9 Biotechnology: Principles and Processes

Fastrack Revision

- **Biotechnology** deals with the techniques of using living organisms or enzymes from organisms to produce products and processes useful to humans.
- The processes like *in vitro* fertilisation leading to a 'test-tube' baby, synthesizing a gene and using it, developing a DNA vaccine or correcting a defective gene, are all parts of biotechnology.
- **Principles of Biotechnology**
  The two core techniques that enabled birth of modern biotechnology are:
  - Genetic engineering
  - Maintenance of sterile conditions
  - **Genetic engineering** is the technique of altering the chemistry of DNA and RNA so that it can be introduced into the host organism to change the phenotype of the host organism.
  - **Sterile conditions** should be maintained to enable growth of only the desired microbe or eukaryotic cell in large quantities for the manufacture of antibiotics, vaccines, enzymes, etc.
- Hybridisation procedures often lead to inclusion and multiplication of undesirable genes along with the desired genes.
- The techniques of genetic engineering, which include creation of recombinant DNA, use of gene cloning and gene transfer, allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.
- Three basic steps in genetically modifying an organism are:
  - Identification of DNA with desirable genes.
  - Introduction of the identified DNA into the host.
  - Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.
- **Tools of Recombinant DNA Technology**
  Important tools of recombinant DNA technology are:
  - **Restriction Enzymes:** Restriction enzymes are called as molecular scissors because these enzymes cut DNA at specific sites.
  - **Cloning Vector:** Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.
  - **Competent Host:** The host should be competent enough to take up the foreign DNA.
  - **Bioreactors:** Bioreactor is the cylindrical vessel in which biological processes are carried out on a large scale.

Restriction Enzymes

- Restriction enzymes belong to a larger class of enzymes called **molecular scissors.**

Knowledge BOOSTER

The first restriction endonuclease is ***Hind*** **II.**

- The restriction enzymes cut DNA at specific base sequence, and these specific base sequences are known as the **recognition sequence.**
- The convention for naming restriction enzymes include the following:
  - The first letter of the name comes from the genus.
  - The second two letters come from the species of the prokaryotic cell from which they were isolated, *e.g.*, *Eco* RI comes from *Escherichia coli* RY 13.
  - In *Eco* RI, the letter 'R' is derived from the name of strain.
  - Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.
  - 900 restriction enzymes have been isolated from over 230 strains of bacteria.
- Restriction enzymes are of two kinds—Exonucleases and Endonucleases.
- **Exonucleases** remove nucleotides from the ends of the DNA whereas **endonucleases** make cuts at specific positions within the DNA.
- Each restriction endonuclease recognises a specific **palindromic nucleotide sequences** in the DNA.
- The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

Example: The following sequences read the same on the two strands in 5′ → 3′ direction and this is also true if read in the 3′ → 5′ direction.

5′ ···· ···· GAATTC ···· ···· 3′
3′ ···· ···· CTTAAG ···· ···· 5′

- 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 which leaves a single stranded portions at the ends. The overhanging stretches are called **sticky ends** on each strand.
- When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky-ends' and, these can be joined together using DNA ligases.
- **Cloning Vectors**
  - A cloning vector is a small piece of DNA, taken from any organism into which a foreign DNA fragment can be inserted for cloning purposes.
  - Plasmids and bacteriophages have the ability to replicate within bacterial cells independent of the control of chromosomal DNA.

- If an alien piece of DNA is linked with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage.

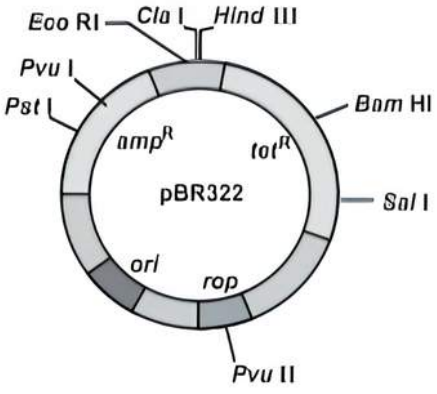
The features that are required to facilitate cloning into a vector are:

1. Origin of Replication (*ori*)

- This is the sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells.
- The sequence is responsible for controlling the copy number of the linked DNA.

2. Selectable Marker

- It helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.
- **Transformation** is a procedure through which a piece of DNA is introduced in a host bacterium.
- The genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc. are useful selectable markers for *E. coli* as the normal *E. coli* cells do not carry resistance against any of these antibiotics.
- Antibiotic resistance genes help in selecting recombinants from non-recombinants by a method called **insertional inactivation** where a recombinant DNA is inserted within the coding sequence of an enzyme β-galactosidase in the presence of a chromogenic substrate which results into inactivation of the enzyme.
- The presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert.
- Presence of insert results into insertional inactivation of the β-galactosidase and the colonies do not produce any colour. These are identified as **recombinant colonies.**



3. Cloning Sites

- Cloning sites are the recognition sites of the restriction enzymes.
- The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes.

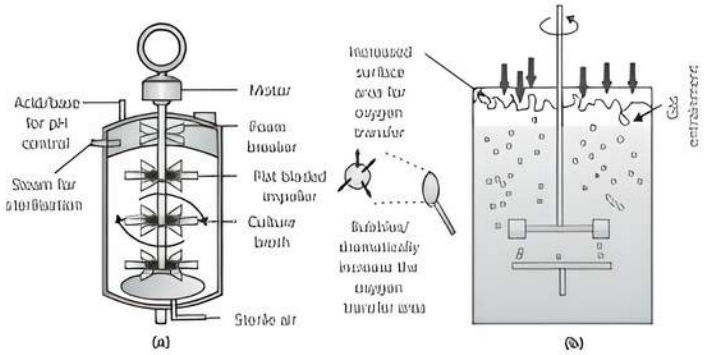
Example: Ligation of a foreign DNA at the *Bam* HI site of tetracycline resistance gene in the vector pBR322.

4. Vectors for Cloning Genes

- Vector for cloning genes in plants is *Agrobacterium tumifaciens,* a pathogen of several dicot plants which delivers a piece of DNA known as 'T-DNA' to transform normal plant cells into a **tumor** and direct these tumor cells to produce the chemicals required by the pathogen.
- The tumor inducing (Ti) plasmid of *Agrobacterium tumifaciens* has now been modified into a cloning vector.
- Vector for cloning genes in animals is **retrovirus** which transforms normal cells into cancerous cells.
- Retroviruses have been disarmed and used to deliver desirable genes into animal cells.

Competent Host

- Since DNA is a hydrophilic molecule, it cannot pass through cell membranes so the bacterial cells must first be made 'competent' to take up DNA.
- Several methods are followed to make the bacterial cells competent:
  - Treating them with a specific concentration of a divalent cation, such as calcium, this increases the efficiency with which DNA enters the bacterium through pores in its cell wall.
  - Recombinant DNA can, then, be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42°C (heat shock), and then putting them back on ice which enables the bacteria to take up the recombinant DNA.
  - Recombinant DNA can be directly injected into the nucleus of an animal cell by a method called **micro-injection**.
  - In **biolistics** or **gene gun** method, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA.
  - Disarmed pathogen vectors can be allowed to infect the cell to transfer the recombinant DNA into the host.
- **Bioreactors**
  - **Bioreactor** is the cylindrical vessel in which biological processes are carried out on a large scale.
  - The recombinant cells can be multiplied in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain the cells.
  - Bioreactor vessels are used 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 such as temperature, pH, substrate, salts, vitamins, oxygen.
  - Bioreactors are of two types:
    - Simple stirred-tank bioreactor.
    - Sparged stirred-tank bioreactor.
  - A **stirred-tank bioreactor** is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents and the stirrer facilitates even mixing and oxygen availability throughout the bioreactor.
  - In **sparged stirred-tank bioreactor,** sterile air is sparged through the reactor.
  - The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.



▶ **Processes of Recombinant DNA Technology**

Recombinant DNA technology involves various steps in specific sequence which are as follows:

I. Isolation of the Genetic Material (DNA)

- The cells are broken and opened to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids which can be achieved by treating the cells with enzymes such as **lysozyme** (bacteria), **cellulase** (plant cells), **chitinase** (fungus).
- The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. The purified DNA ultimately precipitates out after the addition of chilled ethanol which can be seen as collection of fine threads in the suspension.

II. Cutting of DNA at Specific Location

- Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme, at the optimal conditions for that specific enzyme which results in the fragments of DNA.
- The fragments are 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 agarose.
- The DNA fragments separate according to their size through sieving effect provided by the agarose gel.
- The smaller the fragment size, the farther it moves and 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.
- Bright orange coloured bands of DNA can be observed in an ethidium bromide stained gel exposed to UV light.
- The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece by the process known as **gel electrophoresis.**

III. Amplification of Gene of Interest using PCR

- PCR stands for **Polymerase Chain Reaction**.
- Multiple copies of the gene of interest are synthesized *in vitro* using two sets of primers and the enzyme DNA polymerase.
- Primers are small chemically synthesized oligonucleotides that are complementary to the regions of DNA.
- PCR includes three major steps:
  - Denaturation
  - Annealing
  - Extension
  - **Denaturation** is the process of heating of target DNA at 94°C to separate the two strands of DNA.
  - **Annealing** is the process of pairing of primers with complementary base sequences of the two separated strands.
  - **Extension** is the process of adding complementary deoxyribonucleotides one by one to the 3'OH ends of primers by the activity of DNA polymerase and as a result, new DNA strand is synthesized.
- If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times by the use of a thermostable DNA polymerase isolated from a bacterium, *Thermus aquaticus*.
- The amplified fragment can be used to ligate with a vector for further cloning.

IV. Insertion of Recombinant DNA into the Host Cell/ Organism

- Recipient cells, after making them 'competent' to receive, take up DNA present in its surrounding.
- If a recombinant DNA bearing gene for resistance to an antibiotic is transferred into *E. coli* cells, the host cells get transformed into ampicillin-resistant cell.

V. Obtaining the Foreign Gene Product

- The foreign gene when gets expressed under appropriate conditions, produces desirable proteins.
- If any protein encoding gene is expressed in a heterologous host, it is called a **recombinant protein**.
- The cells harboring cloned genes of interest may be grown on a small scale in the laboratory or on a large scale in a bioreactor.

VI. Downstream Processing

- Downstream processing is the separation and purification of the product.
- The product has to be formulated with suitable preservatives and the formulation has to undergo thorough clinical trials as in case of drugs.

Practice Exercise

Multiple Choice Questions

Q 1. An enzyme catalysing the removal of nucleotides from the ends of DNA is:

a. endonuclease
b. exonuclease
c. DNA ligase
d. Hind II

Q 2. The term 'molecular scissors' refers to:

a. recombinant DNA
b. restriction enzymes
c. *Taq* polymerase
d. palindromic nucleotide sequences

Q 3. The term 'recombinant DNA' refers to:

a. DNA of the host cell
b. DNA with a piece of foreign DNA
c. DNA with selectable marker
d. DNA with more than one recognition sites

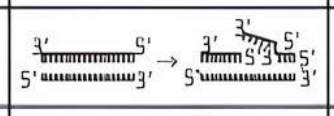
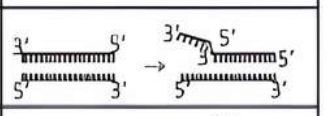
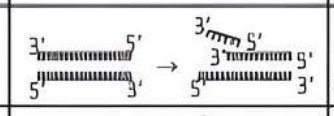
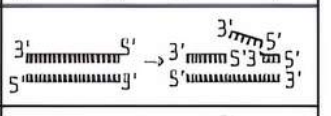
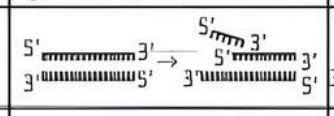
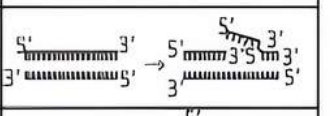
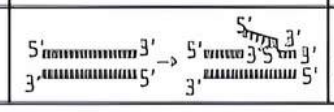
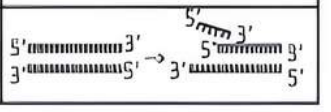
Q 4. The term 'chimeric DNA' refers to:

a. DNA with overhanging stretches
b. DNA with palindromic sequence
c. a recombinant DNA
d. molecular scissors

Q 5. The first restriction endonuclease isolated was:

a. *Eco* RI
b. *Bam* HI
c. *Sal*I
d. *Hind* II

Q 6. Identify the activity of endonuclease and exonuclease in the given image. *(CBSE SQP 2023-24)*

	Endonuclease	Exonuclease
a.		
b.		
c.		
d.		

Q 7. Which of the following tools of recombinant DNA technology is incorrectly paired with its use?

a. *Eco* RI – Production of sticky ends
b. DNA ligase – Multiplication of *r*DNA molecules
c. *ori* – copy number
d. Selectable marker – Identification of transformants

Q 8. Which one of the following characteristic is generally not preferred for a cloning vector?

a. An origin of replication
b. An antibiotic resistance marker
c. Multiple restriction sites
d. A high copy number

Q 9. pBR322 was the first artificial cloning vector to be constructed. What does "BR" stands for?

a. Bacteriophage and recombinant
b. Bolivar and Rodriguez
c. Boyer and replicative
d. None of the above

Q 10. In agarose gel electrophoresis, DNA molecules are separated on the basis of their:

a. charge only
b. size only
c. charge to size ratio
d. All of these

Q 11. During isolation of genetic material, the chemical used to precipitate out the purified DNA is:

a. bromophenol blue
b. chilled ethanol
c. Ethidium bromide
d. Both a. and c.

Q 12. Which of the following has popularised the PCR (Polymerase Chain Reactions)?

a. Easy availability of DNA template
b. Availability of synthetic primers
c. Availability of cheap deoxyribonucleotides
d. Availability of 'thermostable' DNA polymerase

Q 13. A device in which large volume of living cells are cultured in order to get a specific product is called:

a. PCR
b. agitator
c. bioreactor
d. assimilator

Q 14. Process used for amplification or multiplication of DNA in DNA fingerprinting is:

a. polymerase chain reaction
b. Southern blotting
c. Northern blotting
d. None of the above

Q 15. One of the key factors, which makes the plasmid the vector in genetic engineering is:

a. its resistance to antibiotics
b. its resistance to restriction enzymes
c. its ability to carry a foreign gene
d. its ability to cause infection in the host

Q 16. Given below is the restriction site of a restriction endonuclease *Pst*-I and the cleavage sites on a DNA molecule.

5′ C – T – G – C – A ↓ G 3′
3′ G ↑ A – C – G – T – C 5′

Choose the option that gives the correct resultant fragments by the action of the enzyme *Pst*-I.

(CBSE 2023)

a. 5′ C ··· T ··· G
3′ G ··· A ··· C ··· G ··· T
C ··· A ··· G 3′
C 5′

b. 5′ C ··· T
3′ G ··· A ··· G ··· C
G ··· C ··· A ··· G 3′
T ··· C 5′

c. 5′ C — T — G — C
3′ G — A — C — G
A — G 3′
T — C 5′

d. 5′ C — T — G — C — A
3′ G
G 3′
A — C — G — T — C 5′

Q 17. The correct sequence of different steps of polymerase chain reaction is:

a. annealing → denaturation → extension
b. denaturation → extension → annealing
c. denaturation → annealing → extension
d. extension → denaturation → annealing

Q 18. Which of the following is required to perform polymerase chain reaction?

a. Primers, dNTPs and DNA polymerase
b. DNA, $CaCl_2$ and nuclease
c. Mg^{2+}, DNA
d. Both a. and c.

Q 19. Enzyme 'Taq polymerase' used in PCR, has been isolated from bacterium:

a. *Agrobacterium tumefaciens*
b. *Thermus aquaticus*
c. *Streptomyces albus*
d. *Escherichia coli*

Q 20. Which of the following is not used to transfer the recombinant DNA into the host?

a. Micro-injection method
b. Gene gun method
c. Bioreactors
d. Disarmed pathogen vectors

Assertion & Reason Type Questions

Directions (Q. Nos. 21-27): *Each of the following questions consists of two statements, one is Assertion (A) and the other is Reason (R). Select the correct answer to these questions from the codes a, b, c and d as given below:*

a. Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
b. Both Assertion and Reason are true but Reason is not the correct explanation of Assertion.
c. Assertion is true but Reason is false.
d. Assertion is false but Reason is true.

Q 21. Assertion (A): Vector DNA and foreign DNA are cut by same restriction endonuclease.
Reason (R): Digestion of vector DNA and foreign DNA with same enzyme produces complementary sticky ends.

Q 22. Assertion (A): Restriction endonuclease recognises palindromic sequence in DNA and cuts them.
Reason (R): Palindromic sequence has two unique recognition sites *Pst* I and *Pvu* I recognised by restriction endonuclease.

Q 23. Assertion (A): Bacteriophage vectors are more advantageous than plasmid vectors.
Reason (R): Bacteriophage vectors can be easily detected at the time of cloning experiments.

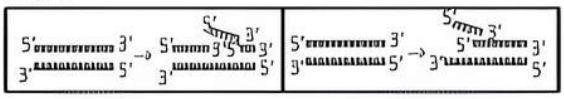
Q 24. Assertion (A): Type I restriction endonucleases are not used in recombinant DNA technology.
Reason (R): Type I restriction endonucleases recognise specific sites within the DNA but do not cut these sites.

Q 25. Assertion (A): Amplification of a gene of interest can be done by polymerase chain reaction.
Reason (R): It is possible to amplify DNA segment approximately 1 billion times within a span of one day.

Q 26. Assertion (A): PCR is a powerful technique to identify genetic disorders.
Reason (R): PCR can detect mutations in low amounts of DNA. *(CBSE 2023)*

Q 27. Assertion (A): Synthetic oligonucleotide polymers are used during annealing in a PCR.
Reason (R): The primers bind to the double stranded DNA at their complementary regions.
(CBSE 2023)

Answers

1. (b) exonuclease
Exonuclease remove nucleotides from the terminal ends (either 5' or 3') of DNA of one strand of duplex. Endonucleases make cut at specific position within the DNA. DNA ligases are known as joining or sealing enzymes. Ligases form phosphodiester bonds between adjacent nucleosides and covalently link two individuals fragments of DNA. *Hind* II is restriction endonuclease. It cuts the DNA and produces blunt ends.
2. (b) restriction enzymes
The term 'molecular scissor' refers to restriction enzyme which is a endonuclease enzymes cleaves the DNA molecule into fragments at specific recognition site within a molecule called restriction site then the foreign DNA has been inserted into chopped DNA and the polymerase is used to attach the plasmid DNA and foreign DNA together to form recombinant DNA.
3. (b) DNA with a piece of foreign DNA
4. (c) a recombinant DNA
After cutting the source DNA and the vector DNA with a specific restriction enzyme, the cut out 'gene of interest' from the source DNA and the cut vector with space are mixed and ligase enzyme is added. This results in the formation of rDNA or hybrid DNA or chimeric DNA.
5. (d) *Hind* II
6. (d) 
7. (b) DNA ligase – Multiplication of rDNA molecules
8. (c) Multiple restriction sites
9. (b) Boliver and Rodriguez
pBR322 was the first artificial cloning vector constructed in 1977 by Boliver and Rodriguez. It is widely used in gene cloning experiments.
In pBR322 plasmid vector,
   - p-denotes that it is a plasmid;
   - BR-stands for Boliver and Rodriguez who constructed this plasmid;
   - 322 - is a number given to distinguish this plasmid from others developed in the same laboratory.

E. coli cloning vector pBR322 has restriction sites (*Hind* II, *Eco* RI, *Bam* HI, *Sal* II, *Pvu* II, *Pst* I, *Cla* I), ori (origin of replication) and antibiotic resistance genes (amp^R and tet^R). Rop codes for the proteins involved in the replication of the plasmid.
10. (b) size only
11. (b) chilled ethanol
The purified DNA, after treatment with various enzymes, precipitates out after the addition of chilled ethanol.
This is viewed as a collection of fine threads in the suspension and is easily collected. The process is known as DNA spooling.
12. (d) Availability of 'thermostable' DNA polymerase
13. (c) bioreactor
A bioreactor is a device in which large volumes (100-1000 liters) of living cells are cultured to get a specific product.
These are the vessels in which raw materials are biologically converted to specific products.
It provides the optimal conditions for achieving the desired product by providing optimum growth conditions such as pH, substrate, salts, vitamins, oxygen content and enzymes.

14. (a) polymerase chain reaction
Process used for amplification or multiplication of DNA in DNA fingerprinting is Polymerase Chain Reaction (PCR). It is done in *in vitro* system.
15. (c) its ability to carry a foreign gene
16. (d) 5′ C — T — G — C — A G 3′
3′ G A — C — G — T — C 5′
17. (c) denaturation → annealing → extension
18. (d) Both a. and c.
19. (b) *Thermus aquaticus*
The final step of PCR is the extension. Wherein *Taq* DNA polymerase is isolated from a thermoplastic bacterium *Thermus aquaticus*.
20. (c) Bioreactors
Bioreactors are considered as vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or their enzymes. To produce large quantities of these products, bioreactors are used where large volumes (100-1000 litres) of culture can be processed. Bioreactor provides the optimal conditions for obtaining the desired product by providing optimum growth conditions such as temperature, pH, substrate, vitamins, oxygen and salts.
21. (a) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
22. (c) Assertion is true, but Reason is false.
23. (a) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
Bacteriophages are a better vectors than the plasmids due to the following reasons:
(i) It can clone the DNA segment of a relatively large size (24 kbp).
(ii) Every bacteriophage produces one plaque area in the culture through which testing is comparatively easy.
24. (a) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
25. (b) Both Assertion and Reason are true but Reason is not the correct explanation of Assertion.
26. (a) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
27. (a) Both Assertion and Reason are true and Reason is the correct explanation of Assertion.

Case Study Based Questions

Case Study 1

Biotechnology Principles

Gene manipulation is a fast-emerging science. It started with development of recombinant DNA molecule. It is named variously as DNA manipulation biotechnology, recombinant DNA technology and genetic engineering. This technology, that mostly involves cutting and pasting of desired DNA fragments, is based on two important discoveries in bacteria, *i.e.*, presence of plasmid in bacteria and restriction endonucleases. Paul Berg was able to introduce a gene of SV-40 into a bacterium. The science of recombinant DNA technology took birth when Cohen and Boyer (1973) were able to introduce a piece of gene containing foreign DNA into plasmid of *E.coli*.

Q 1. Biotechnology is also known as:
a. DNA manipulation biotechnology
b. recombinant DNA technology
c. genetic engineering
d. All of the above

Q 2. A bacterial plasmid is a/an:
a. extrachromosomal material that do not replicate
b. extrachromosomal material that undergo replication with or without chromosomal DNA
c. tubular structures that help in conjugation
d. bristle like solid structure that help in adhesion

Q 3. Father of genetic engineering is:
a. Paul Berg b. Arber c. Nathan d. Smith

Q 4. Which of the following is used by Paul Berg to introduce a gene of SV-40 in a bacterium?
a. *E.coli* b. Cos-plasmids
c. Lambda phage d. None of these

Q 5. Assertion (A): Biotechnology started with the development of recombinant DNA molecule.
Reason (R): Biotechnology mostly involves cutting and pasting of desired DNA fragments.
a. Both Assertion and Reason are true, and Reason is the correct explanation of Assertion.
b. Both Assertion and Reason are true, but Reason is not the correct explanation of Assertion.
c. Assertion is true, but Reason is false.
d. Assertion is false, but Reason is true.

Answers

1. (d) 2. (b) 3. (a) 4. (c) 5. (b)

Case Study 2

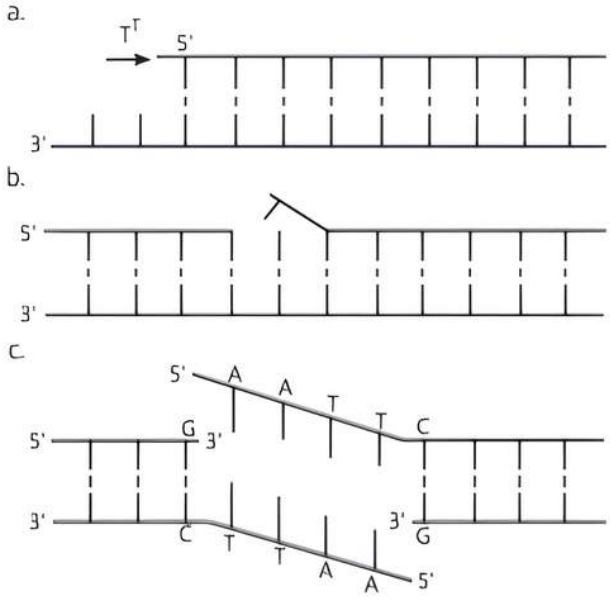
Tools of Recombinant DNA Technology

Tools used in the formation of recombinant DNA are of three types. These are enzymes, cloning vectors and competent host. Lysing enzymes are used to extract DNA for experimental purpose from the cells. Cleaving enzymes break the DNA molecules. They are of three types: exonucleases, endonucleases, and restriction endonucleases. A competent host is required for transformation with recombinant DNA and cloning vectors help to propagate DNA.

Q 1. Which of the following is an example of natural lysing activity in a human body?
a. Lysozyme present in tears dissolve the bacterial cell wall
b. Conversion of starch to maltose in the buccal cavity
c. Absorption of digested food into the intestinal cells
d. Conversion of protein molecules into amino acids in the stomach

Q 2. Which of the following depicts exonuclease activity?

a.



d. All of the above

Q 3. Cloning vectors are the DNA molecules that:

a. carry foreign DNA segment but do not replicate inside the host cell
b. carry foreign DNA segment and replicate inside the host cell
c. transfer nuclear DNA from nucleus to the cytoplasm of the same cells
d. help in sealing gaps in DNA segments

Q 4. Transfer of DNA into a eukaryotic cell is called:

a. transformation
b. transduction
c. transfection
d. electroporation

Q 5. Assertion (A): Type I restriction enzymes are not used in *r*DNA technology.

Reason (R): Type I restriction endonucleases consist of two different subunits and require ATP for restriction activity.

a. Both Assertion and Reason are true, and Reason is the correct explanation of Assertion.
b. Both Assertion and Reason are true, but Reason is not the correct explanation of Assertion.
c. Assertion is true, but Reason is false.
d. Assertion is false, but Reason is true.

Answers

1. (a) 2. (a) 3. (b) 4. (c) 5. (c)

Case Study 3

Restriction Enzymes

The foundations of recombinant DNA (*r*DNA) were laid by the discovery of restriction enzymes. These enzymes are present in many bacterias where they function as a part of their defence mechanism called the Restriction Modification system (RM system). Molecular basis of this system was explained first by Werner Arber in 1962.

The restriction modification system consists of two components:

(i) A restriction enzyme (called restriction endonuclease) identifies the introduced foreign DNA and cuts it into pieces.

(ii) The second component is a modification enzyme (methylase) that adds a methyl group to DNA at specific site to protect it from the restriction enzyme cleavage.

Q 1. Restriction endonucleases are enzymes present in: (i) ..... where they function as a part of (ii) ..... mechanism.

a. (i) bacteria (ii) digestive
b. (i) protists (ii) transcription
c. (i) plant cells (ii) replication
d. (i) prokaryotes (ii) defence

Q 2. Which of the following statements regarding modification enzyme is correct?

a. It adds methyl group to one or two bases usually within the host DNA sequence to protect it from the restriction enzyme
b. It adds ethyl group to one or two bases usually within the sequence recognised by the restriction enzymes
c. It adds methyl group to only one of bases within the foreign DNA sequence that is recognised by the restriction enzymes
d. None of the above

Q 3. Which of the following is a type II restriction enzyme?

a. *Alu* I
b. *Eco* RI
c. *Bam* HI
d. All of these

Q 4. Which of the following is the first discovered restriction endonuclease?

a. *Sal*I
b. *Eco* RI
c. *Hind* II
d. *Eco* R2

Q 5. Components of restriction modification system include:

a. restriction enzyme
b. modification enzyme
c. Both a. and b.
d. lysing enzyme

Answers

1. (d) 2. (a) 3. (d) 4. (c) 5. (c)

Case Study 4

Restriction Endonucleases

Restriction endonuclease was isolated for the first time by W. Arber in 1962 in bacteria. Restriction endonucleases cut the DNA duplex at specific points, therefore they are also called as molecular scissors or biological scissors. Three types of restriction endonucleases are Type I, Type II and Type III but only Type II restriction endonucleases are used in recombinant DNA technology.

Restriction endonuclease *Eco* RI recognises the base sequence GAAT TC in DNA duplex and cut strands between G and A.

Q 1. Only type II restriction enzymes are used in gene manipulation because:

a. ATP is not required for cleaving
b. it consists of three different subunits
c. it makes cleavage or cut in both the strands of DNA molecule
d. Both a. and c.

Q 2. Which of the following ions are used by restriction endonucleases for restriction?

a. Mg^{2+} ions b. Mn^{2+} ions
c. Na^{+} ions d. K^{+} ions

Q 3. Restriction endonuclease was isolated for the first time in a:

a. plant cell b. animal cell
c. prokaryotic cell d. germinal cell

Q 4. Restriction endonucleases are also called as molecular or biological scissors because:

a. they cleave base pairs of DNA only at their terminal ends.
b. they cleave one or both the strands of DNA
c. they act only on single stranded DNA
d. None of the above

Q 5. Select the option that correctly states the working action of restriction endonuclease *Eco* RI on DNA sequence GAATTC.

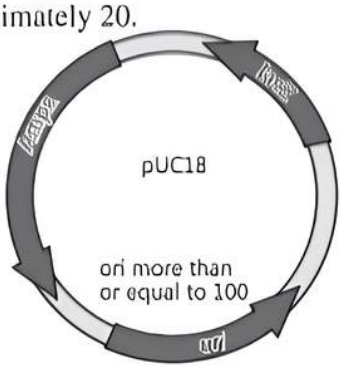
a. 5'-GAATTC-3'
3'-CTTAAG-5
b. 5'-GAATTC-3'
3'-CTTAAG-5'
c. 5'-GAATTC-3'
3-CTTAAG-5
d. 5'-AATTC-3'
3'-CTTAAG-5

Answers

1. (d) 2. (a) 3. (c) 4. (b) 5. (b)

Case Study 5

The structure below shows pUC18 which is similar to pBR322 in its function. However, they differ in some of their restriction sites and number of *ori*. The *ori* number for pBR322 is approximately 20.



Read the given passage carefully and give the answer of the following questions:

Q 1. How are puc18 and pBR322 used in biotechnological studies?

Ans. Plasmids which can be used to insert the gene of interest from a desired organism into a host/they act as vectors to transfer gene of interest into the host.

OR

What will be the impact if ori in the above structure gets damaged?

Ans. *Ori*-Origin of replication (*ori*)-No replication will take place resulting in no copies of linked DNA.

Q 2. The *lac* Z gene has many recognition sites. Study the segment of DNA given below and answer the questions:

5'... ATC GTA AAG CTT CAT ... 3'

3'... TAG CAT TTC GAA GTA ... 5'

(i) Applying your knowledge of palindrome sequences identify and mark the possible region where the restriction enzyme X will act.

(ii) Restriction enzyme Y was used to extract gene of interest from a plant. This gene needs to be inserted in the given DNA segment which has been treated with restriction enzyme X. Will there be a successful recombination? Explain with a reason.

Ans. (i) 5' ... ATC GTA/AAG CTT / CAT ... 3'
3' ... TAG CAT/TTC GAA/GTA ...5'

OR

5' ... AAG CTT ... 3'
3' ... TTC GAA ... 5'

(ii) **No**, as the restriction enzymes need to be the same which cut the DNA of the plasmid and the gene of interest from the plant.

Q 3. Which one of the two (pUC18 and pBR322) would you prefer for biotechnological studies? Justify.

(CBSE SQP 2023-24)

Ans. pUC18 as it has a higher copyrate.

Very Short Answer Type Questions

Q 1. How is the action of exonuclease different from that of endonuclease? *(CBSE 2018)*

Ans. Exonucleases cleave the DNA molecules at their ends whereas endonucleases cleave DNA molecules internally.

Q 2. What are cloning vectors?

Ans. Cloning vectors are carriers or vehicles of desired DNA fragments which can replicate independently to increase copies of desired genes in the host cell *e.g.*, plasmids, bacteriophages.

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

Ans. Selectable markers are essential to identify and eliminate non-transformants, by selectively permitting the growth of the transformant.

Q 4. Mention the uses of cloning vector in biotechnology.

Ans. Cloning vectors are used for transferring fragments of foreign DNA into a suitable host. They are also used to select recombinants from non-recombinants.

Q 5. Mention the type of host cells suitable for the gene guns to introduce an alien DNA.

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

Q 6. Name the host cells in which micro-injection technique is used to introduce an alien DNA.

Ans. Animal cells are the host cells in which micro-injection technique is used to introduce an alien DNA.

Q 7. Write the name of the enzymes that are used for isolation of DNA from bacterial and fungal cells respectively for recombinant DNA technology.

Ans. Bacterial cell is treated with enzyme lysozyme and fungal cell is treated with chitinase.

TiP

If question comes in 2 marks, then student has to write about the cell wall too as bacterial cells have peptidoglycan cell wall while fungi have chitin cell wall.

Q 8. Why is the enzyme cellulase needed for isolating genetic material from plant cells and not from the animal cells?

Ans. The enzyme cellulase breaks down cellulose which is present in cell walls of plants but absent in animal cells.

Q 9. How can bacterial DNA be released from the bacterial cell for biotechnology experiments?

(CBSE 2016)

Ans. The bacterial cell wall is digested by the enzyme lysozyme to release DNA from the cell.

Q 10. What is gene gun?

Ans. The instrument for bombarding micro-projectile particles (gold/tungsten particles) coated with foreign DNA, with great velocity, into a target cell is called gene gun.

Q 11. How does an alien DNA gain entry into a plant cell by 'biolistics' method?

Ans. In biolistics method, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA.

Q 12. Write the two components of the first artificial recombinant DNA molecule constructed by Cohen and Boyer.

Ans. The two components were—antibiotic resistance gene and plasmid vector of *Salmonella typhimurium*.

Q 13. What is the host called that produces a foreign gene product? What is this product called?

Ans. The host that produces a foreign gene product is called competent host. The product is called recombinant protein.

Q 14. Why is it 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* or origin of replication site to start replication.

Q 15. Expand the terms: *r*DNA, BACs, YACs.

Ans. *r*DNA—Recombinant DNA
BACs—Bacterial Artificial Chromosomes
YACs—Yeast Artificial Chromosomes

TiP

Student must revise all the expanded terms that are important for 1 mark as well as for mcqs.

Q 16. Biotechnologists refer to *Agrobacterium tumifaciens* as a natural genetic engineer of plants. Give reasons to support the statement.

Ans. This is because *A. tumifaciens* can transfer genes naturally by delivering a piece of T-DNA to plant cells. It has a tumor inducing plasmid.

Q 17. What are palindromes?

Ans. Palindromes are group of letters (sequences) that read same both in forward and backward direction.

Q 18. Suggest a technique to a researcher who needs to separate fragments of DNA.

Ans. Gel electrophoresis is used to separate DNA fragments.

Q 19. Name the compound used for staining the isolated DNA in the gel electrophoresis.

Ans. Ethidium bromide is used for staining the isolated DNA in the gel electrophoresis.

Q 20. How is repetitive/satellite DNA separated from bulk genomic DNA for various genetic experiments?

Ans. They can be separated by density gradient centrifugation.

Short Answer Type Questions

Q 1. (i) What are "molecular scissors"? Give one example.
(ii) Explain their role in recombinant DNA technology.

OR

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

Ans. (i) The restriction endonucleases are called molecular scissors, as they cut the DNA segments at particular locations, *e.g.*, *Eco* RI.

(ii) The restriction enzymes cut the DNA strands a little away from the center of the palindromic sites, but between the same two bases on the opposite strands. This leaves single stranded portions with overhanging stretches called sticky ends on each strand as they form hydrogen bonds with their complementary cut counterparts. This stickiness at the ends facilitates the action of the enzyme DNA ligase.

Q 2. Insertional inactivation is a method to detect recombinant DNA. Explain the method. *(CBSE 2023)*

Ans. Insertional inactivation is a technique used in recombinant DNA technology. In this procedure, a bacteria carrying recombinant plasmids or a fragment of foreign DNA is made to insert into a restriction site inside a gene to resist antibiotics, hence causing the gene to turn non-functional or in an activated state.

Q 3. Explain palindromic nucleotide sequence with the help of a suitable example. *(CBSE 2015)*

Ans. The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.

For example, the following sequences read the same on the two strands in 5' → 3' direction. This is also true if it is read in the 3' → 5' direction.

5' — — GAATTC — — 3'

3' — — CTTAAG — — 5'

TiP

Always write sequence with 5' and 3' annotation. If proper sequence with enzyme is not written in answer then definitely marks will be deducted.

Q 4. Explain with the help of an example the relationship between restriction endonuclease and a palindromic nucleotide sequence. *(CBSE 2016)*

Ans. Restriction endonuclease recognises a specific palindromic nucleotide sequence in the DNA. Restriction endonuclease cuts the strand of DNA a little away from the center of palindromic nucleotide sequence but between the same two bases on the opposite strands, leaving single stranded portions at the end or sticky ends.

Q 5. What is *Eco* RI? How does *Eco* RI differ from an exonuclease?

Ans. *Eco* RI is restriction endonuclease enzyme. Exonuclease removes nucleotides from the ends of DNA while *Eco* RI makes cut at specific position within the DNA.

Q 6. Explain how recombinant DNA technology is used to detect a disease even before any clinical symptom appears. *(CBSE 2023)*

Ans. Recombinant DNA procedures have now been applied to the problem of the identification of molecular defects in man that account for heritable diseases, somatic mutations associated with neoplasia, and acquired infectious disease. Thus, recombinant DNA technology has rapidly expanded our ability to diagnose disease.

Q 7. A wine maker and a molecular biologist who has developed a recombinant vaccine, both claim themselves to be biotechnologist. Who, in your opinion, is right? *(CBSE 2016)*

Ans. Both are right because biotechnology is a very wide area which deals with techniques of using a 'natural' organism (or its parts) as well as genetically modified organism to produce products and processes useful for mankind. A wine maker employs a strain of yeast to produce wine by fermentation (a natural phenomenon), while the molecular biologist has cloned gene for the antigen (that is used as vaccine) in an organism which allows the production of the antigen in large amount.

Q 8. (i) State the principle involved in separation of DNA fragments using gel electrophoresis.

(ii) How are DNA fragments visualised once they are separated by gel electrophoresis?

(CBSE 2023)

Ans. (i) DNA is negatively charged, therefore, when an electric current is applied to the gel, DNA will migrate towards the positively charged electrode. Shorter strands of DNA move more quickly through the gel than longer strands resulting in the fragments being arranged in order of size.

(ii) 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 as we cannot see pure DNA fragments in the visible light and without staining.

Q 9. What do you mean by clones?

Ans. The cell or organism derived from the same parents by asexual means which are genetically identical to each other and to the parent are called clones.

Q 10. What are 'cloning sites' in a cloning vector? Explain their role. Name any two such sites in pBR322.

Ans. Cloning sites are the recognition sites on plasmid. The restriction enzymes recognise these sites for cutting and ligation of alien DNA at this place. *Eco* RI and *Bam* HI are two such sites in pBR322.

Q 11. (i) Write the scientific name of the source organism of the thermostable DNA polymerase used in PCR.

(ii) State the advantage of using Thermostable DNA polymerase. *(CBSE 2023)*

Ans. (i) The source organism of the thermostable DNA polymerase used in PCR is the bacterium, *Thermus aquaticus*. The DNA polymerase enzyme isolated from this organism is commonly known as *Taq* polymerase. Its scientific name is *Taq* DNA polymerase.

It is widely used in PCR because it is stable at high temperatures, allowing it to withstand the high temperatures used during the denaturation step of PCR.

(ii) The advantage of using a thermostable DNA polymerase, such as *Taq* polymerase, in PCR is that it can withstand the high temperatures required for the denaturation step of the PCR cycle.

Q 12. Why is *Agrobacterium*-mediated genetic transformation described as natural genetic engineering in plants?

Ans. *Agrobacterium tumifaciens* is a pathogen of several dicot plants which exhibit natural genetic engineering in plant.

The reasons are:

(i) It is able to deliver a piece of DNA called 'T-DNA' to transform normal plant cell into a tumor cell.

(ii) The DNA transforms the normal cells into tumor cells which direct them to produce the chemical essential for the pathogen.

As this process occurs in nature, it is called natural genetic engineering.

Q 13. Write any four ways used to introduce a desired DNA segment into a bacterial cell in recombinant technology experiments.

Ans. The four ways are:

(i) The desired DNA segment is inserted into a cloning vector and the bacterial cell can be made to take it up after making them competent by treating them with specific concentration of divalent cations such as calcium.

(ii) Microinjection.

(iii) Biolistics.

(iv) Disarmed pathogen vector.

Q 14. Explain any two methods of vectorless gene transfer.

Ans. The two methods of vectorless gene transfer are:

(I) Micro-injection: The technique of introducing foreign gene in a target cell by injecting the DNA, directly into the nucleus, by micro-needle is called micro-injection.

(II) Electroporation: It is the process in which transient holes are produced in the plasma membrane of the target cell, to incorporate foreign DNA.

Q 15. What is the significance of adding proteases at the time of isolation of genetic material (DNA)?

Ans. Role of proteases is to degrade the proteins present inside a cell (from which DNA is being isolated). If the proteins are not removed from DNA preparation, then they could interfere with any downstream treatment of DNA.

Q 16. How is a continuous culture system maintained in bioreactors and why? *(CBSE 2019)*

Ans. In continuous culture system, the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase. Continuous culture method produces a larger biomass leading to higher yield of desired protein.

Q 17. Differentiate between *r*DNA and *c*DNA.

Ans.

S. No.	Basis of difference	*r*DNA	*c*DNA
(I)	Other name	*r*DNA is also called as recombinant DNA.	*c*DNA also called as complimentary DNA.
(II)	Definition	It is the DNA which is formed by joining together the DNA from two different organisms.	It is the DNA which is obtained from RNA template catalysed by reverse transcriptase enzyme.

TiP

Learn the differences in tabular form for easy remembrance.

Long Answer Type-I Questions

Q 1. (i) Explain the significance of palindromic nucleotide sequence in the formation of recombinant DNA.

(ii) Write the use of restriction endonuclease in the above process. *(CBSE 2017)*

Ans. (I) The palindromic sequences *i.e.*, the sequence of base pairs read the same on both the DNA strands, when orientation of reading is kept the same, *e.g.*

5' —— GAATTC —— 3'

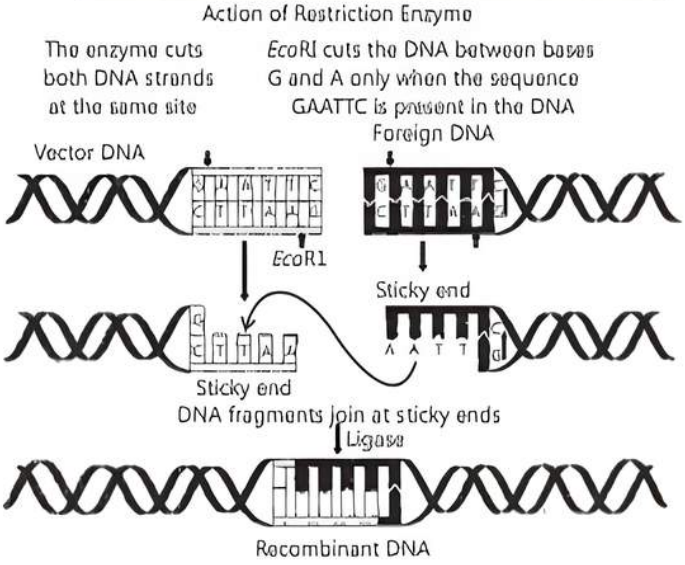
3' ····· CTTAAG ····· 5'

Every endonuclease inspects the entire DNA sequence for palindromic recognition sequence.

(II) On finding the palindrome, the endonuclease binds to the DNA. It cuts the opposite strands of DNA, but between the same bases on both the strands and forms sticky ends. These sticky ends facilitate the action of enzyme DNA ligase and helps in the formation of recombination DNA.

Q 2. Describe the formation of recombinant DNA by the action of *Eco* RI. *(CBSE 2019)*

Ans. Restriction endonuclease enzyme *Eco* RI is used in the molecular biology to cut the foreign DNA and vector DNA to form overhangs (called sticky ends).



These sticky ends then form hydrogen bonds with their complementary counterparts. The segments with the help of DNA ligases are joined to produce recombinant DNA.

Q 3. (i) Explain the significance of 'palindromic nucleotide sequence' in the formation of recombinant DNA.

(ii) Write the use of restriction endonuclease in the above process.

Ans. (i) Palindromic nucleotide sequence is the recognition (specific) sequence present both on the vector and on a desired or alien DNA for the action of the same (specific) restriction endonuclease to act upon.

(ii) The use of restriction endonuclease as:

(a) Every endonuclease inspects the entire DNA sequence for the palindromic recognition sequence.

(b) On finding the palindrome, the endonuclease binds to the DNA.

(c) It cuts the opposite strands of DNA in the sugar–phosphate backbone; a little away from the center of the palindrome sites but between the same bases on both strands.

(iii) This results in the formation of single stranded overhanging stretches at the end of each strand called sticky ends.

(iv) The sticky ends facilitate the action of the enzyme DNA ligase by readily forming hydrogen bonds with complementary strands.

Q 4. Why are genes encoding resistance to antibiotics considered useful selectable markers for *E. coli* cloning vector? Explain with the help of one example.

Ans. Genes encoding resistance to antibodies are considered useful selectable markers for *E.coli* cloning vector is explained using this example. If a recombinant DNA bearing gene for resistance to an antibiotic (*e.g.*, ampicillin) is transferred into *E.coli* cells, the host cells become transformed into ampicillin-resistant cells. If these transformed cells are spread on agar plates containing ampicillin, only transformants will grow, and the non-transformed recipient cells will die as they do not contain the gene for ampicillin resistance. Thus, transformed cells can be selected. The gene for ampicillin resistance, in this case, is a useful selectable marker.

Q 5. Explain the roles of the following with the help of an example each in recombinant DNA technology:

(i) Restriction Enzymes

(ii) Plasmids *(CBSE 2018)*

Ans. (i) Restriction Enzymes:

(a) Restriction enzymes belong to class of enzymes nucleases which breaks nucleic acids by cleaving their phosphodiester bonds.

(b) Since restriction endonucleases cuts DNA at specific recognition site, they are used to cut the donor DNA to isolate the desired gene.

(c) The desired gene has sticky ends which can be easily ligated to cloning vector cut by same restriction enzymes having complementary sticky ends to form recombinant DNA.

(d) An example is *Eco* R1 which is obtained from *E. coli* bacteria 'R' strain that cuts DNA at specific Palindromic Recognition site.

5′—GAATTC—3′

3′—CTTAAG—5′

(ii) Plasmids:

(a) Plasmids are autonomous, extra chromosomal circular double stranded DNA of bacteria.

(b) Since they are small and self-replicating, they are used as cloning vectors in genetic engineering.

(c) Some plasmids have antibiotic resistance genes which can be used as marker genes to identify recombinant plasmids from non-recombinant ones.

(d) The plasmids are cut and ligated with desired genes and transformed into host cell for amplification to obtain the desired products.

(e) An example of artificial modified plasmids is pBR322 (constructed by Bolivar and Rodriguez) or pUC (constructed at university at California).

COMMON ERROR

Students get confused and sometimes interchange the roles of both.

Q 6. (i) How has the development of bioreactor helped in biotechnology?

(ii) Name the most commonly used bioreactor and describe its working. *(CBSE 2018)*

Ans. (i) 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.

(ii) The most commonly used bioreactors are of stirring type. A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. The bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.

Q 7. How can be a host made competent? Explain the different methods. *(CBSE 2015)*

Ans. A bacterial cell or host can be made competent by the following methods:

(i) Chemical Method:

(a) The cell is treated with specific concentration of a divalent cation such as calcium to increase pore size in cell wall.

(b) The cells are incubated with recombinant DNA on ice, followed by placing them briefly at 42°C and then putting it back on ice. This is called heat shock treatment.

(c) The bacteria now take up the recombinant DNA.

(ii) Physical Methods:

The physical methods include:

(a) Micro-injection Method: Recombinant DNA is directly injected into the nucleus of an animal cell.

(b) Biolistics or Gene Gun Method: Cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA in plants.

Q 8. A mixture of fragmented DNA was electrophoresed in agarose gel. After staining the gel with ethidium bromide, no DNA bands were observed. What could be the reason?

Ans. The reasons that could be possible are as follows:

(i) DNA sample that was loaded on the gel may have got contaminated with nuclease (exo- or endo- or both) and completely degraded.

(ii) Electrodes were put in opposite orientation in the gel assembly, *i.e.*, anode towards the wells (where DNA sample is loaded). Since DNA molecules are negatively charged, they move towards anode and hence move out of the gel instead of moving into the matrix of gel.

(iii) Ethidium bromide was not added at all or was not added in sufficient concentration and so DNA was not visible.

(iv) After staining with ethidium bromide, it was not observed under UV.

Q 9. Write the steps you would suggest to be undertaken to obtain a foreign-gene-product.

Ans. Recombinant DNA technology involves the following steps:

(i) Isolation of DNA.

(ii) Fragmentation of DNA by restriction endonucleases.

(iii) Isolation of a desired DNA fragment.

(iv) Amplification of the gene of interest.

(v) Ligation of the DNA fragment into a vector.

(vi) Insertion of recombinant DNA into the host.

(vii) Culturing the host cells on a suitable medium at a large scale.

(viii) Extraction of the desired gene product.

(ix) Downstream processing of the products as finished product, ready for marketing.

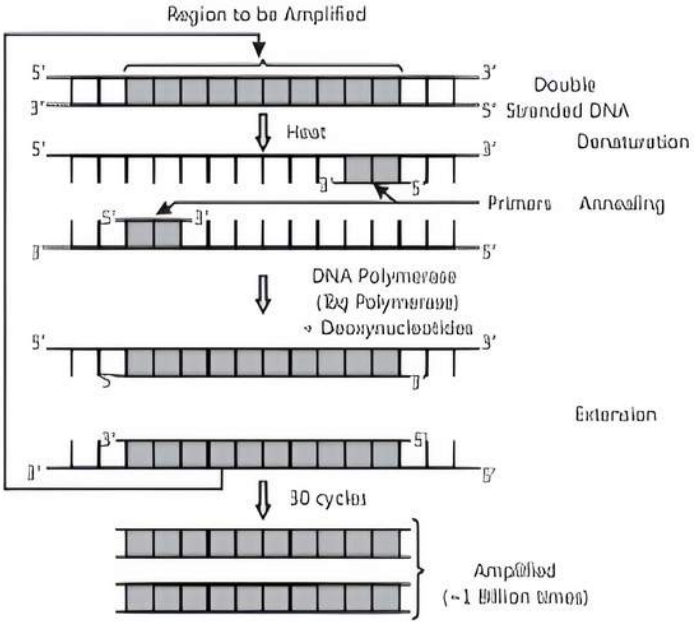
Q 10. Describe the process of amplification of "gene of interest" using PCR technique. *(CBSE 2019)*

OR

In a pathological lab, a series of steps were undertaken for finding the gene of interest. Describe the steps, or make a flow chart showing the process of implication of this gene of interest.

(CBSE SQP 2022, Term-2)

Ans. To amplify the gene segment of the interest, we should know the sequence of the gene of interest. Primers are designed for amplifying the gene of interest. Two sets of primers (chemically synthesised oligonucleotide stretches) that are complementary to the gene of interest, DNA polymerase enzyme, and deoxynucleotides are added. PCR can then be carried out for its amplification.



PCR consists of three steps:

(i) Denaturation: Double-helical DNA is denatured by providing high temperature (95-degree Celsius). DNA polymerase does not get degraded in such high temperatures. The DNA polymerase used in this reaction is thermostable and is isolated from the thermophilic bacteria, Thermus aquaticus(Taq).

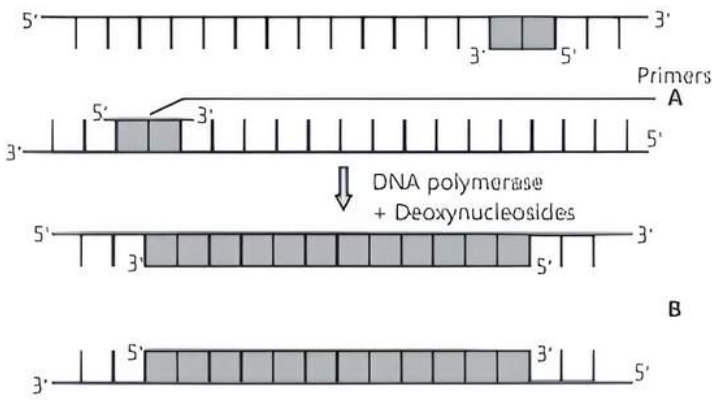
(ii) Annealing: It is the step in which primers are annealed to single-stranded DNA templates. Two sets of primers are used. The temperature of the reaction mixture is lowered to 50-65°C for some seconds to allow annealing of primers. DNA polymerase extends the primer in 5' to 3' direction.

(iii) Extension: Replication of DNA occurs in vitro. This cycle is repeated several times to generate up to 1 billion identical copies of the DNA.

COMMON ERROR

Students often write irrelevant answer. It seems that they are unaware of the mechanism of PCR.

Q 11. (i) Identify steps A and B in a cycle of Polymerase Chain Reaction given below: *(CBSE 2017, 19)*



(ii) State the specific characteristic feature of the enzyme in carrying step B.

Ans. (i) **A. Annealing of Primer:** It is the step in which primers are annealed to single-stranded DNA templates. Two sets of primers are used. The temperature of the reaction mixture is lowered to 50-65°C for some seconds to allow annealing of primers. DNA polymerase extends the primer in 5' to 3' direction.

B. Extension/Polymerisation: DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. Taq polymerase is used in the reaction, which can tolerate heat.

(ii) The enzyme is used to automate the repetitive steps in PCR technique, an extremely important method of amplifying specific DNA sequences.

Q 12. Name and explain the technique used for separating DNA fragments and making them available for biotechnology experiments.

OR

How are the DNA fragments separated and isolated for DNA fingerprinting? Explain. *(CBSE 2017)*

Ans. Separation and Isolation of DNA Fragments (Gel Electrophoresis):

(i) Gel electrophoresis is a technique for separating DNA fragments based on their size. Firstly, the sample DNA is cut into fragments by restriction endonucleases.

(ii) The DNA fragments being negatively charged can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.

(iii) Commonly used matrix is agarose, which is a natural linear polymer of D-galactose and 3, 6-anhydro-L-galactose which is extracted from seaweeds.

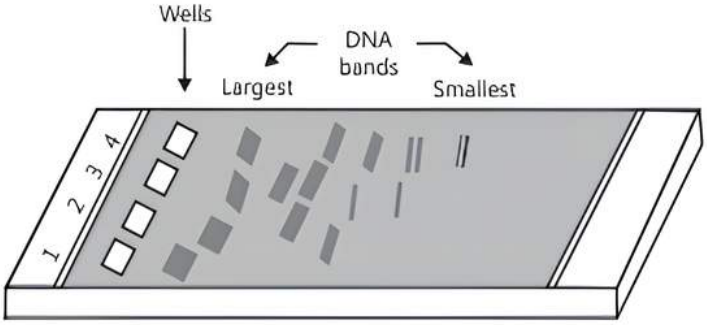
(iv) The DNA fragments separate out (resolve) according to their size because of the sieving property of agarose gel. Hence, smaller the fragment size, the farther it will move.

(v) The separated DNA fragments are visualised after staining the DNA with ethidium bromide followed by exposure to UV radiation.

(vi) The DNA fragments are seen as orange coloured bands.

(vii) The separated bands of DNA are cut out and extracted from the gel piece. This step is called elution.

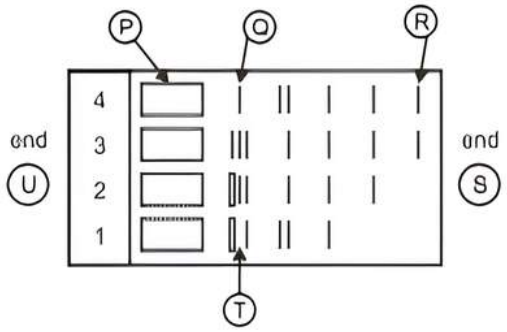
(viii) The purified DNA fragments are used to form recombinant DNA which can be joined with cloning vectors.



TiP

Learn the steps involved in the technique of separating DNA fragments step-wise and practice making its diagram too.

Q 13. (i) Given below is the stepwise schematic representation of the process of electrophoresis. Identify the 'alphabets' representing (a) Anode end (b) Smallest/Lightest DNA strand in the matrix (c) Agarose gel. *(CBSE 2022, Term-2)*



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

Ans. (i) (a) Anode end → (S)

(b) Smallest/lightest DNA strand in the matrix → (R)

(c) Agarose gel → (T)

(ii) Elution is the process of separating and extracting a material from another. Ethidium dye is used to visualise the DNA fragments. The DNA fragments are only visible in UV radiation.

The separated DNA fragments are further stained and used for several techniques like DNA fingerprinting, etc.

Long Answer Type-II Questions

Q 1. Answer the following questions:

(i) Why are engineered vectors preferred by biotechnologists for transferring the desired genes into another organism?

(ii) Explain how do "ori", "selectable markers" and "cloning sites" facilitate cloning into a vector.

Ans. (i) Engineered vectors are preferred by biotechnologists because they help in easy linking of foreign DNA and selection of recombinants from non-recombinants.

(ii) Origin of Replication (*ori*):

(a) This is a DNA sequence that is responsible for initiating replication. Any piece of DNA when linked to this sequence can replicate within the host cells.

(b) *Ori* also controls the copy numbers of the linked DNA.

Selectable Marker:

(a) It helps to select the host cells which contain the vector (transformants) and eliminate the non-transformants.

(b) Transformation is defined as the procedure by which a piece of DNA is introduced into a bacterial host.

(c) Genes encoding resistance to antibiotics like ampicillin, chloramphenicol, tetracycline or kanamycin, are useful selectable markers for *E. coli*.

(d) The normal *E.coli* cells do not carry resistance against any of these antibiotics.

Cloning Sites:

(a) To link the alien DNA, the vectors require very few (mostly single) recognition sites for the restriction enzymes.

(b) More than one recognition sites within the vector can complicate the gene cloning as it will generate several fragments.

(c) Ligation of alien DNA can be carried out at a restriction site present in one of the two antibiotic resistance genes.

COMMON ERROR

Student do not write correct functions of ori, selectable markers and cloning sites or interchange them and lose their marks.

Q 2. If a desired gene is identified in an organism for some experiments, explain the process of the following:

(i) Cutting this desired gene at specific location.

(ii) Synthesis of multiple copies of this desired gene.

Ans. (i) Cutting of desired gene at specific location is done by incubating the DNA with specific restriction endonuclease. Restriction enzyme recognises a particular palindromic nucleotide sequence and cuts the DNA at that site.

(ii) Synthesis of multiple copies of desired gene is carried out by Polymerase Chain Reaction (PCR).

Amplification of gene is done using Polymerase Chain Reaction (PCR).

It is carried out in the following steps:

(i) Denaturation: The double stranded DNA is denatured by applying high temperature of 95°C for 15 seconds. Each separated strand acts as a template.

(ii) Annealing: Two sets of primers are added, which anneal to the 3' end of each separated strand.

(iii) Extension: DNA polymerase extends the primers by adding nucleotides complementary to the template provided in the reaction. *Taq* polymerase is used in the reaction, which can tolerate heat. All these steps are repeated many times to get several copies of the desired DNA.

Q 3. Bioreactors are the containment vehicles of any biotechnology-based production process. For large scale production and for economic reasons the final success of biotechnological process depends on the efficiency of the bioreactor.

Answer the following questions w.r.t. the given paragraph:

(i) List the operational guidelines that must be adhered to so as to achieve optimisation of the bioreactor system. Enlist any four.

(ii) Mention the phase of the growth we refer to in the statement "Optimisation of growth and metabolic activity of the cells".

(iii) Is the biological product formed in the bioreactor suitable for the intended use immediate? Given reason in support of your answer. *(CBSE 2023)*

Ans. (i) Four operational guidelines that must be adhered to for achieving optimization of the bioreactor system are as follows:

(a) Monitoring and control of key parameters.

(b) Optimization of mixing and aeration.

(c) Minimizing contamination.

(d) Maintenance and cleaning of the bioreactor.

(ii) The phase of the growth referred to in the statement "Optimization of growth and metabolic activity of the cells" is the exponential phase of growth.

During the exponential phase, the microorganisms, animal or plant cells, or other biological systems in the bioreactor are actively dividing and growing at their maximum rate. This phase is characterised by a steady increase in biomass and metabolic activity, as nutrients are being efficiently utilised for growth and reproduction.

Optimizing the growth and metabolic activity of the cells during the exponential phase is critical for achieving high yields of the desired product, as it is during this phase that the biological system is most productive. Maintaining optimal conditions such as temperature, pH, nutrient availability, and aeration during this phase is essential for achieving maximum productivity and minimising the duration of the production process.

(iii) Whether or not the biological product formed in the bioreactor is suitable for immediate use depends on the specific product and its intended use.

In many cases, further processing and purification of the biological product are required before it can be used for its intended purpose. This is because the product may contain impurities or other components that could be harmful or interfere with its activity. Purification may involve various techniques such as filtration, chromatography and centrifugation, among others.

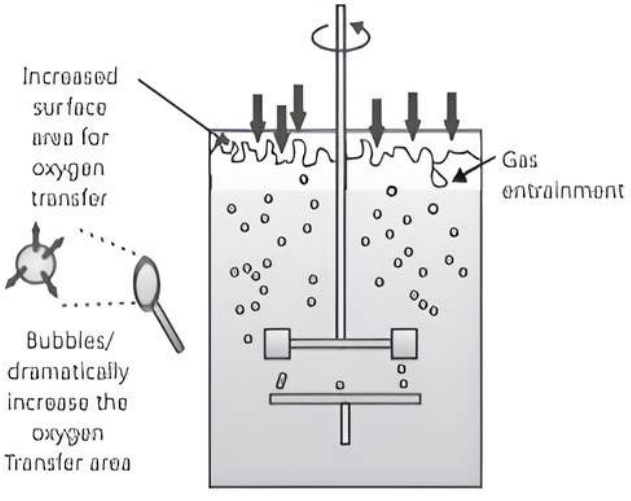
Additionally, some biological products may require testing and validation to ensure that they meet the required quality standards and regulatory requirements for their intended use. This may involve performing assays and tests to determine the product's purity, activity and safety.

Therefore, while the biological product formed in the bioreactor may contain the desired compound or molecule, it is typically not suitable for immediate use without further processing and validation.

Q 4. What is a bioreactor? Draw a labelled diagram of a sparged stirred tank bioreactor. Explain its functioning.

Ans. Bioreactors are large vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or human cells. It works by providing optimal conditions to process the culture as well as the production of desired product by maintaining optimum pH, temperature, oxygen and other growth conditions required.

Functioning of Sparged Stirred Bioreactor: In the sparged stirred-tank bioreactor, sterile air bubbles are sparged. This increases the surface for oxygen transfer, thus, facilitating the growth and metabolism of cells and hence, production of recombinant products as well.



TiP

Practice drawing the diagram of bioreacting with correct labelling and functioning.

Q 5. Some restriction enzymes break a phosphodiester bond on both the DNA strands, such that only one end of each molecule is cut and these ends have regions of single stranded DNA. *Bam* H1 is one such restriction enzyme which binds at the recognition sequence, 5'-GGATCC-3' and cleaves these sequences just after the 5'-guanine on each strand.

(i) What is the objective of this action?

(ii) Explain how the gene of interest is introduced into a vector.

(iii) You are given the DNA show below.

5' ATTTTGAGGATCCGTAATGTCCT 3'

3' TAAAACTCCTAGGCATTACAGGA 5'

If this DNA was cut with *Bam* H1, how many DNA fragments would you expect? Write the sequence of these double-stranded DNA fragments with their respective polarity.

(iv) A gene M was introduced into *E.coli* cloning vector pBR322 at *Bam* H1 site. What will be its impact on the recombinant plasmids? Given a possible way by which you could differentiate non-recombinant to recombinant plasmids.

(CBSE SQP 2022, Term-2)

Ans. (i) The two different DNA molecules will have compatible ends to recombine.

(ii) Restriction enzyme cuts the DNA of the vector and then ligates the gene of interest into the DNA of the vector.

(iii) 2 fragments

5' ATTTTGAG 3' 5'GATCCGTAATGTCCT 3'

3' TAAAACTCCTAG 5' 3' GCATTACAGGA 5'

(iv) *Bam* H1 site will affect tetracycline antibiotic resistance gene, hence the recombinant plasmids will olose tetracycline resistance due to inactivation of the resistance gene.

Recombinats can be selected from non-recombinants by plating into a medium containing tetracycline, as the recombinants will not grow in the medium because the tetracycline resistance gene is cut.

Q 6. (i) '*Eco* RI' has played very significant role in *r*DNA technology.

(a) Explain the convention for naming *Eco* RI.

(b) Write the recognition site and the cleavage sites of this restriction endonuclease.

(ii) What are the protruding and hanging stretches of DNA produced by these restriction enzymes called? Describe their role in formation of *r*DNA. *(CBSE 2023)*

Ans. (i) (a) *Eco* RI comes from *Escherichia coli* RYB strain. In *Eco* RI, 'E' comes from the genus '*Escherichia*' and 'co' comes from the species '*coli*'. The letter 'R' is derived from the name of the strain RYB. Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

(b) The restriction endonuclease enzyme *Eco* RI recognises the ssDNA sequence 5'-GAATTC'-3, and introduces a single-strand cut between the G and A nucleotides. This recognition site is a palindrome: the opposite strand also reads 5'-GAATTC'-3 and will be cut in the same manner.

(ii) *Eco* RI cuts the DNA between bases G and A only when the sequence GAATTC is present in the DNA. This leaves single-stranded portions at the end which are overhanging stretches called sticky ends.

Sticky ends form hydrogen bonds with their complementary cut counterparts. This stickiness of the end facilitates the action of enzyme DNA ligase. Thus, recombinant DNA is formed.

Q 7. (i) Mention the role of vectors in recombinant DNA technology. Give any two examples.

(ii) With the help of diagrammatic representation only, show the steps of recombinant DNA technology.

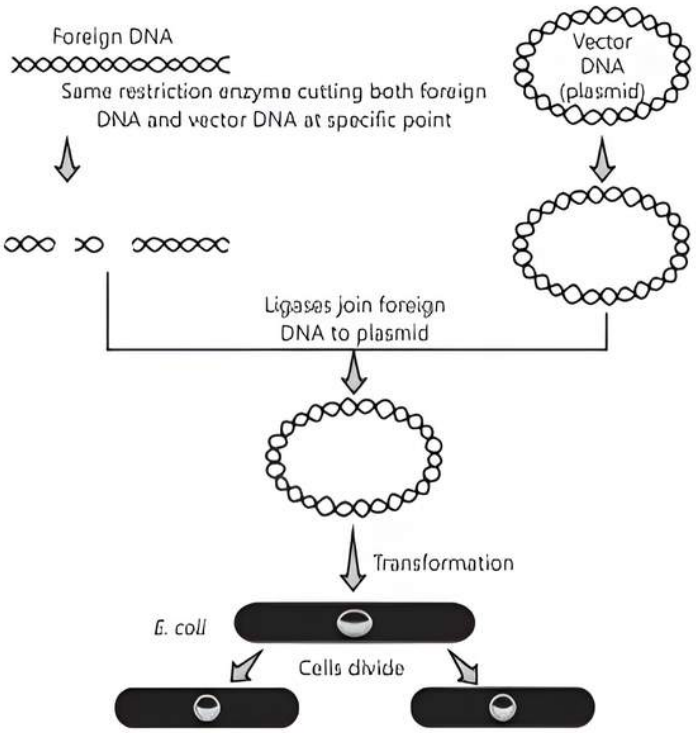
Ans. (i) Role of Vectors:

(a) The vectors have the ability to replicate within the bacterial cells independent of the control of chromosomal DNA.

(b) If an alien piece of DNA is linked to the vector like bacteriophage or plasmid DNA, it can be made to multiply its number being equal to the copy number of the vector.

(c) Vectors are also used in the selection of recombinants from non-recombinants. Plasmids and bacteriophages are the most commonly used vectors.

(ii) Diagrammatic Representation of Recombinant DNA Technology:



Chapter Test

Multiple Choice Questions

Q 1. The source of the restriction enzyme *Hind* III is:

a. *Escherichia coli* RY 13
b. *Haemophilus influenzae* Rd
c. *Bacillus amyloliquefaciens* H
d. *Streptomyces albus*

Q 2. A restriction endonuclease breaks bonds between the:

a. base pairs of a DNA molecule
b. base pairs of a DNA-RNA hybrid molecule
c. sugar and phosphate components of a nucleic acid molecule
d. exons and introns of a DNA molecule

Q 3. The sticky ends of a fragmented DNA molecule are made of:

a. calcium salts
b. endonuclease enzyme
c. unpaired bases
d. methyl groups

Assertion and Reason Type Questions

Directions (Q.Nos. 4-5): *Each of the following questions consists of two statements, one is Assertion (A) and the other is Reason (R). Select the correct answer to these questions from the codes a, b, c and d as given below:*

a. Both Assertion and Reason are true and Reason is the correct explanation of Assertion.
b. Both Assertion and Reason are true, but Reason is not the correct explanation of Assertion.
c. Assertion is true but Reason is false.
d. Both Assertion and Reason are false.

Q 4. Assertion (A): YAC vectors have been exploited extensively in the mapping of large genomes.

Reason (R): YAC vectors have a composite structure made of bacteriophage and plasmid.

Q 5. Assertion (A): *c*DNA is copy DNA which is synthesised in vivo on a DNA template using DNA polymerase.

Reason (R): *c*DNA of all possible genes are ligated with different plasmids and maintained in either plant or animal cells.

Case Based Questions

Case Study 1

Q 6. Bioreactors are considered as vessels in which raw materials are biologically converted into specific products by microbes, plant and animal cells or their enzymes. They are used for large scale production as they provide optimum growth

conditions such as temperature, pH, substrate, vitamins, oxygen and salts for obtaining desired product. Most commonly used bioreactors are of stirring type which include simple stirred tank bioreactor and sparged stirred-tank bioreactor.

(i) **Bioreactor are useful in:**
   a. amplifying a gene
   b. isolation of genetic material
   c. processing large volume of culture
   d. infecting DNA in a cell

(ii) **Which of the following is essential to obtain desired product in a bioreactor?**
   a. Size of the bioreactor
   b. Sterile condition
   c. Quantity of the raw material
   d. All of the above

(iii) **Growth condition that could affect the quality of obtained product in a bioreactor are:**
   a. temperature and pH only
   b. pH and oxygen supply only
   c. temperature and oxygen supply only
   d. temperature, pH and oxygen supply

(iv) **Vessels in which raw materials are biologically converted into specific products are:**
   a. bioreactors b. fermentors
   c. Both a. and b. d. gene guns

Case Study 2

Q 7. 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 and 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.

Read the given passage carefully and give the answer of the following questions:

(i) **What is *r*DNA stands for?**

(ii) **What do you mean by recombinant DNA (*r*DNA)?**

(iii) **Why is it difficult for DNA to pass through the membrane?**

OR

What type of ions are used for DNA mediated gene transfers?

Very Short Answer Type Questions

Q 8. **What does competent refer to incompetent cells used in transformation experiment?**

Q 9. **Explain the role of lysing enzymes in biotechnology.**

Q 10. **What is meant by elution in electrophoresis?**

Short Answer Type Questions

Q 11. **Name the natural source of agarose. Mention one role of agarose in biotechnology.**

Q 12. **Illustrate briefly downstream processing.**

Long Answer Type-I Question

Q 13. **Describe the roles of heat, primers and the bacterium *Thermus aquaticus* in the process of PCR.**

Long Answer Type-II Question

Q 14. **Discuss with your teacher and find out how to distinguish between:** *(NCERT)*

(i) Plasmid DNA and Chromosomal DNA.

(ii) RNA and DNA.

(iii) Exonucleases and Endonucleases.