

**Chapter 11**  
**Biotechnology Principles and Processes**

**1 Marks Questions**

**1. A restriction enzyme digests DNA into fragments. Name the technique used to check the progression of this enzyme and separate DNA fragments.**

**Ans.** Gel electrophoresis

**2. Name two commonly used vectors in genetic engineering.**

**Ans.** Plasmid and Bacteriophage.

**3. Some enzymes are considered as molecular scissors. in genetic engenrring. What is the name assigned to such enzymes?**

**Ans.** Restriction Enzymes.

**4. Write conventional nomenclature of EcoRI.**

**Ans.** E. – Escherichia; co – coli; R – Name of Strain; I – order in which enzyme isolated from strain of bacteria.

**5. A linear DNA fragment and a plasmid has three restriction sites for EcoRI, how many fragments will be produced from linear DNA and plasmid respectively.**

**Ans.** Number of fragments of linear DNA = 4

Number of fragments of plasmid = 3

**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?**

**Ans.** Plasmid.

**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'

**Ans.** 5-G↓AATTC35-G↓AATTC3

3-CTTAA↑G53-CTTAA↑G5

**8.Name the substance used as a medium in gel electrophoresis.**

**Ans.**Agarose

**10.Name the bacterium that yields thermostable DNA polymerase.**

**Ans.**Thermusaquaticus.

**11.Which enzymes are known as “molecular Scissors”?**

**Ans.**Restriction Endonuclease.

**12.Name the commonly used vector for trans formation in plant cell?**

**Ans.**Agrobacterium tumefaciens.

**13.Name the technique used for amplification of DNA?**

**Ans.**Polymerase Chain Reaction.

**14.Name the enzyme responsible for removal of 5 – phosphate group from nucleic acid?**

**Ans.**Alkaline Phosphatase.

**15.Who isolated Restriction enzymes for the first time?**

**Ans.**Warner Arber & Hamilton Smith.

**16.Why do eukaryotic cells do not contain restriction enzymes?**

**Ans.**Because in eukaryotic cell, DNA is heavily methylated.

**17.Why does DNA moves towards anode in gel electrophoresis.**

**Ans.**Because of presence of phosphate group, DNA is negatively charged & ∴ moves towards anode.

## **2 Marks Questions**

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

**Ans.**Separation and Purification

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

## 2. How does plasmid differ from chromosomal DNA?

**Ans.**

	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.

## 4. Mention two classes of restriction enzymes. Suggest their respective roles.

**Ans.** Exonucleases and endonucleases

- Exonucleases remove nucleotides from the ends of the DNA.
- Endonucleases cut DNA at specific sites between the ends of DNA.

## 5. 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.**

**Ans.** B – Gel Electrophoresis

E – Elution

## 6. Write any two properties of restriction endonuclease enzymes?

**Ans.** (i) Each Restriction endonuclease functions by inspecting the length of DNA sequence & binds to DNA at the recognition sequence.

(ii) It cuts the two strands of DNA at specific point in their sugar – phosphate backbone.

## 7. What are 'Selectable marker'? What is their use in genetic engineering?

**Ans.** A selectable marker is a gene which helps in selecting those host cells which contain the vector & eliminating the non-transformant – gene encoding resistance to antibiotics are useful selectable markers as they allow selective growth of transformants only.

**8. How can the desired product formed after genetic engineering be produced on a commercial scale?**

**Ans.** The product obtained from genetic engineering is subjected to a series of processes collectively called downstream processing before it is made into final products. The processes involved in downstream processing are :- Separation & purification.

**9. What is "Insertional Inactivation"?**

**Ans.** If a recombinant DNA is inserted within the coding sequence of enzyme  $\beta$ -galactosidase. This results into inactivation of enzyme which is referred to as "Insertional Inactivation". The presence of chromogenic substrate gives blue-coloured colonies if the plasmid in bacteria does not have an insert. The presence of insert results into insertional inactivation & the colonies do not produce any color.

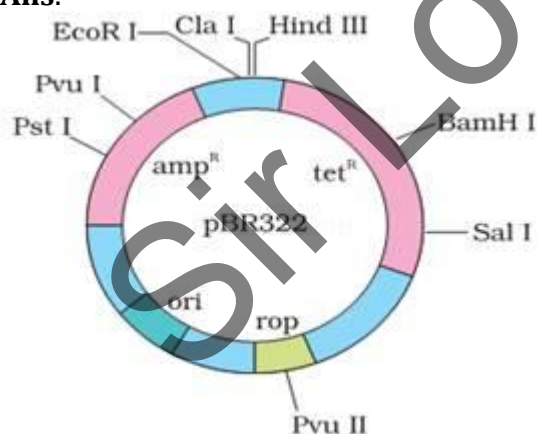
**10. What are the two basic techniques involved in modern Biotechnology?**

**Ans.** The two basic techniques involved in modern Biotechnology are:-

- a) Genetic Engineering is the technique of altering the nature of genetic material or introduction of it into another host organism to change its phenotype.
- b) Techniques to facilitate the growth & multiplication of only the desired microbes or cells in large number under sterile conditions for manufacture

**11. Represent diagrammatically the E. coli. Cloning vector  $\beta$  PBR 322.**

**Ans.**



**12. Differentiate between plasmid DNA and chromosomal DNA?**

**Ans.** Plasmid DNA is extranuclear DNA, found in the cytoplasm, whereas chromosomal DNA is the nuclear or genetic DNA which is found within the nucleus.

**13.What is the role of enzyme “Ligase” in genetic Engineering?**

**Ans.**Enzyme “Ligase” acts as molecular Suture which helps in joining two pieces of DNA. The Joining process requires ATP as it derive energy to construct phosphodiester bond between cohesive ends.

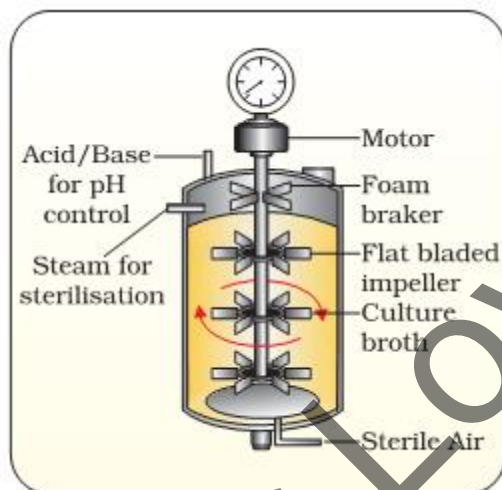
**16.How is gene Z used as a marker?**

**Ans.**Lac Z gene codes for enzyme B-galactosidase, if a recombinant DNA is inserted within the coding sequence of an enzyme B-galactosidase. This results into inactivation of enzyme. The bacterial colonies whose plasmid does not have an insert produce blue colour but those with an insert do not produce any colour.

**17.What is Bioreactor? What are the advantages of Stirred tank Bioreactor over Shake flask. Show diagrammatically a simple Stirred tank Bioreactor?**

**Ans.**Bioreactors are large vessels in which raw materials are biologically converted into specific proteins using microbial, plant, animal or human cells. The advantages of Bioreactor over shake flask are :-

- a) It provides optimal conditions for achieving desired product by providing optimum growth conditions eg. temp, pH etc.
- b) Small volume of cultures can be withdrawn periodically from bioreactor to test the sample.
- c) It has an agitation system, temp control system, foam control system & pH control system



**Chapter 11  
Biotechnology Principles and Processes**

**3 Marks Questions**

**1. Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. Name and explain the technique with which the DNA is forced into (i) a bacterial cell (ii) a plant cell (iii) an animal cell.**

**Ans. (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.

**2. How will you obtain purified DNA from a cell?**

**Ans.**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.

**3. 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.**

**Ans.**(i) Have origin of replication(Ori)  
(ii) Have a selectable marker  
(iii) Have at least one recognition site.

**4. Why is “ Agrobacterium– mediated genetic engineering transformation” implants considered as natural genetic engineering?**

**Ans.**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.

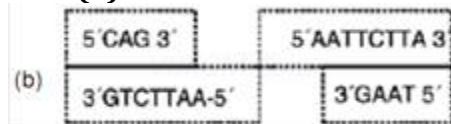
**5. Observe the given sequence of nitrogenous bases on a DNA fragment and answer the following question -**

5' - CAGAATTCTTA - 3'

3' - GTCTTAAGAAT - 5'

- (a) Name a restriction enzyme which can recognise this DNA sequence.  
(b) Write the sequence after digestion.  
(c) Why are the ends generated after digestion called sticky ends?

**Ans. (a)** EcoRI



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

**6. A selectable marker is used in the selection 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?

**Ans. (a)** Insertional inactivation

(b)  $\beta$ -galactosidase.

(c) Selection of recombinants due to inactivation of antibiotics requires simultaneous plating on two plates having different antibiotics.

**7. Mention the important properties which a good vector must possess?**

**Ans.**The important properties which a good vector must possess are :-

- Size :- The vector must have small size so that it is easier to purify & isolate.
- Origin of replication :- This is a sequence of base pairs where replication starts. Any piece of DNA linked to this sequence can be made to replicate within its host cell & thus, controls the copy number of linked DNA.
- Selectable Marker :- A marker is a gene which helps in selecting those host cells which contain the vector & eliminating the non - transformants Common Selectable marker include gene encoding resistance to antibiotics.
- Cloning Sites :- The vector Should have a few or at least one unique recognition site

to link the foreign / alien DNA. Presence of a particular recognition site enables the particular restriction enzyme to cut the vector.

**8. Describe any three vectors less method of introducing the rDNA into a competent host cell?**

**Ans.** i) Transformation :- In order to force bacteria to take up the plasmid, the bacterial cell must first be made competent to take up DNA. This is done by treating them with specific concentration of divalent cationeg.  $\text{Ca}^{2+}$  which 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 at  $420^\circ\text{C}$  & then putting them back into ice. This enables the bacteria to take up the recombinant DNA.

ii) Microinjection :- recombinant DNA is directly injected into the nucleus of an animal cell using a micro – needle of tip with diameter ( $\sim 4\text{mm}$ )

iii) Biolistics / Gene gun :- cells are bombarded with high velocity micro – particles of gold or tungsten coated with DNA.

**9. Why is Agrobacterium mediated genetic transformation described as Natural Genetic engineering in plants?**

**Ans.** Agrobacterium tumefaciens, a natural pathogen of several dicot plants is able to deliver a piece of DNA known as “T – DNA” to transform normal plant cell into a tumor & direct gene transfer transform tumor cells to produce chemicals required by pathogen . The tumor inducing (Ti) plasmid of

Agrobacterium tumefaciens has now been modified into a cloning vector which is no more pathogenic to plant but is still able to use the mechanism to deliver genes of our interest into a variety of plants Since Agrobacterium tumefaciens has the natural ability to donate a part of its DNA to the plant during infection. This property of Agrobacterium is exploited and a gene of interest is ligated into T-DNA so that it automatically gets transformed into plant cell thus, Agrobacterium tumefaciens is known as “Natural Genetic Engineer” of plants.

**10. Mention the important tools required for genetic engineering technology?**

**Ans.** The process of genetic engineering is accomplished only when we have following key tools :-

a) Restriction enzymes:- Restriction enzymes are a group of endonucleases which cut the DNA at specific position anywhere in its length. Each restriction endonuclease functions by inspecting the length of DNA & binds to DNA at the recognition Sequence.

b) Cloning Vector:- The DNA molecule which carry the desired DNA Segment of an organism & transfer it to cell or DNA of another organism is called cloning vector.

c) Desired foreign DNA:- The segment of DNA containing genes having desired characters & which are being transferred into genome of another cell with the help of vector is called foreign DNA.