BTNY-MM: XII

12. BIOTECHNOLOGY & ITS APPLICATIONS

Biotechnology has a wide range application such as biopharmaceuticals, therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, energy production etc.

❖ 3 critical research areas in Biotechnology:-

a. Providing the best **catalyst** in the form of improved organism usually a microbe or pure enzyme.

b. Creating **optimal conditions** through engineering for a catalyst to act.

c. **Downstream processing technologies** to purify the protein/organic compound.

APPLICATION IN AGRICULTURE

3 options for increasing food production

1. Agro-chemical based agriculture
2. Organic agriculture
3. Genetically engineered crop-based agriculture

Genetically Modified Organisms (**GMO**) or **transgenic organisms** whose genes are altered by manipulation.

Advantages of GM plants:

- **Increase tolerance** against abiotic stresses (cold, drought, salt, heat etc).
- **Pest-resistant crops** reduce the use of chemical pesticides.
- **Reduced post harvest loss**.
- **Increased efficiency of mineral usage** by plants (this prevents early exhaustion of fertility of soil).
- **Enhances nutritional value of food**. E.g. Vitamin 'A' enriched rice.
- **Creation of tailor-made plants** to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Development of Pest Resistant Plants

- It reduces the need for pesticides.
  E.g. Bt cotton, Bt corn, rice, tomato, potato, soyabean etc.

A. Bt Cotton:

- **Insect resistance of *Bt***
  Some strains of ***Bacillus thuringiensis*** (a soil bacterium) produce insecticidal crystal proteins / **Bt toxin-(**coded by ***cry*** gene**)** that kill insects like **coleopterans** (beetles) **lepidopterans** (tobacco budworm, armyworm) & **dipterans** (flies, mosquitoes).

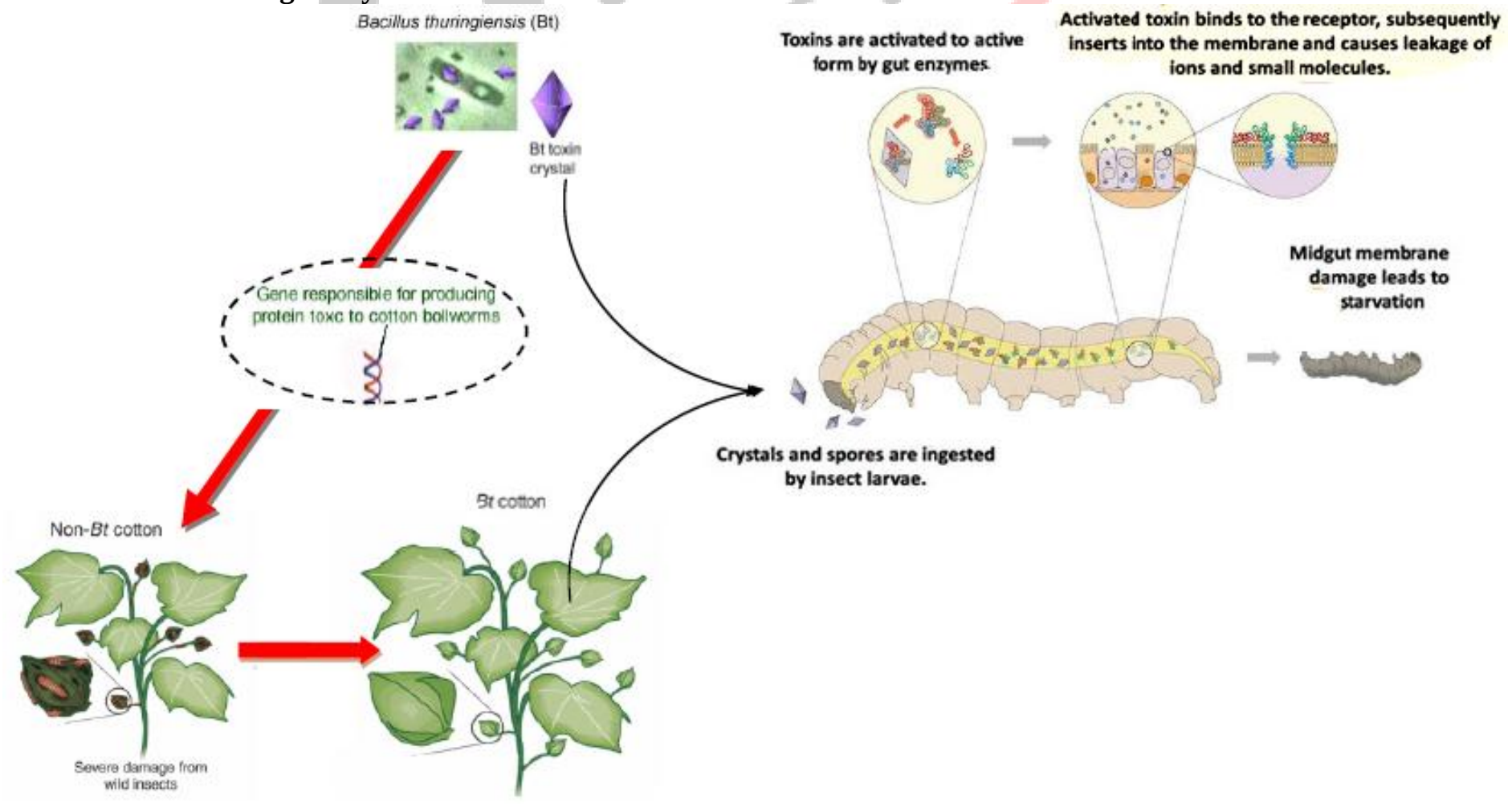
Step 1. Bt produces **insecticidal protein**. (It does not kill the *Bacillus* itself as it exists as inactive ***protoxins***).

Step 2. **Insect ingest** the protoxin

Step 3. In the gut of insect, due to the **alkaline pH,** crystal protoxin solubilise to form **active toxin**.

Step 4. The toxin **binds to the surface of midgut** epithelial cells and creates pores.

Step 5. It causes **cell swelling** and **lysis** leads to the death of the insect.



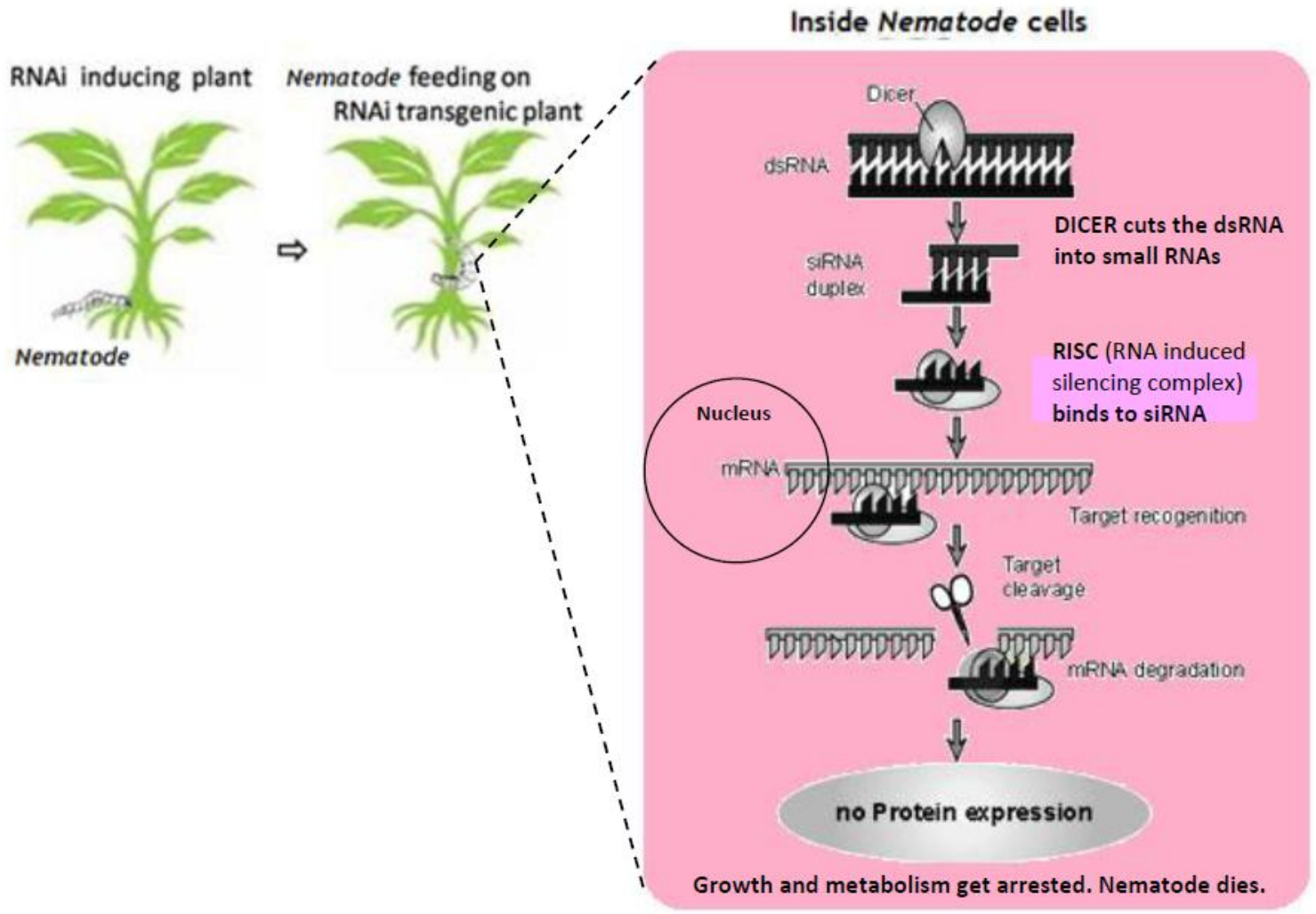
- Bt toxin genes were isolated from *B. thuringiensis* and incorporated into crop plants such as cotton is called **Bt-cotton**.
- Most Bt toxins are insect-group specific.
  E.g. the proteins encoded by the genes-
  cryIAb control corn borer.
  cryIAc and ***cryIIAb*** control the cotton bollworms

B. Nematode resistance in tobacco plants:

- A nematode ***Meloidegyne incognitia*** infects the roots of tobacco plants and causes a great reduction in yield.
- **RNA interference** (RNAi) strategy is used to prevent this infestation.
  RNAi is a method of cellular defense in all eukaryotic organisms. It prevents translation of a specific mRNA (*silencing*) due to a complementary dsRNA molecule.
  - ♥ The source of this complementary RNA is from an infection by **RNA viruses** or **mobile genetic elements** (*transposons*) that replicate via an RNA intermediate.

➔ Using *Agrobacterium* vectors, nematode-specific genes (DNA) were introduced into the host plant.

- ✓ It produced both sense & anti-sense RNA in host cells.
- ✓ These 2 RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of nematode.
- ✓ Thus the parasite cannot survive in a transgenic host expressing specific interfering RNA.



APPLICATION IN MEDICINE

- About 30 recombinant therapeutics have been approved for human-use.
- **Advantages** of rDNA technology in medicine:-
  a. Helps for the mass production of safe and more effective drugs.
  b. The recombinant therapeutics does not induce unwanted immunological responses as products isolated from non-human sources.

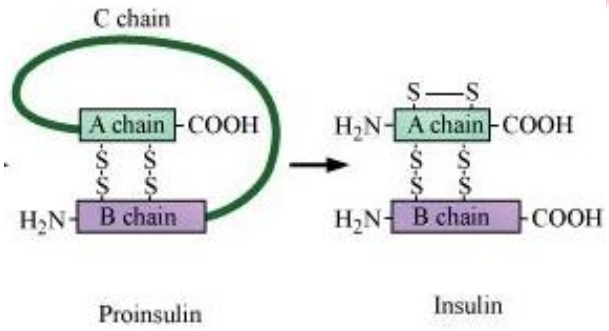
A. Genetically Engineered Insulin:

- Management of adult-onset diabetes is possible by taking insulin at regular time intervals.

Insulin from the pancreas of animals (cattle & pigs) causes allergy or other types of reactions to the foreign protein.

- Now, it is possible to produce human insulin using bacteria.
- Insulin consists of 2 short polypeptide chains (**chain A** of 21 amino acids & **chain B** of 30) that are linked together by disulphide bridges (**1st S-S bond** is between 7th amino acids of each chain and **2nd** is between 20th amino acid of A and 19th of B).
- In mammals, insulin is synthesized as a pro-hormone containing an extra stretch called **C-chain** (of 33 amino acids).
  The pro-hormone needs processing (removal of **C chain)** before it becomes a fully mature and functional hormone.

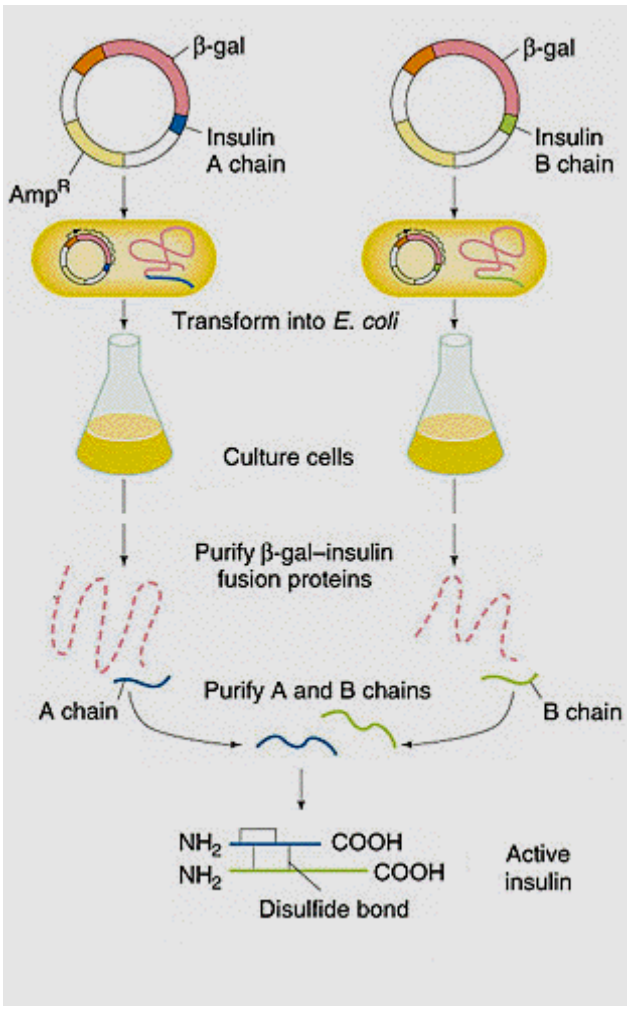
➔ ***Challenge:*** Getting insulin assembled into a mature form.



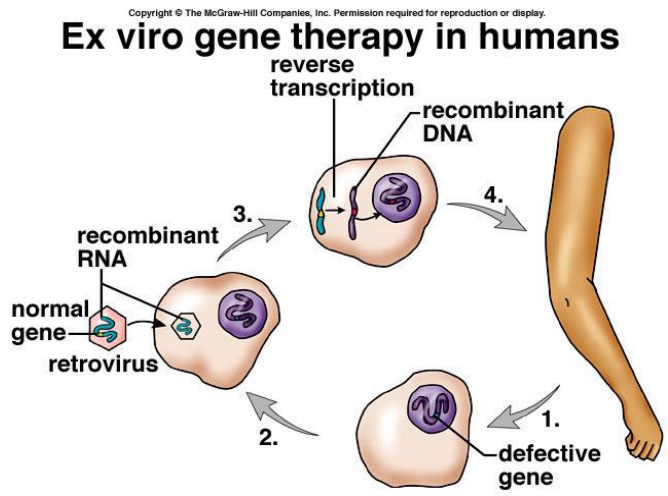
- In 1983, *Eli Lilly* an American company prepared human insulin.

(Step-1) Prepared 2 DNA sequences corresponding to A & B chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains.

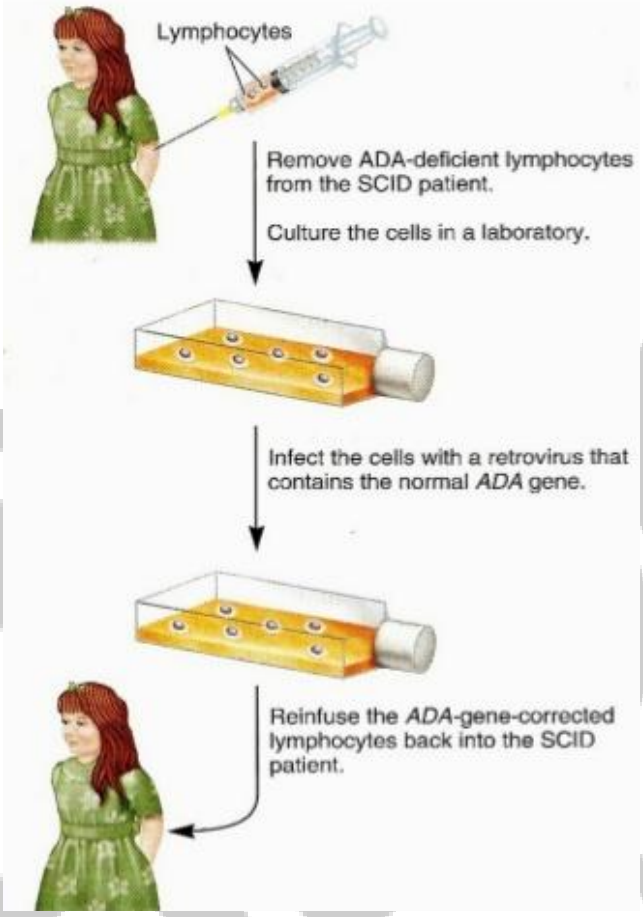
(Step-2) Chains A & B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.



B. Gene Therapy:



- It is a method to correct a gene defect diagnosed in a child/embryo.
- By inserting normal gene, the defective genes are replaced and non-functional genes are compensated.
- First clinical gene therapy was given in 1990 to **Ashanti DeSilva** of U.S, a 4-year old girl with **adenosine deaminase (ADA) deficiency.**
  Severe Combined Immunodeficiency (SCID) is caused due to the deletion of the gene for ***adenosine deaminase*** (the enzyme crucial for the immune system to function). The patient have non-functioning T-lymphocytes. So, they cannot produce immune responses against invading pathogens.
  This can be cured by –
  1. **Bone marrow transplantation**
  2. **Enzyme replacement therapy** (injection of functional ADA).
     But these approaches are not completely curative. But, a permanent cure can be achieved through gene therapy.



(Step-1) Lymphocytes are collected from the patient and grown in a culture.
(Step-2) Then, a functional ADA cDNA (using a retroviral vector) is introduced into these lymphocytes.
(Step-3) Then, they are returned to the patient's body.
This should be periodically repeated as these cells are mortal.

➔ For permanent cure, the ADA gene (from marrow cells) is introduced into cells at early embryonic stages.

C. Molecular Diagnosis:

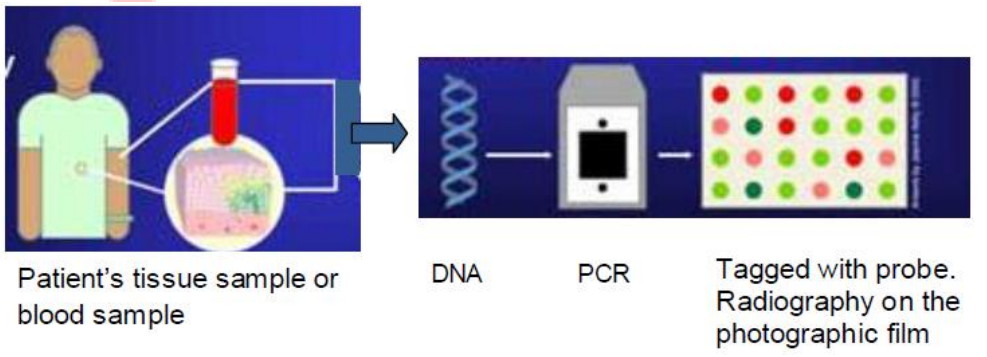
✓ Presence of a pathogen (bacteria, virus etc.) is normally suspected only when the pathogen has produced a symptom. By this time the concentration of pathogen is already very high in the body.

Conventional methods of disease diagnosis like serum and urine analysis are not able to detect low con. of pathogens.

- **Recombinant DNA technology, PCR & ELISA** are some techniques for early diagnosis.

1. Recombinant DNA technology

➢ **Probe** (*'Detective'*-A single stranded DNA or RNA, tagged with a radioactive molecule) is allowed to hybridise to its complementary DNA in a clone of cells
➢ Cells are detected using autoradiography.
➢ The cells having the mutated gene will hence not appear on the photographic film, because the probe will not have complimentary with the mutated gene.

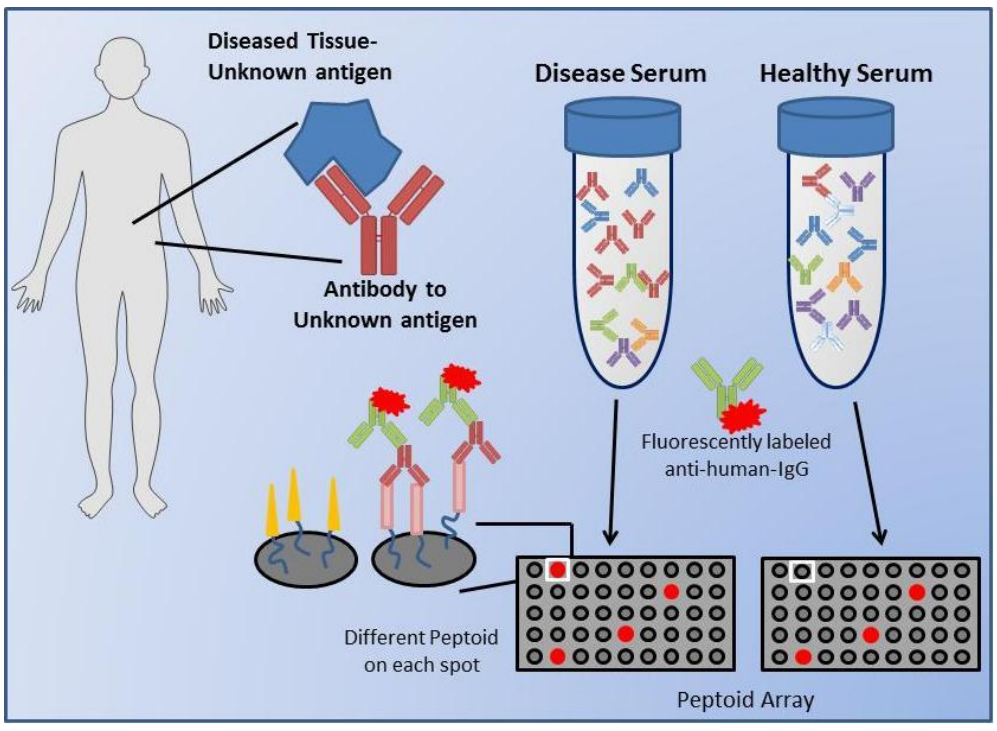


2. PCR (Polymerase Chain Reaction)

Very low concentration of a pathogen can be detected by amplification of pathogen's nucleic acid by PCR.

Applications of PCR

- It is a powerful technique to **identify many genetic disorders**.
- Used to detect HIV in suspected AIDS patients.
- It is used to detect mutations in genes in suspected cancer patients.



3. ELISA (Enzyme Linked Immuno-sorbent Assay)

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

TRANSGENIC ANIMALS

♥ These are the animals whose genome has been altered by introduction of an extra (foreign) gene by manipulation.
E.g. Transgenic rats, rabbits, pigs, sheep, cows and fish.

Reasons for developing transgenic animals:-

(a) To study normal physiology & development:

Transgenic animals are used to study how genes are regulated, and how they affect the normal body functions and its development.
E.g. Study of complex factors such as insulin-like growth factor. Genes (from other species) that alter the formation of this factor are introduced and the biological effects are studied. This gives information about the biological role of the factor in the body.

(b) To Study the contribution of genes in the development of a disease:

Transgenic models help for investigation of new treatments for human diseases.
E.g. transgenic models for many human diseases such as cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.

(c) Biological products:

Transgenic animals are used to produce useful biological products by introducing genes which codes for a particular product.
E.g. 1. human protein **(α-1-antitrypsin**) used to **treat emphysema**.
2. products for **treatment of phenylketonuria** (PKU) and **cystic fibrosis** etc.
3. produced **human protein-enriched milk**
In 1997, Rosie (first transgenic cow) produced human protein-enriched milk (2.4 gm per litre). It contains the human **α -lactalbumin** and is nutritionally more balanced product for human babies than natural cow-milk.

(d) Vaccine safety testing:

Transgenic mice are used to test the safety of the polio vaccine.
If it is found to be reliable, they can replace the use of monkeys to test the safety of batches of the vaccine.

(e) Chemical safety testing (toxicity testing):

Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals.
They are exposed to the toxic substances and the effects studied. It gives immediate results.

ETHICAL ISSUES

♥ **Problem of unpredictable results:** Genetic modification may cause unpredictable results when such organisms are introduced into the ecosystem.
Indian Government has set up organizations like **GEAC** (Genetic Engineering Approval Committee), which make decisions about the validity and safety GM products.

♥ **Problems of patent:** Certain companies have got patents for products and technologies that make use of the genetic materials, plants etc that have been identified, developed and used by farmers and indigenous people of a specific country.
E.g. Basmati rice, herbal medicines like turmeric, neem etc.
Basmati rice: This had actually been derived from Indian farmer's varieties. In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. Other people selling Basmati rice could be restricted by the patent.

♥ **Biopiracy:** It is the use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.
Indian Parliament has cleared the second amendment of the **Indian Patents Bill** that takes such issues into consideration.
It prevents unauthorized exploitation of bio-resources and traditional knowledge.