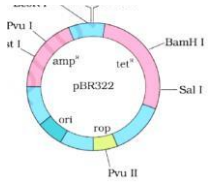
CHAPTER 9



BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

(A) NCERT QUESTIONS & SOLUTIONS

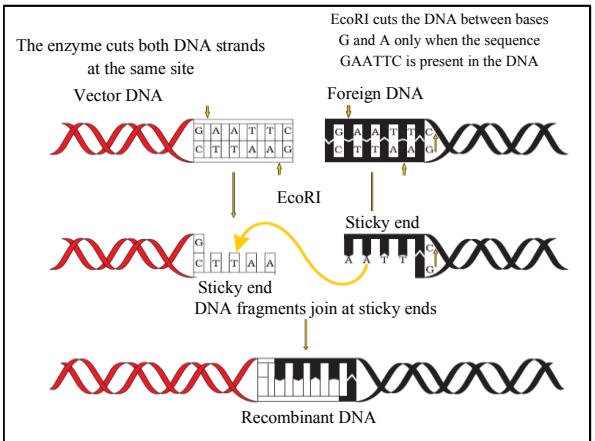
1. Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).

Ans.

S.No.	Recombinant Proteins	Therapeutic uses
1.	Human Insulin (Humulin)	Treatment of diabetes type-1
2.	Human growth hormone	Replacement of missing hormone in short stature people.
3.	Platelet Growth factor	Stimulation of wound healing
4.	Calcitonin	Treatment of rickets
5.	Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilla A/B.
6.	Hirudin	Used as an anticoagulant
7.	Interferon	Treatment of viral infection and cancer
8.	Chorionic Gonadotropin	Treatment of infertility
9.	Interleukins	Enhancing activity of immune system
10.	Tissue Plasminogen Activator	Treatment for acute myocardial infarction, dissolves blood clot after heat attack and stroke.

2. Make a chart (with diagrammatic representation) showing a restriction enzyme, the substrate the DNA on which it acts, the site at which it cuts DNA and the product it produces. [IMP.]

Ans.



Step in formation of recombinant DNA by action of restriction endonuclease enzyme - EcoRI

3. From what you have learnt, can you tell whether enzymes are bigger or n molecular size? How did you know? [IMP.]

Ans. DNA is bigger in molecular size. DNA is made up of sugar, phosphate and nitrogenous bases. An enzyme is made up of only one or few polypeptides.

Enzyme is synthesized from a portion of DNA.

4. What would be the molar concentration of human DNA in a human cell? Consult your teacher.

Ans. The molar concentration of human DNA in a human diploid cell as follow:

- Total number of chromosomes × 6.023×10^{23}
- $46 \times 6.023 \times 10^{23}$
- 277.06×10^{23} Moles.

Hence, the molar concentration of DNA in each diploid cell in human is 277.06×10^{23} moles.

5. Do eukaryotic cells have restriction endonucleases? Justify your answer. [IMP.]

Ans. Eukaryotic cells have no restriction enzymes as the DNA molecules of eukaryotes are heavily methylated.

It is present in prokaryotic cell (like bacteria) where these act as defense mechanism to restrict the growth of bacteriophages.

6. Besides better aeration and mixing properties, what other advantages do stirred-tank bioreactors have over shake flasks?

Ans. Shake flask is used for a small–scale production but the stirred-tank bioreactors are used for large scale production of biotechnological products.

Advantages of stirred - tank bioreactors over shake flasks are that these facilitate –

- ✓ Temperature control system,
- ✓ pH control system,
- ✓ Foam control system and
- ✓ Sampling ports from where small, volume of the cultures can be obtained and tested time to time.

7. Collect 5 examples of palindromic DNA sequences by consulting your teacher. better try to create a palindromic sequence by following base - pair rules.

Ans. (i) 5' GAATTC 3'
3' CTTAAG 5'

(ii) 5' GGATCC 3'
3' CCTAGG 5‘

(iii) 5' ACTAGT 3'
3' TGATCA 5'

(iv) 5' AAGCTT 3'
3' TTCGAA 5'

(v) 5' AGGCCT 3'
3' TCCGGA 5'

8. Can you recall meiosis and indicate at what stage a recombinant DNA is

Ans. Meiosis is the cell division process of gamete formation. It occurs in two steps –
Meiosis-I and Meiosis-II

During pachytene stage of prophase–I of meiosis-I, crossing over takes place between non-sister chromatids of homologous chromosomes and a recombinant DNA is made.

9. Can you think and answer how a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker? [IMP.]

Ans. A reporter gene is used to monitor the transformation of host cells by foreign DNA. They act as a selectable marker to determine whether the host cell has taken up the foreign DNA or the foreign gene gets expressed in the cell.

Here, the reporter gene is used as a selectable marker to find out the successful uptake of gene of interest.

An example of reporter gene includes lac Z gene which encodes β- galactosidase enzyme.

10. Describe briefly the following:

(a) Origin of replication (b) Bioreactors (c) Downstream processing

Ans. (a) Origin of replication:- It is a DNA sequence that initiates any piece of linked DNA to replicate and is also called ori site. It controls the copy numbers of the linked DNA**.**

(b) Bioreactors:-Bioreactors are vessels of large volumes (100-1000 liters) in which raw materials are biologically converted into specific products.

- It provides the optimal conditions for achieving the desired product by providing optimal growth conditions like temperature, pH, substrates, salts vitamins and oxygen.

Stirred tank bioreactors are commonly used bioreactors.

A bioreactor has the following components.

(i) An agitator system
(ii) An oxygen delivery system
(iii) Foam control system
(iv) Temperature control system
(v) pH control system
(vi) Sampling ports to withdraw cultures periodically

(c) Downstream processing - All the process to which a product has to be subjected through a series of processes before it is ready for marketing as a finished product called downstream processing.

- It includes separation of the product from the reactor.
- Purification of the product.
- Formulation of the product with suitable preservatives.
- Quality control testing and trials in case of drugs.

11. Explain briefly.

(a) PCR (b) Restriction enzymes and DNA (c) Chitinase

Ans. (a) PCR - PCR stands for polymerase chain reaction, which is a method for amplification of small segments of DNA**.**

(b) Restriction enzymes and DNA- Restriction enzymes are called 'molecular scissors' because they cut the helix of DNA at a specific site. DNA is the genetics material, which carries and pass the genetic characters or information from one generation to other.

(c) Chitinase - Chitinase is an enzyme which is used to degrade the cell wall of fungi to release its cellular components.

12. Discuss with your teacher and find out how to distinguish between. [IMP.]

(A) Plasmid DNA and chromosomal DNA (B) DNA and RNA

(C) Exonuclease and endonuclease

Ans. (A) Plasmid DNA and chromosomal DNA

S.No.	Plasmid DNA	Chromosomal DNA
1.	This is present in prokaryotic cells. eg -bacteria	This is present in both prokaryotic and eukaryotic cells.
2.	This is the circular extra-chromosomal DNA not associated with histone proteins.	It is linear and associated with histones proteins in eukaryotes, but it is double stranded and circular in prokaryotes.
3.	It gives extra characters to prokaryotes like antibiotic resistance.	It contains genes for characters essential for life of organism.

(B) DNA and RNA

S.No.	DNA	RNA
1.	It has deoxyribose sugar .	It has ribose sugar.
2.	It has A, G, C, T nitrogen base in its nucleotide.	It has A, G, C, U nitrogen base in its nucleotide.
3.	It is double stranded.	It is mostly single stranded.
4.	It acts as a genetic material in almost all organism.	It acts as genetic material in only some viruses.
5.	DNA can't be catalytic.	It can be catalytic.(Ribozyme)

(C) Exonuclease and Endonuclease

S.No.	Exonuclease	Endonuclease
1.	Remove nucleotides from ends of the DNA	Cut at specific positions within the DNA
2.	They are end specific.	They are site specific.
3.	e.g : Exonuclease - I	e.g. EcoRI

(B) PREVIOUS YEAR QUESTIONS

1. **Assertion (A) :** Synthetic oligonueleotide polymers are used during Annealing in a PCR.

[CBSE 2023]

Reason (R) : The primere bind to the double stranded DNA at their complementary regions.

Ans. (c) Assertion (A) is true but Reason (R) is false

2. (a) Write the scientific name of the source organism of the thermostable DNA polymerase used in PCR. **[CBSE 2023]**

(b) State the advantage of using Thermostable DNA polymerase.

Ans. (a) Thermus aquaticus

(b) Thermostable polymerase (Taq polymerase) is used in the PCR due to their advantage to remain active at very high temperatures. Unlike other polymerases. Taq polymerase do not get denature in the process of PCR thus, effectively perform the task of DNA strand polymeriozation even at 94 - 95ºC temperature.

3. (a) State the principle involved in separation of DNA fragments using gel electrophoresis.

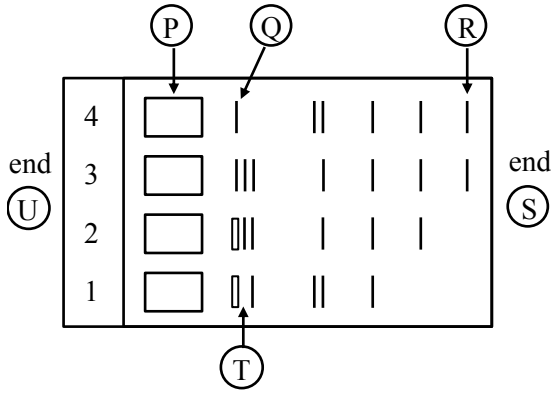
(b) How are DNA fragments visuaised once they are separated by gel electrophoresis?

[CBSE 2023]

Ans. (a) DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.

(b) The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation .You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light.

4. (a) Given below is the stepwise schematic representation of the process of electrophoresis.



Identify the 'alphabets' representing
(i) Anode end
(ii) Smallest/lightest DNA strand in the matrix
(iii) Agarose gel

(b) What is elution? State the importance of elution in this process.

[CBSE Term-II 2022]

Ans. (a) (i) Anode- S end (ii) R (iii) T

(b) The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution.

Importance:- The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

5. (a) Read the paragraph given below and answer the questions that follow:

Enzyme Taq polymerase, is extracted from a eubacterial microorganism *Thermus aquaticus* from Yellowstone National Park in Montana, USA and isolated by Chien at al. (1976). Taq polymerase successfully replaced the DNA polymerase from E.coli that was being used in PCR earlier and this shift revolutionised the PCR technique.

(i) Taq polymerase after its discovery replaced *E.coli* DNA polymerase in PCR technique. Explain giving reasons why was the need felt for the change?

(ii) What is a primer and its importance in PCR?

(ii) What the importance of PCR as a diagnostic tool. **[CBSE Term-II 2022]**

Ans. (a) (i) Thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus*), which remain active during the high temperature induced denaturation of double stranded DNA.

(ii) primer is a small segment of DNA that binds to a complementary strand of DNA. Primers are necessary to start the functioning of DNA polymerase enzyme and therefore are necessary in polymerase chain reaction.

(iii) PCR is important because it can generate several copies of a DNA sequence in a very short time period. It is also important in forensic science as a tool for genetic engineering. It helps in analyzing the gene expression

6. Name the commonly used vector for cloning genes into higher organisms.

[CBSE IMP Questions]

Ans. Retrovirus/ Adenoviruses/Papilloma virus/Cauliflower mosaic virus/Tobacco mosaic virus

7. Assertion: *E. coli* having pBR322 with DNA insert at BamHI site cannot grow in medium containing tetracycline. **[CBSE IMP Questions]**

Reason: Recognition site for Bam HI is present in tet^R region of pBR322.

(A) Both assertion and reason are true, and the reason is the correct explanation of the assertion.

(B) Both assertion and reason are true, but the reason is not the correct explanation of the assertion.

(C) Assertion is true but reason is false.

(D) Both assertion and reason are false

Ans. (A) Both assertion and reason are true, and the reason is the correct explanation of the assertion.

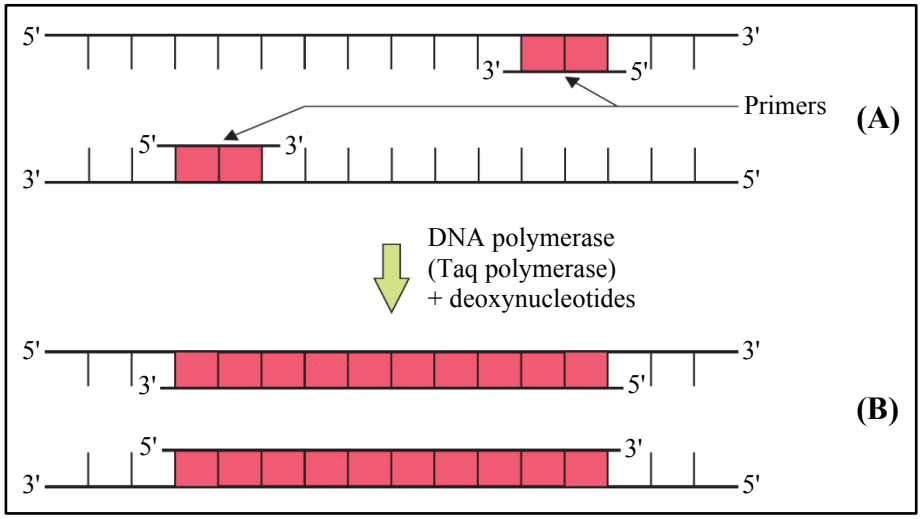
8. What are sticky ends? State their significance in recombination DNA technology.

[CBSE IMP Questions]

Ans.

- Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded portions at the ends. These overhanging stretches on each strand are called sticky ends.
- They form hydrogen bonds with their complementary counterparts and facilitate the action of DNA ligase enzyme.

9. (a) Identify step A and B in a cycle of polymerase chain reaction given bel



(b) State the specific characteristic feature of the enzyme in carrying step B. [CBSE 2020]

Ans. (a) Step A- Annealing, B- Extension

(b) Thermostable DNA polymerase (isolated from a bacterium, ***Thermus aquaticus***), which remain active during the high temperature induced denaturation of ds DNA. It is known as **Taq DNA Polymerase.**

10. How is a continuous culture system maintained in bioreactors and why? [CBSE 2019]

Ans. Used medium is drained out from one side of the bioreactor and fresh medium is added from the other side.

- This type of culturing method produces a larger biomass leading to higher yields of desired protein.

11. How are DNA fragments visualized during gel electrophoresis? What is elution?

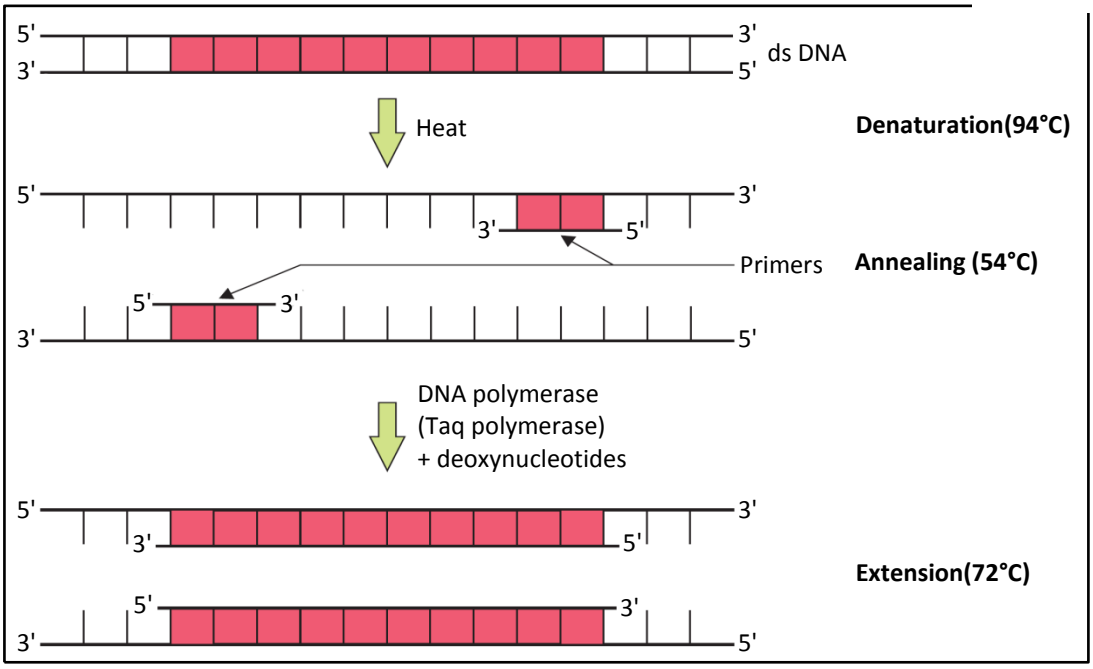
[CBSE 2019]

Ans. Separated DNA fragments by agarose gel electrophoresis are stained with ethidium bromide, followed by exposure to UV radiations and bright orange coloured DNA bands are visualized.

- The removal of DNA band by cutting a piece of gel with knife from agarose gel, this step is called as elution.

12. Diagrammatically represent the process of amplification of "gene of interest" using PCR technique. [CBSE 2018]

Ans. The process of amplification of "gene of interest" using PCR technique explain through following structure.



13. Briefly explain the roles of the following with the help of an example each in recombinant DNA technology.

(a) Restriction Enzymes

(b) Plasmids **[CBSE 2017]**

Ans. (a) Restriction Enzymes - It recognizes a specific sequence of base pairs / palindromes, and cuts the DNA strand at a specific site. eg. EcoRI

(b) Plasmids - Plasmids are **extra-chromosomal** and **autonomously replicating** circular ds DNA in bacteria. They act as vector to transfer desired gene into the host cell.

eg. -pBR322, Ti plasmid of Agrobacterium.

14. Briefly explain the role (s) of the following in Biotechnology :

1. Restriction endonuclease

2. Gel – electrophoresis

3. Selectable markers in pBR322 **[CBSE 2017]**

Ans. (1) Restriction endonuclease - The enzyme which cuts at specific position within the DNA known as restriction enzyme.

(2) Gel – electrophoresis-Separation of DNA fragments under the influence of electric field. **Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.**

(3) Selectable markers in pBR322- Helps in identifying and eliminating non-transformants from transformants or selection of transformants .

Eg- (i) Ampicillin resistance gene

(ii) Tetracycline resistance gene

(C) MULTIPLE CHOICE QUESTIONS

1. The term "Biotechnology" was given by –

(1) Karl Ereky (2) Stanley Cohen (3) Herbert Boyer (4) Paul Berg

Ans. (1) Karl Ereky

2. Genetic engineering is:-

(1) Study of extra nuclear gene (2) Manipulation of genes by artificial method

(3) Manipulation of RNA (4) Manipulation of enzymes

Ans. (2) Manipulation of genes by artificial method

3. Who transferred gene of SV-40 virus into E.coli by λ–bacteriophage?

(1) Karl Ereky (2) Stanley Cohen (3) Herbert Boyer (4) Paul Berg

Ans. (4) Paul Berg

4. First Recombinant DNA produced by linking an antibiotic resistance gene with native plasmid of

(1) *Salmonella typhimurium* (2) *Agrobacterium tumefaciens*

(3) *Escherichia coli* (4) *Haemophilus influenzae*

Ans. (1) *Salmonella typhimurium*

5. Which of the following enzyme are known as molecular scissors?

(1) DNA Ligase (2) DNA Polymerase

(3) Reverse Transcriptase (4) Restriction endonuclease

Ans. (4) Restriction endonuclease

6. Restriction endonucleases are used in genetic engineering because :-

(1) They can degrade harmful proteins (2) They can join DNA fragments

(3) They can cut DNA at variable site (4) They can cut DNA at specific base sequences

Ans. (4) They can cut DNA at specific base sequences

7. According to EFB, "The integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services," is known as–

(1) Biochemistry (2) Bioinformatics (3) Biotechnology (4) Biology

Ans. (3) Biotechnology

8. Which of the following lytic enzyme used to isolation of DNA from fungal cell?

(1) Lysozyme (2) Cellulose (3) Pectinase (4) Chitinase

Ans. (4) Chitinase

9. The enzyme which remove nucleotides from ends of the DNA are known as

(1) Exonuclease (2) Endonuclease (3) Polymerase (4) Ligase

Ans. (1) Exonuclease

10. Which of the bond of DNA molecules cut by Restriction endonuclease?

(1) Phosphodiester bond (2) Hydrogen bond

(3) Glycosidic bond (4) Phosphoanhydride bond

Ans. (1) Phosphodiester bond

11. In enzyme EcoRI, the letter R indicate –
(1) Indicates species of bacteria
(2) Indicates genus of bacteria
(3) Indicates strain of bacteria
(4) Order in which the enzymes were isolated from bacteria
Ans. (3) Indicates strain of bacteria
12. The first isolated restriction endonuclease is-
(1) EcoR I (2) BamH I (3) Hind II (4) Hind III
Ans. (3) Hind II
13. The technique to check the progression of a restriction enzyme digestion is-
(1) Gel electrophoresis (2) PCR-technique
(3) Centrifugation (4) Southern blotting
Ans. (1) Gel electrophoresis
14. In agarose gel electrophoresis, DNA molecules are separated on the basis of their
(1) charge only (2) size only (3) charge to size ratio (4) all of the above
Ans. (4) all of the above
15. The separated bands of desired DNA are cut out from the agarose gel and extracted from the gel piece is known as
(1) Southern blotting (2) Centrifugation
(3) Elution (4) Gel electrophoresis
Ans. (3) Elution
16. Which of the given statements is correct in the context of observing DNA separated by agarose gel electrophoresis?
(1) DNA can be seen in visible light
(2) DNA can be seen without staining in visible light
(3) Ethidium bromide stained DNA can be seen in visible light
(4) Ethidium bromide stained DNA can be seen under exposure to UV light
Ans. (4) Ethidium bromide stained DNA can be seen under exposure to UV light
17. Which of the following is the example of molecular scissors.
(1) EcoRI (2) Hind - III (3) Bam – I (4) All the above
Ans. (4) All the above
18. Which of the following is the example of direct gene transfer?
(1) Microinjection (2) Electroporation (3) Particle gun (4) All the above
Ans. (4) All the above
19. *Agrobacterium tumefaciens* contains a large plasmid, which induces tumour in the plants it is termed as -
(1) Ti plasmid (2) Ri plasmid
(3) Recombinant plasmid (4) pBR322
Ans. (1) Ti plasmid
20. The DNA molecules that can carry a foreign DNA segment into the host cell is known as
(1) Cloning vector (2) Selectable marker
(3) Recombinant DNA (4) Foreign DNA
A vector

(D) ASSERTION & REASON QUESTIONS

Directions: In the following questions, a statement of assertion is followed by a statement of reason. Mark the correct choice as:

(1) If both Assertion and Reason are true and Reason is the correct explanation of Assertion.

(2) If both Assertion and Reason are true but Reason is not the correct explanation of Assertion.

(3) If Assertion is true but Reason is false.

(4) If both Assertion and Reason are false.

1. **Assertion:** Bacterial cells are made competent by treating them with specific concentration of a divalent cation.
Reason: Treatment of bacterial cell with a divalent cation increases the efficiency with which DNA enters the bacterium through pores in its cell wall.
Ans. (1)

2. **Assertion**: Both the passenger and vehicle DNAs are treated separately with separate restriction endonuclease.
Reason: Ligation is done by the use of alkaline phosphatase and DNA ligase.
Ans. (4)

3. **Assertion** : Vector DNA and foreign DNA are cut by same restriction endonuclease.
Reason: Digestion of vector DNA and foreign DNA with same enzyme produces complementary sticky ends.
Ans. (1)

4. **Assertion**: Selectable marker is meant for distinguishing a recombinant from non-recombinant.
Reason: Every recombinant can flourish in medium having both ampicillin and tetracycline, while the non- recombinants cannot.
Ans. (3)

5. **Assertion**: Restriction endonuclease recognises palindromic sequence in DNA and cuts them.
Reason: Palindromic sequence has two unique recognition sites Pst I and Pvu I recognised by restriction endonuclease.
Ans. (3)

6. **Assertion**: Bacteriophage vectors are more advantageous than plasmid vectors.
Reason: Bacteriophage vectors can be easily detected at the time of cloning experiments.
Ans. (1)

7. **Assertion** : The separated DNA fragments can be visualised only after staining the DNA with ethidium bromide in gel electrophoresis.
Reason: We can see pure DNA fragments in the visible light without staining.
Ans. (3)

8. **Assertion**: The ori site of vector responsible for controlling the copy number of the linked DNA.
Reason: Ori is a sequence from where replication starts.
Ans. (2)

9. **Assertion**: Retroviruses in animals have the ability to transform normal cells into cancerous cells.
Reason: The retrovirus should have been disarmed whenever it used to deliver desirable genes into animal cells.
Ans. (2)

10. **Assertion**: Amplification of a gene of interest can be done by polymerase chain reaction.
Reason: It is possible to amplify DNA segment approximately 1 billion times within a span of 30 cycles.
Ans. (2)

(E) VERY SHORT ANSWER QUESTIONS

1. Mention the type of host cells suitable for the gene guns to introduce an alien DNA.

Ans. The host cells suitable for the gene guns to introduce an alien DNA are plant cells.

2. Name the host cells in which microinjection technique is used to introduce an alien DNA.

Ans. The microinjection technique to introduce alien DNA is usually carried out in animal cell, i.e. directly into the nucleus.

3. Why is it essential to have a selectable marker in a cloning vector?

Ans. Selectable marker in cloning vector helps in identifying and selecting the recombinants and eliminating the non-recombinants.

4. Why do DNA fragments move towards the anode during gel electrophoresis?

Ans. DNA fragments are negatively charged molecules and hence, moves toward the anode during gel electrophoresis.

5. How is the action of exonuclease different from that of endonuclease.

Ans. Exonuclease removes nucleotides from the ends of DNA, while endonuclease cuts the DNA at specific positions.

6. Mention the role of molecular scissors in recombinant DNA technology.

Ans. Molecular scissors or restriction enzymes cut DNA at specific site, thus allowing to extract desired gene and like it with DNA of host.

7. Name the technique used for separating DNA fragments in the laboratory.

Ans. Gel electrophoresis is used for separating DNA fragments in the laboratory.

8. What is the role of ethidium bromide during agarose gel electrophoresis?

Ans. The separated DNA fragments during agarose gel electrophoresis are visualised after staining the DNA with ethidium bromide, in UV light. This staining imparts DNA a bright orange colour.

9. Why it is not possible for an alien DNA to become part of a chromosome anywhere along its length and replicate normally ?

Ans. Alien DNA must be linked to ori / origin of replication / site to start replication.

10. Which main technique and instrument is used to isolate DNA from a plant cell ?

Ans. Centrifugation and centrifuge.

(F) SHORT ANSWER QUESTIONS

1. Describe a palindrome with the help of an example.

Ans. A DNA sequence that reads the same, on the two strands from 5'– 3' direction or 3' – 5' direction.

5'–GAATTC–3'

3'–CTTAAG–5'

2. List the key tools used in recombinant DNA technology.

Ans. Restriction enzymes / Polymerase enzymes / Ligase enzymes / Vectors / Host organisims/ *E.coli/ Agrobacterium.*

3. Name two commonly used vectors in genetic engineering.

Ans. Plasmid and Bacteriophage.

4. Name the bacterium that yields thermostable DNA polymerase.

Ans. *Thermus aquaticus.*

5. Write any two biochemical / molecular diagnostic procedures for early detection of viral infection. Explain the principle of any one of them.

Ans. ELISA

ELISA – antigen antibody interaction / PCR – amplification of nucleic acid for its identification

6. Retroviruses have no DNA. However, the DNA of the infected host cell does possess viral DNA. How is it possible?

Ans. Retrovirus have RNA as genetic material whenever it enter into host cell formed cDNA through process of reverse transcription so that host cell possess viral DNA.

7. Why is 'plasmid' an important tool in biotechnology experiments?

Ans. Plasmids are commonly used to multiply or express particular genes and act as vectors to transfer piece of foreign DNA attached to them.

8. Why is it essential to have 'selectable marker' in cloning vector ?

Ans. Selected marker helps in the identification and elimination of non-transformants and permitting the growth of the transformants. Therefore, they are considered. Therefore, they are considered essential in cloning vacter.

9. Why are molecular scissors so called? Write their use in biotechnology.

Ans. The restriction enzymes are known as molecular scissors as they cut the DNA at specific sites or locations.

They help (in genetic engineering) to form recombinant molecules of DNA, which are composed of DNA from different genomes.

10. What if EcoRI? How does EcoRI differ from an exonuclease?

Ans. EcoRI is restriction endonuclease enzyme.

Exonuclease removes nucleotides from the ends of DNA.

EcoRI makes cuts at specific position within the DNA.

(G) LONG ANSWER QUESTIONS

1. Briefly explain the roles of the following with the help of an example each in recombinant DNA technology.

(a) Restriction Enzymes

(b) Plasmids

Ans. **(a) Restriction Enzymes -** It recognizes a specific sequence of base pairs / palindromes, and cuts the DNA strand at a specific site. eg. EcoRI

(b) Plasmids - Plasmids are **extra-chromosomal** and **autonomously replicating** circular ds DNA in bacteria. They act as vector to transfer desired gene into the host cell.

e.g., -pBR322, Ti plasmid of Agrobacterium.

2. Briefly explain the role (s) of the following in Biotechnology:

1. Restriction endonuclease

2. Gel – electrophoresis

3. Selectable markers in pBR322

Ans. **(a) Restriction endonuclease** The enzyme which cuts at specific position within the DNA known as restriction enzyme.

(b) Gel-electrophoresis-Separation of DNA fragments under the influence of electric field. **Agarose gel electrophoresis is employed to check the progression of a restriction enzyme digestion.**

(c) Selectable markers in pBR322- Helps in identifying and eliminating non-transformants from transformants or selection of transformants .

Eg- (i) Ampicillin resistance gene

(ii) Tetracycline resistance gene

3. (a) Why must a cell be made 'competent' in biotechnology experiments? How does calcium ion help in doing so?

(b) State the role of 'biolistic gun' in biotechnology experiments.

Ans. **(a)** To take up the (hydrophilic) DNA from the external medium.

- Divalent calcium ions increase the efficiency of DNA entering the cell through pores in the cell wall.

(b) Biolistic gun helps to introduce alien DNA into the plant cell by bombarding high velocity microprojectile (gold or tungsten) coated with DNA.

4. (a) How has the development of bioreactor helped in biotechnology ?

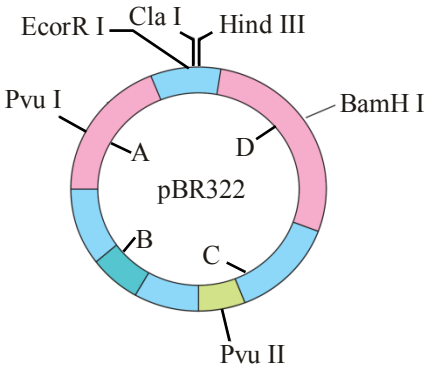
(b) Name the most commonly used bioreactor and describe its working.

Ans. **(a)** Larger biomass / large volume of culture can be processed leading to higher yields of desired specific products (protein / enzymes),under controlled condition.

(b) Stirring type

- Mixing of reactor contents evenly (with agitator system or a stirrer)
- Facilitates oxygen availability
- Temperature / pH / foam control/under optimum conditions

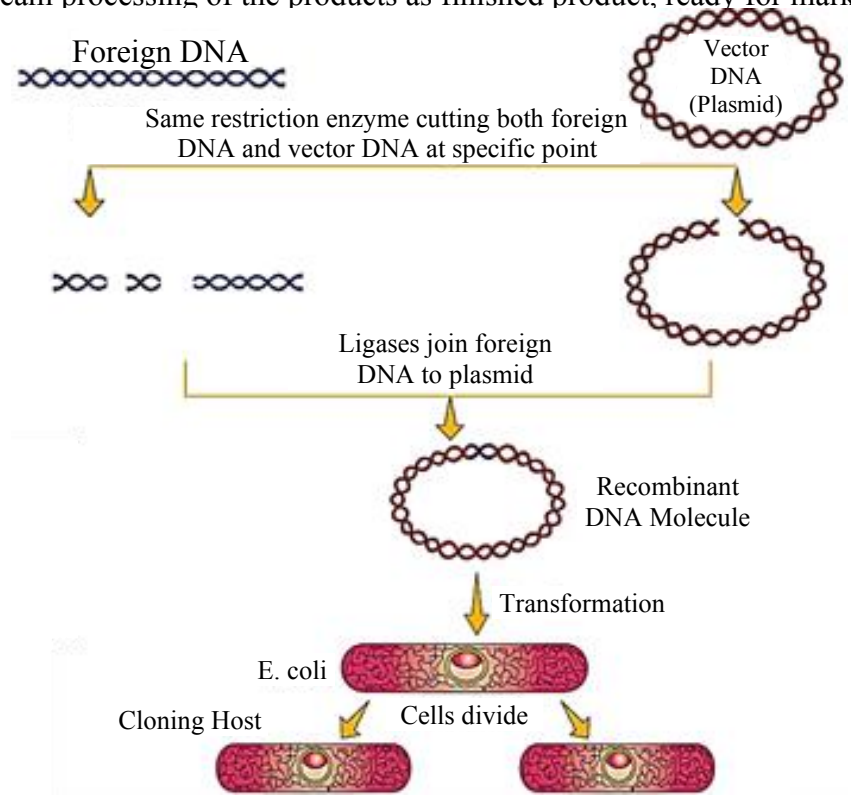
5. Study the figure of vector pBR322 given below. Identify A, B and C and s in cloning a vector.



Ans. **A** - antibiotic resistance genes, the ligation of alien DNA is carried out at a restriction site on this gene / acts as selectable marker present in this antibiotic resistance gene.
B - ori, the sequence where replication starts
C - rop, codes for proteins involved in the replication of the plasmids

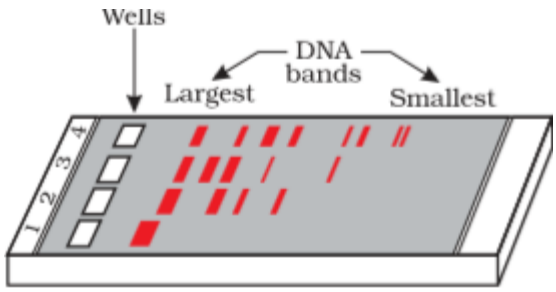
6. With the help of diagrammatic representation only, show the steps of recombinant DNA technology.

Ans. Recombinant DNA technology involve the following steps:
(a) Isolation of DNA.
(b) Digestion of DNA by restriction endonuclease.
(c) Isolation of a desired DNA fragment.
(d) Amplification of the gene of interest.
(e) Ligation of the DNA fragment into a vector.
(f) Insertion of recombinant DNA into the host.
(g) Culturing the host cells on a suitable medium at a large scale.
(h) Extraction of the desired gene product.
(i) Downstream processing of the products as finished product, ready for marketing.



Diagrammatic representation of recombinant DNA technology

7. A typical agarose gel electrophoresis showing migration of DNA fragme it and give the answer of question that follow.



(a) How are the separated DNA fragments visualised?

(b) What are the criteria for separation of DNA fragments in gel electrophoresis.

(c) What is elution?

Ans. (a) The separated DNA fragments can be visualised only after staining the DNA with ethidium bromide followed by exposure of UV radiation.

(b) The DNA fragments separate (resolve) according to size and length along with charge through sieving effect provided by the agarose gel.

(c) The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece called as elution.

8. (a) Why should a bacterium be made 'competent' ?

(b) Explain the role of `microinjection' and 'gene gun' in biotechnology.

Ans. (a) The bacterial cells must first be made competent in order to receive the hydrophilic rDNA/ plasmid which cannot otherwise pass through the cell membrane.

(b) Microinjection - rDNA is directly injected into the animal cell nucleus

Biolistics (gene gun) - Plant cells are bombarded with high velocity micro-particles of gold /tungsten coated with DNA.

(H) CASE-STUDY BASED QUESTIONS

1. Read the following and answer the questions given below:

The first restriction endonuclease–Hind II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised five years later. It was found that Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II. Besides Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

(i) What distinguish exonuclease activity from Endonuclease?

Ans. Exonuclease remove nucleotides from the ends of DNA whereas, endonucleases make cuts at specific positions within the DNA.

(ii) When a restriction enzyme discovers its recognition sequence, what happens?

Ans. Once restriction enzyme finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the two strands of the double helix at specific points in their sugar - phosphate backbone.

(iii) Why are molecular scissors so called? write their use in biotechnology.

Ans. The restriction enzymes are as molecular scissors as, They cut the DNA at specific sites or locations. They Help (in genetic engineering) to from recombinant molecules of DNA, which are composed of DNA from different genomes.

(iv) In accordance with restriction enzyme naming conventions, expand Hind II.

Ans. In Hind II enzyme, H stands for *Haemophilus*, in stands for influenzae, d stands for strain, II stands for type II restriction ezymes.

2. Read the following and answer the questions given below:

Rajat is a student of biotechnology. His professor tells him that for transformation with recombinant DNA the bacterial cells must be made capable of taking up DNA as DNA do not pass through membrane. While doing experiment in the lab, Rajat noticed that bacterial cells were not taking up the foreign DNA even after treating it with sodium ion. He asked his professor, the **Reason** behind this. His professor explained that he should check the valency and charge of the ion that he is using for the treatment.

(i) Mention the type of host cells suitable for the gene guns to introduce an alien/foreign DNA.

Ans. Plant host cells are suitable for the gene guns to introduce an alien/foreign DNA.

(ii) Why DNA cannot pass through the cell membrane? Explain.

Ans. Since, DNA molecules are hydrophilic, they cannot pass through cell membranes. For recombinant DNA to be Integrated into vector or host genome, it is necessary for the DNA to be inserted in the cell.

(iii) How is a bacterial cell made competent to take up recombinant DNA from

Ans. The two ways by which cells can be made competent to take up DNA are

(1) Chemical action :- The host cell is treated with a specific concentration of Divalent cation, i.e., calcium Increases the pore size in the cell membrane.

(2) Heat-shock treatment :- Incubating the cells with recombinant DNA on ice, followed by brief Treatment of heat at 42ºC and again putting them back on ice.

(iv) Write any four ways used to introduce a desired DNA segment into a bacterial cell in recombinant technology experiments.

Ans. (i) Microinjection

(ii) Disarmed Pathogen vectors

(iii) Biolistic or gene gun

(iv) Treatment of host cell by bivalent cation such as calcium.

3. Read the following and answer the questions given below:

The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as gel electrophoresis. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix. The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light.

(i) In Gel-electrophoresis the separation of DNA fragments take place upon the basis of.

Ans. Charge and length of fragments.

(ii) The Agarose gel which used as a medium is electrophoresis obtained from.

Ans. See weeds.

(iii) What is the role of ethidium bromide during agarose gel-electrophoresis?

Ans. The separated DNA fragments during agarose gel-electrophoresis are visualised after staining the DNA with ethidium bromide, in UV light, this staining imparts DNA a bright orange colour.

(iv) The separation of DNA fragments into electrophoresis take plane by which effect?

Ans. Sieving effect.

(v) Why do DNA fragments move towards the anode during gel electrophoresis?

Ans. Because DNA is negatively charged.

4. Read the following and answer the questions given below :

Small volume cultures cannot yield appreciable quantities of products. To produce in large quantities, the development of bioreactors, where large volumes (100-1000 litres) of culture can be processed, was required. Thus, bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen). The most commonly used bioreactors are of stirring type,which include simple stirred-tank bioreactor and sparged stirred - tank bioreactor.

(i) Which of the apparatus used in large scale production of recombinant product?

Ans. Bioreactor

(ii) Which of the factors affect the quality of obtained product in a bioreactor?

Ans. These are (i) Temperature (ii) pH (iii) Oxygen supply

(iii) Write the name of most commonly used bioreactor.

Ans. Stirred type

(iv) The sparged stirred-tank bioreactor is well suited for large scale production why?

Ans. In sparged stirred tank bioreactor surface area for oxygen transfer is increased.

(v) Define bioreactor

Ans. A large vessel with stirring arrangement in which organic raw material are biologically converted into specific product under optimum condition.