA, or protein to cells. By coating particles of a heavy metal with a gene of interest and firing these micro-projectiles into cells using mechanical force, an integration of desired genetic information can be induced into cells.

Ultrasonication : based on sonoporation (the rupture of cellular membranes by acoustic waves). It is a non-invasive way to introduce DNA molecules into cells via acoustic cavitation that temporarily changes the permeability of the cell membrane.

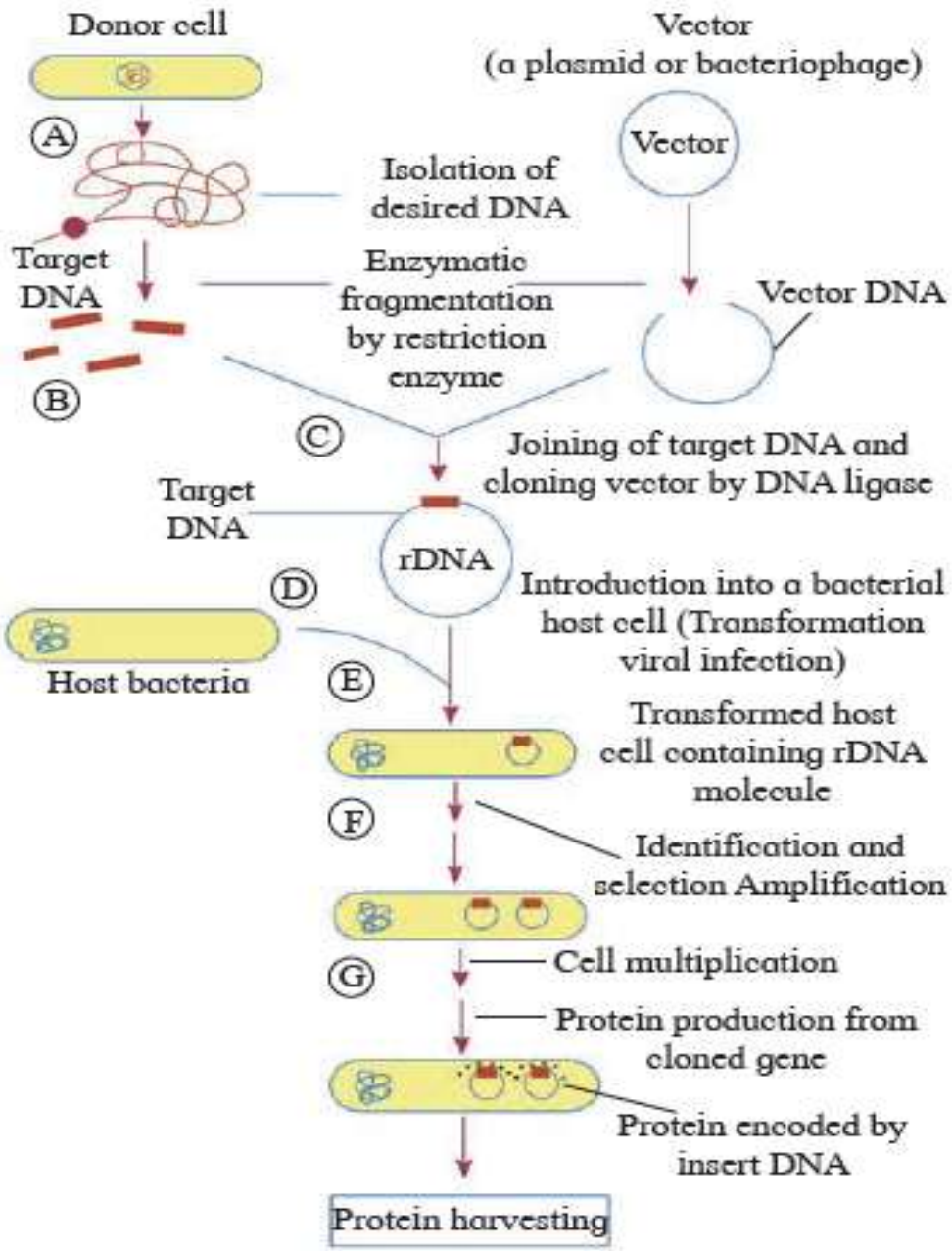


Fig. 12.5 : Outline of the process of recombinant DNA technology

d. Selection of the transformed host cell

- *The transformation process generates a mixed population of transformed (recombinant) and non-transformed (non-recombinant) host cells.*
- ***For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.***
- ***For example, pBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene).***
- ***When pst1 RE is used, it knocks out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.***

e. Multiplication of transformed host cell

- *Once transformed, host cells are separated by the screening process.*
- *In this step the transformed host cells are introduced into fresh culture media.*
- *At this stage the host cells divide and re-divide along with the replication of the recombinant DNA carried by them.*

f. Expression of the gene to obtain the desired product

- *The next step involves the production of desired products like alcohol, enzymes, antibiotics, etc.*

- *Finally the desired product is separated and purified through downstream processing using suitable bioreactor.*

APPLICATIONS OF BIOTECHNOLOGY

Biotechnology is an umbrella term covering a broad spectrum of scientific applications used in many sectors, such as health and agriculture, industry, environment and genomics.

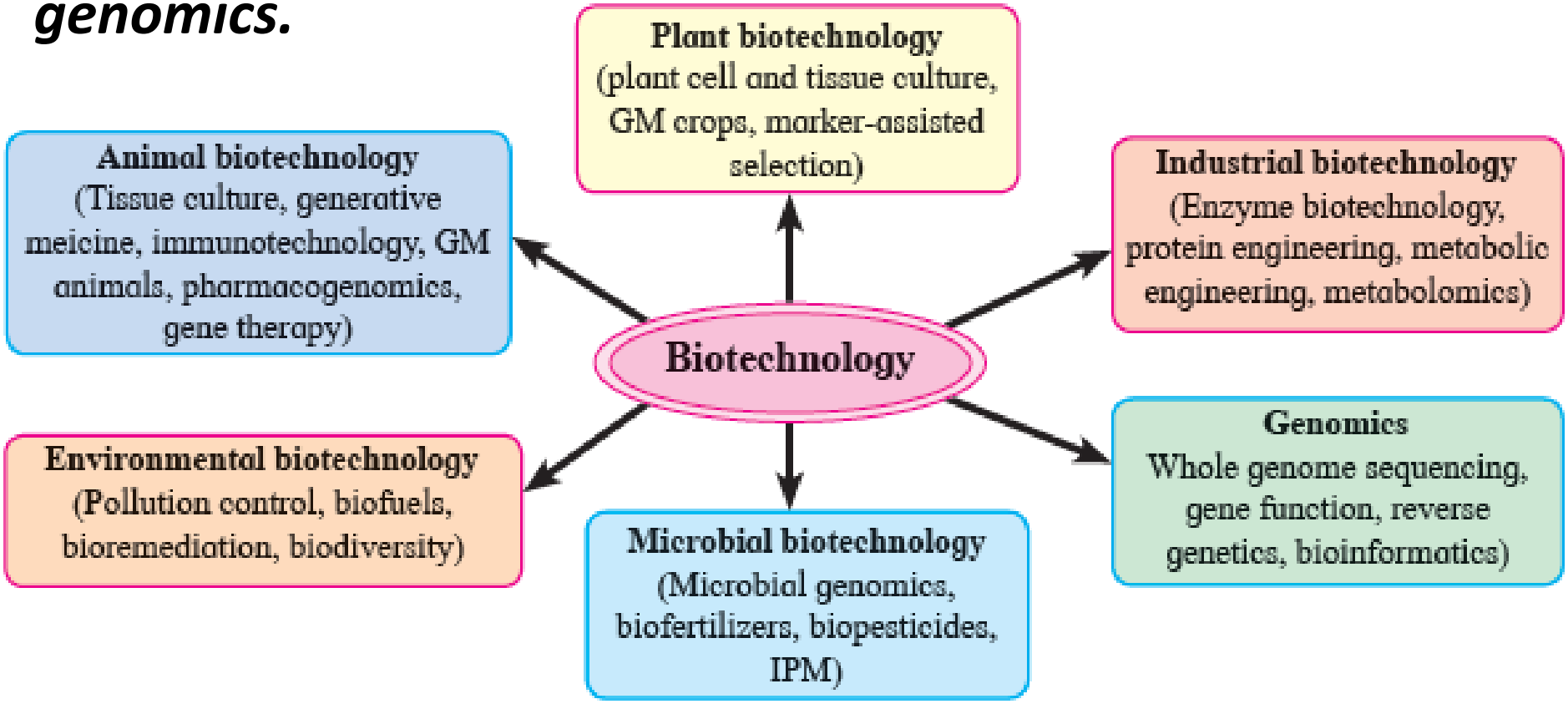


Fig. 12.6 : Applications of Biotechnology

a. Healthcare Biotechnology

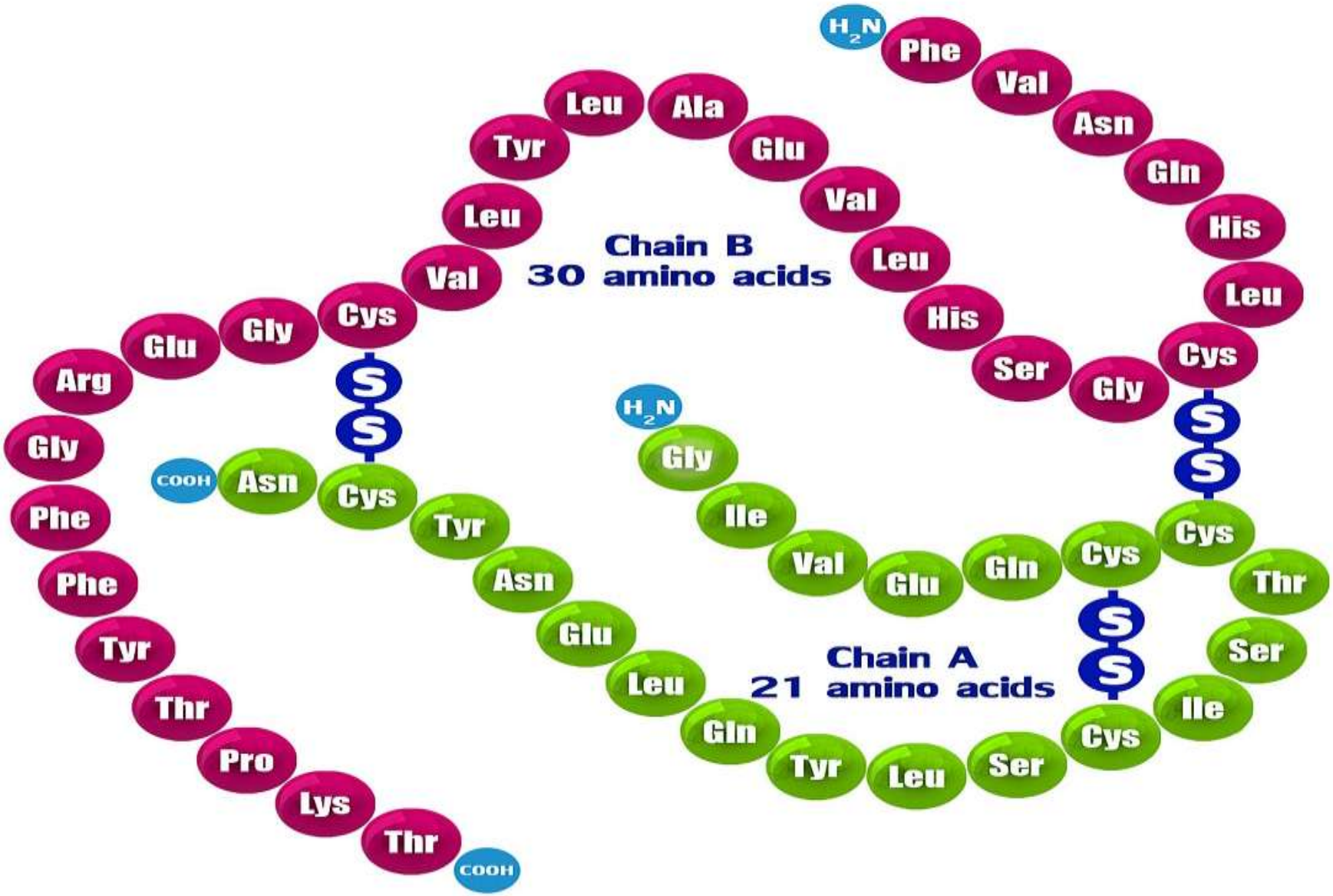
- ***It refers to a medicinal or diagnostic product or a vaccine.***
- ***This technology has a tremendous impact on meeting the needs of patients.***
- ***Biotechnology offers patients a variety of new solutions such as:***

- ***Unique, targeted and personalized therapeutic and diagnostic solutions for organ transplant.***
- ***Stem cell technology.***
- ***Genetic counselling,***
- ***Forensic medicine.***
- ***Gene probes.***
- ***Genetic fingerprinting and***
- ***Karyotyping are outcomes of biotechnology.***

HUMAN INSULIN

- *Insulin is a peptide hormone produced by β-cells of islets of Langerhans of pancreas.*
- *It was discovered by sir Edward Sharpey Schafer (1916).*
- *Insulin is essential for the control of blood sugar levels.*
- *Diabetes mellitus is a disease in which some people cannot make insulin themselves.*
- *Hakura et al (1977), chemically synthesized DNA sequence of insulin for two chains A and B and separately inserted into two PBR322 plasmid vector.*

Human Insulin



- ***Insulin production by recombinant DNA technology is designed by Gilbert and Villokomaroff in 1978.***

- ***The genes are inserted by the side of β-galactosidase gene of the plasmid.***

- ***The recombinant plasmids were then separately transformed into E. coli host.***

- ***The host produced penicillinase + pre-pro insulin.***

- ***Insulin is later separated by trypsin treatment.***

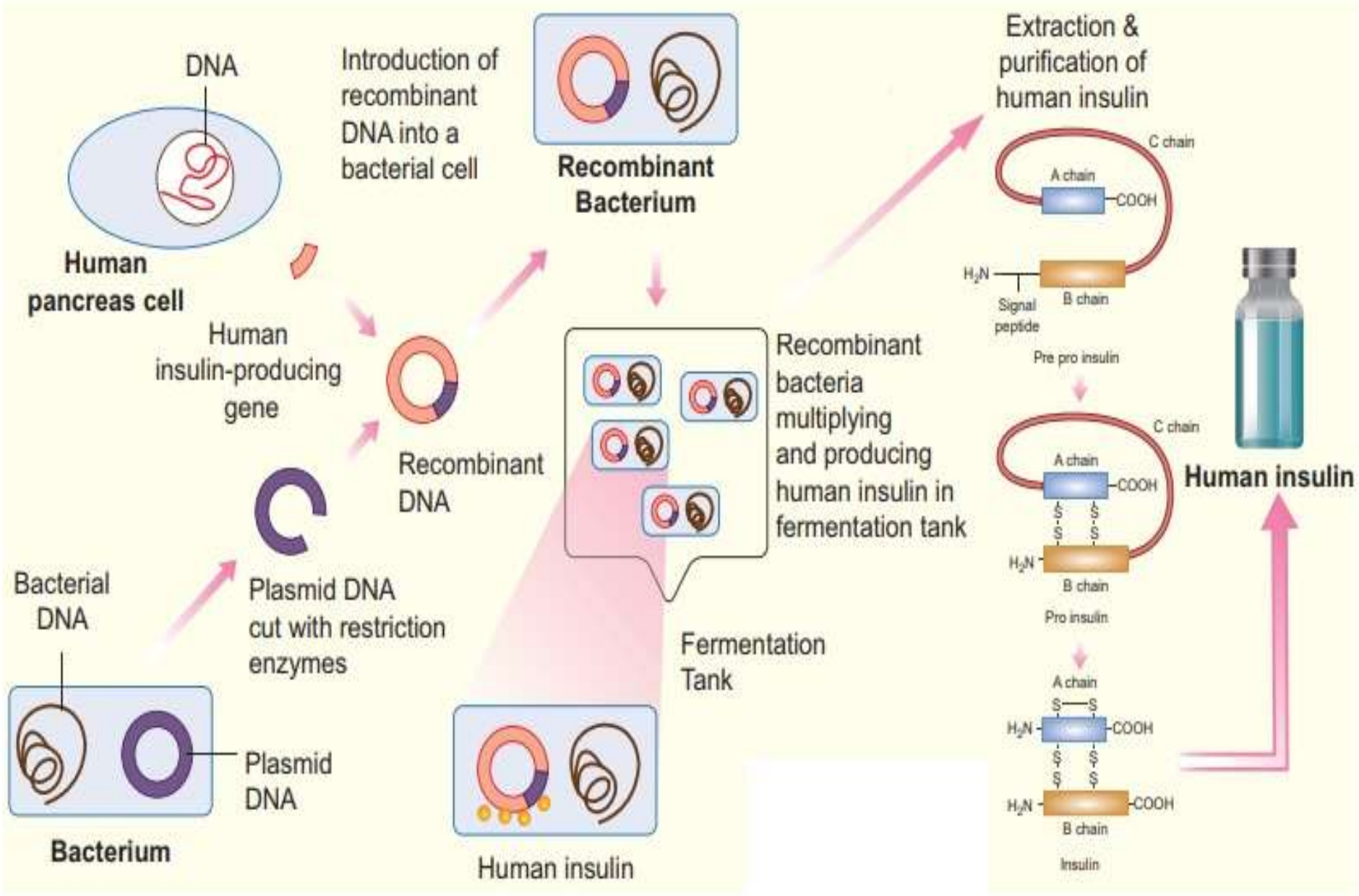


Fig. 10.1 Human Insulin Production

Tabel 12.7 : Human proteins produced by rDNA technology to treat human diseases

Disorder/ Diseases/ Health condition	Recombinant protein(s)
1. Anaemia	Erythropoeitin
2. Asthma	Interleukin-1 receptor
3. Atherosclerosis	Platelet derived growth factor
4. Parturition	Relaxin
5. Blood clots	Tissue plasminogen Activator (TPA) Urokinase
6. Cancer	Interferons, tumour necrosis factor interleukins, macrophage activating factor
7. Diabetes	Insulin
8. Emphysemo	α_1- Antitrypsin
9. Haemophilia A	Factor VIII
10. Haemophilia B	Factor IX
11. Hepatitis B	Hepatitis B vaccine

VACCINE PRODUCTION

- ***A vaccine is a biological preparation that provides active acquired immunity against a certain disease.***
- ***Usually it consists of a biological agent that represents the disease causing microorganism.***
- ***Often made from a weakened or killed form of the microorganism, its toxins or one of its surface protein antigens.***
- ***Biotechnology has offered modern diagnostic test kits- rickettsial, bacterial and viral vaccines along with radio-labelled biological therapeutics for imaging and analysis.***

Journey of vaccine: a complex manufacturing process

1 BACTERIA, VIRUS OR CELL CULTURE

2 HARVESTING

3 PURIFICATION

4 INACTIVATION

5 VALENCE ASSEMBLY

6 FORMULATION

7 FILLING

8 FREEZE-DRYING

9 PACKAGING

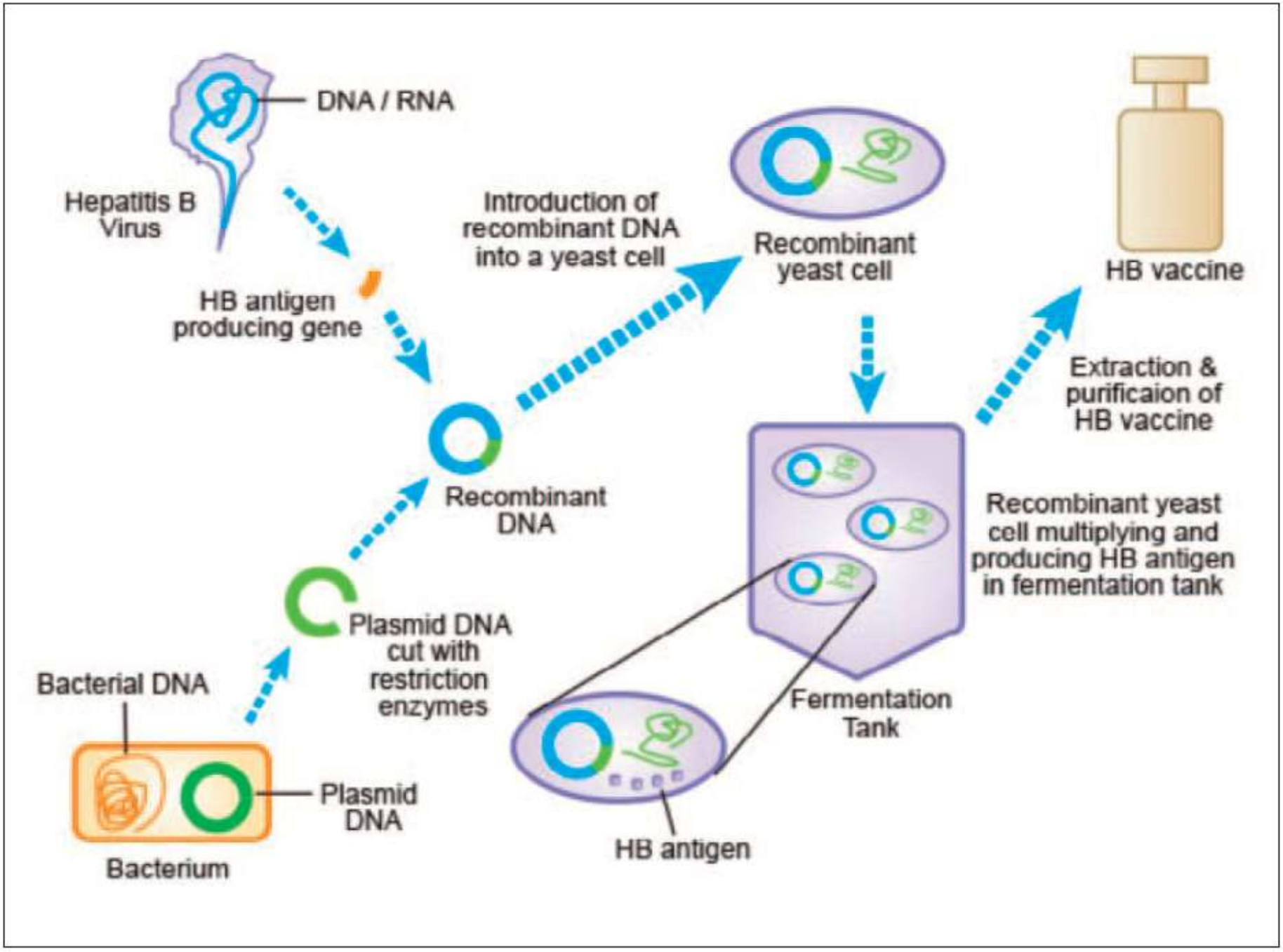
10 BATCH RELEASE

11 TRANSPORT

- ***Vaccines have eliminated small pox, polio and other deadly diseases for the last several decades.***

- ***Biotechnology has made advancements in vaccination by making recombinant vaccines that have the potential to eradicate non-communicable diseases like cancer.***

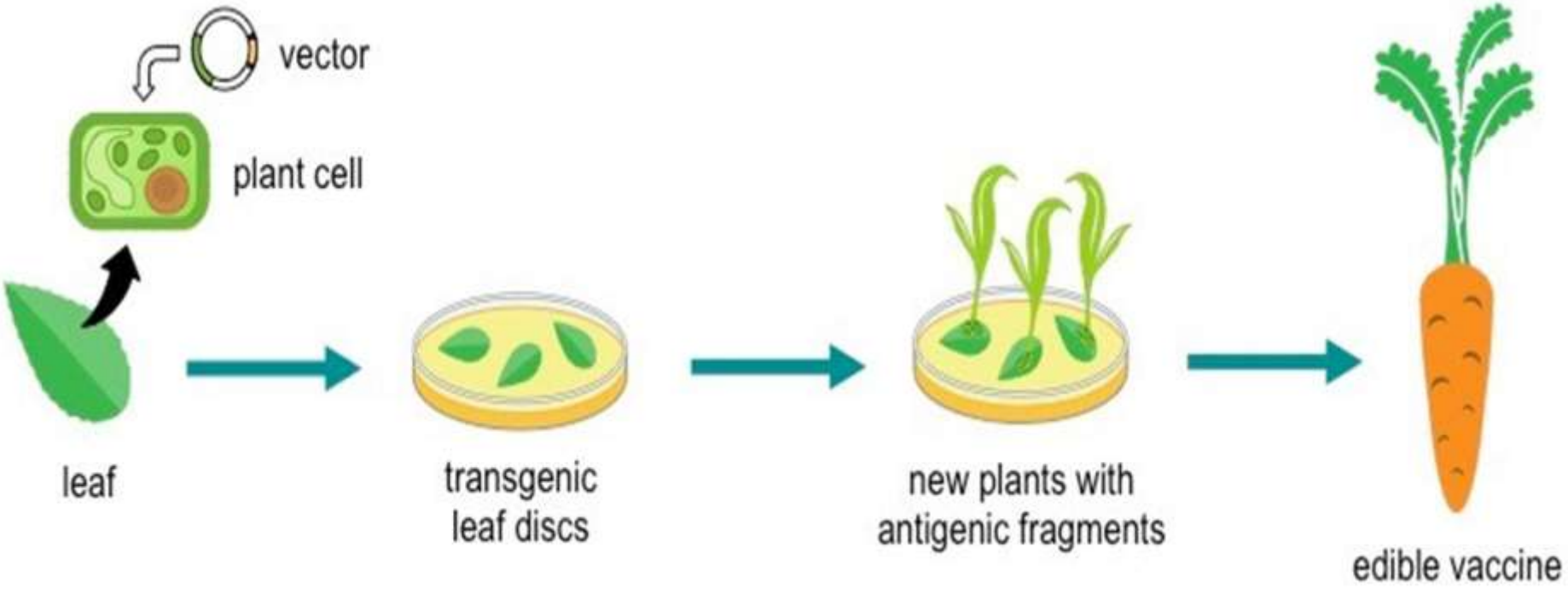
- ***Naked DNA vaccines, viral vector vaccines and plant-derived vaccines are found to be most effective against a number of bacterial and viral disorders.***


DNA / RNA
Hepatitis B Virus
HB antigen producing gene
Introduction of recombinant DNA into a yeast cell
Recombinant yeast cell
HB vaccine
Extraction & purificaion of HB vaccine
Recombinant DNA
Recombinant yeast cell multiplying and producing HB antigen in fermentation tank
Plasmid DNA cut with restriction enzymes
Bacterial DNA
Fermentation Tank
Plasmid DNA
Bacterium
HB antigen

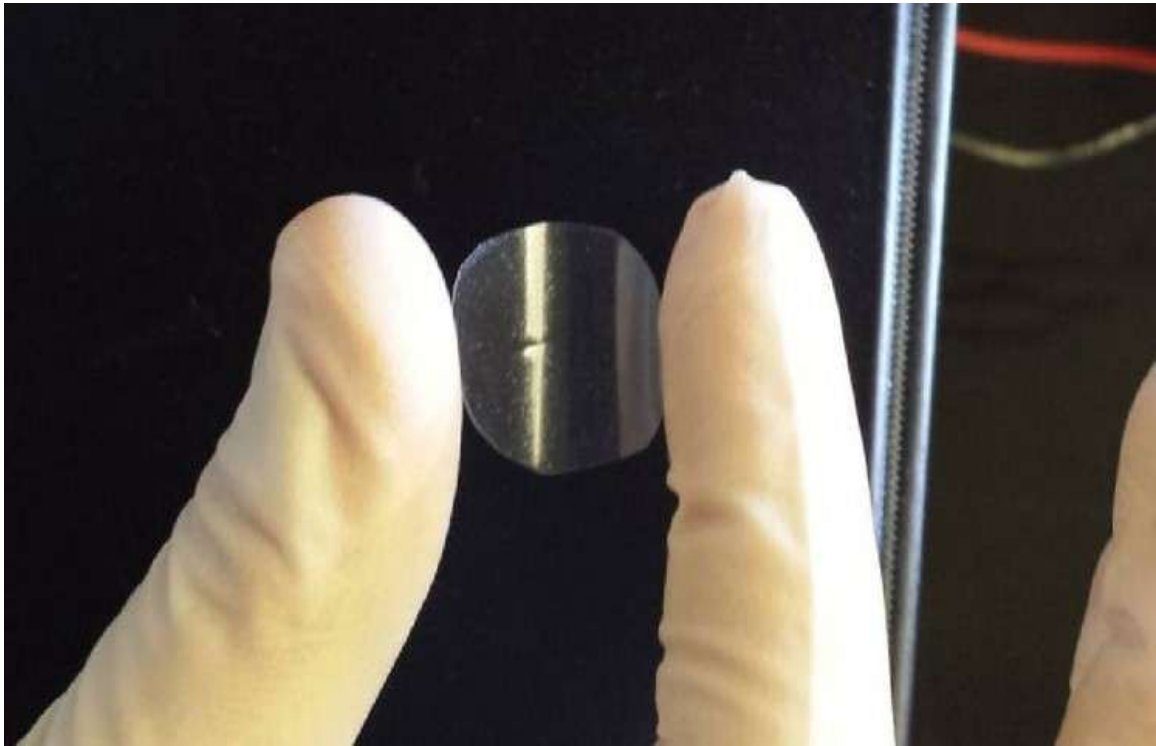
ORAL VACCINES: A NOVEL APPROACH

- ***The latest hot spot in the field of vaccine research is the development of vaccine which can be taken orally.***
- ***Immunogenic protein of certain pathogens is found to be active when administered orally.***
- ***The gene corresponding to such proteins is isolated and a gene construct is produced.***
- ***This is introduced and expressed in a plant genome, which results in production of such immunogenic proteins in the parts of the plant where it is expressed.***
- ***These when fed into animals or mainly humans, the person becomes vaccinated against certain pathogen.***
- ***Such vaccines are also known as edible vaccines.***

Production of edible vaccines



- ***An exciting invention is production of 'melt in the mouth' vaccines that can be administered by placing them under your tongue that delivers it into the blood stream.***
- ***The most important example is the production of flu vaccine by Bacillus which melts in the mouth.***
- ***The tremendous benefit of such vaccines, is the comfort of administration, low cost and ease of storage.***



A Vaccine Mouth Strip

- Like breath strips
- Quick dissolving film
- Melts into a liquid that children and infants would swallow easily
- First designed by undergraduates at Johns Hopkins University at Biomedical Engineering Design Day

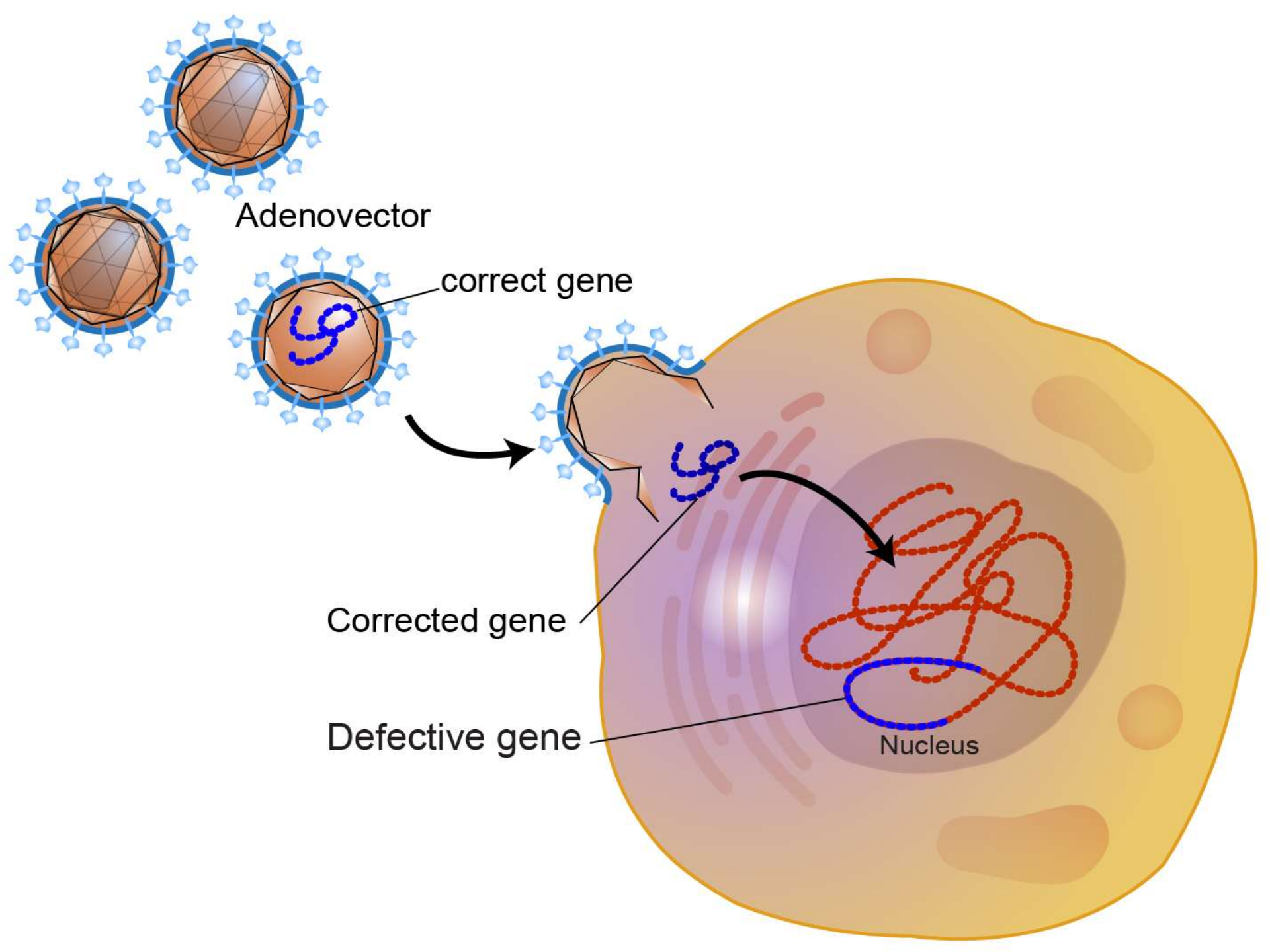
b. Agriculture

- ***Application of Biotechnology in Agriculture involves scientific techniques such as Genetically Modified Organisms, Bt Cotton, Pest Resistant Plants.***
- ***It helps in modifying plants, animals, and micro-organisms and improve their agricultural productivity.***
- ***Tissue Culture is used in Micro-propagation i.e. large-scale propagation of plants in very short durations.***
- ***Tissue culture technique is also best method for storing germplasm & maintaining a specific genetic type(Clone).***
- ***This technique is used in those plants, which produce recalcitrant seeds or produce highly variable seeds.***

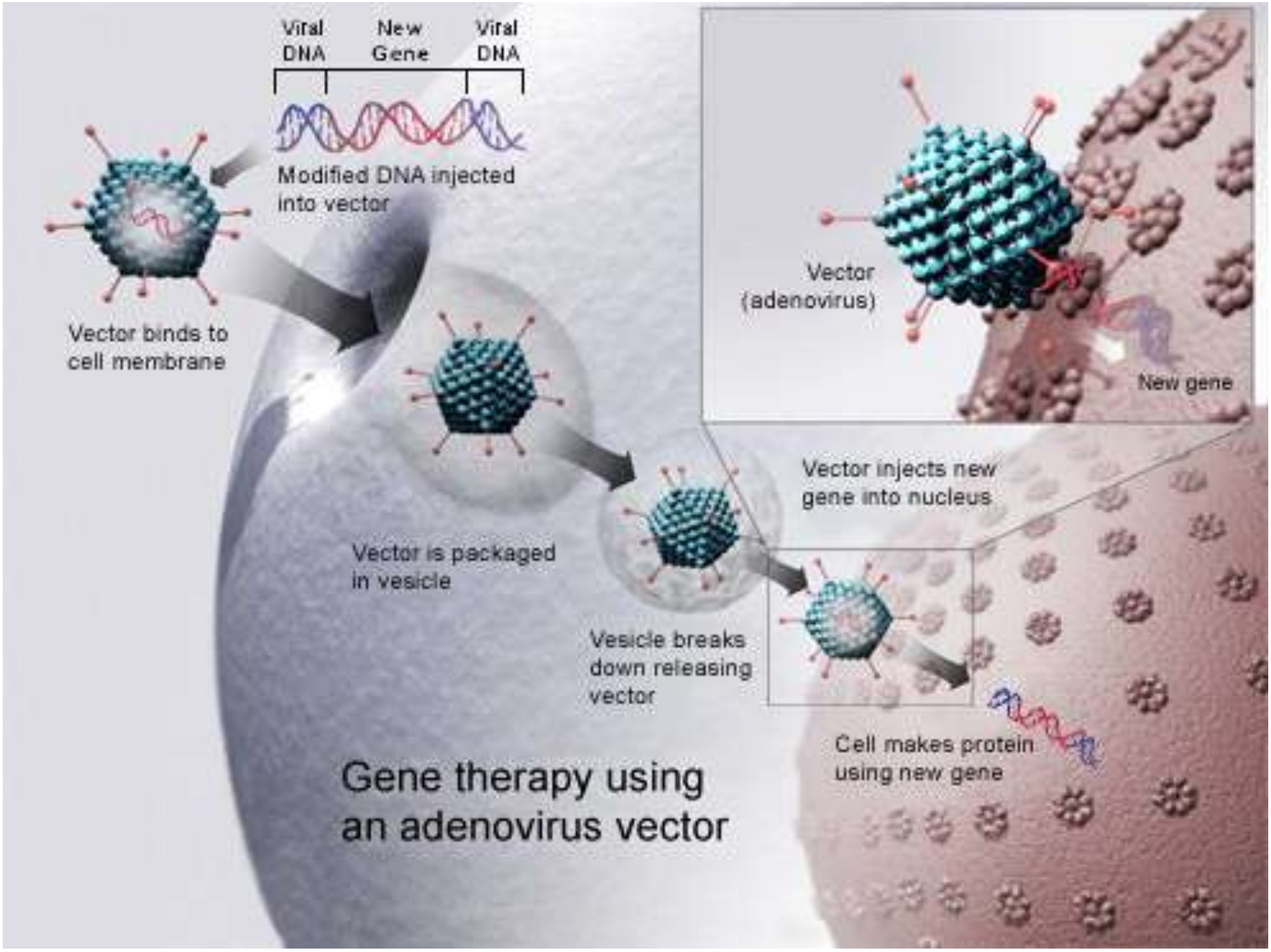
*** Recalcitrant seeds are seeds that do not survive drying and freezing during ex-situ conservation. By and large, these seeds cannot resist the effects of drying or temperatures less than 10 °C (50 °F); thus, they cannot be stored for long periods like orthodox seeds because they can lose their viability.*

c. Gene therapy

- ***A gene is a stretch of DNA required to make a functional product such as part or all of a protein.***
- ***During gene therapy, DNA that codes for specific genes is delivered to individual cells in the body.***
- ***Gene therapy is the treatment of disease by replacing, altering or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for the disease.***


Adenovector
correct gene
Corrected gene
Defective gene
Nucleus

- *Most diseases have a genetic factor which can be wholly or partially responsible for the disease.*
- ***For example,** in disorders such as cystic fibrosis, haemophilia, and muscular dystrophy, changes in a gene directly result in the disorder.*
- *In other conditions, such as high cholesterol and high blood pressure, genetic and environmental factors interact to cause disease.*
- *There are more than 5000 different human genetic diseases known to be caused by single gene defects e.g. sickle cell anaemia, thalassemia, Tay-sach's disease, cystic fibrosis, Huntington's chorea, haemophilia, alkaptonuria, albinism, etc.*


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Vector injects new gene into nucleus
Vector (adenovirus)
New gene
Cell makes protein using new gene
Gene therapy using an adenovirus vector

Gene therapy is being used in many ways.

For example, to:

- ***Replace missing or defective genes.***
- ***Deliver genes that speed the destruction of cancer cells.***
- ***Supply genes that cause cancer cells to revert back to normal cells.***
- ***Deliver bacterial or viral genes as a form of vaccination.***
- ***Deliver DNA to antigen expression and generation of immune response.***
- ***Supply of gene for impairing viral replication.***
- ***Provide genes that promote or impede the growth of new tissue and***
- ***Deliver genes that stimulate the healing of damaged tissue.***

FORMS OF GENE THERAPY

A. Germ line gene therapy

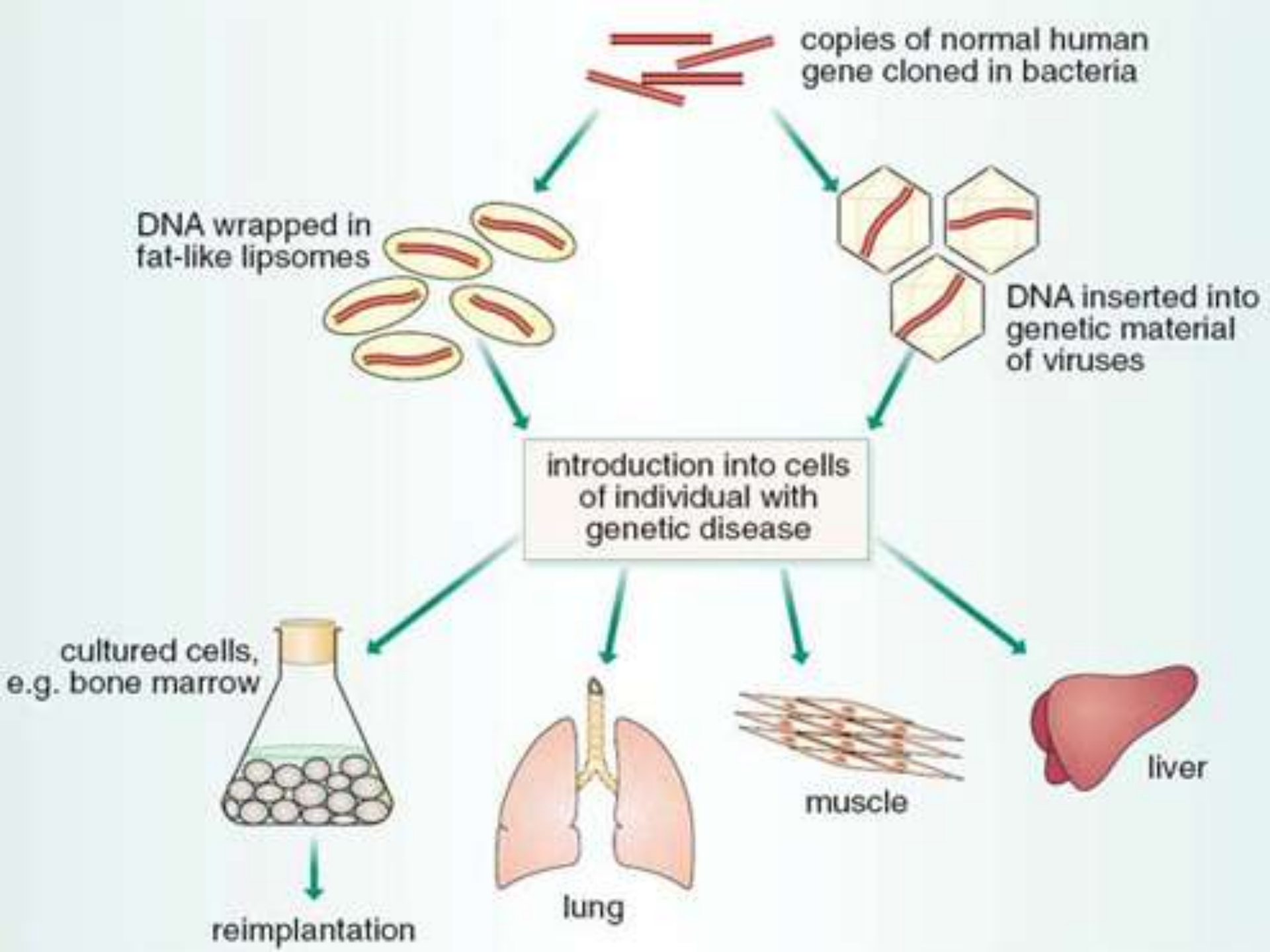
- ***In this method healthy genes can be introduced into germ cells like sperms, eggs, early embryos.***

- ***It allows transmission of the modified genetic information to the next generation.***

- ***Though it is highly effective in counteracting the genetic disorders, it is not encouraged for application in human beings due to a variety of technical and ethical reasons.***

B. Somatic cell gene therapy

- ***In this type the gene is introduced only in somatic cells like bone marrow cells, hepatic cells, fibroblasts, endothelium and pulmonary epithelial cells, central nervous system, endocrine cells and smooth muscle cells of blood vessel walls.***
- ***Modification of somatic cells only affects the person being treated and the modified chromosomes cannot be passed on the future generations.***
- ***Somatic cell gene therapy is the only feasible option and the clinical trials have already employed for the treatment of acquired disorders such as cancer and rheumatoid arthritis and blood disorders including SCID, Gaucher's disease, familial hypercholesterolemia, haemophilia, phenylketonuria, cystic fibrosis, sickle cell anaemia, Duchenne muscular dystrophy, emphysema, thalassemia etc.***

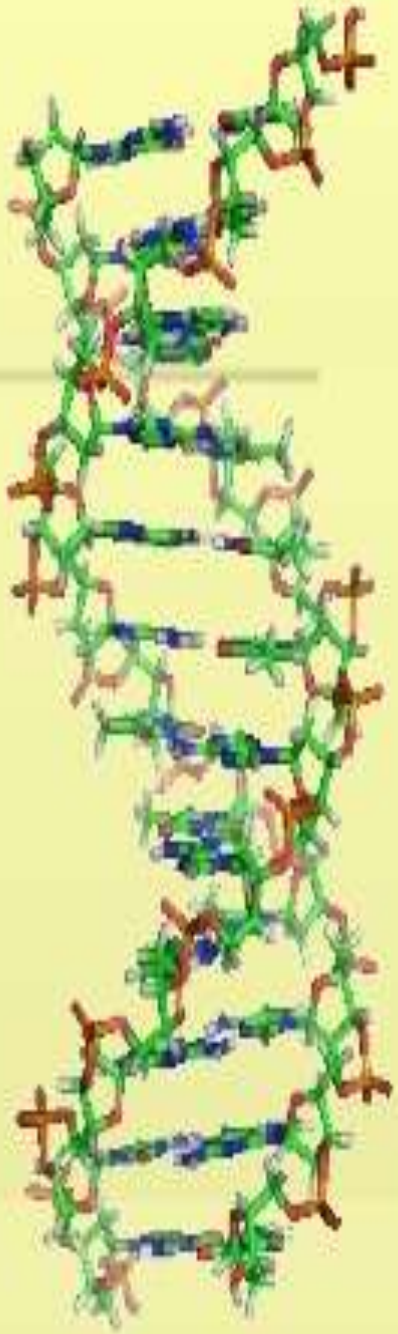

copies of normal human gene cloned in bacteria
DNA wrapped in fat-like lipsomes
DNA inserted into genetic material of viruses
introduction into cells of individual with genetic disease
cultured cells, e.g. bone marrow
reimplantation
lung
muscle
liver

d. Genetically Modified Organisms (GMOs)

- *Living organisms whose genetic material is artificially manipulated in lab through genetic engineering.*
- *This creates combinations of plant, animal, bacteria, and virus genes that do not occur in nature or through traditional crossbreeding methods.*
- *Most GMOs are engineered to withstand the direct application of herbicide and/or to produce an insecticide.*
- *New technologies are now being used to artificially develop other traits in plants, such as a resistance to browning in apples, and to create new organisms using synthetic biology.*
- *Despite biotech industry promises, there is no evidence that any of the GMOs currently on the market, offer increased yield, drought tolerance, enhanced nutrition, or any other consumer benefit.*

What is a GMO?

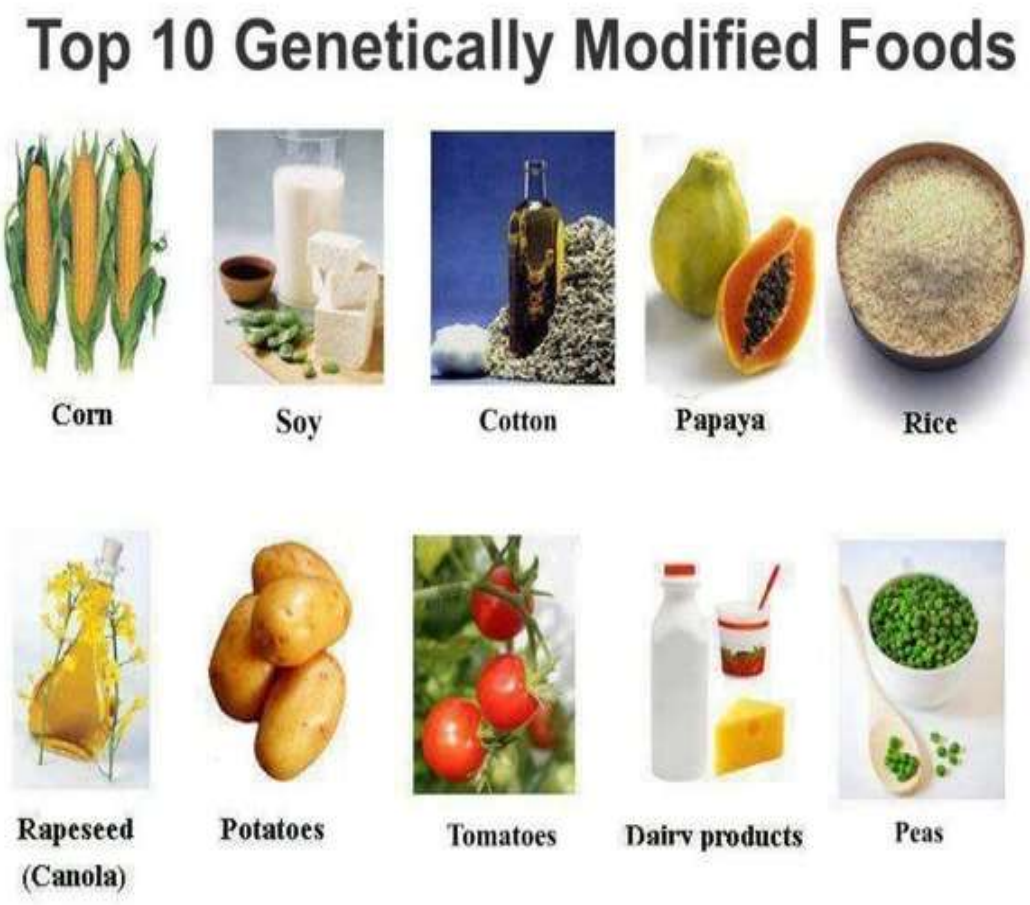
- A genetically modified organism is one whose genetic material has been altered using genetic engineering.
- Genetically modified organisms, or GMOs, are commonly used in foods and medicines. This has led to concern about the dangers they might cause to the environment and to human health



1. Transgenic Plants

- *The human race is dependent on agriculture.*
- *As world populations continue to increase, there is a need to optimize the effeciency of agricultural practices.*
- *Humans have forever sought to improve the quality and productivity of agriculturally important plants.*
- *This was done by selection and traditional breeding procedures that were slow and difficult.*
- *Traditional breeding programmes involve sexual crosses, which resulted in the high quality of present day food plants such as wheat, rice, corn, potato, etc.*
- *More recently, biotechnological approaches have been applied to these plants to create genetic variations that are beneficial for mankind.*

- *First transgenic plant produced was tobacco.*
- *More then 60 transgenic dicot plants and several monocot plant like maize, oat, rice, wheat are known.*
- *Tomato, soybean, potato, sugar beet, grapes, brinjal, cotton are other transgenic plants.*
- *Transgenic plants are being looked up as bioreactors for molecular farming - for production of novel drugs like interferons, edible vaccines, antibodies, amino acids, immunotherapeutic drugs, etc.*



GMO Foods

For more information go to
olmag.co/gmo-foods

Tomato

Tomatoes have been genetically modified, but they are not being grown commerically at thia time

Rice

GMO rice has been approved but is not yet being used commercially

Sweet Corn

More than 70 percent of corn grown in the United States has been genetically engineered

Summer Squash

Farmers don't like GMO squash but some experts say GM squash have blended with wild squash

Salmon

GMO salmon has not been approved by the FDA, but it will be very soon

Canola Oil

87% of canola grown commercially, and 80% of wild canlola is GMO

Yeast

GMO yeast for wine has been approved

Alfalfa

GMO alfalfa is contaminating non GMO alfalfa crops at a rapid rate

Wheat

Unapproved GMO has contaminated wheat fields, and we don't yet know the extent of it

Sugar Beets

90% of Sugar Beets (used to make 50% of our sugar) are GMO

Soy

More than 93% of soybeans the United States produces are genetically modified

Peas

Peas have been genetically modified but are not approved or availlable

Hawaiian Papaya

Most Hawaiian papaya is GMO, even many organic crops are contaminated

Cotton

At least half of cotton grown in the world is GMO

organic lifestyle MAGAZINE

Advantages of GM food-plants

Genetically modified plants can help in the following ways :

a. Insect pest resistance

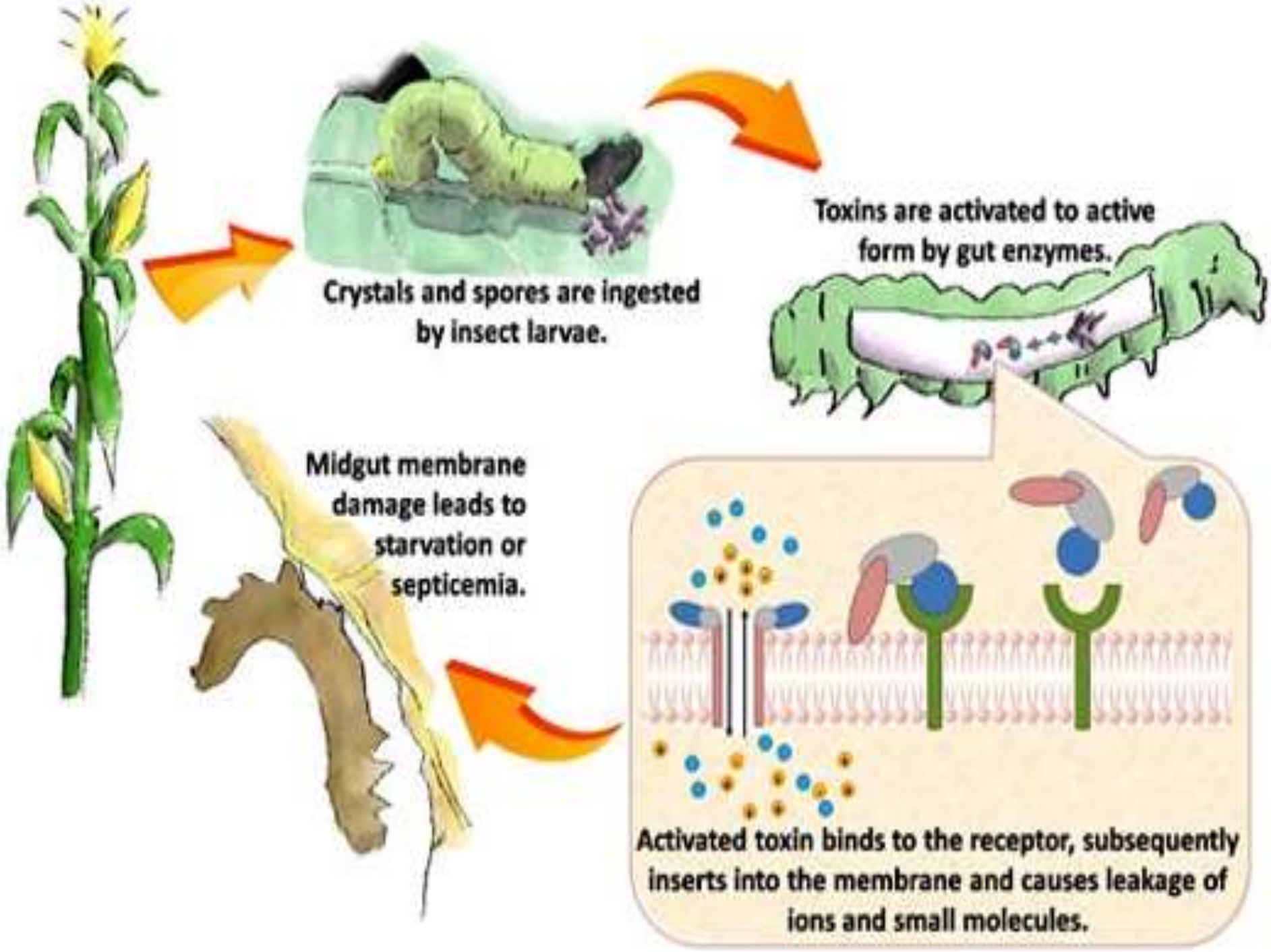
- ***It can help farmers to reduce their use of chemical pesticides, which in turn can reduce the cost of producing food.***
- ***An alternative has been available for more than 30 years which is a biological insecticide from the bacterium, Bacillus thuringiensis (Bt).***
- ***However, the use of B. thuringiensis sprays is limited because of low stability of the protein in the field.***

*****Bt cotton is one of the best transgenic plants known for its insect resistance property.***

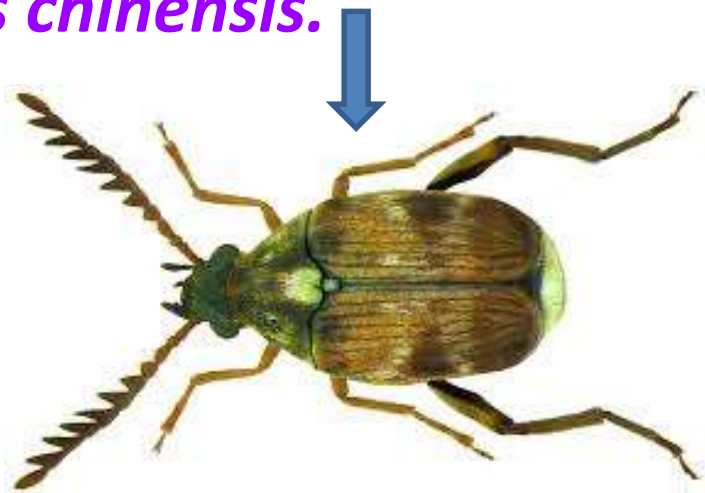
Advantages	Disadvantages
Potent insecticidal activity	Rapidly inactivated by UV light, heat and extreme pH
Specific host range	Susceptible to proteases in leaf exudates
Harmless to humans and other mammals	Easily removed from plant surface by wind and rain
Biodegradable	Therefore needs to be reapplied for full effect

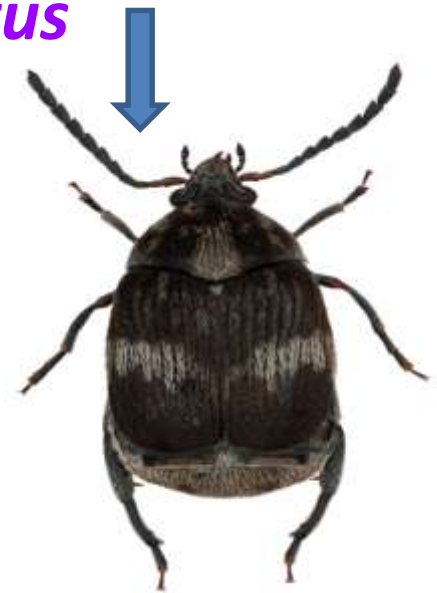
Advantages and disadvantages of B. thuringiensis sprays

- *Insect resistant plants contain either a gene from Bt. or the cowpea trypsin inhibitor gene.*
- *The gene called cry gene present in B. thuringiensis produces a protein that forms crystalline inclusions in bacterial spores.*
- *When ingested by a susceptible insect, a combination of high pH and enzyme proteinase of the insect's midgut, processes them hydrolytically to release the core toxic fragments.*


Crystals and spores are ingested by insect larvae.
Toxins are activated to active form by gut enzymes.
Midgut membrane damage leads to starvation or septicemia.
Activated toxin binds to the receptor, subsequently inserts into the membrane and causes leakage of ions and small molecules.

- ***The effect of these fragments is seen within minutes of ingestion, beginning with midgut paralysis and ending with disruption of midgut cells of insect.***
- ***Bt toxin activity has been against many species of insects within the orders of Lepidoptera (moths and butterflies), Diptera (fly), and Coleoptera (beetles).***
- ***Similarly, the gene of α-amylase inhibitor (αAI-Pv) has been isolated from adzuki bean (Phaseolus vulgaris – red moong bean) and transferred to tobacco and this gene works against pests like Zabrotes subfasciatus and Callosobruchus chinensis.***





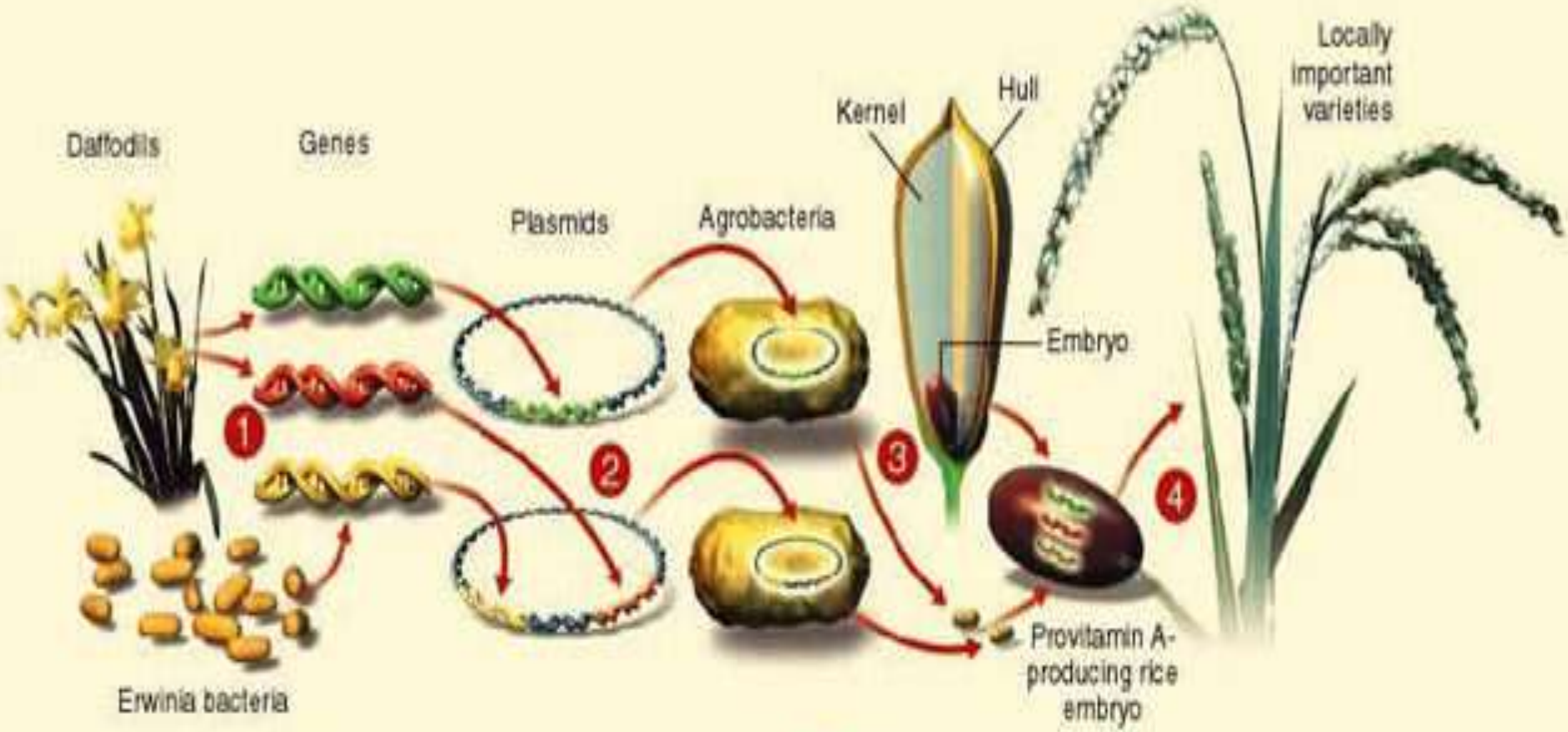
b. Improved nutritional qualities (biofortification)

Transgenic plants have also been produced to provide functional food and nutraceuticals.

Nutraceuticals : Any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to promote general well-being, control symptoms and prevent malignant processes.

The term "nutraceutical" combines two words – "nutrient" (a nourishing food component) and "pharmaceutical" (a medical drug)

- *For millions of people in developing countries, rice is the main item in their diet.*
- *Because rice does not contain many essential nutrients, malnutrition is very common in these countries.*
- *Especially the blindness that results from a lack of vit-A.*
- *This vitamin is abundant in milk and vegetables such as carrots, which poor people of the world cannot afford.*
- *To solve this problem, Swiss researchers created transgenic rice (golden rice) and transgenic mustard (golden mustard) varieties that are high in vitamin A.*
- *The golden colour is due to vitamin A.*
- *They hoped that this rice, if grown and eaten in developing countries, would reduce the diseases associated with vitamin A deficiency (VAD).*



1. The genes that give golden rice its ability to make beta-carotene in its endosperm (the interior of the kernel) come from daffodils and a bacterium called Erwinia uredovora

2. These genes, along with promoters (segments of DNA that activate genes), are inserted into plasmids (small loops of DNA) that occur inside a species of bacterium known as Agrobacterium tumefaciens

3. These agrobacteria are then added to a Petri dish containing rice embryos. As they "infect" the embryos, they also transfer the genes that encode the instructions for making beta-carotene

4. The transgenic rice plants must now be crossed with strains of rice that are grown locally and are suited to a particular region's climate and growing conditions

HOW TO MAKE GOLDEN RICE?

1. The genes that give golden rice its ability to make beta-carotene in its endosperm (the interior of the kernel) come from **Daffodils** & a bacterium called ***Erwinia Uredovora.***

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4. The transgenic rice plants must now be crossed with strains of rice that are grown locally and are suited to a particular region's climate and growing conditions.

FINAL PRODUCT:

Daffodils

&

Erwinia uredovora

Genes

Plasmids

Provitamin-A producing Rice seed

Locally Grown Variety

- ***Improvement in oil content and oil quality of oil crops like soybean, oil palm, rapeseed and sunflower, have been achieved by transfer of "Arabidopsis genes'***

- ***Iron deficiency is also a serious nutritional problem, affecting an estimated 30% of the world population.***
- ***For production of transgenic crops that will produce food rich in iron, an iron storage protein ferritin found in many animals, plants and bacteria is targeted.***
- ***The genes for ferritin protein isolated from soybean and Phaseolus (bean) have been transferred to rice.***

Table 12.8 : Some transgenic plants produced for functional food and neutraceuticals

Substance	Potential benefit	Crop	Transgene
Provitamin A	Anti-oxidant	Rice	Phytoene synthase, lycopene cyclase
Vitamin E	Anti-oxidant	Canola	γ- tocopherol methyl trasnferase
Flavonoids	Anti-oxidants	Tomato	Chalone isomerase
Fructants	Low calories	Sugarbeet	1-sucroese: sucrase fructosyl transferase
Iron	Iron fortification	Rice	Ferritin, metallothioein, phytase

c. Modification in Post-harvest characteristics

- *Diseases & pests, bruising on soft fruits & vegetables, heat and cold storage, over-ripeness, loss of flavours and odours, lead to great losses during storage and transport of crops.*
- ***These physiological changes are mostly** due to endogenous enzyme activity.*
- *Genetic engineering has made it possible to slow down these activities.*
- *In tomato, the enzyme polygalacturonase breaks down the cell wall constituent- pectin, leading to softening of fruit during ripening*.
- ***Hence fruits are easily bruised and damaged on shipment.***
- *By inhibiting the polygalacturonase by antisense genes, the tomato (GM-Flavr savr tomatoes) can remain on the vine until mature and be transported in a firm solid state.*

d. Plants as factories

- *To produce novel biochemicals and vaccines (Biopharmaceuticals), plants are potential factories or bioreactors for high value biochemicals like starch, sugar, lipids, proteins, and products like fine chemicals, perfumes and adhesive compounds as well as industrial lubricants, biodegradable plastic and even 'renewable' energy crops to replace fossil fuels.*

- *Biopharmaceuticals are proteins, hormones, antibodies, vaccines or enzymes isolated from transgenic plants.*

Some of the proteins that are being produced by transgenic crop plants:

- ***Human growth hormone* with the gene inserted into the chloroplast DNA of tobacco plants.**
- ***Humanized antibodies* against such infectious agents as *HIV, Respiratory syncytial virus (RSV), Herpes simplex virus (HSV)*, the cause of "cold sores".**
- ***Protein antigens* to be used in vaccines for e.g. *Patient-specific anti-lymphoma* (a cancer) vaccines. B-cell lymphomas are clones of malignant B cells, expressing on their surface a unique antibody molecule.**

Currently many products have been commercially exploited for their products such as:

a. *A 'superglue' produced by tobacco plants with genes encoding for powerful adhesive proteins, enables marine mussels to stick to rocks. It will be especially valuable as a biochemical glue for body repairs during surgery.*
b. *Transgenic plants, containing oil encoding gene from marine algae, produce oil that has nutritional value similar to cod- liver oil.*
c. *Plant that will produce the antimalarial drug, Artemisinin.*
d. *Genetically engineered opium poppy to produce more powerful painkillers.*

e. Transgenic plants producing edible vaccines

- *Genetically altered plants can provide protection from infectious diseases.*
- *Plant products acting as vaccines would be cheaper to produce and thus, can easily be made available in developing countries.*
- *Potatoes, tomatoes, bananas, soybeans, alfalfa and cereals are the most common foods proposed for edible vaccine delivery.*

II. Transgenic animals

- ***Many transgenic animals** such as mice, rats, rabits, pig, sheep, cows, fowls, fish have been **produced through rDNA technology.***
- ***The term transgenic animal refers to an animal in which there has been a **deliberate modification of the genome** in contrast to spontaneous mutation.***
- ***Foreign DNA is introduced into the animal**, using recombinant DNA technology, and then must be **transmitted through the germ line** so that every cell, including germ cells, of the animal contain the same modified genetic material.*

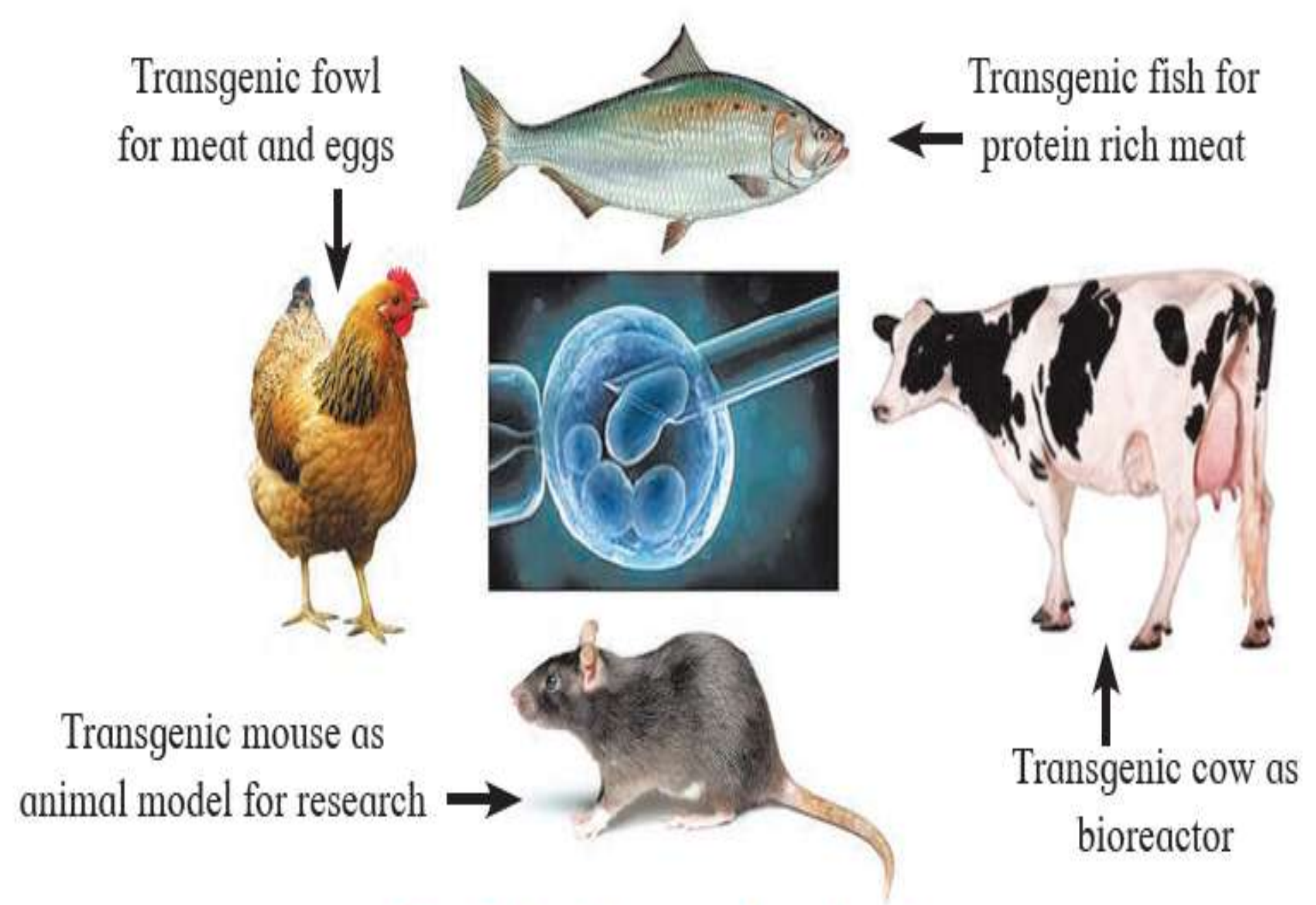


Fig. 12.9 : Transgenic animals

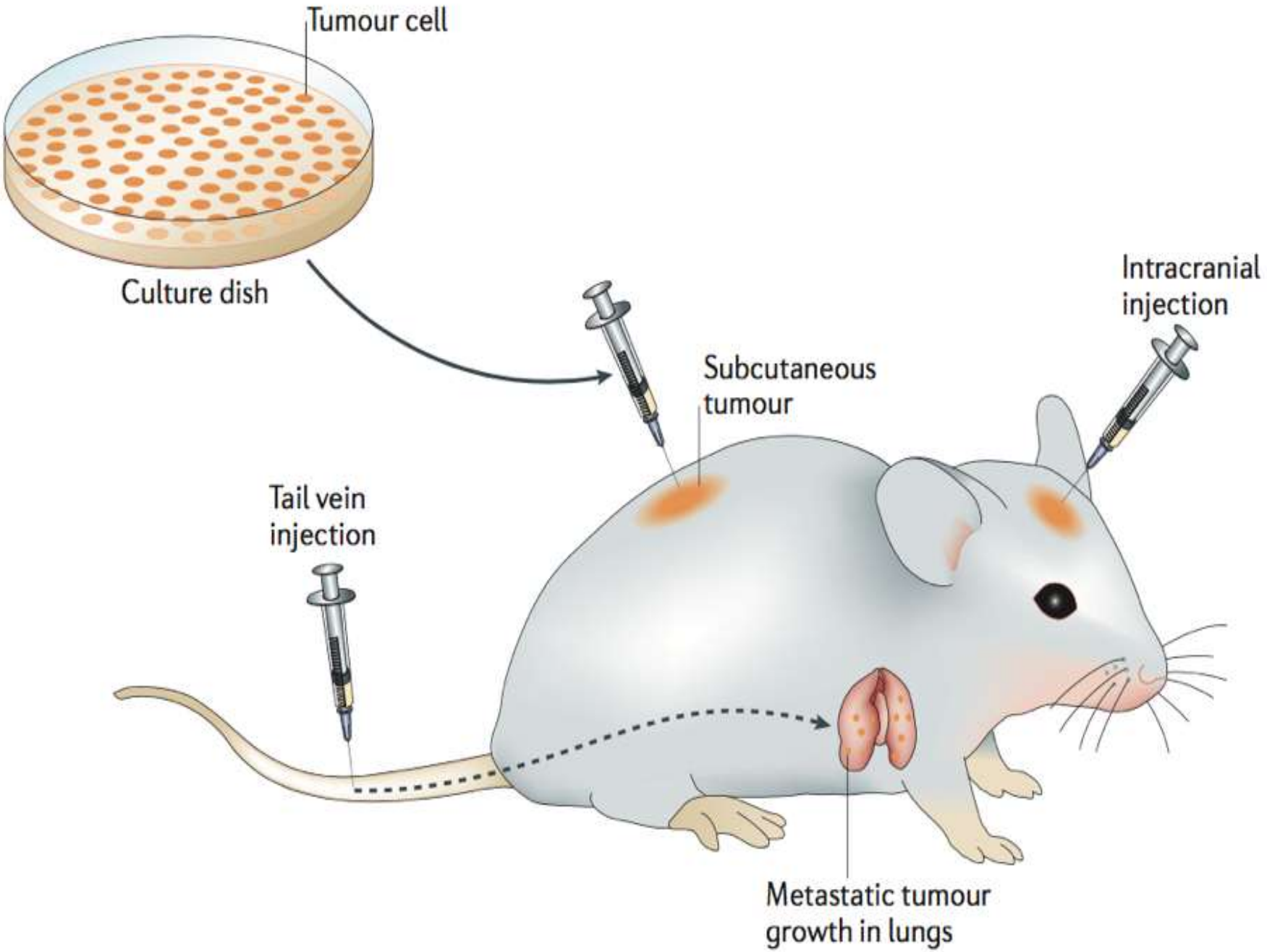
A list of purposes for which transgenic animals have been used, indicates wide-ranging application of this biotechnology:

- ***In medical research, transgenic animals are used to identify the functions of specific factors in complex homeostatic systems through over- or under-expression of a modified gene (the inserted transgene).***
- ***In toxicology, as responsive test animals (detection of toxicants).***
- ***In mammalian developmental genetics.***
- ***In molecular biology, the analysis of the regulation of gene expression makes use of the evaluation of a specific genetic change at the level of the whole animal.***

- *In the pharmaceutical industry, production of pharmaceutical proteins, drug production and product efficacy testing.*
- *In biotechnology: as producers of specific proteins.*
- *Genetically engineered hormones to increase milk yield, meat production.*
- *Genetic engineering of livestock and in aquaculture for modification of animal physiology and/or anatomy.*
- *Cloning procedures to reproduce specific blood lines.*
- *Developing animals specially created for use in xenografting.*

a. Transgenic mice and cancer research

- ***Through laboratory investigations with transgenic mice modified using a particular oncogene (cancer causing gene) and thus developed a certain type of cancer, questions concerning the relationship between oncogenes and cancer development could be answered.***
- ***Theoretically, such animals can also be used for research into cancer treatment and prevention of malignancy.***
- ***In the laboratory of Philip Leder in Harvard (USA) the transgenic mouse model for the investigation of the breast cancer was developed.***
- ***The oncogenes myc and ras were analysed to find out if they lead to breast cancer in mice transformed with these genes.***


Tumour cell
Culture dish
Intracranial injection
Subcutaneous tumour
Tail vein injection
Metastatic tumour growth in lungs

Why Use Transgenic Mouse Models?

- Model the development of human diseases in a controlled environment
- Test possible new drug treatments and get faster results

- Target specific genes to study
- Replicate specific characteristics, symptoms, or pathology of the disease

ingenious targeting laboratory

b. Transgenic farm animals

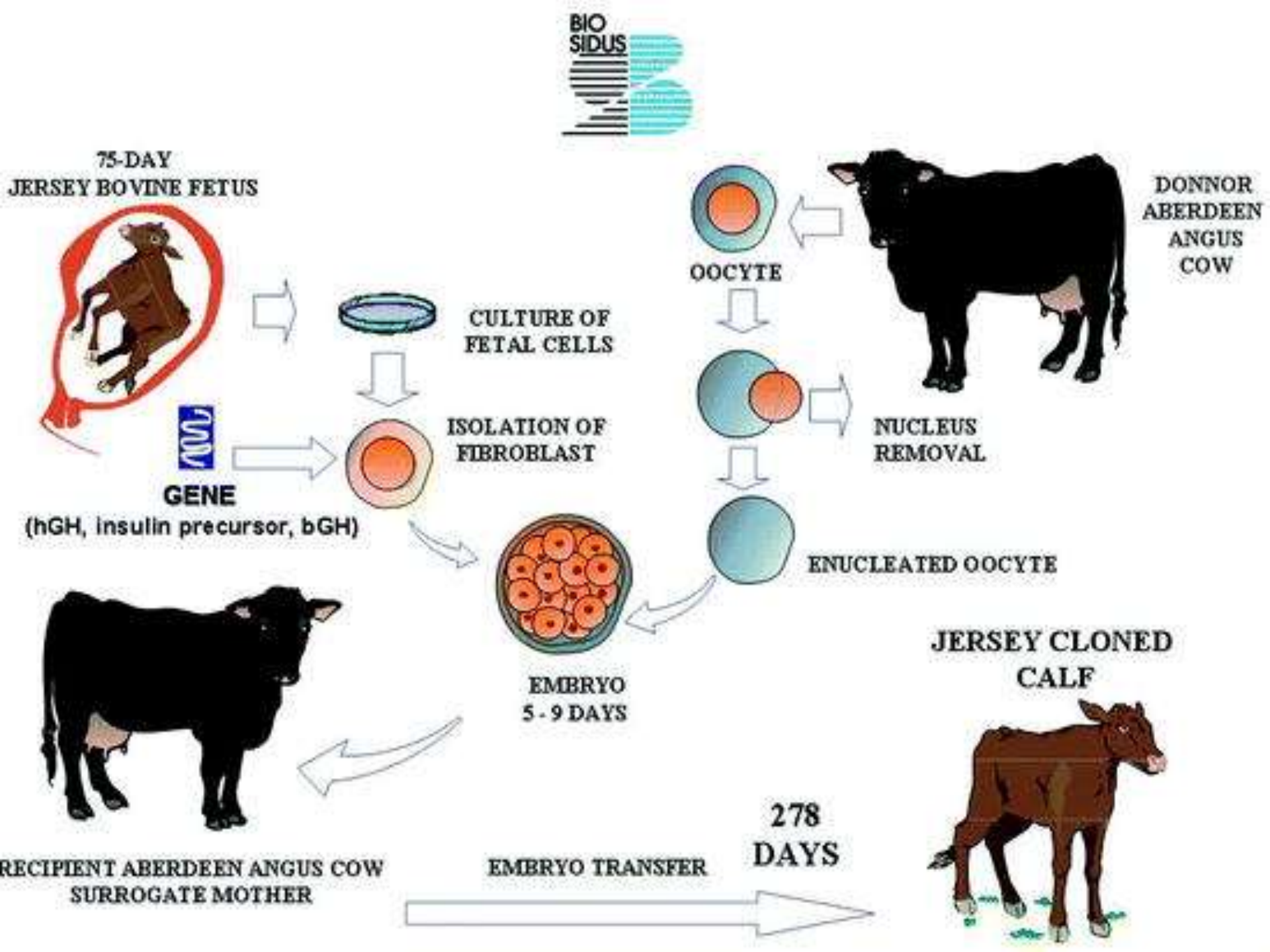
- *With the advent of technology, the intention of researchers is to produce transgenic farm animals from which we can derive greater benefits.*
- *Many of the farm animals are improved for their meat production ability while some of them are improved for milk yields and quality, and disease-free status.*
- *At the beginning of the century, a dairy cow provided 2,000 to 3,000 liters of milk a year.*
- *Today, Holstein cow provides 6,000 liters on average and up to 8,000 – 10,000 for the best ones.*
- *A century ago, a hen laid about 70 eggs a year whereas today the best races lay up to 250 eggs per year.*
- *This could be possible because of the advent of biotechnology.*

The main objectives for improved animal breeding programmes coupled with this new technology of gene transfer are given below.

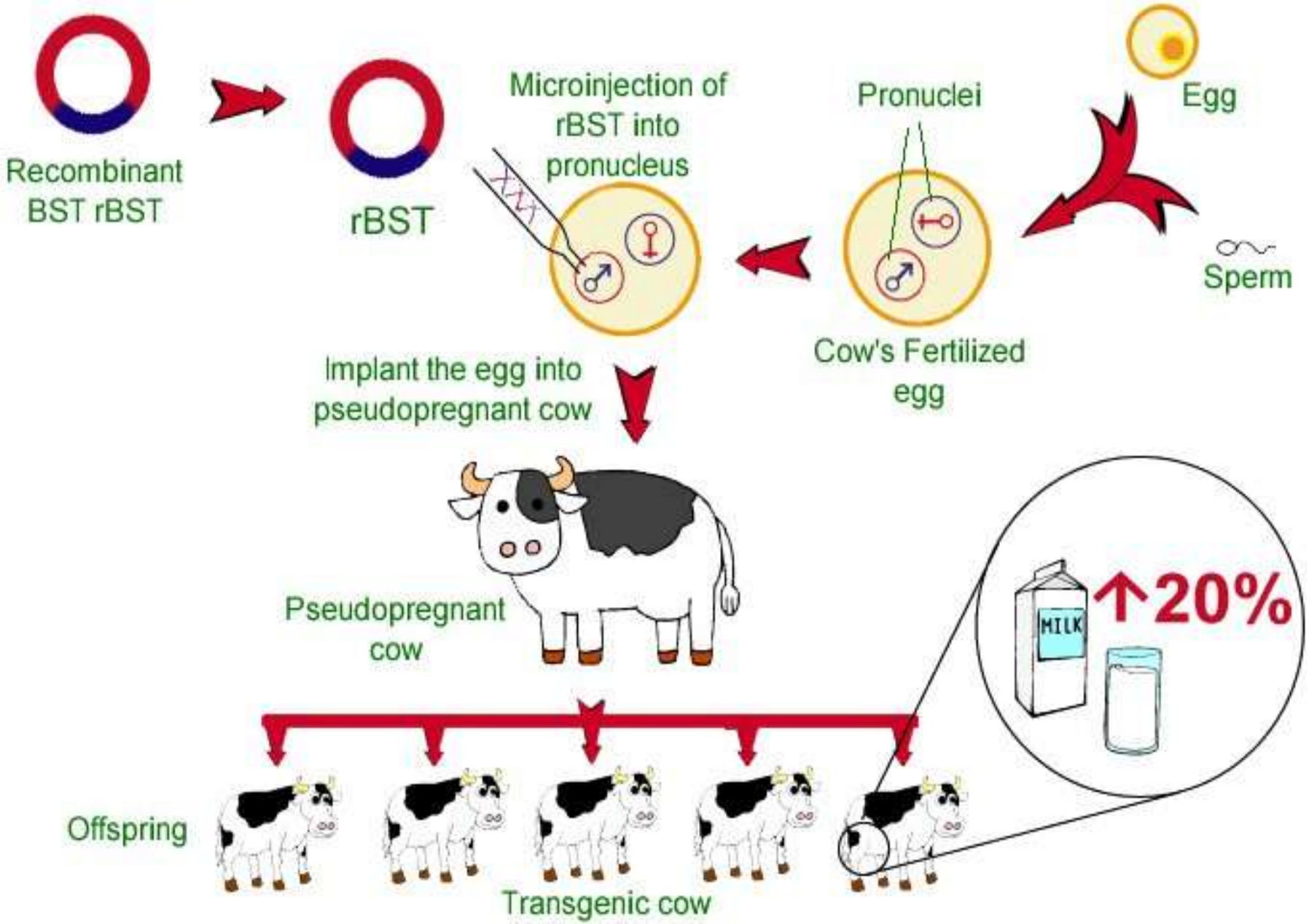
- ***Efficiency of meat production***
- ***Improved quality of meat***
- ***Milk quality and quantity***
- ***Egg production***
- ***Wool quality and quantity***
- ***Disease resistance in animals***
- ***Production of low-cost pharmaceuticals and biologicals.***

c. Transgenic cattle for food production

- *Of the few research reports describing the use of transgenic technologies in cattle, only one is directed towards a food production application.*
- *Researchers introduced additional copies of bovine beta or kappa casein into dairy cattle and evaluated the effect on milk production and composition.*
- *Transgenic offspring had an 8 to 20% increase in beta casein and a two-fold increase in kappa casein.*


BIO SIDUS
75-DAY
JERSEY BOVINE FETUS
DONNOR
ABERDEEN
ANGUS
COW
OOCYTE
CULTURE OF
FETAL CELLS
ISOLATION OF
FIBROBLAST
NUCLEUS
REMOVAL
GENE
(hGH, insulin precursor, bGH)
ENUCLEATED OOCYTE
EMBRYO
5 - 9 DAYS
JERSEY CLONED
CALF
278
DAYS
RECIPIENT ABERDEEN ANGUS COW
SURROGATE MOTHER
EMBRYO TRANSFER

Increase the Yield of Milk



d. Transgenic cattle for human therapeutic production

- A second application for genetically modified cattle is the production of human therapeutic proteins.
- Human proteins that have been expressed in milk include human lactoferrin, human alpha lactalbumin, human serum albumin and human bile salt stimulated lipase.
- The mammary gland in dairy cows is an excellent protein production factory.
- On the other hand, one transgenic cow would be more than sufficient for production of annual world supply of factor IX (plasma thromboplastin component) that is used in the treatment of haemophilia.

Transgenic cow

- 1990: World's 1st transgenic cow – "Herman" the bull (Netherlands)
- Herman carried the human lactoferrin gene (pronuclear microinjection)
- Cow's milk & artificial formula do not naturally contain lactoferrin
- Other RPs in transgenic cow's milk – lysozyme, human lactalbumin, rATIII, Factors VIII & IX etc

Transgenic cow

- "ROSIE" was the first transgenic cow , born in 1997.
- Produced human protein enriched milk at 2.4g/lt contains human gene Alpha lactalbumin.

- ***Antibodies are currently used for many different human clinical applications, including treatment of infectious disease, cancer, transplanted organ rejection, autoimmune diseases and for use as antitoxins.***

- ***To make a human antibody product, the genetically modified cows are immunized with a vaccine containing the disease agent.***

e. Transgenic Sheep

- *Gene transfer technology is applied to sheep to produce transgenic sheep which are able to achieve better growth and meat production as well as to serve as bioreactors.*

- *In 1990 Tracy, the transgenic sheep was born in Scotland, and could produce a human protein in her milk for human therapeutics.*

Tracy, a transgenic sheep, 1999

- Her milk produced a human protein called alpha antitrypsin, a potential treatment for the disease cystic fibrosis.

- *Human growth hormone gene is introduced in sheep for promoting the growth and meat production.*
- ***Bacterial genes, cys E and cys M, are concerned with biosynthesis of cysteine amino acids involved in formation of keratin protein found in wool.***
- ***Both these genes are identified, cloned and introduced in sheep to increase wool production and to improve the quality of wool.***

Transgenic sheep & lamb

- 1996: 'Dolly' the sheep was cloned using cells from a 6 yr old sheep's udder by Somatic Cell Nuclear Transfer (SCNT) technology.

- 1997: World's 1st transgenic lamb, 'Polly' was also created using SCNT. Contained human gene for blood clotting factor IX

f. Transgenic pigs

- ***The objective of gene transfer in pigs is to increase growth and meat production and to act as bioreactors.***
- ***Pigs are regarded as the most suitable animals to be bred for heart transplant because a pig's heart is about the same size as a human heart, and pig heart valves have been used in human heart surgery for over a decade.***
- ***The pig clone is the first step towards providing animal organs and tissues for human transplants (xenotransplantation).***

Xenotransplantation

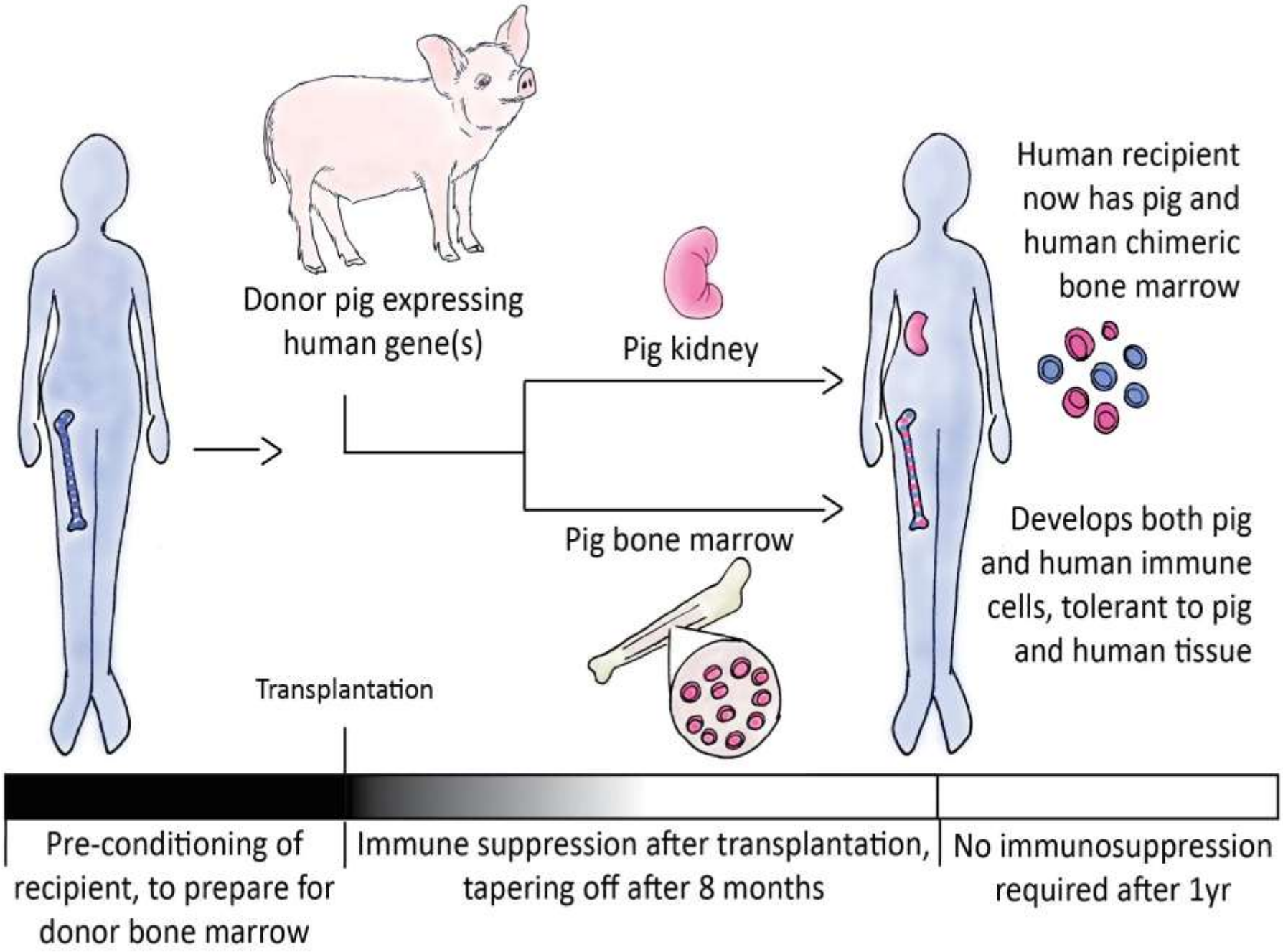
Xenotransplantation - transplantation of living cells, tissues, and organs from one species to another.

Xenograft - is an organ transplanted from one species to another.

Allotransplantation – refers to same-species transplant.

Xenozoonosis – disease transmission.

Definition: Genetically alter an animal organ with human genes to trick a patient's immune system into accepting it


Donor pig expressing human gene(s)
Pig kidney
Pig bone marrow
Transplantation
Human recipient now has pig and human chimeric bone marrow
Develops both pig and human immune cells, tolerant to pig and human tissue
Pre-conditioning of recipient, to prepare for donor bone marrow
Immune suppression after transplantation, tapering off after 8 months
No immunosuppression required after 1yr

g. Transgenic fish

- *The commercially important fish like Atlantic salmon, catfish, goldfish, Tilapia, zebra-fish, common carp, rainbow trout, etc. are transfected with growth hormone, chicken crystalline protein and E.coli hygromycin resistance gene.*
- *Transgenic fish showed increased cold tolerance and improved growth and the quantity and quality of fish proteins as well as its preservation, are the factors affecting the economic value of fish.*

TRANSGENIC FISH

- **Superfish**
  - Increased growth and size
  - Growth hormone gene inserted into fertilized egg.
  - Transgenic salmon grows about 10 – 11 times faster than normal fish.

- **Glo fish**
  - GM freshwater zebra fish (*Danio rerio*)
  - Produce by integrating a fluorescent protein gene from jelly fish into embryo of fish.

h. Transgenic chicken

- ***Chickens also carry and express foreign genes.***

- ***They could be used to improve the genetic make-up of existing strains with respect to built-in (in vivo) resistance to viral and coccidial diseases, better feed efficiency, lower fat and cholesterol levels and high protein containing eggs, and better meat quality.***

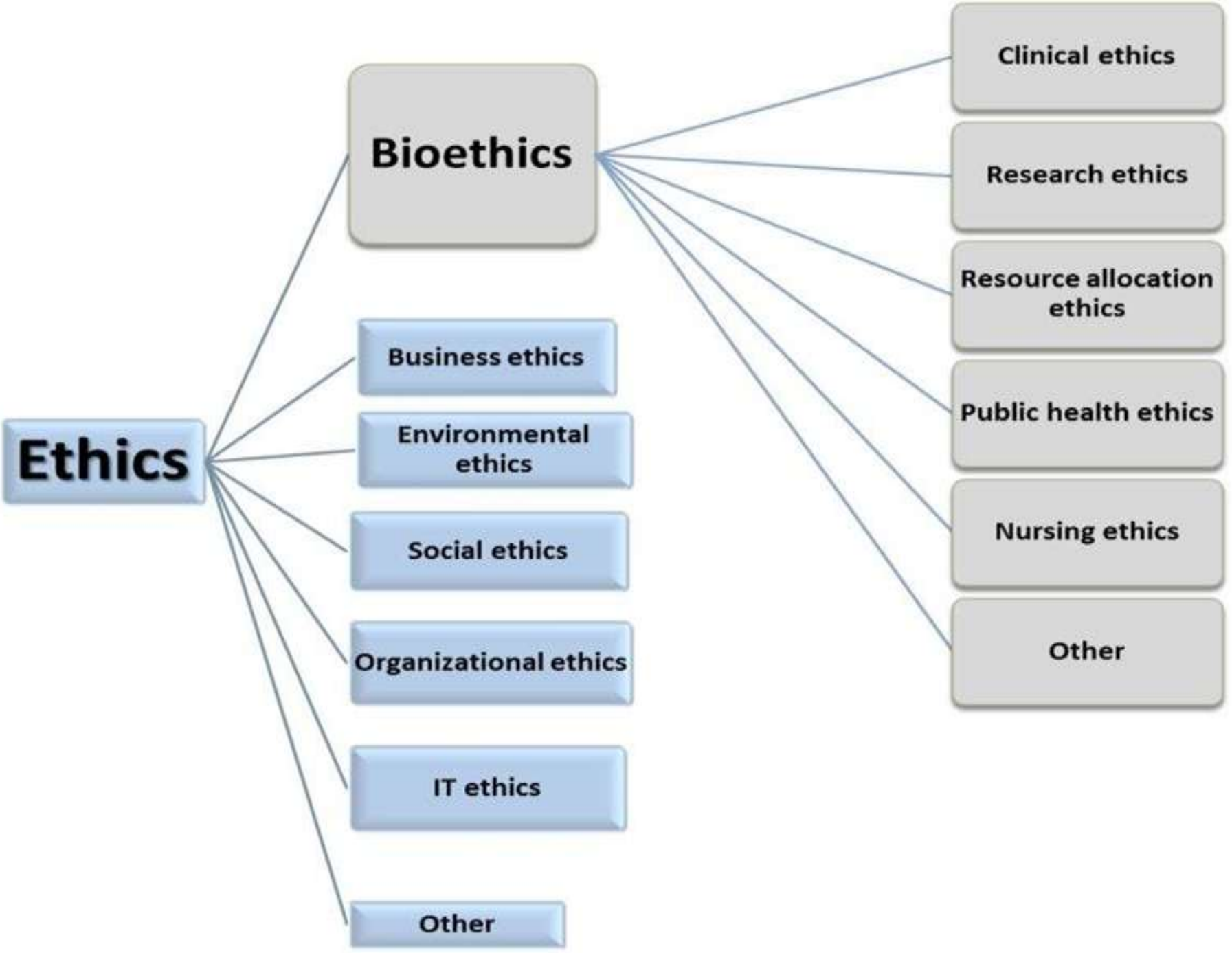
Transgenic chickens

- Methods:
  - Infecting embryos with a viral vector carrying the transgene
  - Transfer human gene into rooster sperm
- Produces 0.1g of human protein in each egg
- Advantages:
  - Cost effective
  - Add correct sugars to glycosylated proteins (unlike E.coli)

BIOETHICS, BIO-PIRACY AND BIO-PATENT

1. Bioethics:

- ***Ethics usually deals with the matters related to socially acceptable moral duty, conduct and judgement.***
- ***It helps to regulate the behaviour of community by some set of standards.***
- ***Concepts differ according to culture & traditions.***
- ***Concepts change with the time due to shifting of perception of values which are affected by progress in science and technology.***


Ethics
Bioethics
Business ethics
Environmental ethics
Social ethics
Organizational ethics
IT ethics
Other
Clinical ethics
Research ethics
Resource allocation ethics
Public health ethics
Nursing ethics
Other

- ***Bioethics helps to study moral vision, decision and policies of human behaviour in relation to biological phenomena or events.***
- ***Ethics deals with 'Life' e.g. in vitro fertilization, sperm bank, gene therapy, cloning, gene manipulations, euthanasia, death, maintaining those who are in comatose state, prenatal genetic selection, etc.***
- ***The era of biotechnology has brought wide spectrum on new topics like cloning, transgenic, gene therapy, eugenics, rDNA technology, etc.***
- ***The use of all these has drawn a wide range of reactions in the society.***

- *The reactions are based on individual's own perception and morals.*
- ***Ethical aspects pertaining to the use of biotechnology seems to be more controversial and frightening.***
- ***These concerns are broadly summarized below :***

❖ *Use of animals causes great sufferings to them.*

❖ ***Voilation of integration of species caused due to transgenosis.***

❖ *Transfer of human genes into animals and vice versa.*

❖ ***Indiscriminate use of biotechnology pose risk to the environment, health and biodiversity.***

- *The introduction of Genetically Modified Organisms (GMOs) has led to a wider debate on bioethical concerns affecting social, economic and environmental spheres.*

- *These include the effects on non-target organisms, insect resistance crops, gene flow and the loss of diversity as well as the issue on interfering with nature in which the modification process itself is disrupting the natural process of biological entities.*

- ***Ethics in biotechnology also includes the general subject of what should and should not be done in using recombinant DNA techniques***

BIOETHICS IN BIOTECHNOLOGY

"Morality is a
private and costly luxury."
Henry B. Adams,

- Who should control technology?
- What should be banned or permitted and who should decide?
- Who should profit?
- Should access to novel and expensive technology be provided to those who cannot afford it?

EFFECTS OF BIOTECHNOLOGY ON THE ENVIRONMENT

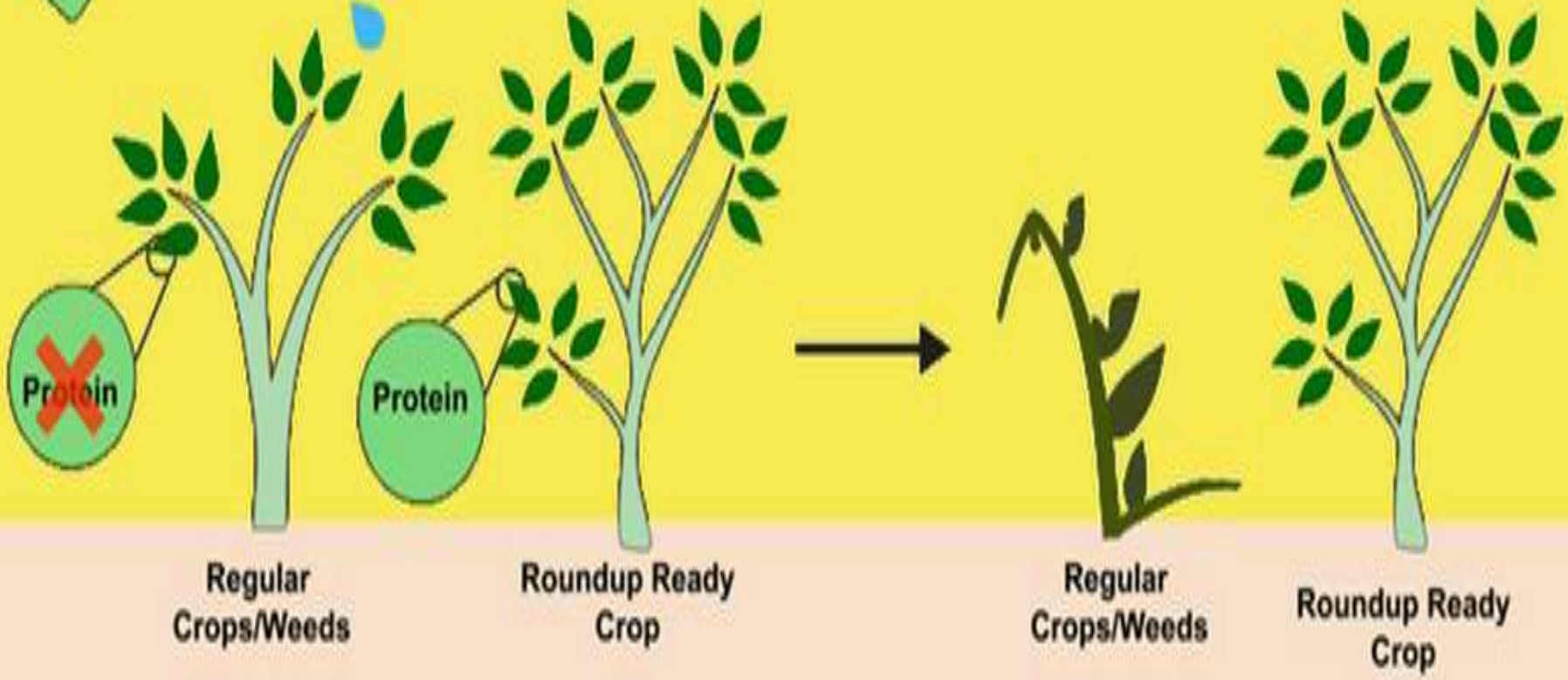
a. Herbicide Use and Resistance

- *Effects on the environment are a particular concern with regard to GMO crops and food production.*
- ***One area of development involves*** *adding the ability to produce pesticides and resistance to specific herbicides.*
- ***These traits are helpful in food production,*** *allowing farmers to use fewer chemicals, and to grow crops in less than ideal conditions.*

- ***However, herbicide use could be increased, which will have a larger negative effect on the surrounding environment.***
- ***Unintended hybrid strains of weeds and other plants can develop resistance to these herbicides through cross-pollination, thus negating the potential benefit of the herbicide.***
- ***One such herbicide that has already been added is RoundUp.***
- ***Crops of RoundUp ready soybeans have already been implemented into agricultural practices, possibly conferring RoundUp resistance to neighboring plants.***

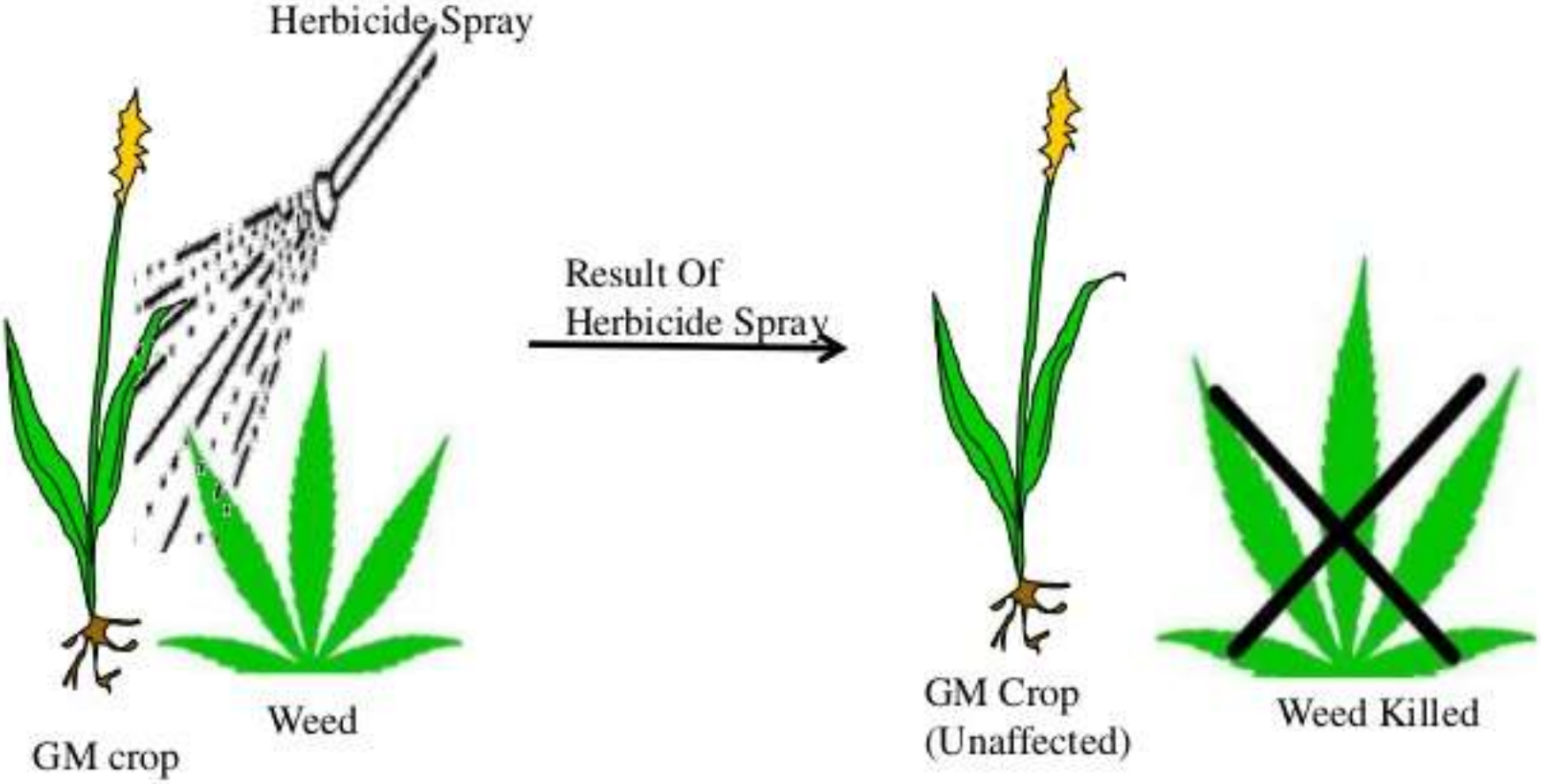

ROUNDUP


ROUNDUP


Protein
Protein
Regular Crops/Weeds
Roundup Ready Crop
Regular Crops/Weeds
Roundup Ready Crop

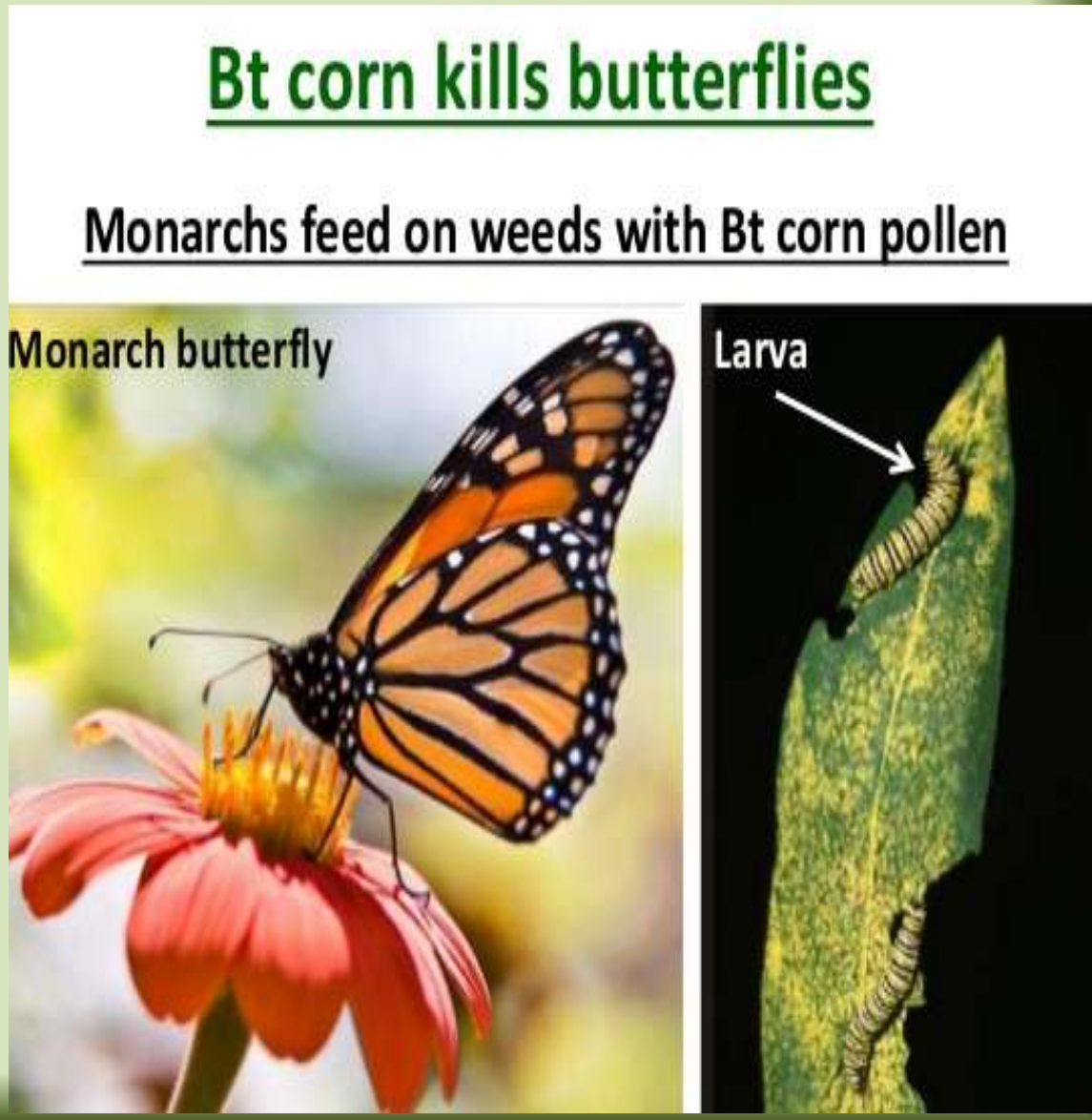
Herbicide Tolerance

- Over 63% of Gm crops grown globally have herbicide tolerance traits.
- Herbicide tolerance is achieved through the introduction of a gene from a bacterium conveying resistance to some herbicides. In situations where weed pressure is high, the use of such crops has resulted in a reduction in the quantity of the herbicides used.



b. Effects on Untargeted Species

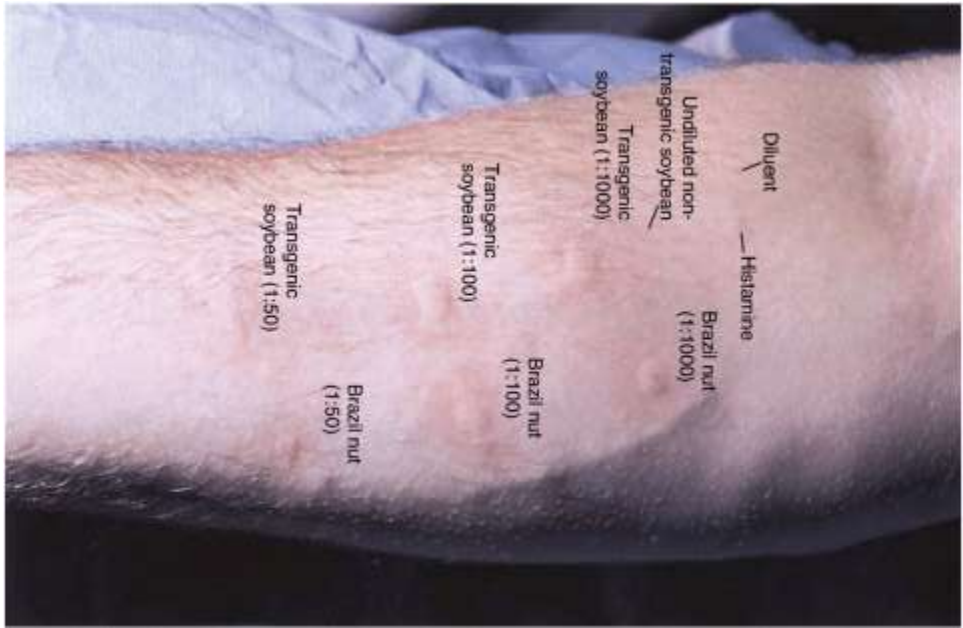
- ***Bt corn**, which produces its own pesticide, is also in use today.*
- *It has adverse effects on Monarch butterfly populations, which are not the original target of the pesticide.*
- *It can also have unintentional effects on neutral or even beneficial species.*



EFFECTS OF BIOTECHNOLOGY ON HUMAN HEALTH

a. Allergies :

- *GMO crops could potentially have negative effects on human health as well.*
- *Consumers have developed unexpected allergic reactions.*
- *e.g. Researchers used a gene from the Brazil nut to increase the production of Methionine in soya beans.*
- *The insertion of this gene inadvertently caused allergic reactions to the soya bean in those with known nut allergies ("Biotech Soybeans").*



b. Long-Term Effects :

- Because GMO technology has been available for such a short amount of time, there is relatively little research which has been conducted on the long-term effects on health which we cannot anticipate at this point.

c. New Proteins :

- Proteins that have never been ingested before by humans are now part of the foods that people consume every day.
- Their potential effects on the human body are unknown so far.

d. Food Additives :

- ***GMOs also present us with possibilities of introducing additional nutrients into foods, as well as antibiotics and vaccines.***
- ***This availability of technology can provide nutrition and disease resistance to those countries that don't have the means to provide these, otherwise.***
- ***However, there is possibility of the creation of antibiotic and vaccine-resistant strains of diseases.***

- *This shows that the vast advances in life sciences and our multicultural and pluralistic modern societies create numerous bioethical problems requiring some stringent regulation.*
- *In terms of GMOs, the Indian Government has set up the Genetic Engineering Approval Committee (GEAC).*
- *This organization makes decisions regarding the validity of research involving GMOs and addresses the safety of GMOs introduced for public use.*

BIOPATENT AND BIOPIRACY

a. Biopatent :

- *Patent is a special right granted to the inventor by the government.*
- *Patent is a personal property of inventor.*
- *It can be sold like any other property.*
- *A patent consists of three parts :*

1. *Grant* ***(aggrement with the inventor),***
2. *Specification* ***(subject matter of invention) and***
3. *Claims* ***(scope of invention to be protected).***

- *Biopatent is a biological patent.*
- *Biopatents are awarded for strains of microorganisms, cell lines, genetically modified strains, DNA sequences, biotechnological processes, product processes, product and product applications.*

- ***Biopatents are awarded to recognize real innovative contributions made by the inventor to the cause of human welfare.***
- ***The awards are given to inculcate encouragement and values in developing scientific culture and in emphasizing the role of biology in shaping human society.***
- ***Indian patent allows 'process patent' and not the 'product patent'.***
- ***Biopatent allows the patent holder to exclude others from making, using, selling or importing protected invention for a limited period of time.***
- ***Duration of biopatents is five years from the date of the grant or seven years from the date of filing the patent application, which ever is less.***

- ***First biopatent was patented pertaining to genetically engineered bacterium 'Pseudomonas' used for clearing oils spills.***
- ***Patent under the title 'control of plant gene expression' was issued jointly to Delta and Pineland company and U. S. department of agriculture.***
- ***Patent is based on a gene that produces a protein toxic to plant thus, do not allow seeds to germinate.***
- ***However, this patent was not granted by Indian Govt.***
- ***Such a patent is considered morally unacceptable and fundametally unequitable.***
- ***This is because financially powerful corporations would aquire monopoly over biotechnological process.***
- ***This in turn would pose a threat to global food security.***

b. Biopiracy :

- ***Pirates in general terms are those who steal and kill others to enrich themselves.***
- ***Biopirates are those who do not kill but steal the patent (misuse the patent).***
- ***Biopiracy is defined as 'theft of various natural products and then selling them by getting patent without giving any benefits or compensation back to the host country'.***
- ***In short, it is unauthorized misappropriation of any biological resource and indigenous knowledge.***

- ***Most developed, industrialized and financially rich nations are poor in biodiversity or traditional knowledge.***
- ***Developing and underdeveloped nations have ample of biodiversity and they traditionally know better use of their bio-resources.***
- ***Traditional Knowledge naturally includes a deep understanding of ecological processes and the ability to sustainably extract useful products from the local habitat.***
- ***Most Traditional Knowledge is handed down through generations.***
- ***This helps to develop modern, commercial applications that save the makers time, money and effort.***

Components of Traditional Knowledge that are especially relevant to our global survival include knowledge of :

- ***Food, crop varieties and agricultural/ farming practice.***
- ***Sustainable management of natural resources and conservation of biological diversity.***
- ***Biologically important medicines.***

- ***The conservation of species, habitat, and biodiversity are essential to the continued survival of indigenous and rural people.***
- ***By conserving the customs and habitat of indigenous people, we concurrently reduce emissions from deforestation and ecosystem degradation.***
- ***The opportunity for cultural survival is a basic human right.***
- ***The traditional knowledge is facing a problem of bio-piracy.***

- ***The act of Piracy is unauthorized publication or reproduction of another person's work or material.***

- ***When someone indulges in piracy, the accused is using someone's work illegally or without taking any permission.***

- ***The innovations and discovery of the pharmaceutical and agricultural researches are not new as to qualify as invention as they are based on centuries of knowledge of the traditional societies.***

Examples of Biopiracy

- **Patenting of Neem (Azadirachta indica) :**

- ***Indians, in a variety of ways have used neem, since time immemorial.***
- ***Indians have shared the knowledge of the properties of the neem with the entire world.***
- ***Pirating this knowledge, the USDA and an American MNC W.R. Grace in the early 90s sought a patent from the European Patent Office (EPO) on the "method for controlling on plants by the aid of hydrophobic extracted neem oil."***
- ***The patenting of the fungicidal properties of Neem, was an example of biopiracy.***

1990
- Multinational chemical corporation, W R Grace and Co (Neem-based bio-pesticides)

1994
- European Patent Office (EPO) granted a patent

1995
- Opposed By Vandana Shiva of the Research Foundation for Science, Technology and Ecology(RFSTE)

2000
- EPO revoked patent on the basis of lack of inventive step and novely.

• Patenting of Basmati :

- ***Basmati is a long-grained, aromatic variety of rice indigenous to the Indian subcontinent.***
- ***In 1997 the US Patent and Trademark Office (USPTO) granted a patent to a Texas based American company Rice Tec Inc for "Basmati rice line and grains" having trade name Texmati.***
- ***The patent application was based on 20 very broad claims on having "invented" the said rice.***
- ***Due to peoples movement against Rice Tec in March 2001, the UPSTO rejected all the claims.***

Opposed by Government of India

In 2000 re-examination /indepth analysis of application by high level inter-ministerial group

Patent cancelled in 2000 and India won the case on basis of violation of GI under TRIPS.

• Haldi (Turmeric) Biopiracy :

- *Two American researchers of Indian origin of the University of Mississippi Medical Center, put a claim to the US Patent and Trademark Office, maintaining that they had discovered haldi's healing properties.*
- *Surprisingly, they were granted a patent in March 1995 for something you had known for years and our Ayurveda for centuries.*

(1993) Patent application by University of Mississippi Medical Center

(1995) USPTO granted patent for use of turmeric in wound healing

Opposed by India's Council of Scientific and Industrial Research(CSIR)

1997 Patent cancelled

- *It meant they had exclusive rights over any such haldi drug and were in a position to make millions of dollars.*
- *The Council of Scientific and Industrial Research (CSIR) applied to the US Patent Office for a re-examination and they realized the mistake and cancelled the patent.*
- *This was after Indian scientists strongly objected about how we are losing our traditional knowledge to foreign companies who have started poaching on our ancient healing techniques.*

It is the need of hour to launch genetic literacy movement in Indian school and colleges for better understanding of opportunities and risks related to biotechnology and also to promote the safe and meaningful use of technologies of modern life sciences.