Studybeesofficial

Where Minds Pollinate

Class 12

Biology

Chapter 10 Notes

Follow Our Socials

studybeesofficial

studybeesofficialpak

studybeesofficialpak

Chapter#10 Biotechnology

Introduction to Genetic Engineering:

Genetic engineering, or Recombinant DNA Technology, involves manipulating an organism's genetic material to modify its characteristics for human welfare.

It based on the Recombinant DNA Technology.

- **Recombinant DNA:** The process of combining genes from different sources to create new genetic combinations.
- **Modification:** It allows for the addition, removal, or editing of specific genes within a genome to improve existing traits or eliminate undesirable ones.

Major Applications

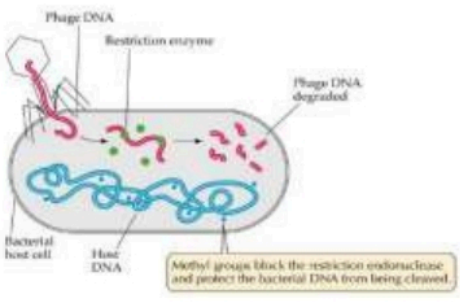
- **Agriculture:** Development of genetically modified (GM) crops that are resistant to pests, diseases, and environmental stresses, as well as more nutritive crops.
- **Medicine:** Development of gene therapies for genetic disorders, synthesis of improved biopharmaceuticals like insulin and antibiotics, and the creation of new vaccines.
- **Industry:** Production of biofuels, biodegradation of industrial waste, and the development of environmentally friendly chemicals.

Molecular Scissors (Restriction Endonuclease):

- **Restriction Endonucleases** are enzymes that act as "molecular scissors."
- **Mechanism:** They cleave the **phosphodiester bonds** of both strands of a DNA duplex at specific sequences.
- **Purpose in Biotech:** They are used to cut a specific "gene of interest" during the process of genetic recombination.

Natural Role in Bacteria

- **Defense System:** They serve as a host-defense mechanism in bacterial cells against infecting viruses (bacteriophages).
- **Why the name "Restriction"?** They "restrict" the growth of viruses by digesting (chopping up) the viral DNA/RNA at specific sites.
- **Self-Protection (Methylation):** Bacteria protect their own DNA from being cut by adding a **methyl group** to the nucleotides in their own recognition sequences. This blocks the endonuclease from attacking the host DNA.



Nomenclature (Naming Convention):

Restriction enzymes are named based on the bacteria from which they were isolated:

- **Genus:** The first part of the name.
- **Species:** The second part.
- **Strain/Order:** The final letters or Roman numerals.

Enzyme	Source Bacteria
EcoRI	*Escherichia coli*
Hind-II / Hind-III	*Haemophilus influenzae*
XhoI	*Xanthomonas holcicola*

Recognition Sites and Palindromes

- **Restriction/Recognition Sites:** Specific sequences (usually 4 to 8 base pairs long) where the enzyme cuts.
- **Palindromic Sequences:** The sequences are symmetrical. This means they read the same on both complementary strands when read in the same direction (e.g., 5' to 3').

Types of DNA Cuts

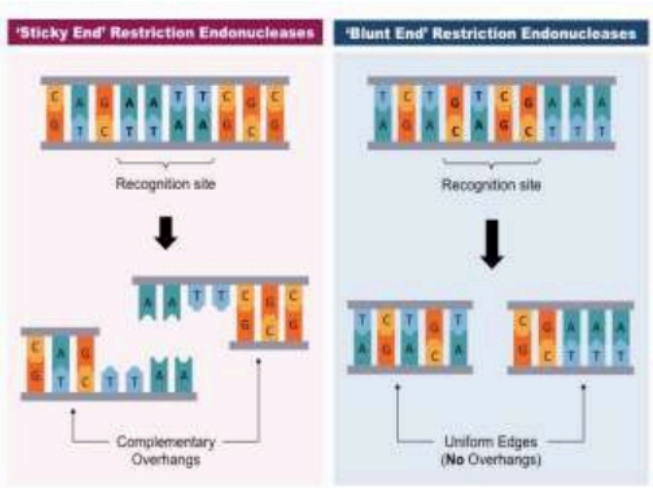
The images describe two primary ways these enzymes cut DNA:

A. Staggered Cut (Sticky Ends)

- The enzyme cuts the strands at different points, leaving overhanging, single-stranded ends.
- **Sticky Ends:** These overhanging ends can easily bond with complementary sequences.
- **Examples:**
  - **EcoRI:** Produces sticky ends at the 5' ends.
  - **Kpn1:** Produces sticky ends at the 3' ends.

B. Blunt Cut (Blunt Ends)

- The enzyme cuts both strands at the same vertical point.
- There are no overhanging single-stranded sequences.
  - **Example: HaeIII** produces blunt ends.



Molecular Carriers or Vectors:

A **vector** is a DNA molecule used as a vehicle to artificially carry foreign genetic material (the gene of interest) into another cell.

Key Functions of Vectors

- **Transport:** They transfer recombinant DNA (rDNA) into a host cell.
- **Multiplication:** They allow the gene of interest to be replicated many times.
- **Expression:** They facilitate the production of specific proteins (e.g., insulin) from the inserted gene.

Types of Vectors

While plasmids are the most common, other examples include:

- **Lambda Phage DNA:** Derived from viruses that infect bacteria.
- **Cosmid:** A hybrid combination of plasmid and phage DNA.
- **YACs:** Yeast Artificial Chromosomes.
- **BACs:** Bacterial Artificial Chromosomes.

Plasmids

Plasmids are the primary tools used in genetic engineering.

Characteristics

- **Structure:** Small, circular DNA molecules.
- **Occurrence:** Naturally found in bacterial cells, but also present in some eukaryotes like Yeast and certain higher plants.
- **Independence:** They are **extra-chromosomal**, meaning they are separate from the main genomic DNA and can replicate independently.
- **Genetic Traits:** They often carry genes for **antibiotic resistance** and fertility.

Common Examples

The text highlights two specific plasmids:

Plasmid	Antibiotic Resistance Provided
pSC-101	Tetracycline
pBR-322	Tetracycline and Ampicillin

Characteristics of Plasmids as Vectors

Plasmids are widely used in biotechnology because of the following key features:

1. Origin of Replication (Ori) Site

- Plasmids contain an **Ori site**, Necessary for self-regulated replication within the host cell.
- Ideal for producing multiple copies of an inserted gene.

2. Selectable Markers (Antibiotic Resistant Genes)

- These markers help identify and select host cells (bacteria) that have successfully taken up the recombinant plasmid.
- Commonly used markers include genes for resistance to antibiotics like **ampicillin** or **tetracycline**.
- Bacteria containing these genes become resistant to the corresponding antibiotic, allowing for easy screening.

3. Restriction Sites

- A vector must have restriction sites for commonly used **endonucleases**.
- At least one restriction site is required to insert a foreign DNA fragment.
- The presence of unique (single) restriction sites provides flexibility and makes the insertion of foreign DNA easier.

4. Promoter Regions

- Plasmids may include **promoter sequences** that enable the transcription of the inserted gene.

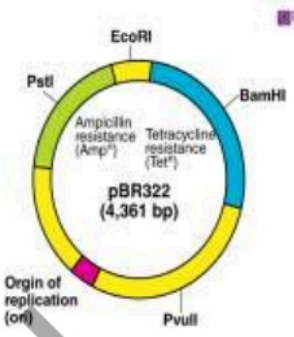
5. Physical Properties

- **Small Size:** Plasmids are much smaller than chromosomal DNA, making them easy to purify as intact molecules and easy to insert into host cells.
- **High Copy Number:** Some plasmids exist in many copies within a single host cell, leading to the production of large amounts of the cloned DNA.

PBR322 Plasmid:

pBR322, which was one of the first widely used artificial cloning vectors in genetic engineering.

- Developed in laboratory by Bolivar and Rodriguez in 1977
- It has origin of replication, restriction site and antibiotic resistant gene



Features:

Origin of replication:

- Carries a fragment of plasmid pMB1 that act as origin of replication.

Size:

- Small size with 4361 bps
- Plasmid transformation efficiency is inversely proportional to size of plasmid.

Copy Number:

- Has high copy number (15 copies per cell)

Cloning sites:

- Has number of unique restriction sites

Production of Human Insulin via Recombinant DNA Technology:

1. Isolation of the Insulin Gene

- **Source:** The insulin gene (INS) is isolated from human DNA, specifically located on chromosome 11 at position 11p15.5 (on short arm p, region 11, Band 15 & sub band 5)
- **Structure:** The gene is approximately 1.5Kbps long, containing three exons and two introns.
- **Function:** Naturally produces insulin by the beta-cells of the pancreas to regulate blood sugar with 2 peptide chains (alpha and beta)

2. Selection of Plasmid Vectors

Plasmids are engineered to carry the insulin gene into host cells like *E. coli*. Common vectors include:

- **pBR322:** Most widely used plasmid that contains resistance genes for ampicillin and tetracycline.
- **pUC18/pUC19:** Derivatives of pBR322 modified for more efficient transformation.
- **Key Features:** These plasmids are chosen for their small size, high copy number, and unique restriction sites.

3. Creation of Recombinant DNA

- **Synthetic Genes:** Because bacteria cannot process complex eukaryotic proteins directly, two separate synthetic genes are created for the alpha and beta chains of insulin.
- **Insertion:** These genes are inserted into separate bacterial plasmids (e.g., pBR322) next to a beta-galactosidase gene.

- **Expression:** The plasmids include a promoter sequence to initiate the production of the insulin chains once inside the bacterial cells.

4. Insertion of recombinant plasmids into host cells

- Recombinant plasmids are inserted into *E. coli* or other bacterial strains via **transformation** that become **recombinant bacteria**.

5. Production of insulin chains

- Bacteria grow in large **fermentation tanks** with optimum culture medium and conditions.
- The insulin gene is multiplied (cloned) as the plasmid DNA replicates.
- Bacteria produce **pro-insulin chains** (A and B) fused with **beta-galactosidase**.
- These **fusion proteins** protect the insulin chains from being destroyed by bacterial protease enzymes.

6. Extraction and Purification

- Bacterial cells are harvested and the fusion proteins are isolated.
- The A and B chains are separated from the beta-galactosidase using **cyanogen bromide**.
- This separation is possible because an extra methionine codon is added at the N-terminal of each gene.

7. Folding and disulphide bridging of both insulin chains

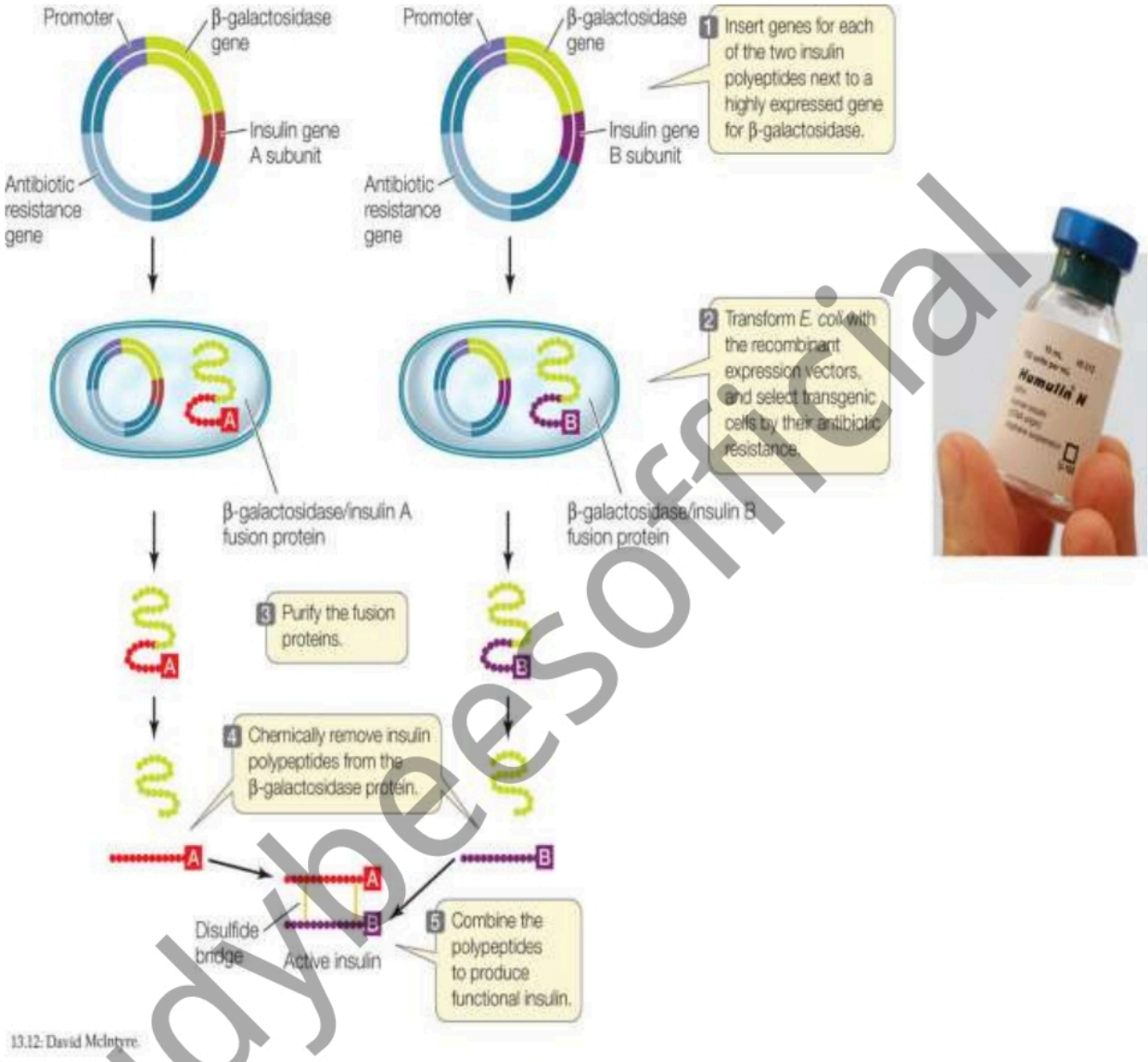
- The detached A and B chains are chemically joined *in-vitro* to form functional insulin.
- **Disulphide bridges** are formed at cysteine residues using sodium disulphonate and sodium sulphite which results in the formation of **biologically active insulin**.

8. Purification and testing

- Insulin is further purified to remove any bacterial impurities or by-products.
- Strictly tested for **quality, effectiveness, safety**, and potential side effects.

9. Packaging for storage, shipment and usage

- Once quality is confirmed, insulin is packaged into **vials or insulin pens**.
- This recombinant version is **purer, safer, and more effective** than insulin formerly derived from animals.



Polymerase Chain Reaction (PCR):

Definition: An *in vitro* replication process used to amplify (clone) a single gene or piece of DNA into thousands or millions of copies.

Inventor: Kary B. Mullis (1983); he was awarded the Nobel Prize in Chemistry in 1993 for this invention.

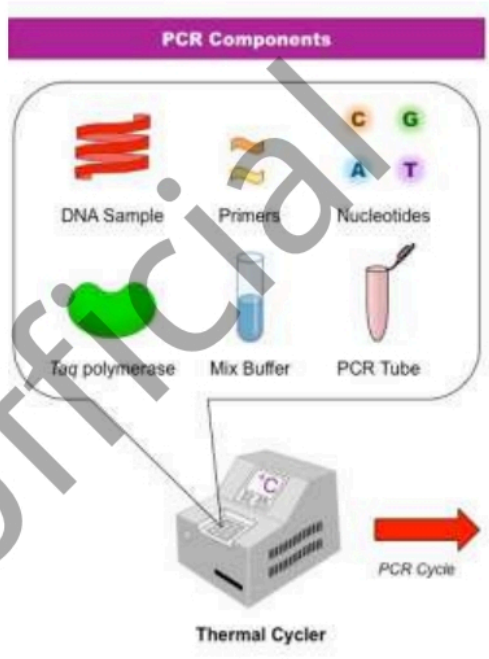
The "Polymerase" name: Named because DNA polymerase is compelled to polymerize a given piece of DNA repeatedly.

Thermocycler: The instrument (PCR machine) that automatically regulates the temperature for each step.

Key Components of the PCR Mixture

To perform PCR, the following are diluted in a **suitable buffer**:

1. **Template DNA:** The specific DNA sequence to be amplified.
2. **dNTPs:** Free nucleotides (deoxynucleoside triphosphates) used as building blocks.
3. **Primers:** Short sequences (Forward and Reverse) that mark the start and end of the target DNA.
4. **Taq Polymerase:** A temperature-tolerant DNA polymerase isolated from the bacterium *Thermus aquaticus* (found in hot springs). It remains stable at near-boiling temperatures.



The Mechanism: Three Main Cyclic Steps

A single PCR cycle doubles the DNA amount. It consists of the following steps:

1. The Denaturation Phase

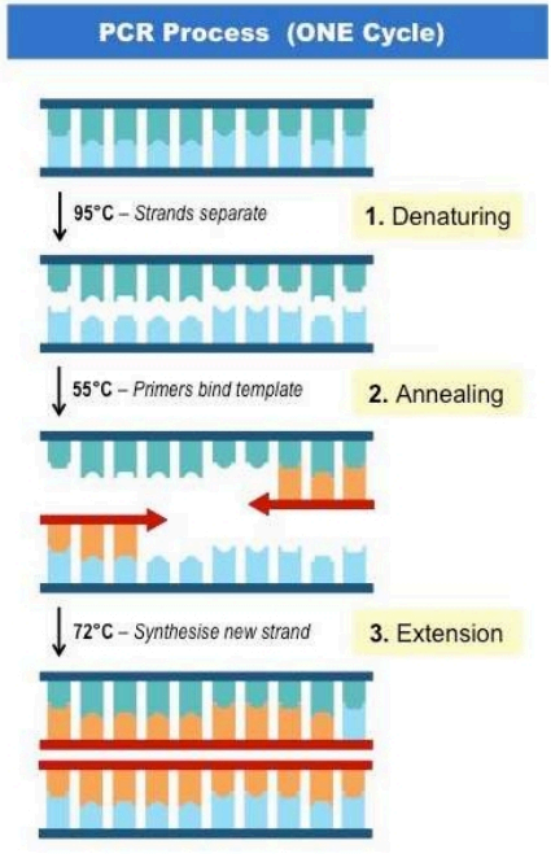
Denaturation occurs in two distinct ways during the process:

a. Initial Denaturation

- **Occurrence**: Performed only once before the cyclic steps begin.
- **Parameters**: Typically set at **94–95°C for 4–5 minutes**.
- **Purpose**: To ensure all DNA strands are completely separated.
- **Importance**: Critical for GC-rich regions or complex structures to break hydrogen bonds fully, allowing proper primer binding.

b. Cyclic Denaturation

- **Occurrence**: Performed at the start of every PCR cycle.
- **Parameters**: Heated to **94°C for one minute**.
- **Result**: Double-stranded DNA (dsDNA) becomes single-stranded (ssDNA), providing the template for synthesis.



2. Primer Annealing:

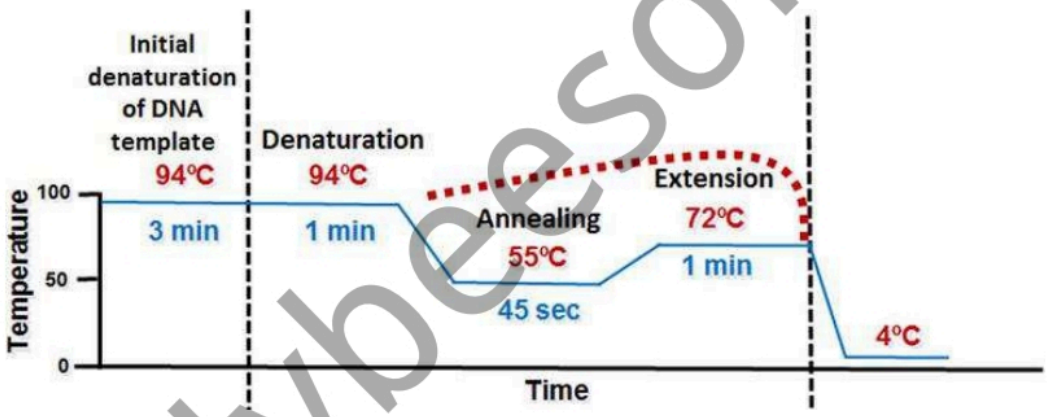
- **Temperature**: Lowered to **55–65°C** depending on primer length and gene size.
- **Duration**: Approximately **2 minutes**.
- **Action**: Primers bind to the complementary regions of the ssDNA.

3. Extension (Polymerization)

a. Cyclic Extension: *Taq* polymerase synthesizes new DNA strands at the 3' ends of primers using dNTPs at an optimum temperature of **72°C** for **1–2 minutes**.

b. Final Extension: Performed once after all cycles are complete at **72°C for 5–7 minutes**.

- **Purpose of Final Extension**: Complete synthesis of all PCR products by Taq Polymerase
- **Reason**: Final extension ensures the formation of complete double stranded products.



How DNA molecules double with each cycle???

- **Cycle Completion:**

  The first cycle finishes by converting one target double-stranded DNA (dsDNA) molecule into two daughter molecules.

- **Continuous Cycling:**

  The second cycle begins immediately with heat denaturation at **94°C**, turning the new molecules back into single-stranded templates.

- **The Triple-Step Process:**

  The process repeats the sequence of **denaturation, primer annealing, and extension** to drive the reaction forward.

- **Exponential Growth:**

  Because the number of DNA molecules doubles after every cycle, the target sequence is amplified at an exponential rate.

- **Support Steps:**

  Initial denaturation and final extension (the "non-cyclic" steps) are crucial for ensuring the overall efficiency, accuracy, and yield of the PCR.

- **Post-Reaction Storage:**

  Once the final extension is complete, the thermal cycler drops to **4°C** to protect the replicated DNA until it can be moved to a refrigerator.

PCR Thermal Profile Summary

The following table summarizes the typical settings programmed into a thermocycler:

Step	Temperature	Duration	Frequency
Initial Denaturation	94–95°C	4–5 minutes	Once (1-hold)
Denaturation	94°C	1 minute	25–35 cycles
Annealing	55–65°C	2 minutes	25–35 cycles
Extension	72°C	1–2 minutes	25–35 cycles
Final Extension	72°C	5–7 minutes	Once (1-hold)
Storage	4°C	Infinity	Once (1-hold)

Genetically Modified Organisms (GMOs):

Definition and Process

- **What they are:** Organisms (like bacteria, plants, or animals) with DNA that has been altered in ways that do not occur naturally through reproduction.
- **The Method:** Genetic engineering techniques are used to **insert** foreign genes, **remove** existing ones, or **modify** specific genes.
- **The Goal:** To convert naturally occurring organisms into versions that possess "desirable traits."

Benefits and Characteristics

GMOs are engineered to provide several advantages, including:

- **Improved Nutrition:** Enhanced vitamins or minerals.
- **Faster Growth:** Increased growth rates for higher productivity.
- **Resilience:** Resistance to pests, diseases, and harsh environmental conditions (like drought).

Applications

Genetically modified and **transgenic organisms** are currently used across four major sectors:

1. **Agriculture** (e.g., pest-resistant crops)
2. **Animal Husbandry** (e.g., livestock)
3. **Medicine** (e.g., insulin production via bacteria)
4. **Research** (e.g., laboratory studies)

1. Transgenic Bacteria

- **Why Bacteria?** They are easily transformed because of their **simple genetics**.
- **Key Milestone (1978):** The human insulin gene was first inserted into *E. coli* to produce synthetic "human" insulin.
- **Applications:**
  - Production of human proteins.
  - Enhancement of plant growth.
  - **Environmental Cleanup:** Removal of pollutants.
  - **Mining:** Extraction of metals from low-grade ores.

2. Transgenic Plants

- **Goal:** To introduce traits that do not occur naturally in the species.
- **Key Milestone (1986):** First field trials occurred in the USA and France using **tobacco plants** engineered for herbicide resistance.
- **Application:**
  - Resistance to pests, diseases, or harsh environmental conditions.
  - Improved nutritional value.
  - Production of pharmaceutical agents.

3. Transgenic Animals

- **Definition:** Animals carrying a foreign gene to improve specific traits (especially in farm animals).
- **Transpharmer Animals:** A specific term for transgenic animals used to produce **human proteins or drugs**.
- **Application:**
  - Improve the traits of Farm animals
  - To produce Drugs