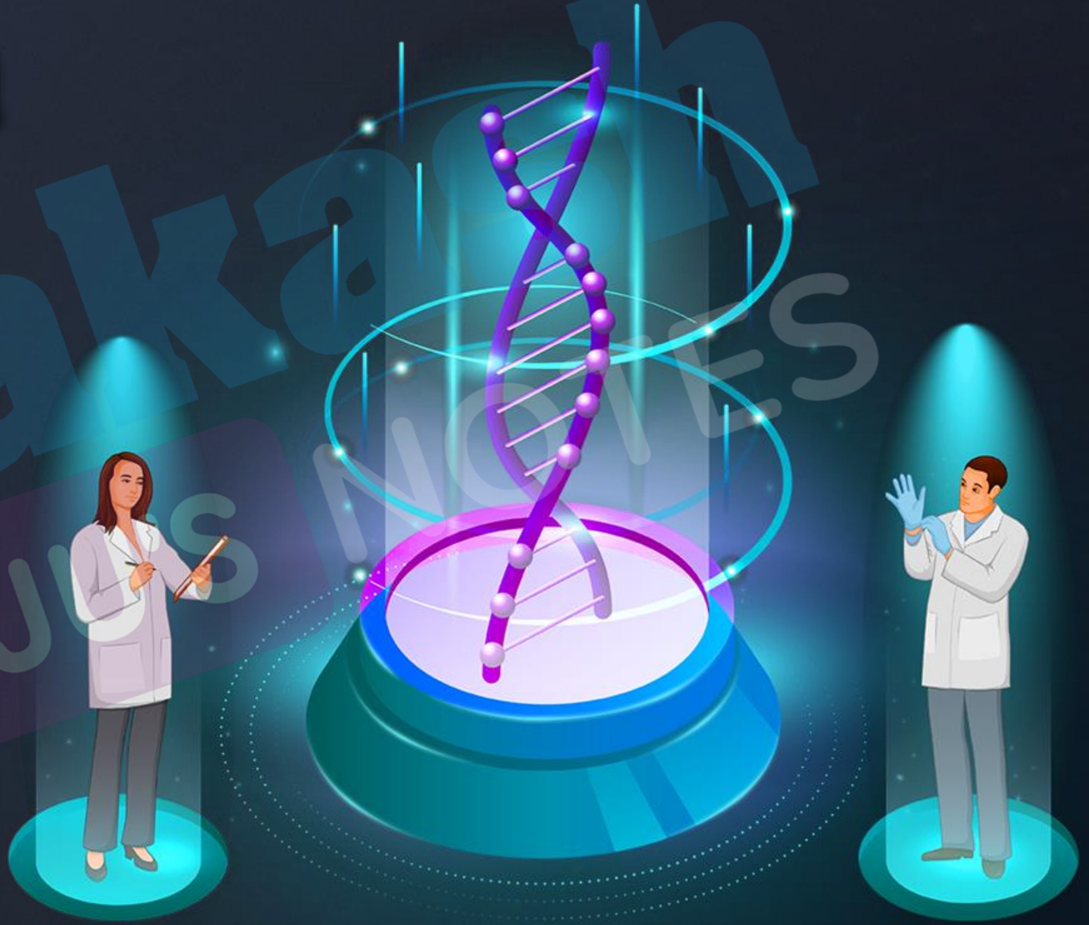
Biotechnology and its Applications



Key Takeaways

1. **Biotechnology**

2. **Application in Agriculture**
   - Genetically modified organisms
   - *Bacillus thuringiensis*
   - RNA interference (RNAi) based pest resistant plants

3. **Application in Medicine**
   - Recombinant therapeutics
   - Gene therapy
   - Molecular diagnosis

4

Transgenic animals

- Medicinal applications
- Agricultural applications
- Industrial applications

5

Ethical Issues

- Bioethics
- Patent
- Biopiracy

Summary

Biotechnology

Three main research areas are :

01

Developing the best catalyst in the form of improved organisms, usually a microbe or pure enzyme

02

Creating optimal conditions through engineering for a catalyst to act

03

Downstream processing technologies to purify the protein/organic compound

Biotechnology

Three options for increasing food production are :

01	02	03
Agro – chemical based agriculture	Organic agriculture	Genetically engineered crop based agriculture
Use of fertilizers and pesticides	**Alternate to agrochemical farming**	**Pioneer was Norman E. Borlaug**
Expensive & causes environment and health related problems	Bacteria along with manure used as biofertilizers	Father of Green Revolution as well

Biotechnology

- The **Green Revolution** succeeded in **tripling the food supply** but yet it was not enough to feed the growing human population.
- **Increased yields** was due to the use of improved crop varieties, better management practices and use of agrochemicals (fertilisers and pesticides).
- However, for farmers in the developing world
  - Agrochemicals are often too expensive
  - Increases in yield with existing varieties are not possible using conventional breeding
  - Fertilizers and pesticides are not cost effective and have harmful effects
- Hence, use of genetically modified crops is a possible solution.

Biotechnological Applications in Agriculture

Genetically modified organisms

- Plants, bacteria, fungi and animals whose genes have been altered by the process of genetic engineering are referred to as genetically modified organisms.
- Genetically modified organisms are:

01 made to be **more tolerant to abiotic stresses** (cold, drought, salt, heat)

02 those that have reduced reliance on chemical pesticides (**pest-resistant crops**)

03 those that have helped to **reduce post harvest losses**

04 noted by **enhanced mineral usage by plants**

05 known for **enhanced nutritional value** of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice

Biotechnological Applications in Agriculture

Genetically modified organisms

Two strategies have been used to enable direct changes in genotype of a plant through gene cloning :

Gene addition

This method involves **altering plant characteristics by insertion of new genes**.

E.g., Bt cotton, Transgenic *Brassica napus*

Gene subtraction

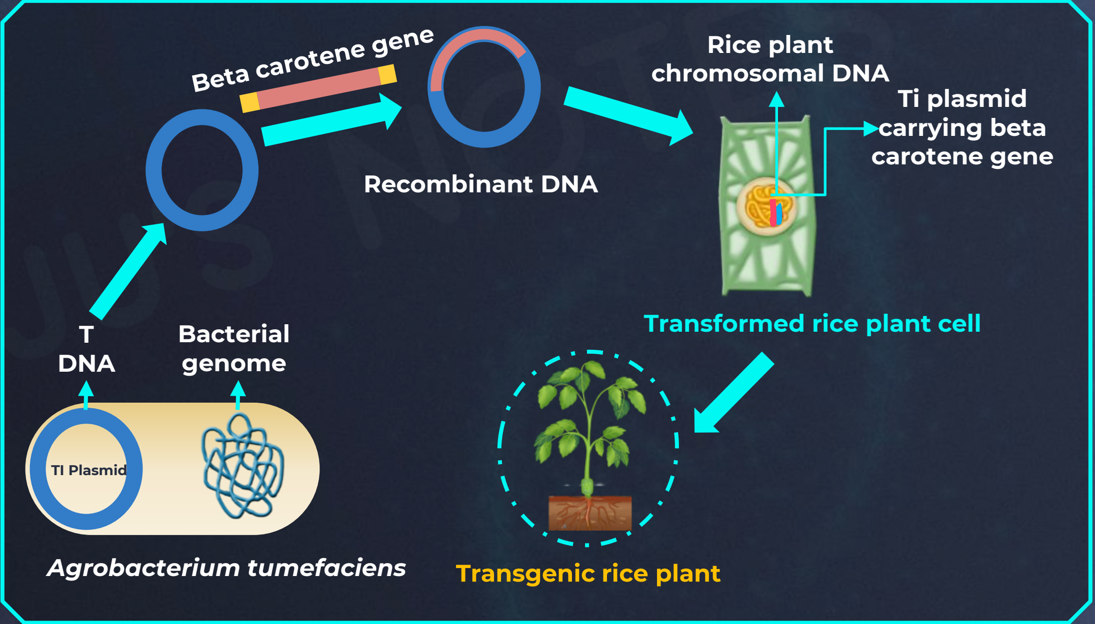
This method involves **inactivation of existing genes**.

E.g., RNA interference in tobacco plant infecting nematode, Flavr Savr tomatoes

Biotechnological Applications in Agriculture

Genetically modified crops

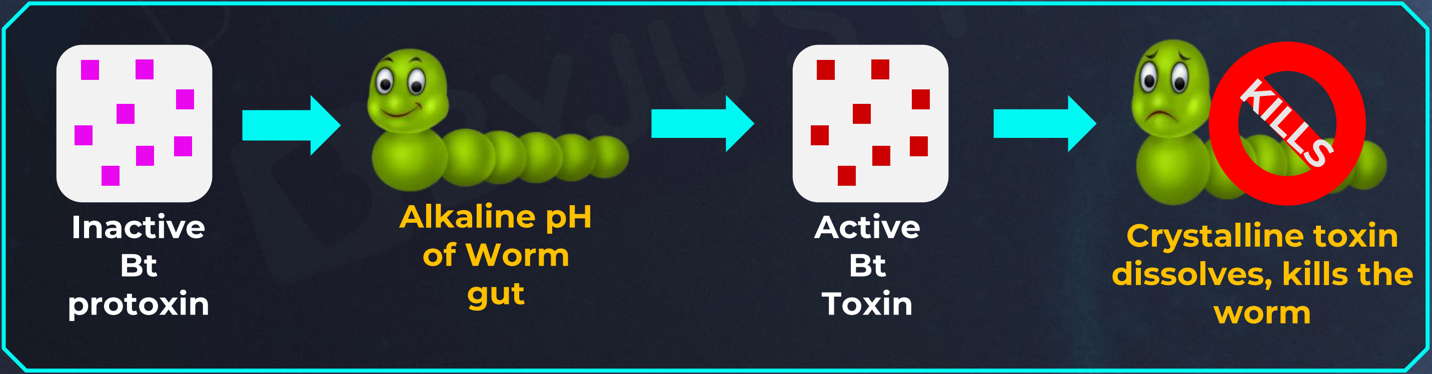
- **Golden rice** is a transgenic variety of rice (*Oryza sativa*).
- Developed by **Ingo Potrykus** and **Peter Beyer** to combat the Vitamin A deficiency which results in poor vision and iron deficiency.
- It contains **beta-carotene** (pro vitamin A) which gives yellow colour to the rice grains.
- The gene for beta-carotene is taken from **daffodil** (*Narcissus pseudonarcissus*) plant.
- The vector used for transfer of gene is *Agrobacterium tumefaciens* vector.



Biotechnological Applications in Agriculture

Bacillus thuringiensis

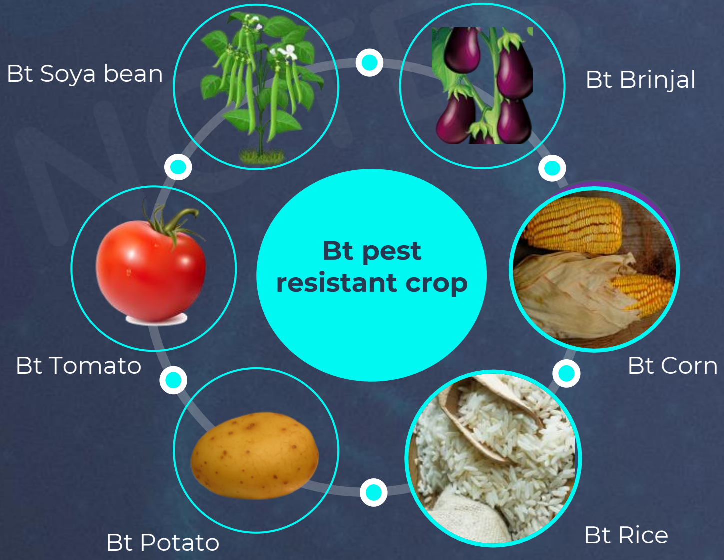
- Defence mechanism against insects - forms **endotoxin** (insecticidal protein).
- The **crystalline protein** encoded by a gene **cry IAc** is present in a **inactive protoxin** form in the bacteria.
- Whenever the worm feeds on the Bt bacteria, this protoxin gets **activated** in **alkaline pH and kills the worm**.
- Toxin binds to the inside of the insects midgut and **damages the surface epithelium by creating pores**.
- These pores cause **swelling** and **lysis**, the insect then **starves to death**.
- This toxin is known as **Bt toxin**.



Biotechnological Applications in Agriculture

Bacillus thuringiensis

- Bt toxin gene is cloned and expressed in many plants like **Bt cotton, Bt tomato, Bt corn, Bt brinjal, Bt rice, Bt soybean, Bt potato**.
- Some strains of ***Bacillus thuringiensis*** produce proteins that kill certain insects such as
  - **Lepidopterans** (tobacco budworm, armyworm)
  - **Coleopterans** (beetles)
  - **Dipterans** (flies, mosquitoes).



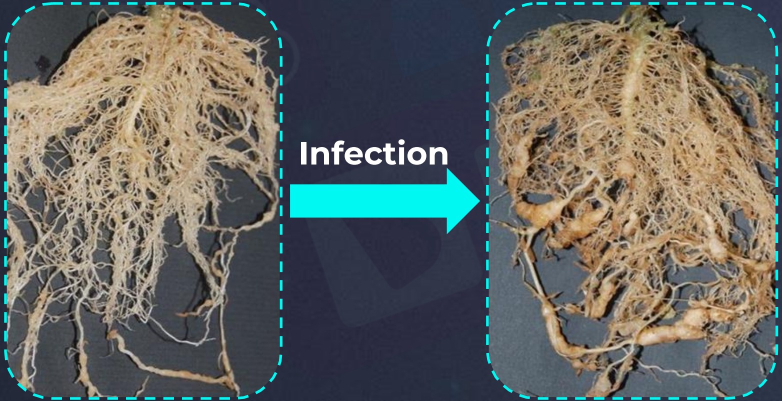
Bt gene - pest resistant crop

Case	Crop	Gene	Pest Resistance
1	Cotton	*cry IAc + cry IIAb*	Cotton Bollworm
2	Corn	*cry IAb*	Corn Borer

Biotechnological Applications in Agriculture

RNA interference (RNAi) based pest resistant plants

- A **nematode** named *Meloidogyne incognita*, commonly known as **root-knot nematode**, causes roots to grow knots and leads to
  - **damaging the crops**
  - **reduction of the yield**



Yield

Prevention strategy:

- **RNA interference strategy** (RNAi) is a
  - **regulatory**.
  - **defence mechanism** in all eukaryotes.
- RNAi technique can **prevent the synthesis of protein** (silence the original mRNA) responsible for the growth of nematode.

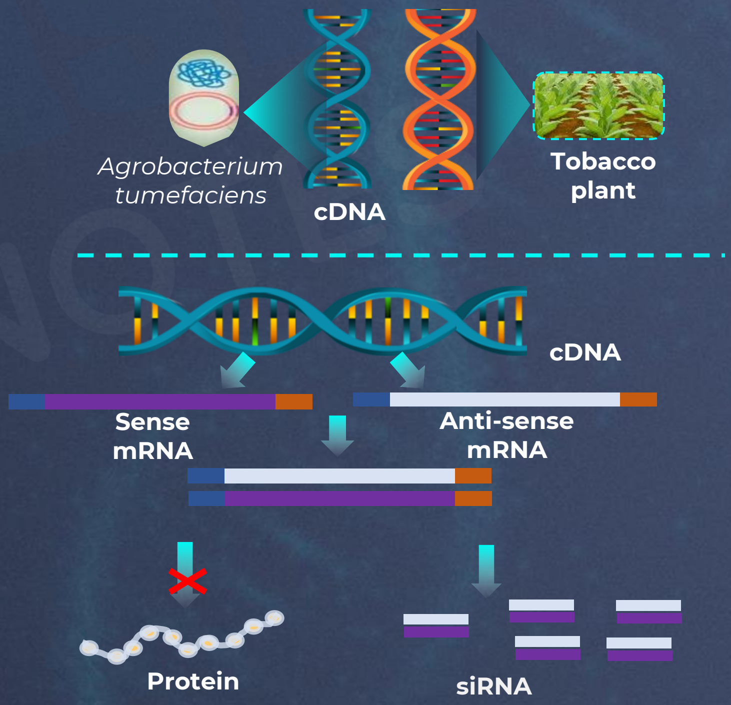
Mechanism of RNAi technique:

- Silences the specific mRNA **due to formation of double stranded RNA (dsRNA)**.
- dsRNA forms by binding with complementary RNA (antisense RNA) to original mRNA.

Biotechnological Applications in Agriculture

RNA interference (RNAi) based pest resistant plants

- Complementary DNA (cDNA) created from the worm is **inserted into plasmid** of *Agrobacterium tumefaciens*.
- This bacterium is used as a **vector to transport cDNA to plant**, which produces both sense and antisense RNA.
- **Sense mRNA** - one which gets translated into protein.
- **Antisense mRNA** - one which is complementary to the sense mRNA and does not code for protein.
- Since, sense and antisense mRNA are **complementary** to each other, they bind and **form double stranded RNA**, also called dsRNA.
- The formation of dsRNA **initiates RNAi** and thus, silences the specific mRNA of the nematode.
- **Causes degradation of the target mRNA of the nematode**.
- The transgenic plant, therefore, is able to protect itself from the parasite.



Biotechnological Applications in Agriculture

RNA interference (RNAi) based pest resistant plants

- Discovered by **Andrew Fire** and **Craig Mello** in 1998
- Discovered in the nematode, ***Caenorhabditis elegans***
- Crops made from to abiotic stresses using Antisense RNA
  - Tomato variety - **Flavr Savr**

Polygalacturonase (PG) enzyme

Pectin

Breakdown of pectin

Enzyme polygalacturonase(PG) promotes softening and ripening of the fruit.

Normal tomato

Natural mRNA (sense)

Protein

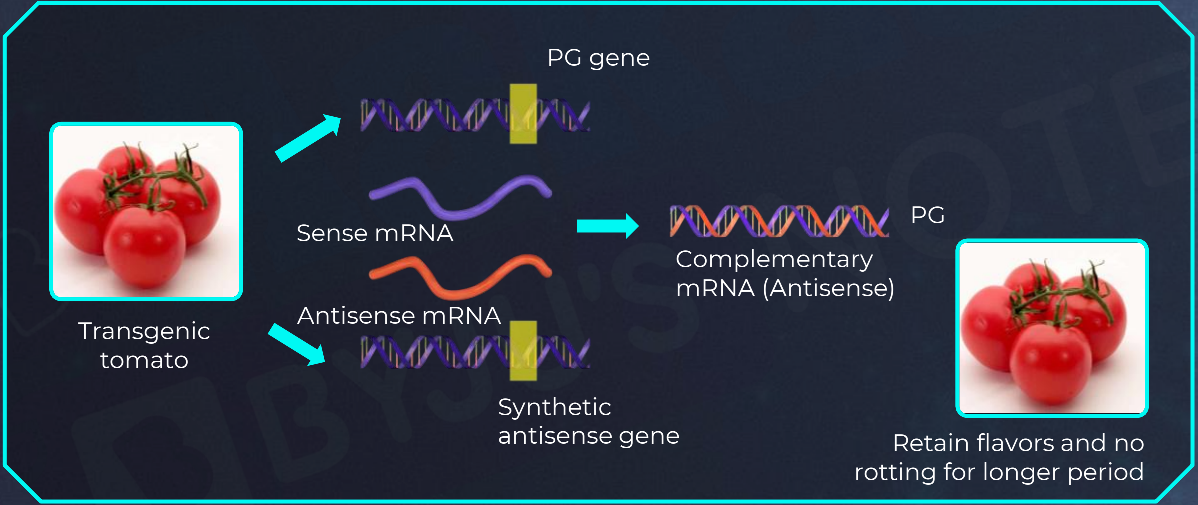
Natural rotting due to PG

In regular tomatoes, the sense mRNA encodes for production of the enzyme **polygalacturonase** (PG)

Biotechnological Applications in Agriculture

RNA interference (RNAi) based pest resistant plants

- Synthetic antisense mRNA helps to prevent rotting of tomatoes by blocking the expression of the polygalacturonase gene.



Silencing of polygalacturonase (PG) gene

Biotechnological Applications in Medicine

Recombinant therapeutics

- **Advantages**
  - enables mass production
  - safe and more effective drugs
  - Does not induce unwanted immunological responses
  - No. of recombinant therapeutics approved globally – **30**.
- First recombinant interferons inventor - **Charles Wiesman**.

Therapeutic products approved in India:		
Human insulin	Streptokinase	Erythropoietin
Hepatitis B vaccine	Human growth hormone	Human interleukin
Granulocyte colony stimulating factor	Granulocyte macrophage	Alpha interferon
Gamma-interferon	Blood factor VIII	Follicle stimulating hormone

Biopharmaceuticals - medical drugs produced using biotechnology

Examples: proteins (antibodies), nucleic acid

Biotechnological Applications in Medicine

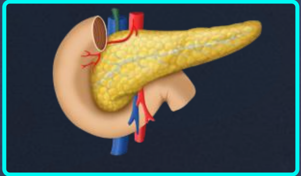
Recombinant therapeutics

Protein	Used in treatment
Erythropoietin	Anaemia
Factor VIII	Haemophilia
Follicle stimulating hormone	Infertility treatment
Granulocyte colony stimulating factor	Cancer
Insulin	Diabetes
Interferon - α	Leukemia and other cancers
Interferon - γ	Cancer, rheumatoid arthritis
Interleukins	Cancer, immune disorders
Somatotropin	Growth disorders
Tissue plasminogen activator	Heart attack

Biotechnological Applications in Medicine

Insulin

- **Insulin synthesis** - **β cells** of **islets of Langerhans** in the pancreas.
- **Controls** - **level of glucose** in the blood.
- **Insulin deficiency** - causes **Diabetes mellitus**.



Decreased insulin production

Increased blood glucose

Diabetes mellitus

Type I	Type II
Pancreas cannot produce enough insulin due to depleted beta cells.	Due to a lack of insulin production, resistance to insulin's peripheral effects, or both

Biotechnological Applications in Medicine

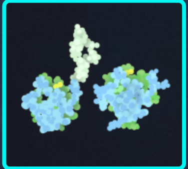
Insulin

External insulin (Based on origin)

Animal

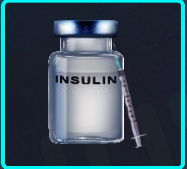
Lots of cattle and pigs slaughtered

Extraction →



Impure insulin

Purification →



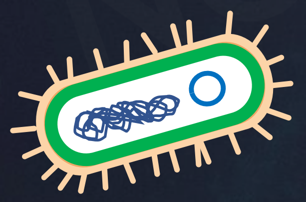
Pure insulin (low yield)

Allergic reactions

Disadvantages

- Caused severe allergic reactions in some patients
- Low insulin yield

Microbial



Bacterium

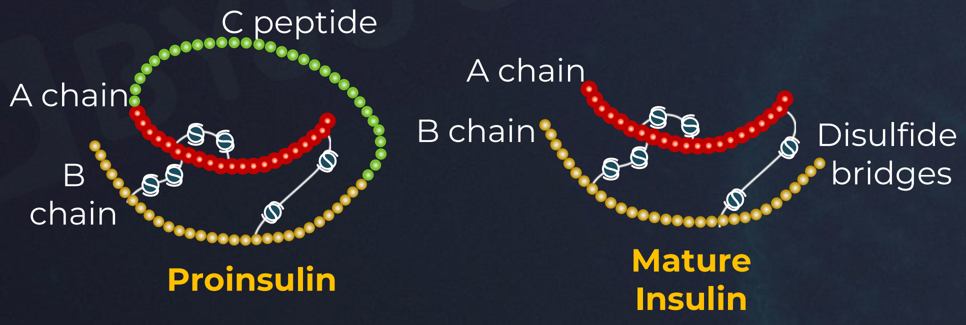
Insulin

Bacteria don't produce insulin

Biotechnological Applications in Medicine

Functional Insulin

- Insulin gene is responsible for synthesis of insulin protein.
- The protein formed from the insulin gene is called **proinsulin (non-functional form)**.
- It has protein chains, **A** chain, **C** peptide and **B** chain.
- **C-peptide** is **not part of functional insulin**, so C-peptide is **removed** to form mature insulin.
- The **A** and **B chains** become **linked together** by two sulphur-sulphur (**disulfide**) bonds which forms the **mature insulin**.



Biotechnological Applications in Medicine

Recombinant insulin production

- In 1983, an American company named **Eli Lilly** helped produce **world's first genetically engineered human insulin or recombinant insulin**.
- It was marketed under the name **Humulin**.

A chain and B chain genes are separately inserted to different plasmid vectors → Two recombinant plasmids are formed → These recombinant plasmids are then introduced into *E.coli* → A chain and B chain peptides are formed → A chain and B chain are joined together by disulfide bonds → Functional recombinant insulin is obtained → Form human insulin

Biotechnological Applications in Medicine

Human growth hormone (hGH) - Somatotropin

- **Secreted by** - anterior pituitary gland.
- **Regulated by :**
  - Somatotropin-releasing hormone.
  - Somatostatin or growth inhibiting hormone.
- Extremely useful to the children born with hypopituitarism (dwarfism).
- **Helpful in healing injuries**.
- Human growth hormone (hGH) deficiency is inherited in about 3% of the cases.
- Scientists have **genetically modified** bacteria by inserting a gene coding for the **production of hGH** and used it **to treat poor growth in children** and **adults**.

Biotechnological Applications in Medicine

Gene therapy

Gene therapy: Treatment of a disease by repairing or reconstructing the defective genes

- Diseased cell is **treated by transferring the deleted gene from the healthy cell into the diseased cell**.
- The **first clinical gene therapy** was given in 1990 to a **4-year old girl** with **adenosine deaminase (ADA) deficiency**.
- This enzyme is **crucial for the immune system** to function because in its absence lymphocyte proliferation is inhibited.
- **SCID** (Severe Combined Immunodeficiency) can be caused due to **mutation of the gene for the ADA**.

Early treatments for ADA deficiency

Enzyme replacement therapy

- Patient is given an intravenous injection of ADA or enzyme lacking in the content.
- Does not completely cure ADA deficiency.

Bone marrow transplantation

- Bone marrow cells are utilised in bone marrow transplantation to treat genetic diseases.
- Does not completely cure ADA deficiency.

Biotechnological Applications in Medicine

Gene therapy

- Gene therapy could be a permanent cure and below are the steps involved.

Lymphocytes from blood of the patient are grown in culture outside the body → Functional ADA cDNA is then introduced into these lymphocytes using a retroviral vector → Periodic infusion of genetically engineered lymphocytes into the patient's body

Extraction of lymphocytes from patient’s blood → Extracted lymphocytes → Preparation of retroviral vector containing ADA complementary DNA → Multiplication of modified lymphocytes → Introduction of modified lymphocytes into the patient and periodic infusion

Biotechnological Applications in Medicine

Molecular diagnosis

- **Diagnosis** : act or process of determining the nature and cause of a disease through evaluation of patient history, examination and lab data review.
- **Pathophysiology** : study of changes in normal, mechanical, physical and biochemical functions caused by a disease.
- **Prognosis** : a medical term denoting the doctor's prediction on patient's progress or recovery.

Methods of diagnosis

Traditional / conventional diagnosis

- Early detection is not possible
- Involves serum and urine analysis
- Time consuming and less effective

Modern / molecular diagnosis

- rDNA technology, PCR, Enzyme Linked Immunosorbent Assay (ELISA)
- Helps in early diagnosis

Biotechnological Applications in Medicine

Molecular diagnosis

It comprises of methods to **detect** and **measure** the **presence of biomolecules** associated with a specific health condition or disease.

Based on the presence of

- **Nucleic acid**
  - **PCR**
  - **Autoradiography**
- **Antigen**
  - **ELISA**

Molecular diagnostics **uses biomolecules** like nucleic acids, proteins sequences that are **specific to specific pathogen** to **detect the disease causing agent**.

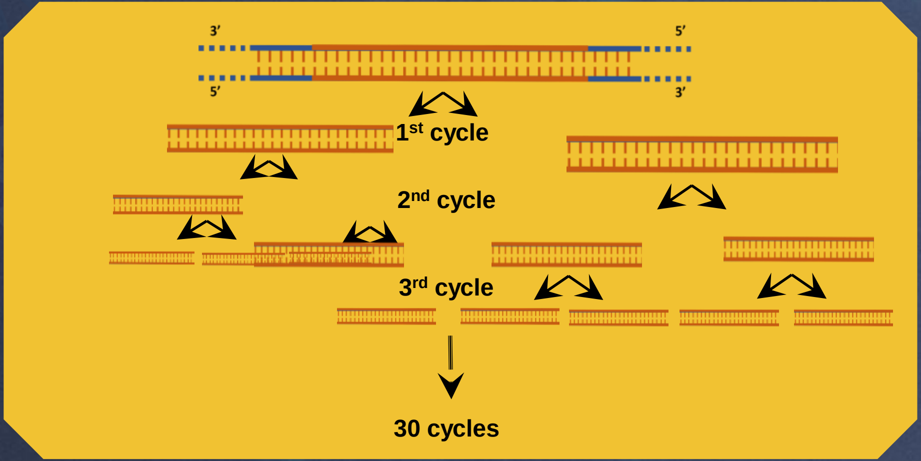
Biotechnological Applications in Medicine

Molecular diagnosis : PCR

A technique used to **create several copies of a certain DNA segment** by amplification of DNA fragment

Applications

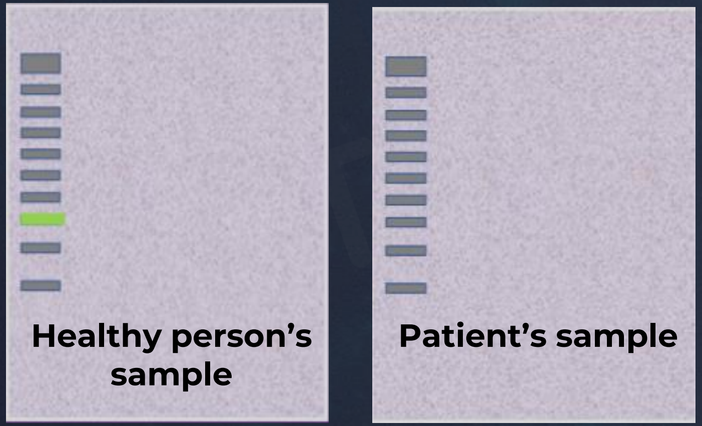
- **Very low concentration** of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected.
- **Mutated genes** in suspected cancer patients can be detected.
- **Abnormal genes** that cause genetic disorders can be detected.
- **HIV in suspected AIDS patients** can be detected.
- Identification of many other genetic disorders.



Biotechnological Applications in Medicine

Molecular diagnosis : Autoradiography

- It is a method allowing the **detection** or **localisation of radioactive isotope** within a biological sample.
- A single stranded DNA or RNA, **tagged with a radioactive molecule** (probe).
- **Probe is allowed to hybridise** its complementary DNA in the clone of cells.
- **Detected using autoradiography**.
- **Clone (mutated gene)** - does not appear on the photographic film, because the probe will not have complementarity with the mutated gene.
- Commonly used molecule - **P^{32}**.

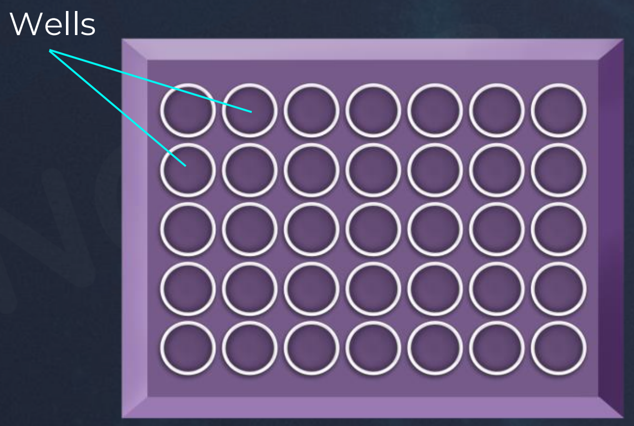


Healthy sample gives out signal while mutated gene does not produce any signal.

Biotechnological Applications in Medicine

Molecular diagnosis : ELISA

- Based on the principle of **antigen-antibody interaction**.
- Rapid, quick and requires a blood sample of the patient.
- Sensitive test, used to **detect** and **measure**
  - infection of pathogen
  - antibodies against pathogen
  - Antibodies
  - Antigens
  - Proteins
  - Glycoproteins
  - Hormones
- It is performed on a special plate with 96 wells called **microtiter**.
- Each well is filled with antibody or antigen depending upon the type of ELISA being performed.



Microtitre plate

Biotechnological Applications in Medicine

Molecular diagnosis : ELISA

Types of ELISA

Direct

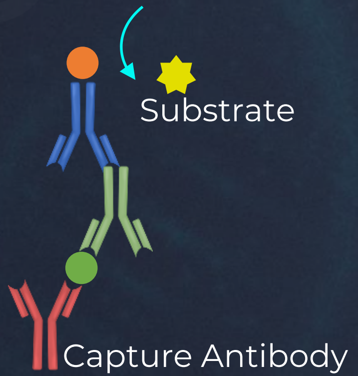
Primary antibody

Indirect

Conjugated secondary antibody

Sandwich

Sandwich ELISA



Transgenic Animals

Introduction

- Animals whose **DNA** are **manipulated to possess** and **express an extra (foreign) gene** are known as transgenic animals.
- **Produced by**
  - DNA microinjection.
  - Retrovirus-mediated gene transfer.
  - Embryonic stem cell mediated gene transfer.
- **Examples:** rats, rabbits, pigs, sheep, cow, monkey and fish.
- 95% transgenic animals are mice.
- **Produced for**
  - specific economic trait.
  - as disease model (genetically modified animals to exhibit disease symptoms for effective treatment study).
- Transgenic animals cloned from adult somatic cell at the **Roslin Institute in Edinburgh**, Scotland
  - **Polly and Molly** (cloned from two ewes).
  - **Dolly, the sheep** (no genetic modification on the adult donor nucleus).
- **Tracy -** First transgenic sheep to produce **α-1-antitrypsin**.
- **ANDi -** First transgenic monkey, **green fluorescent protein** gene was inserted into its chromosome.

Transgenic Animals

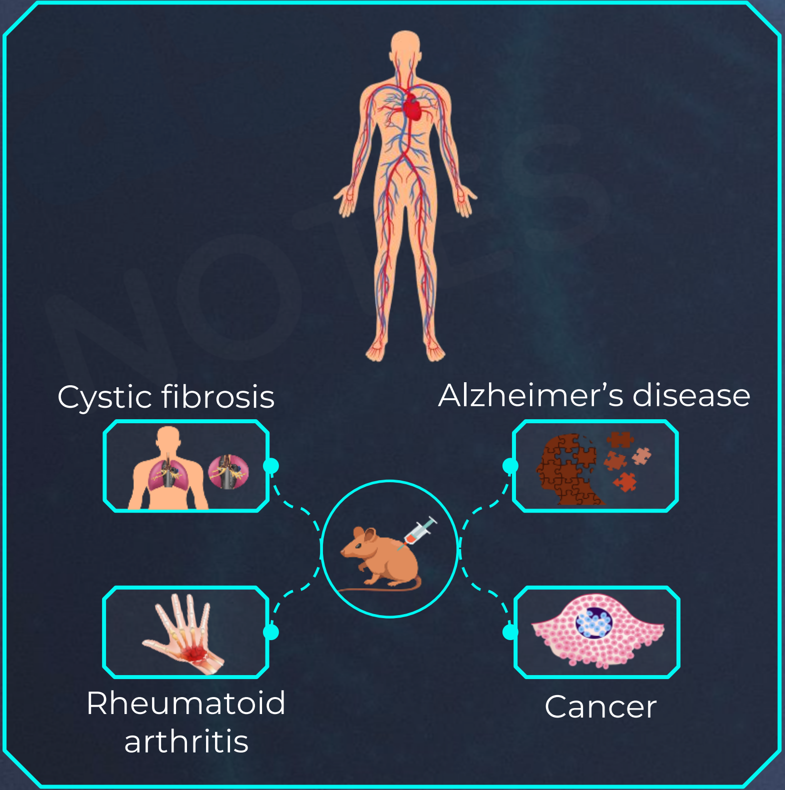
Medicinal applications

Normal physiology and development :

- **Gene regulation** and its **affects normal functioning of the body** and **development**.
- To **obtain information** about the biological role of the factor introduced in the body.

Study of diseases :

- Understanding the **contribution of gene in** disease development.
- To serve as **models for human diseases**.
- To investigate new treatment for diseases.



Transgenic Animals

Medicinal applications

Biology product :

- Introduction of the portion of DNA (or genes) which codes for a particular product such as human protein (α-1-antitrypsin) used to treat emphysema.
- Attempts are also being made for treatment of cystic fibrosis and phenylketonuria.
- First transgenic cow, Rosie, produced human protein-enriched milk (contained alpha-lactalbumin).

Rosie

Transgenic Animals

Medicinal applications

Vaccine safety :

- **Transgenic mice** are used to **test the safety of vaccines** (e.g. - polio vaccine) before testing on humans.
- If successful, they could replace monkeys for testing.

Chemical safety testing :

- **Transgenic animals** are designed to carry genes which make them more sensitive to toxic substances and **toxicity results are obtained in lesser time**.

Transgenic Animals

Agricultural application

Breeding :

- To produce breeds with desirable traits in shorter time and with more precision.
- Provides easy way to increase yields for farmers.

Quality :

- Transgenic cows - They produce more milk and with less lactose or cholesterol.
- Transgenic pigs and cattle - yield more meat.
- Transgenic sheep- grow more wool.

Disease resistance :

- Attempts are being made to produce animals which are resistance to specific diseases, such as resistant pigs showing resistance towards influenza virus.

Transgenic Animals

Industrial application

- Scientists at Nexia Biotechnologies in Canada :
  - **Spliced spider genes** into the cells of **lactating goats**.
  - Goats manufactured - **Silk + milk**.
- **Properties of extracted silk**
  - light.
  - Tough.
  - Flexible.
- The extracted silk, due to its property, is **used in**
  - Military uniforms
  - Medical microsutures
  - Tennis racket strings
- **Toxicity-sensitive** transgenic animals have been produced **for chemical safety testing**.
- Microorganisms have been engineered to produce wide variety of proteins and enzymes to **speed up industrial chemical reactions**.

Transgenic Animals

Other applications of biotechnology

To improve renewable fuel production

- Engineered microorganisms for **fermentation of ethanol, butanol** and **other fuels**.
- Engineered microorganisms or plants are used to manufacture enzyme used in **fuel production**.
- Improved **algal strains** for **biofuel production**.
- Engineered plant species with **favourable traits** for use as **improved biofuel feedstocks**.

Bioremediation

- Use of microorganisms' **metabolism to remove pollutants**.
- **In situ** - involves treating the contaminated material **at the site**.
- **Ex situ** - involves the removal of the contaminated material to be treated **elsewhere**.
- E.g., *Pseudomonas putida* or **super bug**
  - Developed by **A.M Chakravorty**.
  - Used for **clearing oil spills**.

Ethical issues

Bioethics

Bioethics are **set of standards** that may be used to **regulate our activities in relation to biological world**

GEAC (Genetic Engineering Approval Committee)

A committee which **makes decisions regarding the validity of GM research** and the safety of introducing GM organisms for public services

The major bioethical concerns are:

- Suffering inflicted on animals.
- Reduction of animals to the status of a 'factory' where they are used for production of pharmaceutical proteins.
- Violation of integrity of species by transgene introduction from one species to other.
- Transfer of human genes into animals.
- Disrespecting living beings by exploiting them for benefits of human beings.
- Unforeseen risks to the environment and risk to biodiversity.

Example: Bioweapon such as Anthrax

Ethical issues

Patent

Exclusive **rights granted by a state or national government** to inventors or their assignees for a limited period of time in exchange of public disclosure

01	02	03
Novelty	Non-obviousness	Utility
Innovation must be new	Innovation shouldn't be something which may not be documented but otherwise well known	Discovered fact or product should be of a particular use for human beings

Ethical issues

Patent

Patent is granted for :

1. Invention
2. Improvement in an earlier invention
3. Process of generating product
4. Concept or design

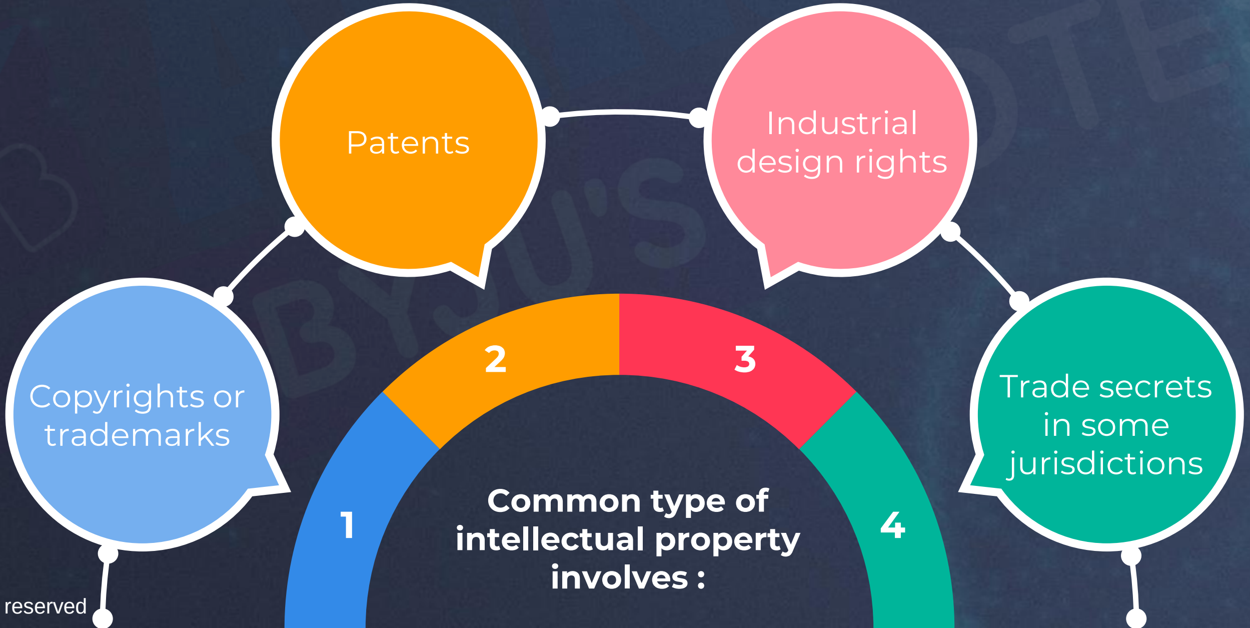
Bio patent : Exclusive rights obtained for a bioresource, or a product derived from it, by an individual or a group or a country.

Ethical issues

Intellectual property

Intellectual property (IP) refers to the **exclusive rights to a variety of intangible assets** such as :

- musical, literary and artist work.
- discoveries and inventions.
- words, phrases, symbol and designs.



Ethical issues

Biopiracy

Biopiracy refers to the **use of bio-resources** by MNCs (multinational companies) and other organisations **without proper authorisation** from other countries and people concerned without compensatory payment

Example: **Basmati rice**

- **200,000 varieties** of rice in India.
- Basmati rice is distinct for its **unique aroma** and **flavor**.
- **27** documented varieties of Basmati **grow in India** itself.
- **Reference** to Basmati rice is present in **ancient texts**, **folklore** and **poetry**, as it has been grown for centuries in India.

- In 1997, an **American company** got patent rights on Basmati rice through the US Patent and Trademark Office.
- This 'new' variety of Basmati had **actually been derived from Indian farmer's varieties**.
- Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty.

New Variety → American company claiming rights → Biopiracy

Ethical issues

Biopiracy

Example: **Turmeric**

Use of turmeric in wound healing → Patented by University of Mississippi Medical Center → Biopiracy

The patent was **revoked by** the efforts of **DR. RA Mashelkar** , an Indian Scientist. It was established that the use of turmeric as a healing agent was well known in India for centuries

Example: **Neem**

Fungicidal uses of neem oil → Patented by W.R Grace & Co. → Biopiracy

The patent was **challenged by Vandana Shiva** and **Ajay Phadke** who pointed out that the company had no “novelty” factor as Indians had known about these properties for long

Summary

RNA interference (RNAi) based pest resistant plants

Synthesis of cDNA from nematode worm

Ligation of cDNA with *Agrobacterium* vector

Transfer of recombinant plasmid from -bacteria into tobacco crop (host)

cDNA integrates with the plant DNA, creating a recombinant plant DNA

Results in genetically modified tobacco crop

Tobacco plant cell produces both sense and antisense mRNA of the worm proteins and forms dsRNA.

A degrading protein binds to this dsRNA and chops it into multiple small interfering RNAs (siRNA) of 20-25 nucleotides.

Summary

Bacillus thuringiensis

Isolation of *Bt* endotoxin gene/ *cry* gene from *Bacillus thuringiensis*
(Endotoxin that accumulates in bacterium remains inactive)

↓

Insertion of *cry* gene into plant DNA

↓

Insect feeds on the plant

↓

Protoxin enters the worm gut

↓

Protoxin gets activated and binds to midgut epithelial cells of worm

↓

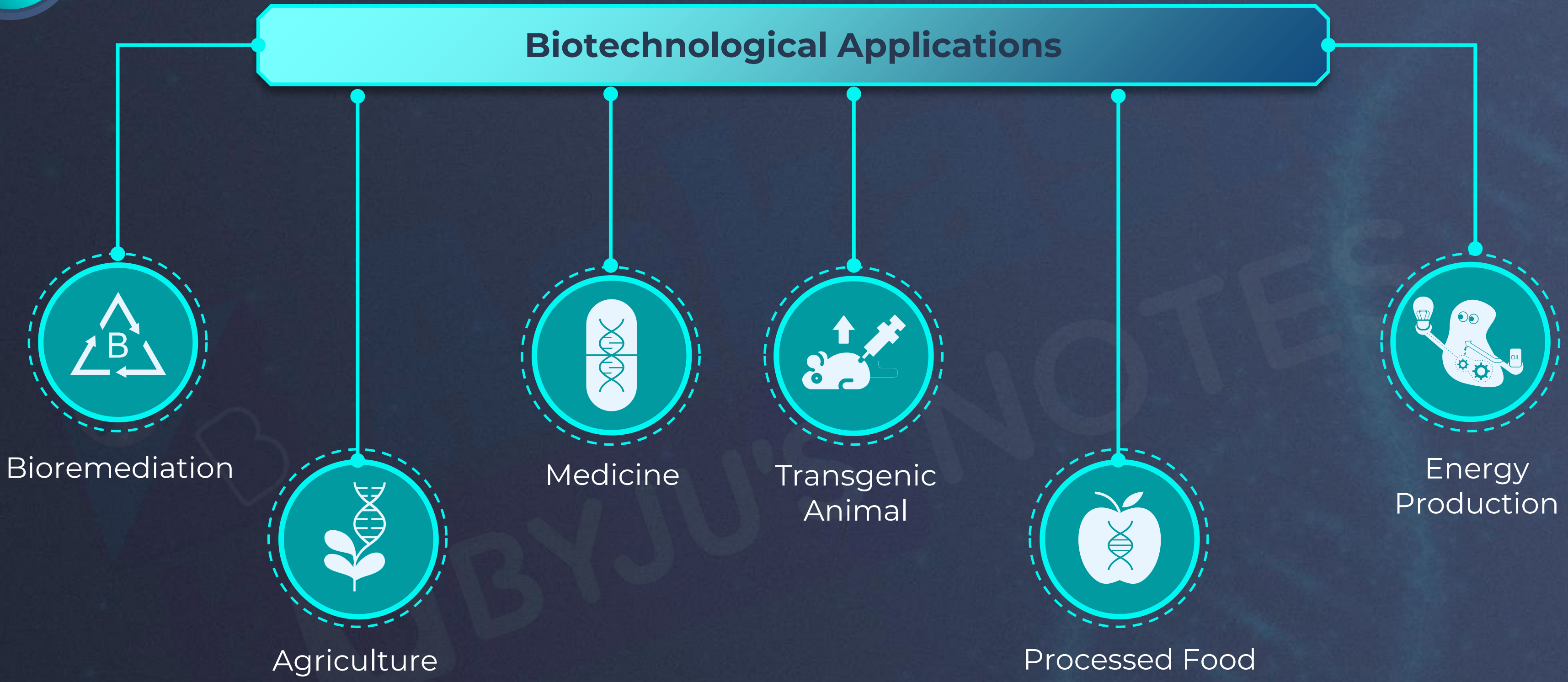
Crystal protein dissolves and creates pores in the gut

Summary

Production of insulin

A chain and **B** chain genes are separately inserted into different plasmid vectors → Two recombinant plasmids are formed → These recombinant plasmids are then introduced into *E.coli* → **A** chain and **B** chain peptides are formed → **A** chain and **B** chain are joined together by disulfide bonds → Functional recombinant insulin is obtained

Summary


Biotechnological Applications
Bioremediation
Agriculture
Medicine
Transgenic Animal
Processed Food
Energy Production