CHAPTER 11

BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

POINTS TO REMEMBER

Bacteriophage : A virus that infects bacteria.

Bioreactor : A large vessel in which raw materials are biologically converted into specific products under optimal conditions such as temperature, pH, substrate, salts, vitamins, oxygen. Stirring type bioreactors are commonly used.

Biotechnology : It deals with techniques of using live organisms (Microbes, plants animals) or components for benefit to humans.

According to EFB (European Federation of Biotechnology) : Biotechnology in the integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services.

Cloning Vectors : A small, self-replicating DNA molecule into which foreign DNA is inserted. It replicates inside the host cell. The vectors that may be used in genetic engineering are plasmids, bacteriophages, animal, plant, virus, YACS and BACs and insome yeasts.

Features of cloning vector: Origin of replication (Ori), selectable marker and cloning sites are the features that are required to facilitate cloning into a vector.

(a) **Origin of Replication (Ori) :** This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is also responsible for controlling the copy number of the linked DNA.

(b) **Selectable Marker :** It is a gene which helps in identifying and eliminating non-transformants from transformants (having recombinant DNA) by selectively permitting the growth of transformants. The process through which a piece of DNA is introduced in a host bacterium is called transformation. The genes encooling resistance to antibiotics are considered useful selectable marker for *E.coli.*

(c) **Cloning Sites :** A location on a cloning vector into where a foreign gene can be introduced is called a cloning site. The vector must have very few (preferably single) recognition sites. The presence of more than one recognition sites within the vector will produce several fragments which will make the process of gene cloning more complicated. Therefore, the foreign DNA is ligated at a restriction site present in one of the two antibiotic resistance gene.

Complementary DNA (cDNA) : A DNA strand formed from mRNA by using the enzyme reverse transcriptase.

Plasmid : Extra chromosomal, self replicating circular DNA molecule found in certain bacteria and in some yeasts. It has a few genes. Plasmids are used as cloning vectrors in genetic engineering.

Genetic Engineering : The techniques to alter the chemistry of genetic material and introduction of it into organisms to change its phenotype.

Ligase : An enzyme used by a genetic engineer to join the cut ends of the double stranded DNA.

Palindromic Sequence : Complementary DNA sequences that are the same when each strand is read in the same direction (5' → 3'). These sequences act as recognition sites for restriction endonucleases.

5' – GAATTC – 3'

3' – CTTAAG – 5'

Restriction Enzymes : The enzyme that cuts out a piece of DNA at a specific site. These are of two types : exonucleases and endonucleases.

Sticky ends : Single stranded portions of DNA which can form hydrogen bonds with their complementary cut DNA segments. These ends can be joined by enzyme ligase.

Taq polymerase : A heat stable DNA polymerase isolated from a thermophilic bacterium *Thermus aquaticus* and is used in PCR.

Ti Plasmid : An extrachromosomal, double stranded and self replicating DNA molecule found in *Agrobacterium tumefaciens* that causes tumor in plants.

Tools of Recombinant DNA Technology : Restriction enzymes, polymerase enzymes, ligases, vectors, and host organisms.

Steps in Formation of rDNA by action of *Eco*RI : *Eco*RI cuts the DNA between bases G and A only → sticky ends of cut DNAs are formed → DNA fragments join at sticky ends → Recombinant DNA is formed.

Recombinant DNA (rDNA) : The hybrid DNA formed by combining DNA segment of two different organisms.

Process of Recombinant DNA Technology : Isolation of DNA → Cutting of DNA using restriction endonuclease → Amplification of Gene using PCR → Making rDNA and insertion of it into host cell/organism → obtaining the foreign gene product → Downstream processing.

(i) ***Isolation of Genetic Material (DNA) :***

- ❑ DNA can be obtained from the cell by treating with enzymes like, Lysozyme for bacteria, Cellulase for plant cell, Chitinase for fungus.
- ❑ Histone protein and RNA can be removed by treating with proteases and ribonuclease
- ❑ Purified DNA ultimately precipitated by the addition of chilled ethanol. Fine threads of DNA are obtained in the suspension.

(ii) **Cutting of DNA at specific location :** The purified DNA is cut by use of restriction enzymes. Agarose gel electrophoresis used to check the progression of restriction enzymes digestion.

(iii) **Amplification of gene of interest using PCR :** Amplification is the process of making multiple copies of desired DNA segment in *vitro*. Polymerase chain reaction involves three steps:

(a) *Denaturation :* The target DNA is heated to high temperature (94°C), resulting the separation of two strands of DNA. Each strand acts as template.

(b) *Annealing :* Two oligonucleotide primers anneal to each of the single stranded DNA template.

(c) *Extension of primers :* DNA polymerase (Taq polymerase) extends the primers using the nucleotides provided in the reaction.

(iv) **Ligation :** The cut out 'gene of interest' from the source of DNA and cut vector with appropriate space, are mixed and ligase enzyme is added. This results recombinant DNA (r-DNA).

(v) **Transfer of recombinant DNA into the host :** The ligated DNA is introduced into the recipient cell. The recipient cell makes itself 'competent' to receive and take up DNA present in the surrounding.

(vi) **Obtaining the foreign gene product :** The cell containing the foreign gene is cultured on suitable medium and the product can be extracted from the medium.

Bioreactors are used for processing large volume of culture for obtaining products of interest in sufficient quantities.

(vii) **Downstream Processing :** The products so obtained undergo a series of processes before putting them in market as a finished product. The processes include separation and purification.

The products are formulated with suitable preservation and subjected to quality control testing and clinical trials. (in case of drugs)

Essential features required to facilitate cloning into vector : Ori, Selectable marker, Recognition site, small size.

Some of the Biotechnological products and processes : rDNA vaccines, Gene therapy, Test tube babies, Synthesis of a gene and introduction of it into a target cell/organism.

Steps in creating GMO : Identification of gene of interest → Introduction of rDNA into host cell/organism → Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Gel Electrophoresis : DNA fragments are regatively charged molecules. They can be separated by forcing them to move towards anode under an electric field through a medium. Agarose gel is used as medium. Ethidium bromide is used as stain for DNA, which on exposure to UV-light appear as orange coloured bands. Separated bands of DNA are cut out from agrose gel. This is called elution. These DNA fragments are used in recombinant DNA by joining them with cloning vectors.

QUESTIONS

VSA (1 MARK)

1. A restriction enzyme digests DNA into fragments. Name the technique used to check the progression of this enzyme and separate DNA fragments.
2. Name two commonly used vectors in genetic engineering.
3. Some enzymes are considered as molecular *scissors*. in genetic engenrring. What is the name assigned to such enzymes?
4. Write conventional nomenclature of *Eco*RI.
5. A linear DNA fragment and a plasmid has three restriction sites for *Eco*RI how many fragments will be produced from linear DNA and plasmid respectively.

6. An extra chromosomal segment of circular DNA of a bacterium is used to carry gene of interest into the host cell. What is the name given to it?

7. Identify the recognition sites in the given sequences at which *E.coli* will be cut and make sticky ends.

   5´–GAATTC–3´

   3´–CTTAAG–5´

SA-II (2 MARKS)

8. Name two main steps which are collectively referred to as down streaming process. Why is this process significant?

9. How does plasmid differ from chromosomal DNA?

10. A bacterial cell is shown in the figure given below. Label the part 'A' and 'B'. Also mention the use of part 'A' in rDNA technology.

11. Mention two classes of restriction enzymes. Suggest their respective roles.

12. In the given process of separation and isolation of DNA fragments, some of the steps are missing, Complete the missing steps –

    A : Digestion of DNA fragments using restriction endonucleases

    ↓

    B : .....

    ↓

    C : Staining with ethidium bromide

    ↓

    D : Visualisation in U.V. light

    ↓

    E : .....

    ↓

    F : Purification of DNA fragments.

SA-I (3 MARKS)

13. Since DNA is a hydrophillic moelcule, it cannot pass through cell membranes. Name and explain the technique with which the DNA is forced into (ii) a bacterial cell (ii) a plant cell (iii) an animal cell.
14. How will you otbain purified DNA from a cell?
15. In recombinant DNA technology, vectors are used to transfer a gene of interest in the host cells. Mention any three features of vectors that are most suitable for this purpose.
16. Why is *"Agrobacterium*–mediated genetic engineering transformation" in plants considered as natural genetic engineering?
17. Observe the given sequence of nitrogenous bases on a DNA fragment and answer the following question –

    5′ – CAGAATTCTTA – 3′

    3′ – GTCTTAAGAAT – 5′

    (a) Name a restriction enzme which can recognise this DNA sequence.

    (b) Write the sequence after digestion.

    (c) Why are the ends generated after digestion called sticky ends?
18. A selectable marker is used in the section of recombinants on the basis of their ability to produce colour in presence of chromogenic substrate.

    (a) Mention the name of mechanism involved.

    (b) Which enzyme is involved in production of colour?

    (c) How is it advantageous over using antibiotic resistant gene as a selectable marker?

LA (5 MARKS)

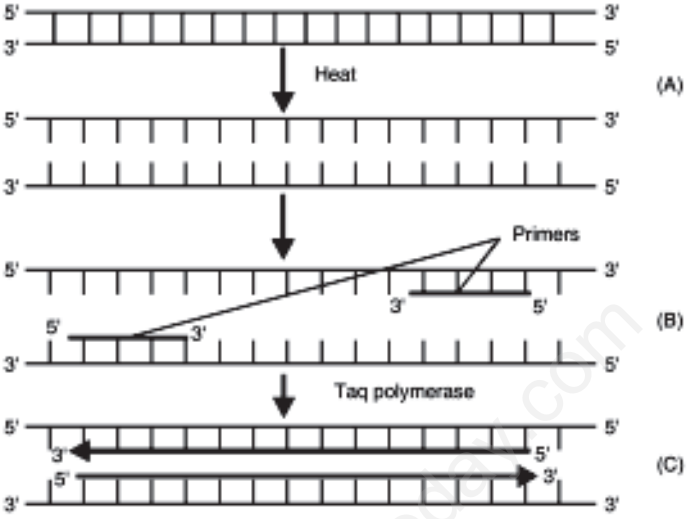
19. The development of bioreactors is required to produce large quantities of products.

    (a) Give optimum growth conditions used in bioreactors.

    (b) Draw a well labelled diagram of simple stirred – tank bioreactor.

    (c) How does a simple stirred – tank' bioreactor differ from sparged stirred – tank' bioreactor?

20. In the given figure, one cycle of polymerase chain reaction (PCR) is shown–

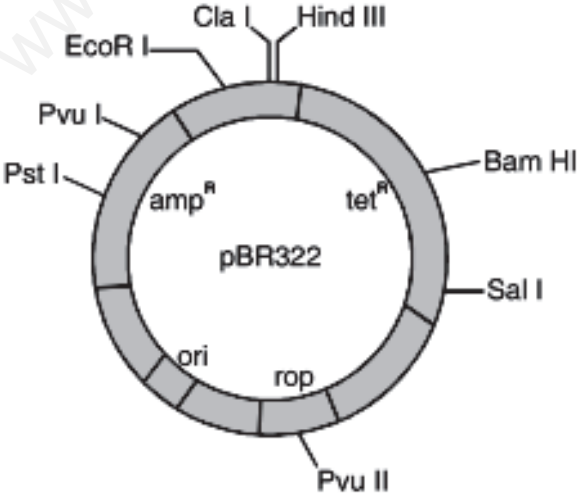


(a) Name the steps A, B and C.

(b) Give the purpose of each of these steps.

(c) State the contribution of bacterium *Thermus aquaticus* in this process.

21. Study the figure of vector pBR322 given below in which foreign DNA is ligated at the Bam H1 site of tetracyline resistance gene.



Answer the following questions :

(a) Mention the function of rop.

(b) What will be the selectable marker for this recombinant plasmid and why?

(c) Explain transformation.

ANSWERS

VSA (1 MARK)

1. Gel electrophoresis
2. Plasmid and Bacteriophage.
3. Restriction Enzymes.
4. E. – *Escherichia*; co – *coli*; R – Name of Strain; I – order in which enzyme isolated from strain of bacteria.
5. Number of fragments of linear DNA = 4

   Number of fragments of plasmid = 3
6. Plasmid.
7. 5′ – G↓AATTC 3′
   3′ – CTTAA↑G 5′

SA-II (2 MARKS)

8. ❑ Separation and Purification
   ❑ This process is essential because before reaching into market, the product has to be subjected for clinical trial and quality control.
9.

	Plasmid DNA		***Chromosomal DNA***
(i)	Circular DNA	(i)	Linear DNA
(ii)	Occurs only in bacterial cells	(ii)	Occurs in nucleus of eukaryotic cells and bacterial cell.
(iii)	Used as Vector in rDNA technology	(iii)	Not used as vector in rDNA technology.

10. A – Plasmid, B – Nucleoid

    Plasmid is used as vector to transfer the gene of interest in the host cell

11. ❑ Exonucleases and endonucleases

    ❑ Exonucleases remove nucleotides from the ends of the DNA.

    ❑ Endonucleases cut DNA at specific sites beween the ends of DNA.

12. ❑ B – Gel Electrophoresis

    ❑ E – Elution

SA I (3 MARKS)

13. (i) Chemical treatment and exposure to cold and high temp. (42°C) alternatively. (Bacterial cell)

    (ii) Biolistics or gene gun. (Plant cell)

    (iii) Micro-injection. (animal cell)

    Explanation – Refer page 200, biology Text Book for class XII.

14. ❑ Cells are treated with appropriate enzymes to release DNA. Lysozyme (bacteria), cellulase (plant cells), chitinase (fungus).

    ❑ RNA and proteins are removed by treatment with ribonuclease and protease enzymes respectively.

15. (ii) Have origin of replication(Ori)

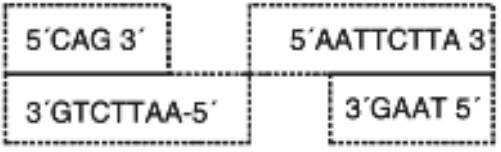
    (ii) Have a selectable marker

    (iii) Have at least one recognition site.

16. *Agrobacterium tumefaciens* is a pathogen in many dicot plants. It is able to deliver a piece of DNA (T–DNA) to transform normal plant cell into a tumor and directs these tumor cells to produce the chemicals required by pathogen.

17. (a) *Eco*RI

    (b)



(c) These are named sticky ends, because they form hydrogen bonds with their complementary cut parts.

18. (a) Insertional inactivation

(b) β-galactosidase.

(c) Selection of recombinants due to inactivation of antibiotics requires simultaneous plating on two plates having different antibiotics. (Refer page 200 NCERT Biology for class XII)

LA (5 MARKS

19. (i) Temperature, pH, susbtrates, salts, vitamins and oxygen.

(ii) Figure 11.7(a) simple stirred–tank bioreactor Page No. 204 NCERT Text book, Biology - XII

(iii) The stirrer facilitates even mixing and oxygen availability throughout simple–stirred tank bioreactor, whereas in case of sparged stirred-tank bioreactor, air is bubbled throughout the reactor for proper mixing.

20. (A) **Denaturation –** Heat denatures DNA to separate complementary strands.

(B) **Annealing :** Primers hybridises to the denatured DNA strands.

(C) **Extension :** Extension of primers resulting in synthesis of copies of target DNA sequence. Enzyme Tag polymerase is isolated from the bacterium *Thermus aquaticus*. This enzyme induces denaturation of double stranded DNA at high temperature.

21. (a) 'rop' codes for the proteins involved in the replication of plasmid

(b) Selectable marker – ampicillin resistance gene. It will help distinguishing transformants from non-transformants after plating them on ampicillin containing medium.

(c) Transformation – It is the phenomenon by which the DNA isolated from one type of cell and introduced into another type and is able to bring about some of the properties of former to the later.