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Maharashtra State Board 12th Biology Solutions Chapter 12 Biotechnology

1. Multiple choice questions

Question 1.
MU The bacterium which causes a plant disease called crown gall is .....
(a) Helicobacter pylori
(b) Agrobacterium tumifaciens
(c) Thermophilus aquaticus
(d) Bacillus thuringienesis
Answer:
(b) Agrobacterium tumtfaciens

Question 2.
The enzyme nuclease hydrolyses ..... of polynucleotide chain of DNA.
(a) hydrogen bonds
(b) phosphodiester bonds
(c) glycosidic bonds
(d) peptide bonds
Answer:
(b) phosphodiester bonds

Question 3.
In vitro amplification of DNA or RNA segment is known as .....
(a) chromatography
(b) southern blotting
(c) polymerase chain reaction
(d) gel electrophoresis
Answer:
(c) polymerase chain reaction

Question 4.
Which of the following is the correct recognition sequence of restriction enzyme hind III.
(a) 5′ —A-A-G-C-T-T— 3′
3′ —T-T-C-G-A-A—5′
(b) 5′ — G-A-A-T-T-C—3′
3′ — C-T-T-A-A-G—5′
(c) 5′ — C-G-A-T-T-C—3′
3′ — G-C-T-A-A-G—5′
(d) 5′ — G-G-C-C—3′
3′ — C-C-G-G—5′
Answer:
(a) 5′ —A-A-G-C-T-T—3′
3′ —T-T-C-G-A-A—5′

Question 5.
Recombinant protein ..... is used to dissolve blood clots present in the body.
(a) insulin
(b) tissue plasminogen activator
(c) relaxin
(d) erythropoietin
Answer:
(b) tissue plasminogen activator

Question 6.
Recognition sequence of restriction enzymes are generally ..... nucleotide long.
(a) 2 to 4
(b) 4 to 8
(c) 8 to 10
(d) 14 to 18
Answer:
(b) 4 to 8

2. Very short answer questions

Question 1.
Name the vector which is used in production of human insulin through recombinant DNA technology.
Answer:
PBR 322

Question 2.
Which cells from Langerhans of pancreas do produce a peptide hormone insulin?
Answer:
cells of islets of Langerhans of a peptide hormone insulin.

Question 3.
Give the role of Ca^{++} ions in the transfer of recombinant vector into bacterial host cell.
Answer:
Ca^{++} ions promotes binding of plasmid DNA to lipo polysaccharides on bacterial cell surface. Then plasmid can enter the cell on heat shock.

Question 4.
Expand the following acronyms which are used in the held of biotechnology:

1. YAC
2. RE
3. dNTP
4. PCR
5. GMO
6. MAC
7. CCMB.

Answer:

1. YAC : Yeast Artificial chromosome
2. RE : Restriction Endonuclease
3. dNTP : Deoxyribonucleoside triphosphates
4. PCR : Polymerase Chain Reaction
5. GMO : Genetically Modified Organisms
6. MAC : Mammalian Artificial Chromosome
7. CCMB : Centre for Cellular and Molecular Biology

Question 5.
Fill in the blanks and complete the chart.

GMO	Purpose
(i) Bt cotton	————
(ii) ————-	Delay the softening of tomato during ripening
(iii) Golden rice	————
(iv) Holstein cow	————

Answer:

GMO	Purpose
(i) Bt cotton	Insect resistance
(ii) Flavr savr Tomato	Delay the softening of tomato during ripening
(iii) Golden rice	Rich in vitamin A
(iv) Holstein cow	High milk productivity

3. Short answer type questions.

Question 1.
Explain the properties of a good or ideal cloning vector for r-DNA technology.
Answer:
Desired characteristics of ideal cloning vector are as follows:

1. Vector should be able to replicate independenly (through ori gene), so that as vector replicates, multiple copies of the DNA insert are also produced.
2. It should be able to easily transferred into host cells.

3. It should have suitable control elements like promoter, operator, ribosomal binding sites, etc.
4. It should have marker genes for antibiotic resistance and restriction enzyme recognition sites within them.

Question 2.
A PCR machine can rise temperature up to 100 °C but after that it is not able to lower the temperature below 70 °C automatically. Which step of PCR will be hampered first in this faulty machine? Explain why?
Answer:

1. If the faulty machine is not able to lower the temperature below 70 °C, then the primer annealing step will be hampered first.
2. Each primer has a specific annealing temperature, depending upon its A, T, G, C content.
3. For most of the primers annealing temperature is about 40-60 °C.
4. Hence, if temperature is more than primers annealing temperature, it will be able to pair with its complementary sequence in ssDNA.

Question 3.
In the process of r-DNA technology, if two separate restriction enzymes are used to cut vector and donor DNA then which problem will arise in the formation of r-DNA or chimeric DNA? Explain.
Answer:
In the process of r-DNA technology, if two separate restriction enzymes are used to cut vector and donor DNA, then it will result in fragments with different sticky ends which will not be complementary to each other.

Question 4.

Recombinent protein	Its use in or for
(1) Platelet derived growth factor	(a) Anemia
(2) a-antitrypsin	(b) Cystic fibrosis
(3) Relaxin	(c) Haemophilia A
(4) Eryhthropoietin	(d) Diabetes
(5) Factor VIII	(e) Emphysema
(6) DNA ase	(f) Parturition
	(g) Atherosclerosis

Answer:

Recombinent protein	Its use in or for
(1) Platelet derived growth factor	(g) Atherosclerosis
(2) a-antitrypsin	(e) Emphysema
(3) Relaxin	(f) Parturition
(4) Eryhthropoietin	(a) Anemia
(5) Factor VIII	(c) Haemophilia A
(6) DNA ase	(b) Cystic fibrosis

4. Long answer type questions.

Question 1.
(i) Define and explain the terms Bioethics.
Answer:

1. Bioethics is the study of moral vision, decision and policies of human behaviour in relation to biological phenomena or events.
2. Bioethics deals with wide range of reactions on new developments like cloning, transgenic, gene therapy, eugenics, r-DNA technology, in vitro fertilization, sperm bank, gene therapy, euthanasia, death, maintaining those who are in comatose state, prenatal genetic selection, etc.
3. Bioethics also includes the discussion on subjects like what should and should not be done in using recombinant DNA techniques.

Ethical aspects pertaining to the use of biotechnology are:

1. Use of animals cause great sufferings to them.
2. Violation of integration of species caused due to transgenosis.
3. Transfer of human genes into animals and vice versa.
4. Indiscriminate use of biotechnology pose risk to the environment, health and biodiversity.
5. The effects of GMO on non-target organisms, insect resistance crops, gene flow, the loss of diversity.
6. Modification process disrupting the natural process of biological entities.

(ii) Define and explain the term Biopiracy.
Answer:

1. Biopiracy is defined as 'theft of various natural products and then selling them by getting patent without giving any benefits or compensation back to the host country'.
2. It is unauthorized misappropriation of any biological resource and traditional knowledge.
3. It is bio-patenting of bio-resource or traditional knowledge of another nation without proper permission of the concerned nation or unlawful exploitation and use of bioresources without giving compensation.

Following are the examples of biopiracy:
(a) Patenting of Neem (Azadirachta indica):

1. Pirating India's traditional knowledge about the properties and uses of neem, the USDA and an American MNC W.R. Grace sought a patent from the European Patent Office (EPO) on the "method for controlling on plants by the aid of hydrophobic extracted neem oil," in the early 90s.
2. The patenting of the fungicidal properties of Neem, was an example of biopiracy.

(b) Patenting of Basmati:

1. Texmati is a trade name of "Basmati rice line and grains" for which Texas based American company Rice Tec Inc was awarded a patent by the US Patent and Trademark Office (USPTO) in 1997.
2. This is a case of biopiracy as Basmati is a long-grained, aromatic variety of rice indigenous to the Indian subcontinent.
3. Very broad claims about "Inventing" the said rice was the basis of patent application.
4. The UPSTO has rejected all the claims due to people movement against Rice Tec in March 2001.

(c) Haldi (Turmeric) Biopiracy:

1. A patent claim about the healing properties of Haldi was made by two American researchers of Indian origin of the University of Mississippi Medical Center, to the US Patent and Trademark Office.
2. They were granted a patent in March 1995.
3. This is an example of biopiracy because healing properties of Haldi is not a new discovery, but it is a traditional knowledge in ayurvedas for centuries.
4. The Council of Scientific and Industrial Research (CSIR) applied to the US Patent Office for a reexamination and they realized the mistake and cancelled the patent.

(iii) Define and explain the term Biopatent.
Answer:

1. Biopatent is a biological patent awarded for strains of microorganisms, cell lines, genetically modified strains, DNA sequences, biotechnological processes, product processes, product and product applications.
2. It allows the patent holder to exclude others from making, using, selling or importing protected invention for a limited period of time.
3. Duration of biopatentis five years from the date of the grant or seven years from the date of filing the patent application, whichever is less.
4. Awarding biopatents provides encouragement to innovations and promote development of scientific culture in society. It also emphasizes the role of biology in shaping human society.
5. First biopatent was awarded for genetically engineered bacterium 'Pseudomonas' used for clearing oils spills.
6. Patent jointly issued by Delta and Pineland company and US department of agriculture having title 'control of plant gene expression', is based on a gene that produces a protein toxic to plant and thus prevents seed germination.

This patent was not granted by Indian government. Such a patent is considered morally unacceptable and fundamentally unequitable. Such patents would pose a threat to global food security as financially powerful corporations would acquire monopoly over biotechnological process.

Question 2.
Explain the steps in process of r-DNA technology with suitable diagrams.
Answer:

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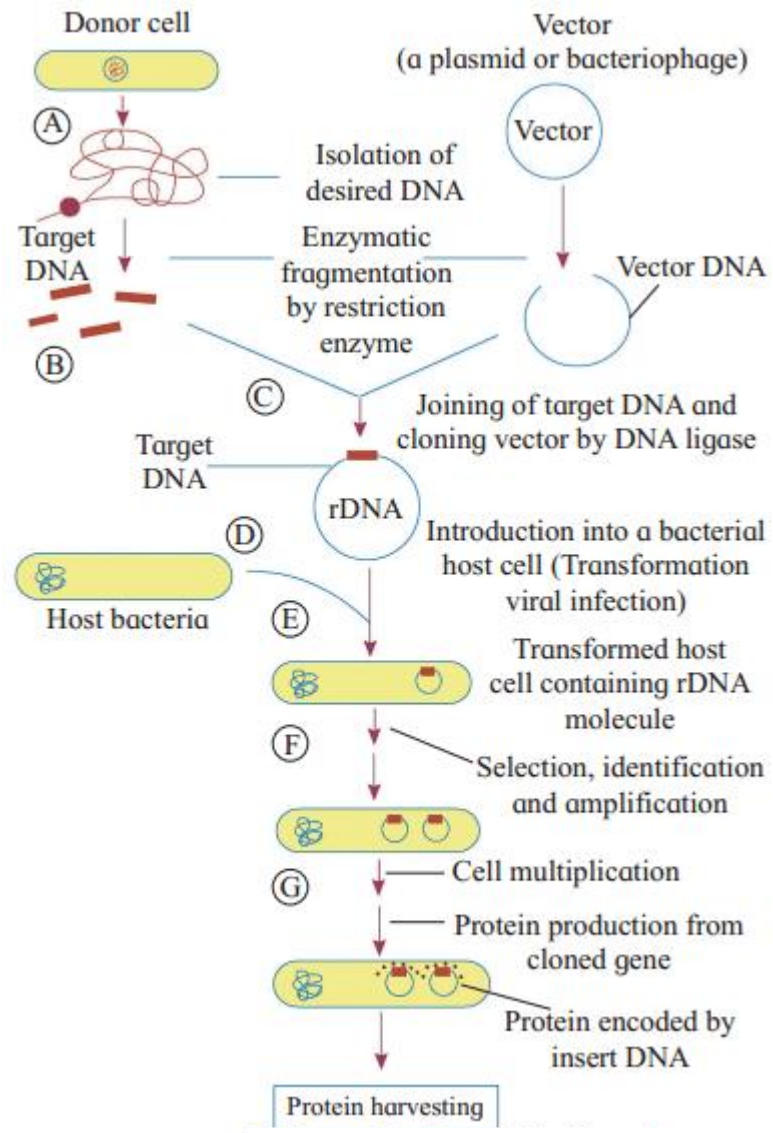


The steps involved in gene cloning are as follows:
(1) Isolation of DNA (gene) from the donor organism:

- To obtain the desired gene to be cloned, the cells of the donor organism are sheared with the blender and treated with suitable detergent. Genetic material is then isolated and purified.
- Isolated purified DNA is then cleaved using restriction Endonucleases.
- Restriction fragment containing desired gene is isolated and selected for cloning. This is now called foreign DNA or passanger DNA.
- A desired gene can also be obtained directly from genomic library or c-DNA library.

(2) Insertion of desired foreign gene into a cloning vector (vehicle DNA):

- The foreign DNA or passanger DNA is inserted into a cloning vector (vehicle DNA) like bacterial plasmids and the bacteriophages like lamda phage and M13. The most commonly used plasmid is pBR 322.
- Plasmids are isolated from the bacteria and are cleaved by using same RE which is used in the isolation of the desired gene from the donor.
- Enzyme DNA ligase is used to join foreign DNA and the plasmid DNA.
- Plasmid DNA containing foreign DNA is called recombinant DNA (r-DNA) or chimeric DNA.

(3) Transfer of r-DNA into suitable competent host or cloning organism:

- The r-DNA is introduced into a competent host cell, which is mostly a bacterium.
- Host cell takes up naked r-DNA by process of 'transformation' and incorporates it into its own chromosomal DNA which finally expresses the trait controlled by passenger DNA.
- The transfer of r-DNA into a bacterial cell is assisted by divalent Ca^{++}.
- The cloning organisms are E.coli and Agrobacterium tumifaciens.
- The competent host cells which have taken up r-DNA are called transformed cells.
- By using techniques like electroporation, microinjection, lipofection, shot gun, ultrasonification, biolistic method, etc. Foreign DNA can also be transferred directly into the naked cell or protoplast of the competent host cell, without using vector.
- In plant biotechnology the transformation is through Ti plasmids of A. tumifaciens.

(4) Selection of the transformed host cell:

- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.
- For example, pBR322 plasmid vector contains different marker genes like ampicillin resistant gene and tetracycline resistant gene. When pstl RE is used, it knocks out ampicillin resistant gene from the plasmid, so that the recombinant cells become sensitive to ampicillin.

(5) Multiplication of transformed host cell:

- The transformed host cells are introduced into fresh culture media where they divide.
- The recombinant DNA carried by them also multiplies.

(6) Expression of gene to obtain desired product. Then desired products like enzymes, antibiotiocs etc. separated and purified through down stream processing using bioreactors.

Question 3.
Explain the gene therapy. Give two types of it.
Answer:
Gene therapy is the treatment of genetic disorders by replacing, altering or supplementing a gene that is absent or abnormal and whose absence or abnormality is responsible for the disease.
Types of gene therapy:
(a) Germ line gene therapy:

1. In this germ cells are modified genetically to correct a genetic defect.
2. Normal gene is introduced into germ cells like sperms, eggs, early embryos.
3. It allows transmission of the modified genetic information to the next generation.
4. Although it is highly effective in treatment of the genetic disorders, its use is not preferred in human beings because of various technical and ethical reasons.

(b) Somatic cell gene therapy:

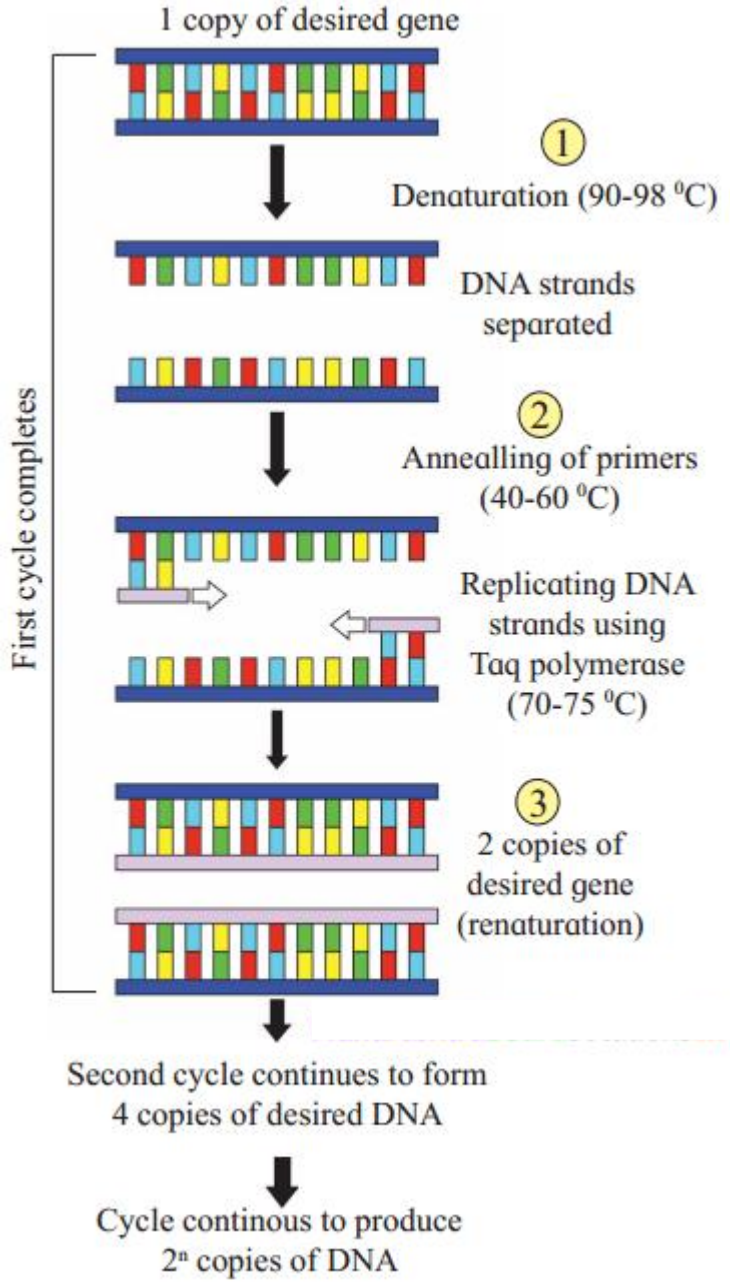
1. In this somatic cells are modified genetically to correct a genetic defect.
2. Healthy genes are introduced in somatic cells like bone marrow cells, hepatic cells, fibroblasts endothelium and pulmonary epithelial cells, central nervous system, endocrine cells and smooth muscle cells of blood vessel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

Question 4.
How are the transgenic mice used in cancer research?
Answer:

1. Transgenic mice are used in various research areas of cancer research.
2. Transgenic mice containing a particular oncogene (cancer causing gene) develop specific cancer.
3. They are used to study the relationship between oncogenes and cancer development, cancer treatment and prevention of malignancy.
4. The transgenic mouse model for the investigation of the breast cancer was developed in the laboratory of Philip Leder in Harvard (USA).
5. Transgenic mice containing oncogenes myc and ras were analyzed to find out role of these genes in the development of breast cancer.

Question 5.
Give the steps in PCR or polymerase chain reaction with suitable diagrams.

Answer:



(1) The DNA segment and excess of two primer molecules, four types of dNTPs, the thermostable DNA polymerase are mixed together in 'eppendorf tube'.

(2) One PCR cycle is of 3-4 minutes duration and it involves following steps:

- Denaturation : The reaction mixture is heated at 90-98°C. Due to this hydrogen bonds in the DNA break and two strands of DNA separate. This is called denaturation.
- Annealing of primer : When the reaction mixture is cooled to 40-60°C, the primer pairs with its complementary sequences in ssDNA. This is called annealing.
- Extension of primer : In this step, the temperature is increased to 70-75°C. At this temperature thermostable Taq DNA polymerase adds nucleotides to 3'end of primer using single-stranded DNA as template. This is called primer extension. Duration of this step is about two minutes.

(3) In an automatic thermal cycler, the above three steps are automatically repeated 20-30 times.
(4) Thus, at the end of 'n' cycles 2n copies of DNA segments, get synthesized.

Question 6.
What is a vaccine? Give advantages of oral vaccines or edible vaccines.
Answer:

1. A vaccine is a biological preparation that provides active acquired immunity against a certain disease.
2. Vaccine is often made from a weakened or killed form of the microorganism, its toxins or one of its surface protein antigens.
3. Edible vaccine is an edible plant part engineered to produce an immunogenic protein, which when consumed gets recognized by immune system.
4. Immunogenic protein of certain pathogens are active when'administered orally.
5. When animals or mainly humans consume these plant parts, they get vaccinated against certain pathogen.
6. Oral or edible vaccines have low cost, they are easy to administer and store.

Question 7.
Enlist different types of restriction enzymes commonly used in r-DNA technology? Write on their role.
Answer:

1. Different restriction enzymes commonly used in r-DNA technology are Alu I, Bam HI, Eco RI, Hind II, Hind III, Pst I, Sal I, Taq I, Mbo II, Hpa I, Bgl I, Not I, Kpn I, etc.
2. They are the molecular scissors which recognize and cut the phosphodiester back bone of DNA on both strands, at highly specific sequences.
3. The sites recognized by them are called recognition sequences or recognition sites.

4. Different restriction enzymes found in different organisms recognize different nucleotide sequences and therefore cut DNA at different sites.
5. Restriction cutting may result in DNA fragments with blunt ends or cohesive or sticky ends or staggered ends (having short, single stranded projections).
6. Restriction endonucleases like Bam HI and EcoRI produce fragments with sticky ends.
7. Restriction endonucleases like Alu I, Hind III produce fragments with blunt ends.
8. Type I restriction endonucleases fuction simultaneously as endonuclease and methylase e.g. EcoK.
9. Type II restriction endonucleases have separate cleaving and methylation activities. They are more stable and are used in r-DNA technology e.g. EcoRI, Bgll. They cut DNA at specific sites within the palindrome.
10. Type III restriction endonucleases cut DNA at specific non-palindromic sequences e.g. Hpal, MboII.
11. In bacterial cells, REs destroy various viral DNAs that might enter the cell, thus restricting the potential growth of the virus.

Question 8.
Enlist and write in brief about the different biological tools required in r-DNA technology.
Answer:
The biological tools used in r-DNA technology are various enzymes, cloning vectors and competent hosts.
(1) Enzymes:

- Enzymes like lysozymes, nucleases (exonucleases and endonucleases), DNA ligase, reverse transcriptase, DNA polymerase, alkaline phosphatases, etc. are used in r-DNA technology.
- The restriction endonucleases are used as biological or molecular scissors. They are able to cut a DNA molecule at a specific recognition site.

(2) Vectors:

- Vectors are DNA molecules which carry foreign DNA segment and replicate inside the host cell.
- Vectors may be plasmids, bacteriophages (M13, lambda virus), cosmid, phagemids, BAC (bacterial artificial chromosome), YAC (yeast artificial chromosome), transposons, baculoviruses and mammalian artificial chromosomes (MACs).
- Most commonly used vectors are plasmid vectors (pBR 322, pUC, Ti plasmid) and bacteriophages (lamda phage, M13 phage).

(3) Competent host cells:

1. They are bacteria like Bacillus haemophilus, Helicobacter pyroliand E. coli.
2. Mostly E. coli is used for the transformation with recombinant DNA.