



# BIOTECHNOLOGY AND ITS APPLICATIONS

## SYLLABUS

Application of Biotechnology in health and agriculture: Human insulin and vaccine production, gene therapy; Genetically modified organisms-Bt crops; Transgenic Animals; Biosafety issues-Biopiracy and patents.

## KEY CONCEPTS

### INTRODUCTION

The applications of biotechnology include therapeutics, diagnostics and genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production. Three critical research areas of biotechnology are;

- \* Providing the best catalyst in the form of improved organism usually a microbe or pure enzyme.
- \* Creating optimal conditions through engineering for a catalyst to act, and
- \* Downstream processing technologies to purify the protein / organic compound.

decided that use of genetically modified crops is a possible solution.

- \* Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO).

### Advantages of Genetic Modification in plants.

- \* Made crops more tolerant to abiotic stresses (cold, drought, salt, heat)
- \* Reduce reliance on chemical pesticides (pest resistant crop)
- \* Helped to reduce post harvest losses.
- \* Increased efficiency of mineral usage by plants.
- \* Enhanced nutritional values of food e.g. vitamin A enriched rice (**golden rice**).

### BIOTECHNOLOGICAL APPLICATIONS

#### IN AGRICULTURE

- \* The three options that can be thought for increasing food production are,
  - Agro-chemical based agriculture
  - Organic agriculture; and
  - Genetically engineered crop-based agriculture.
- \* The Green Revolution has succeeded in tripling the food supply but yet it was not enough to feed the growing human population. Scientists have

#### Bt Cotton:

- \* Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as **lepidopterans** (tobacco budworm, armyworm), **coleopterans** (beetles) and **dipterans** (flies, mosquitoes).
- \* *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic **insecticidal protein**.
- \* These proteins are present in inactive **protoxin** form, but become active toxin in the alkaline pH of insect gut.

- \* The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of insect.
- \* Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and genetically transferred to several plants such as cotton.
- \* Crystal proteins are produced by a gene called cry in *Bacillus thuringiensis*. This Cry protein is toxic to Larvae of certain insects. Each Cry protein is toxic to a different group of insects. The gene encoding cry protein is called **cry gene**. This Cry protein isolated and transferred into several crops.
- \* A crop expressing a cry gene is usually resistant to the group of insects for which the concerned Cry protein is toxic. There are a number of them, for example, the proteins encoded by the genes **cryIac** and **cryIIAb** control the cotton bollworms, that of **cryIAb** controls corn borer.
- \* Bt-tobacco was first cultured to kill hornworm (*Manduca sexta*).

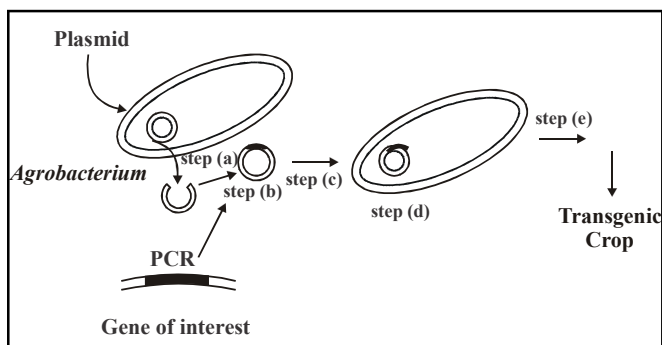
**Pest resistant plants:**

- \* Several **nematodes** parasitize a wide variety of plants and animals including human beings.
- \* A nematode *Meloidogyne incognitia* infects the root of **tobacco plants** and causes a great reduction in yield.
- \* Strategy based on **RNA interference (RNAi)** prevents this infestation.
- \* RNAi takes place in all eukaryotic organisms as a method of cellular defense.
- \* This method involves silencing of a specific mRNA due to a complementary **dsRNA** molecule that binds to and prevents translation of the mRNA (silencing).
- \* The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

**Steps of RNA interference:**

- \* Double stranded RNA is produced endogenously or exogenously.

- \* Using *Agrobacterium* vectors nematode specific genes were introduced into the host plant (tobacco plant).
- \* Introduction of DNA produces both sense and antisense RNA in the host. These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi.
- \* The dsRNA injected into the host plant from outside called exogenous dsRNA.
- \* The dsRNAs are cleaved into 21-23 nt segments ("small interfering RNAs", or **siRNAs**) by an enzyme called **Dicer**.
- \* siRNAs are incorporated into RNA-induced silencing complex (**RISC**).
- \* Guided by base complementarity of the siRNA, the RISC targets mRNA for degradation.
- \* The consequence was that the parasite could not survive in a transgenic host.
- \* **Production of a transgenic crop**



- Step (a) :** Plasmid is removed and cut open with restriction endonuclease.
- Step (b) :** Gene of interest is isolated from another organism and amplified using PCR.
- Step (c) :** New gene is inserted into plasmid.
- Step (d) :** Plasmid is put back into *Agrobacterium*.
- Step (e) :** *Agrobacterium* based transformation.

**BIOTECHNOLOGICAL APPLICATIONS**

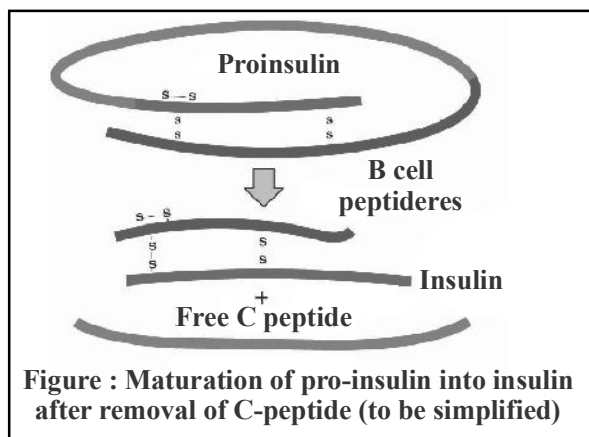
**IN MEDICINE**

- \* Biotechnology enables mass production of safe and more effective therapeutic drugs.
- \* Recombinant therapeutics does not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources.

- \* At present, about 30 recombinant therapeutics have been approved for human use the world over. In India, 12 of these are presently being marketed.
- \* The therapeutic products approved in India are:
  1. Human Insulin
  2. Streptokinase
  3. Erythropoietin
  4. Hepatitis B vaccine
  5. Human growth Hormone
  6. Human Interleukin
  7. Granulocyte colony stimulating factor (GCSF)
  8. Granulocyte macrophage CSF (GMCSF)
  9. Alpha-interferon
  10. Gamma-interferon
  11. Blood factor VIII
  12. Follicle stimulating hormone.

**Genetically Engineered Insulin:**

- \* Taking insulin at regular interval of time is required for adult-onset diabetes.
- \* Previously the source of insulin was the slaughtered cattle & pigs.
- \* This insulin caused allergy in some patients.
- \* Each insulin made of two short polypeptide chains; chain A and chain B that are linked together by disulphide linkage.



- \* Insulin synthesized in pancreas as pro-hormone which is a single polypeptide with an extra stretch called **C-peptide**.
- \* C-peptide is removed during matured insulin.
- \* In 1983 **Eli Lilly** an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of E.coli to produce insulin chains.

- \* Chain A and chain B produced separately, extracted and combined by creating disulfide bonds to form mature human insulin.

**Gene therapy:**

- \* Technique for curing genetic disease through replacing a “Faulty Gene” by a normal healthy functional gene. The first gene therapy used in severe combined immuno deficiency (SCID) patient.
- \* The first clinical gene therapy (by M.Blease and W.F Andresco) was given in 1990 to a 4-yr old girl with adenosine deaminase (**ADA**) **deficiency**.
- \* This enzyme is required for breakdown of deoxyadenosine into uric acids.
- \* In the absence of ADA toxic deoxyadenosine is accumulated and destroy the infection fighting immune cells called **T-cells and B-cells**.
- \* This disorder is caused due to the deletion of the gene for adenosine deaminase in chromosome 20.
- \* **Treatment:** In some children ADA deficiency can be cured by bone marrow plantation. Lymphocytes from the blood of patient are grown in a culture. A functional ADA cDNA is then introduced into these lymphocytes using retroviral vector. The lymphocytes are transferred into the body of patients. As these cells are not immortal, the patient required periodic infusion of such genetically engineered lymphocytes. If a functional gene is introduced into a bone marrow cells at early embryonic stage, It could be a permanent cure of ADA deficiency.

**Molecular diagnosis:**

- \* Early detection of disease is not possible by conventional methods (serum and urine analysis)
- \* Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

**PCR:**

- \* A very low concentration of a bacteria or virus can be detected by amplification of their nucleic acid by PCR. It is now routinely used to detect HIV in suspected AIDS patients.

- \* It is being used to detect mutations in genes in suspected cancer patients too.

**Recombinant DNA technology :**

- \* A single stranded DNA or RNA tagged with radioactive molecule (probe) is allowed to hybridize to its complementary DNA in a clone of cells followed by detection using autoradiography.
- \* The clone having mutated gene unable make complementary bonding of probe, hence not appears in photographic film.

**ELISA**

- \* It is based on the principle of antigen-antibody interaction.
- \* Infection by pathogen can be detected by presence of antigens or by detecting the antibodies synthesized against the pathogen.

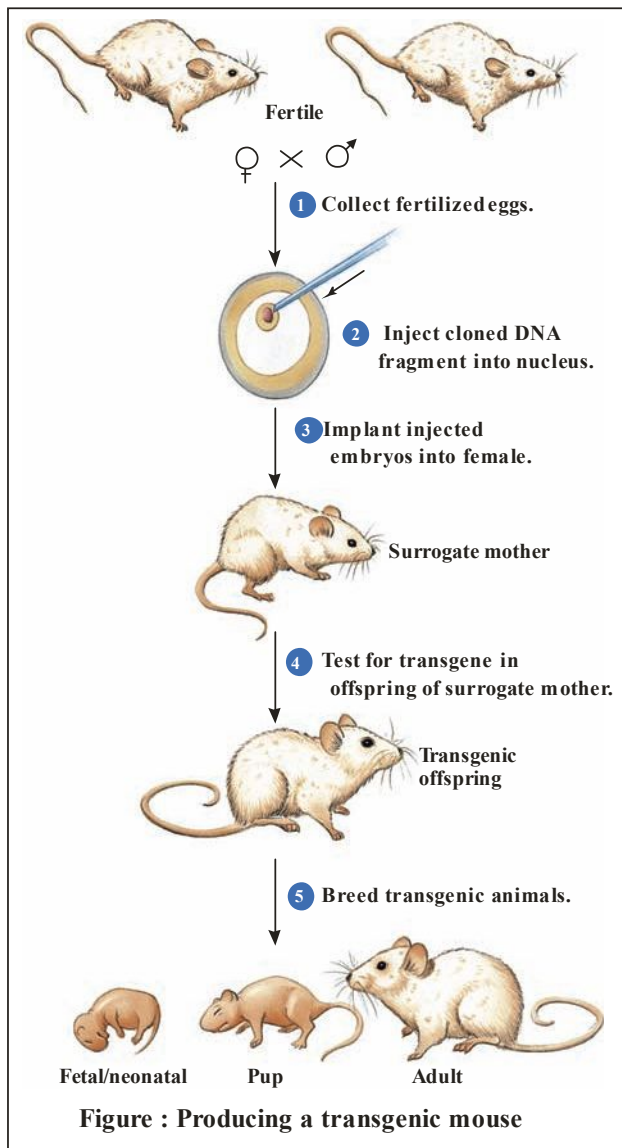
**TRANSGENIC ANIMALS**

Animals that have an **alien DNA** which able to express in it is called **transgenic animals**.

**Reasons for creation of transgenic animals:**

- (i) **Normal physiology and development:** Transgenic animals are specifically designed to allow study of:
  - \* How the genes are regulated.
  - \* How the gene affects normal functioning of body
  - \* How it affects growth and development. E.g. insulin like growth factor.
  - \* The animals made transgenic to know the biological effect and result.
- (ii) **Study of disease:** Transgenic animals are designed to understand how genes contribute to the development of disease like cancers, cystic fibrosis, rheumatoid arthritis and Alzheimer's.
- (iii) **Biological products:** Transgenic animals are used to produce biological product of human interest:
  - \*  $\alpha$ -1-antitrypsin used to treat emphysema.
  - \* The first transgenic sheep to produce  $\alpha$ -1-antitrypsin was Tracy.
  - \* Proteins for treatment for PKU and cystic fibrosis.

In 1997, the **first transgenic cow, Rosie**, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human alpha-lactalbumin and was nutritionally a more balanced product for human babies than natural cow-milk.



- (iv) **Vaccine safety:**
  - \* Transgenic mice are being developed and use in testing the safety of vaccines before they are used for humans.
  - \* Polio vaccine is tested in mice.
- (v) **Chemical safety testing:**
  - \* This is also known as toxicity/safety testing.
  - \* Transgenic animals are made to know the effect of toxic chemicals.

## ACHIEVEMENTS OF GENETIC ENGINEERING

- \* The DNA recombinant technology or genetic engineering provides great benefits for advancement of science and society.
- \* A new system of medicine gene therapy, may develop to treat hereditary diseases such as haemophilia. Genetic disorder can be over come by introducing specific gene.
- \* Bacteria may be used as "Living factories" for synthesizing vitamins, hormones and antibodies.
- \* Human insulin (Humulin) was first genetically engineered product produced by an American firm Eli Lilly - 5th July 1983.
- \* Charles weismann of university of Zurich, obtained interferon through recombinant E.coli (1980) Microbes have been engineered to produce Human growth Hormone (HGH) for curing dwarfism.
- \* Vaccines which are produced by genetic engineering e.g., for Hepatitis-B and Herpes virus).
- \* Nitrogen fixation genes may be transferred from bacteria to the major food crops to boost food production without using expensive fertilizers.
- \* Transgenic plant obtained through recombinant DNA technology. First transgenic plant was tobacco. It contains resistant gene against weedicide (Glycophosate).
- \* First transgenic animal was mouse contain gene for growth hormone.
- \* First introduced transgenic crop in India (2002) is Bt-cotton.  
It is resistant for boll worm (*Helicoperpa armigera* - Larva of insect). It is formed by transfer of pest resistant gene from *Bacillus thuringiensis* (bt-2 gene encoding Bt-toxin).  
*Bacillus thuringiensis* produces a toxic protein called crystal protein (Cry-Protein) this protein is toxic for larva of certain insect.  
This protein kills the insect by inhibiting ion transport in midgut (bt 2 gene is called cry-gene)
- \* In pollution control, microbes have been engineered to break up the crude oil spills.

- \* Dr. Ananda Mohan chakraborti introduced plasmid from different strains in to single cell of *pseudomonas putida*. The result was new genetically engineered bacterium which would be cleaning oil spills called "Super bug" (oil eating bug.)

## ETHICAL ISSUES

- \* GEAC (Genetic Engineering Approval Committee) set up by Indian Govt, which will make decisions regarding validity of GM research and safety of introducing GM-organisms for public services.
- \* A patent is the right granted by a government to an inventor to prevent others from commercial use of his invention.
- \* Patents granted for biological entities and for products derived from them; these patents are called biopatents.
- \* 27 documented varieties of Basmati are grown in India.

## BIOPIRACY

- \* Biopiracy is the term used to refer to the use/ exploit or patent, of biological resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment.

## **CONCEPT REVIEW**

- \* **Adenosine Deaminase (ADA) Deficiency :** ADA enzyme is crucial for the immune system to function. This disorder is caused due to the deletion of gene for adenosine deaminase enzyme.
- \* **Bt Cotton :** It is transgenic plant. Bt toxin genes were isolated from *Bacillus thuringiensis* and were incorporated into cotton plant.
- \* **Biopiracy :** Unauthorised use of biological resources and traditional knowledge related to bioresources for commercial benefits without proper compensatory payments.

- \* **Biopesticides** : Biological agents that are used to control weeds, insects and other pests.
- \* **Cry Gene** : The Bt toxins are coded by a gene cry1Ac named cry gene.
- \* **Cry Protein** : The insecticidal protein which is produced by soil bacterium named *Bacillus thuringiensis* is called cry protein.
- \* **C. Peptide** : In mammals, including humans, insulin is synthesised as a prohormone which contains an extra stretch called the c peptide. This c-peptide is not present in the mature insulin and is removed during maturation into insulin.
- \* **ELISA** : Enzyme Linked Immuno-Sorbent Assay is one of the diagnostic technique which is used to detect HIV/AIDS and other diseases.
- \* **Genetically Engineered Insulin** : The human insulin was prepared by recombinant DNA technology. This insulin has two short polypeptide chains A and B which are linked by disulphide bridges.
- \* **Green Revolution** : Crop yield has increased tremendously to feed the growing human population.
- \* **Genetically Modified Organisms (GMO)** : The organisms whose genes have been altered by manipulation are called Genetically Modified Organisms.
- \* **Gene Therapy** : Gene therapy is a collection of methods that allow correction of a gene defect that has been diagnosed in a child or embryo.
- \* **Insecticidal Protein** : A toxin in the protein crystal that is secreted by *Bacillus thuringiensis*. It can kill certain insects such as tobacco budworm, armyworm and beetles.
- \* **Molecular Diagnosis** : Molecular diagnosis is the early detection of diseases which is not possible by traditional diagnostic techniques. These are – Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-Sorbent Assay.
- \* **RNA Interference** : RNA interference is a process which is used to develop pest resistant plants.
- \* **Rosie** : In 1997, the first transgenic cow, Rosie, produced human protein (alpha-lactalbumin) enriched milk (2.4 grams per litre).
- \* **Transgenic organisms** have foreign DNA

incorporated into their genetic material. **Gene targeting** and **mutagenesis screening** in mice help identify the function of a gene and its protein product. Transgenic livestock produce foreign proteins in their milk. Transgenic plants have great potential in agriculture.

- \* **Transgenic Animals** : Animals that have had their DNA manipulated by the process & express an extra gene are known as transgenic animals.
- \* **Transgenic Crops** : Transgenic crops are the crops that contain and express transgene. These crops are also called genetically modified crops or G M Crops. These crops are resistant to insects and capable of producing medical proteins with challenged qualities.
- \* **Cloning** is the process of producing many identical organisms (clone), generally used to produce new plants with similar characteristics. Microbes produce clones through asexual reproduction. In higher animals, clones are produced by nuclear transplantation technique in which the nucleus from a somatic cell is transferred into an unfertilized enucleated egg. The world's most famous sheep 'Dolly' was a clone produced by this method.
- \* **Examples of applications of plant cloning**

Applications	Examples
Herbicide resistant plants	Petunia, tobacco, tomato and corn
Insect resistant plants	Cotton, tobacco and mustard
Virus resistant plants	Tomato, potato, alfalfa, cucumber, rice and papaya
Plants which improved storage proteins	French bean and potato
Plants with improved oil and fats	Rapeseed (rich in oleic acids and sterates) and soybean (rich in cocoa oil)
Stress tolerant plants	Tobacco

- \* **Transgenics and their potential applications**

Transgenic	Useful applications
Bt Cotton	Pest resistance, herbicide tolerance, and high yield.
<i>Flavr Savr</i> Tomato	Increased shelf-life (delayed ripening) and better nutrient quality.
Golden Rice	Vitamin A and Fe – rich.
Cattles (cow, sheep, goat)	Therapeutic human proteins in their milk.
Pig	Organ transplantation without risk of rejection.

\* **Recombinant proteins**

S.No.	Recombinant Proteins	Therapeutic Uses
1.	Human Insulin (Humulin)	Treatment of diabetes type 1
2.	Tissue Plasminogen Activator	Treatment for acute myocardial infarction dissolves blood clot after heart attack and stroke.
3.	DNase	Treatment of cystic fibrosis.
4.	Platelet Growth Factor	Stimulation of wound healing.
5.	Calcitonin	Treatment of rickets.
6.	Reo Pro	Prevention of blood clots.
7.	Hirudin	Used as an anticoagulant
8.	Interferon ( $\alpha, \beta$ and $\gamma$ )	Treatment of viral infection and cancer.
9.	Chorionic Gonadotropin	Treatment of infertility.
10.	Interleukins	Enhancing activity of immune system.

**IMPORTANT POINTS**

- \* Transgenic plants are developed by introducing foreign genes.
- \* cry IAc and cry IAb produce toxins that control cotton bollworms and corn borer respectively.
- \* GM Bt brinjal has been developed in India for insect resistance.
- \* Clot buster obtained from *Streptococcus* and modified by genetic engineering is Streptokinase.
- \* First transgenic plant released for commercial use was tobacco.
- \* Plasmids are extra-chromosomal, self-replicating, usually circular, double-stranded DNA molecules found naturally in many bacteria.
- \* In 1983, Eli Lilly an American company, first prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them in plasmids of E. coli to produce insulin chains. These chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin (humulin).
- \* A nematode *Meloidegyne incognitia* infects the roots of tobacco plants which reduce the production of tobacco.

\* **Application of Genetically Engineered Microbes**

Microbes	Applications
<i>Escherichia coli</i> (gut bacterium)	Production of human insulin, human growth factor interferons, interleukin and so on.
<i>Bacillus thuringiensis</i> (soil bacterium)	Productions of endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide for plant protection.
<i>Rhizobium meliloti</i> (bacterium)	Nitrogen fixation by incorporating "nif" gene in cereal crops.
<i>Pseudomonas putida</i> (bacterium)	Scavenging of oil spills, by digesting hydrocarbons of crude oil.
Bacterial strains capable of accumulating heavy metal	Bioremediation (cleaning of pollutants in the environment).
<i>Trichoderma</i> (fungus)	Production of enzyme chitinases for biocontrol of fungal diseases in plants.

# QUESTION BANK

## EXERCISE - 1 (LEVEL-1) [NCERT EXTRACT]

### SECTION - 1 (VOCABULARY BUILDER)

Choose one correct response for each question.

For Q.1-Q.2

Match the column I with column II.

- |            |                 |                       |
|------------|-----------------|-----------------------|
| <b>Q.1</b> | <b>Column I</b> | <b>Column II</b>      |
| a.         | Golden rice     | i. Armyworm           |
| b.         | Bt toxin        | ii. Rich in vitamin-A |
| c.         | RNAi            | iii. Cry protein      |
| d.         | Lepidopterans   | iv. Gene silencing    |

Codes

- (A) (a) – (ii), (b) – (iii), (c) – (iv), (d) – (i)  
 (B) (a) – (iii), (b) – (iv), (c) – (i), (d) – (ii)  
 (C) (a) – (iv), (b) – (i), (c) – (ii), (d) – (iii)  
 (D) (a) – (ii), (b) – (i), (c) – (iii), (d) – (iv)

**Q.2**

**Column I**

- Bacillus thuringiensis*
- Rhizobium meliloti*
- Escherichia coli*
- Pseudomonas putida*
- Trichoderma*

**Column II**

- Production of chitinases
- Scavenging of oil spill
- Incorporation of nit gene
- Production of Bt toxin
- Production of human insulin

- (A) (a)-(ii), (b)-(iv), (c)-(i), (d)-(v), (e)-(iii)  
 (B) (a)-(ii), (b)-(iv), (c)-(v), (d)-(i), (e)-(iii)  
 (C) (a)-(iv), (b)-(iii), (c)-(v), (d)-(ii), (e)-(i)  
 (D) (a)-(v), (b)-(iv), (c)-(iii), (d)-(i), (e)-(ii)

### SECTION - 2 (BASIC CONCEPTS BUILDER)

For Q.3 to Q.27 :

Choose one word for the given statement from the list.

**Alpha-lactalbumin, Gene therapy, Insulin, *Bacillus thuringiensis*, Transgenic organism, Bioethics, Lymphocytes, Nematodes, Alkaline, cry I Ab, Molecular farming, Autoradiography, Worms, Probe, ADA- Adenosine deaminase, GEAC, dsRNA, Ti-plasmids of Agrobacterium, Disulphide bridges.**

**Q.3** In Bt cotton, a transgenic plant, Bt refers to \_\_\_\_\_

**Q.4** \_\_\_\_\_ is a collection of methods that allows correction of gene defects diagnosed in a child or embryo.

**Q.5** Technique used to detect the DNA in a clone is called \_\_\_\_\_.

**Q.6** 'Silencing of mRNA molecule' in order to control the production of a harmful protein has been used in the protection of plants from \_\_\_\_\_.

**Q.7** The first human drug made by using genetic engineering technique was \_\_\_\_\_.

**Q.8** A single strand of nucleic acid tagged with a radioactive molecule is called \_\_\_\_\_.

**Q.9** Cry I endotoxins obtained from *Bacillus thuringiensis* are effective against \_\_\_\_\_.

**Q.10** The site of the production of ADA in the body is \_\_\_\_\_.

**Q.11** The insecticidal protein produced by *Bacillus thuringiensis* is not toxic to bacteria itself. \_\_\_\_\_ conditions in the gut of insect trigger the protoxin to be active.



- Q.12** In 1997, the first transgenic cow, Rosie produced human protein enriched milk at 2.4 g/L. Human gene present in Rosie's milk was \_\_\_\_\_.
- Q.13** Gene therapy allows a faulty or absent gene to be replaced by a working gene. The gene, which was worked on in first clinical gene therapy is \_\_\_\_\_.
- Q.14** Insulin consists of two short polypeptide chains, which are linked together by \_\_\_\_\_.
- Q.15** The organisation appointed by Indian government for making decisions regarding validity of GM research and the safety of introducing GM-organism for public service \_\_\_\_\_.
- Q.16** A set of standards that may be used to regulate human activities in relation to the biological world can be termed as \_\_\_\_\_.
- Q.17** Cry gene codes for the protein which can control the corn borer effectively is \_\_\_\_\_.
- Q.18** RNA interference (RNAi) technique has been devised to protect the plants from the nematode. In this technique, mRNA of nematode is silenced by \_\_\_\_\_ produced by the host plant.
- Q.19** A genetically manipulated organism containing in its genome one or more inserted gene of another species is called \_\_\_\_\_.
- Q.20** Use of transgenic plants as biological factories for the production of special chemicals is called \_\_\_\_\_.
- Q.21** Vector commonly used in the transfer of gene in a crop plant is \_\_\_\_\_.
- Q.22** Normal genes introduced in the somatic cells with defective gene will eventually be passed on to the next generation. [True / False]
- Q.23** A tobacco plant with a firefly gene is called a transgenic plant. [True / False]
- Q.24** Human genome has about 100,000 protein coding genes. [True / False]
- Q.25** All restriction enzymes leave sticky ends on DNA fragments. [True / False]
- Q.26** Enzyme reverse transcriptase is present in prokaryotic and eukaryotic cells. [True / False]
- Q.27** Gene therapy has been successful in animals other than humans. [True / False]

### SECTION - 3 (ENHANCE PROBLEM SOLVING SKILLS)

Choose one correct response for each question.

#### PART - 1 : BIOTECHNOLOGY APPLICATION IN AGRICULTURE

- Q.28** Blindness is prevented by use of which crop in poor countries?  
 (A) Golden rice (B) Wheat  
 (C) Gram (D) Pea
- Q.29** Which one of the following bacteria has found extensive use in genetic engineering work in plants?  
 (A) *Bacillus coagulans*  
 (B) *Xanthomonas citri*  
 (C) *Clostridium septicum*  
 (D) *Agrobacterium tumefaciens*
- Q.30** Cry IIAb & cry IAb produces toxins that control  
 (A) cotton bollworms and corn borer respectively  
 (B) corn borer and cotton bollworms respectively  
 (C) tobacco budworms & nematodes respectively  
 (D) nematodes and tobacco budworms respectively

- Q.31** Bt toxin kills insects by  
 (A) inhibiting protein synthesis  
 (B) generating excessive heat  
 (C) creating pores in the midgut epithelial cells, leading to cell swelling and lysis  
 (D) obstructing a biosynthetic pathway
- Q.32** Bt toxin is  
 (A) intracellular crystalline protein  
 (B) extracellular crystalline protein  
 (C) intracellular monosaccharide  
 (D) extracellular polysaccharide
- Q.33** The RNAi stands for  
 (A) RNA interference (B) RNA interferon  
 (C) RNA inactivation (D) RNA initiation
- Q.34** Bt toxins are initially inactive protoxins but after ingestion by the insects their inactive toxin becomes active due to the  
 (A) alkaline pH of the gut  
 (B) acidic pH of the gut  
 (C) temperature of the gut  
 (D) hormone present in the gut
- Q.35** Bt cotton is not:  
 (A) A GM plant  
 (B) Insect resistant  
 (C) A bacterial gene expressing system  
 (D) Resistant to all pesticides
- Q.36** Insect resistant transgenic cotton has been produced by inserting a piece of DNA from  
 (A) an insect (B) a bacterium  
 (C) a wild relative of cotton (D) a virus
- Q.37** Bt corn has been made resistant from corn borer disease by the introduction of the gene  
 (A) cry I Ac (B) cry II Ab  
 (C) cry I Ab (D) cry II Ac
- Q.38** A nematode *Meloidogyne incognitia* infects the root of tobacco plant and causes a great reduction in yield. A novel strategy was adopted to present this infection which was based on the process of:  
 (A) DNA interference (B) RNA interference  
 (C) PCR technique (D) DNA test
- Q.39** Why is Bt toxin not toxic to human beings?  
 (A) the toxin recognises only insect specific targets.  
 (B) Bt toxin activation requires temperature above the human body temperature.  
 (C) Bt toxin formation forms pro Bt state which requires pH lower than one present in human stomach.  
 (D) conversion of pro Bt to Bt state takes place only in highly alkaline condition
- Q.40** Golden rice is a transgenic crop of the future with which of the following improved trait.  
 (A) High lysine (essential amino acid) content  
 (B) Insect resistance  
 (C) High protein content  
 (D) High vitamin-A content
- Q.41** Which bacteria was the first to be used as biopesticide on the commercial scale in the world?  
 (A) *Bacillus thuringiensis*  
 (B) *E. coli*  
 (C) *Pseudomonas aeruginosa*  
 (D) *Agrobacterium tumefaciens*
- Q.42** The protein products of the following Bt toxin genes cry IAc and cry IIAb are responsible for controlling  
 (A) bollworm (B) roundworm  
 (C) moth (D) fruitfly

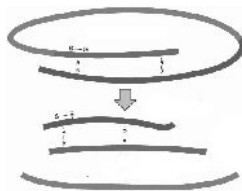
**PART - 2 : BIOTECHNOLOGICAL APPLICATION IN MEDICINE**

- Q.43** C-peptide of human insulin is –  
 (A) a part of mature insulin molecule  
 (B) responsible for the formation of disulphide bridges.  
 (C) removed during the maturation of pro-insulin to insulin.  
 (D) responsible for its biological activity.
- Q.44** An example of gene therapy is  
 (A) production of injectible hepatitis-A vaccine  
 (B) introduction of the genes for adenosine deaminase in a person suffering from SCID.  
 (C) production of test-tube babies by artificial insemination.  
 (D) All of the above

- Q.45** A probe which is a molecule used to locate specific sequences in a mixture of DNA or RNA molecules could be:  
 (A) A single stranded RNA  
 (B) A single stranded DNA  
 (C) Either RNA or DNA  
 (D) Both (A) and (B)
- Q.46** ELISA is based on  
 (A) antigen- antibody interaction.  
 (B) antigen- protein interaction.  
 (C) lectin- antibody interaction  
 (D) All of the above
- Q.47** Which of the following peptide chain is removed during the maturation of proinsulin into insulin?  
 (A) A-chain (21 amino acid)  
 (B) B-chain (30 amino acid)  
 (C) C-chain (33 amino acid)  
 (D) A and B chain
- Q.48** The demerit of using bovine insulin (from cow) and porcine insulin (from pig) in diabetic patients–  
 (A) It leads to hypercalcemia  
 (B) It is expensive  
 (C) It may cause allergic reactions  
 (D) It may lead to mutations in human genome
- Q.49** Full form of ADA is –  
 (A) Adenosine Deoxyaminase  
 (B) Adenosine Deaminase  
 (C) Aspartate Deaminase  
 (D) Arginine Deaminase
- Q.50** In which method, a probe is allowed to hybridise to its complementary DNA in the clone of cells?  
 (A) Gene therapy  
 (B) Recombinant DNA technology  
 (C) Polymerase chain reaction  
 (D) Enzyme Linked Immuno-Sorbent Assay
- Q.51**  $\alpha$ -1 antitrypsin is:  
 (A) An antacid  
 (B) An enzyme  
 (C) Used to treat arthritis  
 (D) Used to treat emphysema
- Q.52** The diagram shows.  
 (A) Maturation of pro-insulin into insulin  
 (B) Method of pro-insulin formation
- (C) Gene therapy  
 (D) Enzyme replacement therapy
- Q.53** PCR is used to –  
 (A) detect HIV in suspected AIDS patients.  
 (B) detect mutations in the genes in suspected cancer patients.  
 (C) diagnose many genetic disorders  
 (D) All of the above
- Q.54** A protoxin is:  
 (A) A primitive toxin  
 (B) A denatured toxin  
 (C) Toxin produced by protozoa  
 (D) Inactive toxin
- Q.55** A functional ADA cDNA can be introduced into the cells of the patients receiving gene therapy by using vector constituted by  
 (A) *E. coli* (B) *Retrovirus*  
 (C) *Bacillus thuringiensis* (D) *Agrobacterium*
- Q.56** The first clinical gene therapy was done for the treatment of:  
 (A) AIDS (B) Cancer  
 (C) Cystic fibrosis (D) SCID

### PART - 3 : TRANSGENIC ANIMALS AND ETHICAL ISSUES

- Q.57** Transgenic animals are those which have foreign  
 (A) DNA in some of its cells  
 (B) DNA in all of its cells  
 (C) RNA in all of its cells  
 (D) RNA in some of its cells
- Q.58** Correct statements about GEAC  
 I. GEAC make decisions regarding the validity of the GM research.  
 II. It checks the safety of introducing GM organisms for the public services for their large scale use.  
 (A) Only I (B) Only II  
 (C) I and II (D) None of these
- Q.59** Which gene was introduced in the first transgenic cow?  
 (A) Human  $\alpha$ -lactalbumin (B)  $\alpha$ -1-antitrypsin  
 (C)  $\beta$ -1-antitrypsin (D) cry-IAc



EXERCISE - 2 (LEVEL-2)

Choose one correct response for each question.

- Q.1** Which of the following would be considered as transgenic?  
 (A) Bacterial plasmid with antibiotic resistance gene.  
 (B) Bacterial chromosome with lac operon.  
 (C) Human cell with growth hormone gene.  
 (D) Bacterial plasmid with insulin gene.
- Q.2** The first clinical gene therapy was given to a 4-year old girl with ADA deficiency in :  
 (A) 1984 (B) 1986  
 (C) 1992 (D) 1990
- Q.3** Toxin present in *Bacillus thuringiensis* does not kill the bacterium because it is inactive form what makes it active inside the insect ?  
 (A) the alkaline pH of the gut, which solubilises the crystals.  
 (B) the acid pH of the gut.  
 (C) the neutral pH of the gut.  
 (D) All of the above.
- Q.4** Bt toxin is produced by *Bacillus thuringiensis* which is:  
 (A) Bacterium (B) Protozoa  
 (C) Fungus (D) Virus
- Q.5** Animals those have had their DNA manipulated to possess and express an extra (foreign) gene are known as:  
 (A) Transgenic animals  
 (B) Genetically modified animals  
 (C) Both (A) and (B)  
 (D) None of the above
- Q.6** Conventional methods to diagnose a disease are:  
 (A) Serum and urine analysis  
 (B) PCR  
 (C) ELISA  
 (D) All of the above
- Q.7** Critical research areas of biotechnology are :  
 (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) Down stream processing technologies to purify the protein/organic compound.  
 (D) All the above.
- Q.8** Process involving silencing of a specific mRNA due to a complementary dsRNA molecule is called :  
 (A) Transcription (B) RNA interference  
 (C) DNA interference (D) None of these
- Q.9** Genetically engineered human insulin is made in  
 (A) Fungus (B) Protista  
 (C) Plants (D) Bacterium
- Q.10** Recombinant DNA Technology prepared vaccine is called  
 (A) First generation vaccine  
 (B) Second generation vaccine  
 (C) Third generation vaccine  
 (D) Synthesized vaccine
- Q.11** 'Flavr Savr' is a transgenic variety of :  
 (A) Potato (B) Tomato  
 (C) Soyabean (D) Rice
- Q.12** Cry1 Ab gene produces proteins which control?  
 (I) Bollworms (B) Corn borer  
 (C) Both (A) and (B) (D) None of the above
- Q.13** By the use of biotechnology in which bacteria production of B<sub>12</sub> vitamins has been increased to about 20,000 times –  
 (A) *Ashbya gossypi*  
 (B) *E. coli*  
 (C) *Pseudomonas denitrificans*  
 (D) *Propionibacterium shermanii*
- Q.14** Crystals of Bt toxin produced by some bacteria do not kill the bacteria themselves because :  
 (A) bacteria are resistant to the toxin  
 (B) toxin is immature  
 (C) toxin is inactive  
 (D) bacteria enclose toxin in a special sac

- Q.15** Over 95% of all existing transgenic animals are:  
 (A) Pigs (B) Cows  
 (C) Fish (D) Mice
- Q.16** Bacterium genetically engineered for cleaning oil spills is:  
 (A) *Eischerichia coli*  
 (B) *Pseudomonas putida*  
 (C) *Salmonella typhimurium*  
 (D) *Agrobacterium tumifaciens*
- Q.17** The organisation set up for making decisions regarding the validity of GM research and the safety of introducing GM organism for public services is :  
 (A) Genetic engineering approval committee  
 (B) Genetic engineering advanced company  
 (C) Genetic engineering applied committee  
 (D) None of these
- Q.18** The first transgenic cow was 'Rosie', produces:  
 (A) Human protein-enriched milk ( $\alpha$ -lactalbumin)  
 (B) Human protein  $\alpha$ -I antitrypsin riched milk.  
 (C) Human protein enriched milk (insulin).  
 (D) All the above
- Q.19** Transgenic mice are being developed for use in :  
 (A) Testing the safety of polio vaccines before they are used on human.  
 (B) Molecular diagnosis of diseases .  
 (C) Production of human protein enriched milk  
 (D) Production of human insulin.
- Q.20** Genetically engineered bacteria are being used commercial production of :  
 (A) melatonin (B) testosterone  
 (C) thyroxine (D) human insulin
- Q.21** Golden rice  
 I. It is a transgenic variety of rice.  
 II. It contains a goods quality of  $\beta$ -carotene (provitamin-A)  
 III.  $\beta$ -carotene is a principal source of vitamin-A.  
 IV. The grains of the rice are yellow in colour due to  $\beta$ -carotene. The rice is commonly called golden rice.
- Which of the statements given above are correct?  
 (A) I, II and III (B) II, III and IV  
 (C) I, III and IV (D) I, II, III and IV
- Q.22** Which of the following statement(s) is/are true?  
 I. Biowar is the use of biological weapons against humans and/or their crops and animals.  
 II. Bioethics is an unauthorised use of bioresources and traditional knowledge related to bioresources for commercial benefits.  
 III. Biopatent is the exploitation of bioresources of other nations without proper authorisation.  
 Choose the correct option.  
 (A) Only II (B) Only I  
 (C) I and II (D) I and III
- Q.23** Which of the following is a transgenic plant?  
 (A) Hirudin (B) Triticale  
 (C) Flavr savr (D) All of these
- Q.24** Consider the following statements about insulin.  
 I. Human insulin is made-up of 51 amino acids arranged in two polypeptide chains.  
 II. The two polypeptide chains are interconnected by two disulphide bridges.  
 III. In mammals including humans, insulin is synthesised as a pro-hormone, which contains an extra stretch called the C-peptide.  
 IV. C-peptide is not present in the mature insulin.  
 Which of the statements given above are correct?  
 (A) I, II and III (B) I, III and IV  
 (C) II, III and IV (D) I, II, III and IV
- Q.25** Choose the correct option regarding Retrovirus:  
 (A) An RNA virus that can synthesise DNA during infection.  
 (B) A DNA virus that can synthesise RNA during infection.  
 (C) A ssDNA virus  
 (D) A dsRNA virus

- Q.26** Find the incorrect statement.
- (A) Gene therapy is a genetic engineering technique used to treat disease at molecular level by replacing defective genes with normal genes.
- (B) Calcitonin is a medically useful recombinant product in the treatment of infertility.
- (C) Bt toxin is biodegradable insecticide obtained from *Bacillus*.
- (D) *Trichoderma* sp. is a biocontrol agent for the fungal diseases of plants.
- Q.27** Silencing of a gene could be achieved through the use of:
- (A) short interfering RNA (RNAi)
- (B) antisense RNA
- (C) by both
- (D) none of the above
- Q.28** Which of the following is/are correct about Adenosine Deaminase (ADA) deficiency?
- I. In the absence of adenosine deaminase enzyme purine metabolism is disturbed and T-Lymphocytes fails to function.
- II. ADA deficiency is caused by the deletion of the gene for ADA.
- III. In some cases, it can be cured by bone marrow transplantation and enzyme replacement therapy. But in both approaches, the patients are not completely cured.
- IV. For permanent cure, genes isolated from the bone marrow cells producing ADA at early embryonic stages can be a possible cure.
- Which of the above statements are correct?
- (A) I, II and III                      (B) II, III and IV
- (C) I, III and IV                      (D) I, II, III and IV
- Q.29** Transgenic crops are modified through genetic engineering to develop the natural resistance to insect and pests. Which one is a transgenic plant?
- (A) Tobacco and cotton
- (B) Tomato and rice
- (C) Maize and sugarcane
- (D) Tomato and wheat

**EXERCISE - 3 (LEVEL-3)**

**Choose one correct response for each question.**

- Q.1** Which animal is being used to test the safety of polio vaccine?
- (A) Transgenic mice      (B) Transgenic pig
- (C) Transgenic cow      (D) Transgenic cat
- Q.2** Bacterium which is known as 'Super bug' is :
- (A) *Pseudomonas putida*
- (B) *Salmonella*
- (C) *Eischerichia*
- (D) *Agrobacterium*
- Q.3** Most widely used bioweapon is –
- (A) *Bacillus subtilis*
- (B) *Pseudomonas putida*
- (C) *Bacillus anthracis*
- (D) None of these
- Q.4** Nematode-specific genes were introduced into the host plant (tobacco plant) by using which vector?
- (A) Plasmid vector
- (B) Cosmid vector
- (C) Bacteriophage vector
- (D) *Agrobacterium tumifaciens*
- Q.5** Select incorrect statement :
- (a) Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as *Lepidopterans*, *Coleopterans* and *Dipterans*.
- (b) RNA interference takes place in all eukaryotic organisms as a method of cellular defence.
- (c) Genetically modified crops are more sensitive to abiotic stresses.
- (d) Golden rice is protein enriched rice.
- (e) *Agrobacterium* is used to deliver desirable genes into animal cell.
- (A) only a                      (B) a, band c
- (C) a, c and d                      (D) c, d and e

- Q.6** Genetically engineered bovine (bSI), sometimes called rbST (recombinant bovine somatotropin) or rbGH (recombinant bovine growth hormone) are used in the –  
 (A) therapeutic drugs (B) agriculture  
 (C) dairy industry (D) DNA fingerprinting
- Q.7** Genetics modified crops (GMC) are useful, in agriculture because :  
 (A) They are more tolerant to abiotic stresses.  
 (B) They increase reliance on chemical pesticide.  
 (C) They have reduced nutritional value.  
 (D) All the above
- Q.8** Golden rice is enriched in :  
 (A) Vitamin C (B) Vitamin D  
 (C) Vitamin A (D) Vitamin E
- Q.9** Which one of the following statements are true regarding genetic modifications ?  
 (A) Genetic modifications reduced reliance on chemical pesticides.  
 (B) Genetic modifications has enhanced nutritional value of food.  
 (C) Genetic modifications made crops more tolerant to abiotic stresses.  
 (D) All are correct
- Q.10** Which one of the following statements about genetically engineered insulin is incorrect.  
 (A) E.coli is used for producing humulin.  
 (B) Chains A, B were produced separately.  
 (C) Eli lily company prepared it for first time.  
 (D) Genetically engineered insulin has C-peptide.
- Q.11** Which peptide is not present in the mature insulin and is removed during maturation into insulin?  
 (A) A-peptide (B) B-peptide  
 (C) C-peptide (D) Both (A) and (B)
- Q.12** For the control of the cotton bollworms, which one of the genes is useful ?  
 (A) Cry 1 Ac (B) Cry 1 Ab  
 (C) Cry 1 Ad (D) All of these
- Q.13** Infection by pathogen can be detected by the presence of antigens or by detecting the antibodies synthesised against the pathogen, on this principle a test is based which is ?  
 (A) PCR (B) ELISA  
 (C) Both (A) and (B) (D) None of the above
- Q.14** Which of the following plants is genetically modified for improved nutritional value of food?  
 (A) Potato (B) Wheat  
 (C) Rice (D) Maize
- Q.15** A transgenic food crop, which may help in solving the problem of night blindness in developing countries is :  
 (A) Bt soyabean (B) Golden rice  
 (C) Flavr savr tomatoes (D) Starlink maize
- Q.16** The name of drug used in cancer treatment produced by biotechnology is –  
 (A) Interferon  
 (B) Human growth hormone [HGH]  
 (C) TSH  
 (D) Insulin
- Q.17** Which of following is not related with bio weapons–  
 (A) Bacillus anthracis (B) Clostridium botulinum  
 (C) E. Coli (D) Yersinia pestis
- Q.18** Find the incorrect match  
 (A) TPA–Blood clot preventer  
 (B) Erythropoitin – Formation of RBCs  
 (C) Reopro– Transplantation rejection  
 (D) Interferon – Treatment of cancer
- Q.19** *Meloidogyne incognitia* which infects the roots of tobacco plants causing a great reduction in yield is a:  
 (A) Nematode (B) Bacterium  
 (C) Virus (D) Alga
- Q.20** The main technique involved in agricultural biotechnology is called –  
 (A) Tissue culture (B) Transformation  
 (C) Plant breeding (D) DNA replication

- Q.21** Golden rice was created by transforming rice with two beta-carotene biosynthesis genes, namely  
(A) Psy and cry I genes (B) LCY-e  
(C) CHY-1 (D) CHY-2
- For Q.22-Q.23 :**
- (A) Statement- 1 is True, Statement-2 is True, Statement-2 is a correct explanation for Statement -1  
(B) Statement-1 is True, Statement-2 is True ; Statement-2 is NOT a correct explanation for Statement-1.  
(C) Statement - 1 is True, Statement- 2 is False.  
(D) Statement -1 is False, Statement -2 is False.
- Q.22** **Statement 1 :** *Agrobacterium tumefaciens* is popular in genetic engineering because this bacterium is associated with the roots of all cereal and pulse crops .  
**Statement 2 :** A gene incorporated in the bacterial chromosomal genome gets automatically transferred to the crops with which bacterium is associated.
- Q.23** **Statement 1 :** Humulin is better than conventions insulin.  
**Statement 2 :** Conventional insulin produces many side effects.

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**EXERCISE - 4 (PREVIOUS YEARS AIPMT/NEET EXAM QUESTIONS)**

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**Choose one correct response for each question.**

- Q.1** Which of the following Bt crops is being grown in India by the farmers? [NEET 2013]  
(A) Brinjal (B) Soybean  
(C) Maize (D) Cotton.
- Q.2** The first human hormone produced by recombinant DNA technology is [AIPMT 2014]  
(A) insulin (B) estrogen  
(C) thyroxin (D) progesterone.
- Q.3** In Bt cotton, the Bt toxin present in plant tissue as protoxin is converted into active toxin due to [AIPMT 2015]  
(A) action of gut microorganisms.  
(B) presence of conversion factors in insect gut  
(C) alkaline pH of the insect gut.  
(D) acidic pH of the insect gut.
- Q.4** Which body of the Government of India regulates GM research and safety of introducing GM organisms for public services? [AIPMT 2015]  
(A) Genetic Engineering Approval Committee  
(B) Research Committee on Genetic Manipulation  
(C) Bio-safety committee.  
(D) Indian Council of Agricultural Research.
- Q.5** The crops engineered for glyphosate are resistant/ tolerant to – [AIPMT 2015]  
(A) insects (B) herbicides  
(C) fungi (D) bacteria
- Q.6** Golden rice is a genetically modified crop plant where the incorporated gene is meant for biosynthesis of – [RE-AIPMT 2015]  
(A) omega 3 (B) vitamin A  
(C) vitamin B (D) vitamin C
- Q.7** The introduction of T-DNA into plants involves [RE-AIPMT 2015]  
(A) exposing the plants to cold for a brief period.  
(B) allowing the plant roots to stand in water.  
(C) infection of the plant by *Agrobacterium tumefaciens*.  
(D) altering the pH of the soil, then heat-shocking the plants.
- Q.8** Which kind of therapy was given in 1990 to a four year- old girl with adenosine deaminase (ADA) deficiency? [NEET 2016 PHASE 2]  
(A) Gene therapy (B) Chemotherapy  
(C) Immunotherapy (D) Radiation therapy



- Q.9** Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes? [NEET 2018]  
(A)  $\lambda$  phage (B) Ti plasmid  
(C) Retrovirus (D) pBR 322
- Q.10** Use of bioresources by multinational companies and organisations without authorisation from the concerned country and its people is called [NEET 2018]  
(A) Biodegradation (B) Biopiracy  
(C) Bio-infringement (D) Bioexploitation
- Q.11** In India, the organisation responsible for assessing the safety of introducing genetically modified organisms for public use is [NEET 2018]  
(A) Research Committee on Genetic Manipulation (RCGM)  
(B) Council for Scientific and Industrial Research (CSIR)  
(C) Indian Council of Medical Research (ICMR)  
(D) Genetic Engineering Appraisal Committee (GEAC)
- Q.12** Which of the following is true for Golden rice? [NEET 2019]  
(A) It is Vitamin A enriched, with a gene from daffodil.  
(B) It is pest resistant, with a gene from *Bacillus thuringiensis*  
(C) It is drought tolerant, developed using *Agrobacterium* vector  
(D) It has yellow grains, because of a gene introduced from a primitive variety of rice
- Q.13** Which of the following features of genetic code does allow bacteria to produce human insulin by recombinant DNA technology? [NEET 2019]  
(A) Genetic code is not ambiguous  
(B) Genetic code is redundant  
(C) Genetic code is nearly universal  
(D) Genetic code is specific
- Q.14** What triggers activation of protoxin to active Bt toxin of *Bacillus thuringiensis* in boll worm? [NEET 2019]  
(A) Body temperature  
(B) Moist surface of midgut  
(C) Alkaline pH of gut  
(D) Acidic pH of stomach

**ANSWER KEY**

**EXERCISE-1(SECTION-1&2)**

- |                                     |                        |  |                        |
|-------------------------------------|------------------------|--|------------------------|
| (1) (A)                             | (2) (C)                | (15) GEAC                                | (16) Bioethics         |
| (3) <i>Bacillus thuringiensis</i> . | (4) Gene therapy.      | (17) <i>cry</i> I Ab                     | (18) dsRNA             |
| (5) Autoradiography                 | (6) Nematodes          | (19) Transgenic organism                 | (20) Molecular farming |
| (7) Insulin                         | (8) Probe              | (21) Ti-plasmids of <i>Agrobacterium</i> |                        |
| (9) Worms                           | (10) Lymphocytes       | (22) False                               | (23) True              |
| (11) Alkaline                       | (12) Alpha-lactalbumin | (24) False                               | (25) False             |
| (13) ADA- Adenosine deaminase       |                        | (26) False                               | (27) False             |
| (14) Disulphide bridges             |                        |  |                        |

**EXERCISE - 1 [SECTION-3]**

Q	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
A	A	D	A	C	A	A	A	D	B	C	B	D	D	A	A	C	B	D	A	C
Q	48	49	50	51	52	53	54	55	56	57	58	59								
A	C	B	B	D	A	D	D	B	D	B	C	A								

**EXERCISE - 2**

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	D	D	A	A	C	A	D	B	D	B	B	B	A	C	D	B	A	A	A	D
Q	21	22	23	24	25	26	27	28	29											
A	D	B	C	D	A	B	C	D	A											

**EXERCISE - 3**

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	A	A	C	D	D	C	A	C	D	D	C	A	B	C	B	A	C	C	A	A
Q	21	22	23																	
A	A	D	A																	

**EXERCISE - 4**

Q	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	D	A	C	A	B	B	C	A	C	B	D	A	C	C

# SOLUTIONS

## EXERCISE-1

- (1) (A) (2) (C)
- (3) *Bacillus thuringiensis*. (4) Gene therapy. (33) (A) (34) (A)
- (5) Autoradiography (6) Nematodes (35) (D). Several transgenic plants have been developed. One such plant is Bt cotton in which the Bt toxin has been used as a biological insecticide.
- (7) Insulin (8) Probe
- (9) Worms (10) Lymphocytes
- (11) Alkaline (12) Alpha-lactalbumin
- (13) ADA- Adenosine deaminase
- (14) Disulphide bridges
- (15) GEAC (16) Bioethics
- (17) *cry* I Ab (18) dsRNA
- (19) Transgenic organism (20) Molecular farming
- (21) Ti-plasmids of *Agrobacterium*
- (22) **False.** Genes introduced in the somatic cells affect that individual. Only genes introduced in the gametic cells are passed on to the next generation.
- (23) **True.** An organism is called transgenic in it has genes from another species.
- (24) **False.** Scientists predicted 100,000 protein coding genes but Human Genome Project showed that there are only 30,000 protein coding genes.
- (25) **False.** Only some restriction enzymes leave sticky ends on DNA fragments.
- (26) **False.** Enzyme reverse transcriptase is isolated from retroviruses
- (27) **False.** Gene therapy has had some success in humans as well.
- (28) (A) (29) (D) (30) (A)
- (31) (C). Bt toxins are initially inactive protoxins but after ingestion by the insects, their inactive toxins becomes active due to the alkaline pH of the gut which solublise the crystals. The activated toxin binds to the surface of the midgut epithelial cells thus, creating pores which causes cell swelling and lysis, further leading to death of the insects.
- (32) (A). Bt toxin is an intracellular crystalline protein. Specific Bt toxin genes obtained from *Bacillus thuringiensis* are used in several crop plants like cotton. Bt toxins are initially inactive protoxins but after ingestion by the insects their inactive toxin becomes active due to the alkaline pH of the gut which dissolves the crystals.
- (36) (B). Insect resistance transgenic cotton has been produced by inserting a piece of DNA from a bacterium *Bacillus thuringiensis*.
- (37) (C)
- (38) (B). RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules. *Meloidogyne incognita* is a nematode (roundworm) in the family Heteroderidae. It is commonly called the "southern root-knot nematode" or the "cotton root-knot nematode". This parasitic roundworm has worldwide distribution and numerous hosts. It is an important plant parasite classified in parasitology as a root-knot nematode, as it prefers to attack the root of its host plant.
- (39) (D). Bt toxin is not toxic to human being because conversion of pro Bt to Bt state takes place only in highly alkaline conditions.
- (40) (D)
- (41) (A). *Bacillus thuringiensis* was the first to be used as biopesticides on the commercial scale in the world.
- (42) (A). The *cry* gene of *Bacillus thuringiensis* produces a protein, which forms crystalline inclusions in the bacterial spores. These crystal proteins are responsible for the insecticidal activities of bacterial strains.

Protein encoded by the genes cry IAc and cry IIAb controls the cotton bollworm and that of cry IAb controls corn borer.

- (43) (C)      (44) (B)      (45) (D)      (46) (A)      (54) (D)
- (47) (C). Insulin contains two short polypeptide chains, chain A and B-chain linked by disulphide bridge. In mammals, insulin is synthesised as prohormone (that needs to be processed to become mature and functional hormone). It contains an extra stretch called-C-peptide. C-peptide is absent in mature insulin and is removed during the maturation into insulin.
- (48) (C). Insulin obtained from the pancreas of cattle and pigs slightly differ from the human insulin in their amino acid sequence. Moreover, the insulin production from pig and cattle is not sufficient to cater the needs of growing number of diabetic patients. Also the injection of insulin into the patients, occasionally produces sensitivity reaction and side effects. These factors led researchers to look for some alternative source of human insulin. The search for new source was soon fulfilled by recombinant DNA technology.
- (49) (B)
- (50) (B). In recombinant DNA technology, a probe is allowed to hybridise to its complementary DNA in the clone of cells. The cells are then detected by autoradiography. The cells with mutated genes will not be observed on the photographic film because the probe was not complementary to the mutated genes.
- (51) (D). Disorders of  $\alpha$ -1-antitrypsin protein include  $\alpha$ -1-antitrypsin deficiency, an autosomal codominant hereditary disorder in which deficiency of  $\alpha$ -1-antitrypsin leads to a chronic uninhabited tissue breakdown. This causes the degradation especially of lung tissue, and eventually leads to charaderstic manifestations of pulmonary emphysema.
- (52) (A). Maturation of proinsulin into insulin after removal of C-peptide.
- (53) (D). PCR can detect very low amounts of DNA. PCR is now usually used to detect HIV in suspected AIDS patients. It is also used to detect mutations in the genes in suspected cancer patients. It is a good technique to identify many other genetic disorders.
- (54) (D)
- (55) (B). A functional ADA cDNA can be introduced into cells of the patients receiving gene therapy by using vector constituted by retrovirus. The SCID patient has a defective gene for the enzyme Adenosine Deaminase (ADA). He/she lacks functional T-lymphocytes and, therefore fails to fight the infecting pathogen. Lymphocytes are extracted from the patient's bone marrow and a normal functional copy of human gene coding for ADA is introduced into these lymphocytes with the help of retrovirus.
- (56) (D). The first clinical gene therapy was given in 1990 to a 4-year old girl with adenosine deaminase (ADA) deficiency. This enzyme is very important for the immune system to function. ADA deficiency can lead to severe combined immune deficiency (SCID).
- (57) (B). Transgenic animals are those, which have foreign DNA in all of its cells.
- (58) (C)
- (59) (A). Gene for human alpha lactalbumin was introduced into the genes of first transgenic cow, which made the milk nutritionally richer.

### EXERCISE-2

- (1) (D). Transgenic organisms have genes from other species.
- (2) (D). ADA: The First Gene Therapy Trial  
A four-year old girl became the first gene therapy patient on September 14, 1990 at the NIH Clinical Center. She has adenosine deaminase (ADA) deficiency, a genetic disease which leaves her defenseless against infections. White blood cells were taken from her, and the normal genes for making adenosine deaminase were inserted into them. The corrected cells were reinjected into her. Dr. W. French Anderson helped develop this landmark clinical trial when he worked at the National Heart, Lung, and Blood Institute.

- (3) (A) (4) (A) (5) (C) (6) (A) (18) (A). In 1997 the first Transgenic cow was Rosie, who produced human protein-enriched milk at (2.4 grams per litre). The milk contained the human protein alpha lactalbumin.
- (7) (D)
- (8) (B). RNA silencing (RNA interference) refers to a family of gene silencing effects by which gene expression is negatively regulated by non-coding RNAs. It may also refer to the introduction of a synthetic antisense RNA molecule used in scientific experiments on gene expression. RNA silencing may also be defined as sequence-specific regulation of gene expression triggered by double-stranded RNA (dsRNA).
- (9) (D). Human insulin is produced in a very controlled and clean environment. Genetically-engineered bacteria are grown in large stainless steel fermentation vessels. The vessel contains all the nutrients needed for growth. When the fermentation is complete, the mixture containing the bacteria is harvested. The bacteria are filtered off and broken open to release the insulin they have produced. It is then purified and packaged into bottles for distribution.
- (10) (B)
- (11) (B). Flavr Savr, a genetically modified tomato, was the first commercially grown genetically engineered food to be granted a license for human consumption.
- (12) (B)
- (13) (A). *Ashbya gossypii* is a riboflavin-overproducing filamentous fungus that is closely related to unicellular yeasts such as *Saccharomyces cerevisiae*.
- (14) (C) (15) (D)
- (16) (B). *Pseudomonas putida* is a rod-shaped, flagellated, gram-negative bacterium that is found in most soil and water habitats where there is oxygen. *Pseudomonas putida* has the ability to produce Poly-3hydroxyalkanoates (PHA) from the aromatic hydrocarbon styrene. PHA is very environmental friendly, oil and grease resistant, and has a long shelf life therefore it is also used in everyday items such as plastic utensils and other disposable items.
- (17) (A)
- (19) (A) (20) (D) (21) (D) (22) (B)
- (23) (C). A transgenic crop is a crop which contains and expresses a transgene. A popular term for the transgenic crop is genetically modified crops or GM crops. *Flavr savr* tomato was the first commercially grown genetically engineered food to be granted a license for human consumption. These tomato can remain fresh for long time than other varieties of tomato.
- (24) (D). Human insulin is made up of 51 amino acids arranged in two polypeptide chains. A having 21 amino acids and B with 30 amino acids. The two polypeptide chains are interconnected by two disulphide bridges or S-S linkages. S-S linkage also occurs in A-chain. The hormone develops from the storage product is called proinsulin. Proinsulin has three chains, A, B and C. C chain with 33 amino acids is removed prior to insulin formation.
- (25) (A). A retrovirus is a ss RNA virus that stores its nucleic acid in the form of an mRNA genome and targets a host cell as an obligate parasite. Once inside the host cells cytoplasm the virus uses its own reverse transcriptase enzyme to produce DNA from its RNA genome (the reverse of usual pattern, thus retro).
- (26) (B). Calcitonin is a hormone secreted from parafollicular cells of thyroid gland. It is chorionic gonadotropin hormone, which is medically useful recombinant product in the treatment of infertility.
- (27) (C) (28) (D)
- (29) (A). Insect resistant transgenic plants contains either a gene from the bacterium *Bacillus thuringiensis* or some other gene. In Bt cotton and Bt tobacco, the insect resistant gene is transferred from *Bacillus thuringiensis*.

### EXERCISE-3

- (1) (A) (2) (A)
- (3) (C). Bio-war or biological war is the misuse of biological organisms (bio-weapons) against human or their crops and animals. e.g., World War-I Germany used anthrax and bacterial diseases to infect sheeps. Bio-terrorism occurred in USA during 2000 through the use of spores of anthrax bacterium.
- (4) (D). Using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells. These two RNA's being complementary to each other formed a double stranded (ds DNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.
- (5) (D)
- (6) (C). These hormones are used in the dairy industry, when injected into cows they would increase the milk production.
- (7) (A)
- (8) (C). Golden Rice is a new type of rice that contains beta carotene, a source of vitamin A. Golden Rice is being developed as a potential new food-based approach to improve vitamin A status.
- (9) (D) (10) (D) (11) (C)
- (12) (A). **Cry1Ac** : Introduced by genetic manipulation and expressed in insect-resistant cotton and tomato
- (13) (B) (14) (C) (15) (B) (16) (A)
- (17) (C) (18) (C) (19) (A) (20) (A)
- (21) (A). Transgenic golden rice was created by transforming rice with the gene *Psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*) and *cry I* from the soil bacterium *Erwinia uredovora*.
- (22) (D). *Agrobacterium tumefaciens* is a pathogenic bacterium that produces crown

galls or tumours in plants on infection. It contains a large Ti-plasmid (tumour inducing plasmid) and it can transfer a part of its plasmid DNA to the host plant. Therefore, the bacterium can be used in the transfer of genes in biotechnological techniques.

- (23) (A). Humulin is the first genetically engineered pharmaceutical product. It is better than conventional insulin because conventional insulin produces many side effects over a long period.

### EXERCISE-4

- (1) (D). Bt cotton is being grown in India by the farmers.
- (2) (A). First human hormone produced by recombinant technology is insulin by Eli Lilly an American company in 1983.
- (3) (C). The Bt toxin is an inactive protoxin, which is activated due to the alkaline pH in the insect gut. Bt toxin dissolve in high pH of insect gut and become active. The toxins then attack the gut cells of the insect, punching holes in the lining.
- (4) (A). Genetic engineering Approval Committee regulates research and safety of Genetically Modified (GM) organisms for public services in India. This committee is governed by the Ministry of Environment, Forest and Climate Change (MOEF).
- (5) (B). Glyphosate (N-phosphonomethyl glycine) is systemic broad spectrum herbicide used to kill weeds especially broad leaves and grasses. It is used to engineer new crop for having herbicides tolerance (resistant).
- (6) (B). Golden rice is nutritionally enriched rich and is meant for biosynthesis of vitamin A.
- (7) (C). When *Agrobacterium tumefaciens* infects the host plant, it will transfer a part of DNA called t-DNA without any human interference so called natural genetic engineer.
- (8) (A). Gene therapy was given in 1990 to a four year old girl child with ADA deficiency.

- (9) (C). Retrovirus is commonly used as vector for introducing a DNA fragment in human lymphocyte. Gene therapy : Lymphocyte from blood of patient are grown in culture outside the body, a functional gene is introduced by using a retroviral vector, into these lymphocyte.
- (10) (B). Biopiracy is term used for or refer to the use of bioresources by multinational companies and other organisation without proper authorisation from the countries and people concerned with compensatory payment.
- (11) (D). Indian Government has setup organisation such as GEAC (Genetic Engineering Appraisal Committee) which will make decisions regarding the validity of GM research and safety of introducing GM-organism for public services.
- (12) (A). Golden rice is vitamin A enriched rice, with a gene from daffodil and is rich in carotene.
- (13) (C). In recombinant DNA technology bacteria is able to produce human insulin because genetic code is nearly universal.
- (14) (C). *Bacillus thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein. These protein exist as inactive protoxins but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to alkaline pH of the gut which solubilize the crystals. The activated toxin binds to the surface of midgut epithelial cells and create pores that cause cell swelling and lysis and eventually cause death of insect.