CLASS: II PUC. **SUBJECT : BIOLOGY.**

UNIT IX .

CHAPTER 11: BIOTECHNOLOGY : PRINCIPLES AND PROCESSES.

ONE MARK QUESTION AND ANSWERS .

1. Define Biotechnology.

ANS : Bio technology deals with the techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans .

2. What are plasmids?

ANS: Autonomously replicating circular extra-chromosomal DNA.

Or

Plasmid is a small, circular, extra chromosomal dsDNA occurring in some bacteria which can undergo replication independently.

3. What are palindromic nucleotide sequences ?

Ans: Palindromic sequences are 'invert repeats' which have the same nucleotide sequences when read in 5' 3' on both the strands.

4. Mention the function of DNA ligase.

Ans: These are enzymes which can join fragments of DNA which have complementary sticky ends or blunt ends.

5. What do you mean by insertional inactivation ?

Å Ans : The inactivation of gene due to insertion of alien DNA is called insertional inactivation.

6. Name the plasmid isolated from Agrobacterium tumifaciens.
   Ans : Ti plasmind or tumor inducing plasmid.
7. What is agarose ?

Ans : Agarose is a natural polymer extracted from sea weeds.

8. Name the stain used in gel- electrophoresis.

Ans : Ethidium bromide

9. What is bioreactor ?

Ans : It is a vessel in which raw materials are biologically converted in to specific products using microbial plants, animal or human cells.

10. What is down stream process ?

Ans : Separation, purification of the products obtained from recombinant DNA technique is called downstream processing (DSP).

11. Name the enzyme used in linking the DNA segments together.

Ans : DNA ligase.

12. Which technique is commonly used to isolate DNA fragments ?

Ans : Gel- electrophoresis.

13. What do mean by Ori ?

Ans : Sequence from where replication starts and any piece of DNA when linked in this sequence can be made to replicate within the host cells.

14. Name the enzyme which is also called “molecular scissors”.

Ans : restriction endonuclease.

15. What is transformation ?

Ans : A process by which a piece of DNA is introduced into a host bacterium.

16. What is elution ?

Ans : In gel-electrophoresis, the separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is called elution.

17. Who constructed the first artificial recombinant DNA molecule ?

Ans : Stanley Cohen and Herbert Boyer .

18. Name the scientists who constructed pBR 322.

Ans : Bolivar and Rodringuez.

19. What is signeficance of selectable marker in plasmids ?

Ans : selectable markers help in identifying and eliminating non-transformants and selectively permitting the growth of the transformants.

20. What is micro-injection ?

Ans : Method of introducing recombinant DNA in to host cell using fine needle is called micro-injection. Or

Recombinant DNA is directly injected in to the nucleus of animal cell.

21. What is biolastics or gene gun ?

Ans : Method of introducing recombinant DNA in to host cell by bombarding high velocity microparticles of gold or tungsten coated with DNA.

22. What is competent host ?

Ans : The cell which is capable of taking up an alien DNA is called competent host.

23. What is recombinant protein ?

Ans : Protein encoding gene is expressed in heterologous host , it is called recombinant protein.

24. Expand EFB.

Ans : European Federation Of Biotechnology.

25. Name the enzyme commonly used to dissolve bacterial cell wall.

Ans : Lysozymes.

26. . Name the enzyme used as an alternate selectable marker.
Ans : β --Galactosidase.

Two marks question and answers :

1. Name the other processes included under biotechnology.

Ans : In vitro fertilization leading to test tube baby, synthesis of gene, developing DNA Vaccine and correcting a defective gene.

2. Name the two types of restriction enzymes .

Ans : 1). Exonucleases. 2). Endonucleases .

3. Name two enzymes used in biotechnological processes.

Ans : Restiction enzymes (REN) , DNA ligase , lysozymes, cellulase,chitinase , ribonuclease, proteases. (any two).

4. Name the tools of recombinant DNA technology.

Ans : Restriction enzymes, Polymerase enzymes , DNA ligases , vectors, host organism and bioreactors.

(Any four)

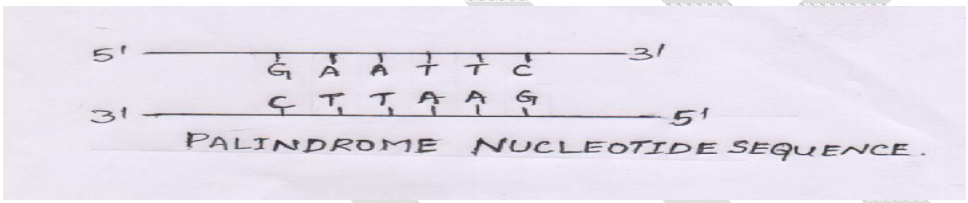
5. Name the selectable markers of E, coli.

Ans : the genes encoding resistance to antibiotics like ampicillin, tetracycline, chloramphenicol and kanamycin are selectable markers in E.coli.

6. What is a palindrome sequence of DNA ? Illustrate with a suitable example.

Ans : Palindromic sequences are 'invert repeats' which have the same nucleotide sequences when read in 5' 3' on both the strands.

Ex :



7. Differentiate between exonuclease and endonuclease .

Ans : Exonucleases remove nucleotides from ends of the DNA.

Endonucleases make cuts at specific positions within the DNA.

8. Mention the function of Ti plasmid. Name the source organism from which it is isolated.

Ans : 1) Ti plasmid is used as a vector for delivering genes of our interest in to variety of plants.

2) Ti plasmid is obtained from bacteria ***Agrobacterium tumifaciens.***

9. Mention the methods of making bacteria capable to take up recombinant DNA.

Ans : 1. Calcium chloride heat treatment.

2. Micro- injection.

3. Gene- gun or biolastics. And

4. Disarmed pathogens .

10. Name any two important sites of a plasmid .

Ans : 1) Ori site. 2) selectable markers. 3) Cloning sites . (any two)

Three marks question and answer :

1. Name the three basic steps involved in genetically modifying an organism.

Ans : a. Identification of DNA with desirable genes.

b. Introduction of indentified DNA in to the host.
c. Maintenance of introduced DNA in the host and transfer of DNA in to its progeny.

2. Draw a neat labeled diagram of pBR322. Plasmid.

Ans :

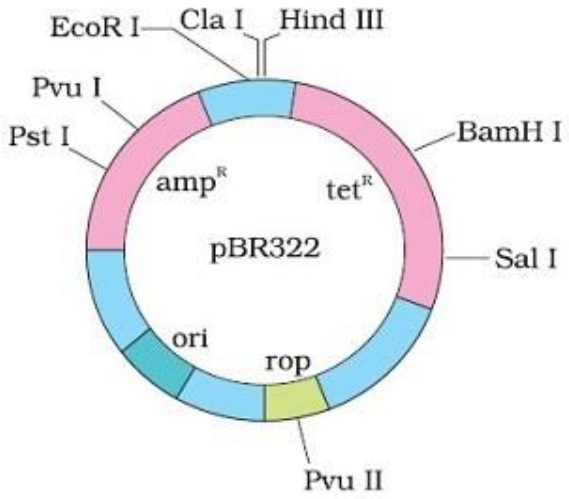


Figure 4. *E. coli* cloning vector pBR322 showing restriction sites (*Hind* III, *Eco*R I, *Bam*H I, *Sa*l I, *Pvu* II, *Pst* I, *Cla* I), ori and antibiotic resistance genes (amp^R and tet^R). Rop codes for the proteins involved in the replication of the plasmid.

3. List the features of a vector required to facilitate cloning.

Ans : : 1) Ori site. 2) selectable markers. 3) Cloning sites .

4. Give a brief account of vectors used for cloning genes in plants and animals.

   Ans :

   1. Tumour inducing Ti plasmid of ***`Agrobactrium tumifaciens ,*** has now been modified into a cloning vector which is no more pathiogenic to the plants but can be used to deliver genes of our interest in to variety of plants.
   2. Retroviruses. Have been disarmed and are used to deliver desirable genes into animal cells.

5. .Explain briefly the process of isolation of DNA.

Ans : In order to cut the DNA with restriction enzymes, It needs to be in pure form , free from other macro-molecules like RNA, histones, chitin, cellulose, etc,. the contents of the cell is treated with enzymes like chitinase, lysozymes, cellulose , ribonuclease and proteases and ultimately chilled ethnol is added to get purified DNA.

6. Mention the steps involved in recombinant DNA terchnology.

   Ans : 1. Isolation of desired DNA .

   2. fragmentation of DNA by restriction endonucleases.

   3. Isolation of a desired DNA fragment.

   4. Ligation of DNA fragment in to vector.

   5.Transferring the recombinant DNA in to host.

   6. culturing the host cell and extraction of desired product.

7. How are restriction enzymes named ?

   Ans : The naming of restriction enzyme is based on the name of bacterium from which they have been isolated. The first letter of the name comes from the genus, and second letter come from the species of the bacterium, the third letter indicate the strain of the organism, Roman numbers following the name indicate the order in which the enzymes were isolated from that strain of bacteria.

8. Explain the action of restriction endinuclease.

Ans : Restriction endonuclease enzyme functions by inspecting the length of a DNA.

It recognizes a specific restriction site on DNA .(palimdromic nucleotide sequence).

It will bind to the DNA and cut each of the two strands of the double helix at specific point in their sugar-phosphate backbones.

Five marks question and answers :

1. Name the tools of recombinant DNA technology . Write a note on restriction enzymes .

Ans : The tools of recombinant DNA technology of Restriction enzymes, Polymerase enzymes , DNA ligases , vectors, host organism and bioreactors.

Å These are enzymes naturally occurring in bacteria (for defence) which recognize specific palindromic sequences in the DNA and cut it at those places. Palindromic sequences are 'invert repeats' which have the same nucleotide sequences when read in 5' 3' on both the strands. Some RENs are Eco RI, Hind III, Sma I, Hae III, etc.,. These RENs are also called **'molecular knives'** or **'molecular scissors'** or **'natures's scalpels'** as they cut DNA. The fragments of DNA produced are called restriction fragments. The fragments of DNA may have **'blunt ends'** or **'staggered ends'** (sticky ends).

Å **Endonuclease :** They breaks DNA double helix at any point except the ends. They produce internal cuts called nick's or cleavage.

Å **Exonuclease :** They breaks or cuts the 5' or 3' ends of DNA molecule i.e. they remove nucleotides from terminal ends of DNA in one strand of double helix.

2. Explain the process of gel electrophoresis.

Ans : **Gel Electrophoresis**

- The fragments obtained after cutting with restriction enzymes are separated by using gel electrophoresis.
- Electric field is applied to the electrophoresis matrix (commonly agarose gel) and negatively charged DNA fragments move towards the anode.
- Fragments separate according to their size by the sieving properties of agarose gel. Smaller the fragment, farther it moves.
- Staining dyes such as ethidium bromide followed by exposure to UV radiations are used to visualise the DNA fragments.
- DNA fragments are visible as bright orange coloured bands in the agarose matrix.
- These bands are cut from the agarose gel and extracted from the gel piece (elution).

- DNA fragments are purified and these purified DNA fragments are used in constructing recombinant DNAs

3. Explain rDNA technology .

Ans : RECOMBINANT DNA (rDNA) TECHNOLOGY:

Ans : Recombinant DNA technology involves;

1. Isolation of DNA.

2. Fragmentation of DNA by restriction enzymes and Isolation of desired gene by electrophoresis.

3. Ligation of desired gene in to plasmid. (creation of recombinant plasmid)

4. Transferring of recombinant plasmid in to the host cell. (transformation)

5. Culturing the transformed cells in a medium at large scale and Extraction of desired product.

1. Isolation of DNA.

DNA is genetic material . It is present in nucleus of the cell. Cells also contain other macromolicules like cellulose, chitin,proteins, carbhydrates, lipids, RNA, etc,. The cellular contents is treated with various enzymes like cellulase, chitenase, proteases, ribonucleases to to hydrolyse these macromolicules and we get pure DNA.

2. Fragmentation of DNA by restriction enzymes and Isolation of desired gene by electrophoresis.

Restriction endonucleases are used to cut DNA at specific palindromic sequences to isolate the desired gene. This DNA fragment which is to be inserted into plasmid for cloning is called passenger DNA. And the desired gene is isolated by a process called gel-electrophoresis.

3. Ligation of desired gene in to plasmid. (creation of recombinant plasmid)

A suitable plasmid is selected. It is treated with the same restriction enzyme to break open the plasmid at specific sites with sticky ends.

Now the desired gene and the plasmid are mixed and enzyme DNA Ligase is added . The desired gene gets incorporated in to the plasmid .

4. Transferring of recombinant plasmid in to the host cell. (transformation)

The recombinant plasmid is introduced in to the host cell by cold calcium chloride method or by using microinjection or by gene gun or using retrovirus.

5. Culturing the transformed cells in a medium at large scale and Extraction of desired product.

The ultimate aim is to produce desirable protein.

There is a need for the recombinant DNA to express . The foreign gets expressed under appropriate conditions.

The host cells are culrured in a vessel called Bioreactor.

The desired protein is extracted and purified by

Using different separation techniques called DOWN STREAM PROCESSING.

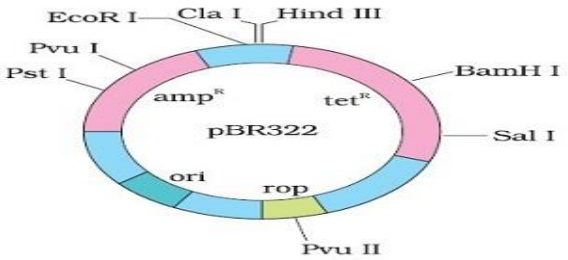
4. Explain with the help of a neat labeled diagram structure of pBR322 plasmid.

Ans : The best known vector which is available commercially is pBR322. (plasmid of Boliver and Rodringuez).

It is modified from natural plasmid of *Escherichia coli* .

It is about 4.3 Kb in size.

It has the following features:



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1. Origin of replication site .(ori)

2. Selectable marker.

3. Cloning site or restriction site .

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

2. Selectable marker. It helps in identifying and eliminating non-transformants and selectively permitting the growth of the transformants. The genes encoding resistance to antibiotics such as ampicillin, chloramphenical , tetracyclin or canamycin , are considered as useful selectable markers for *E.coli*.

3. Cloning site or restriction site :The vector should have single or few recognition site for the commonly used restriction enzymes in order to insert foreign DNA. In pBR322 alian DNA is ligated in the area of Bam HI site of tetracycline resistance gene. The recombinanat plasmid does not possess tetracycline resistance but continues to have ampicilline resistance.

6. Write short notes on :

(a) Bio -reactor . (3 marks)

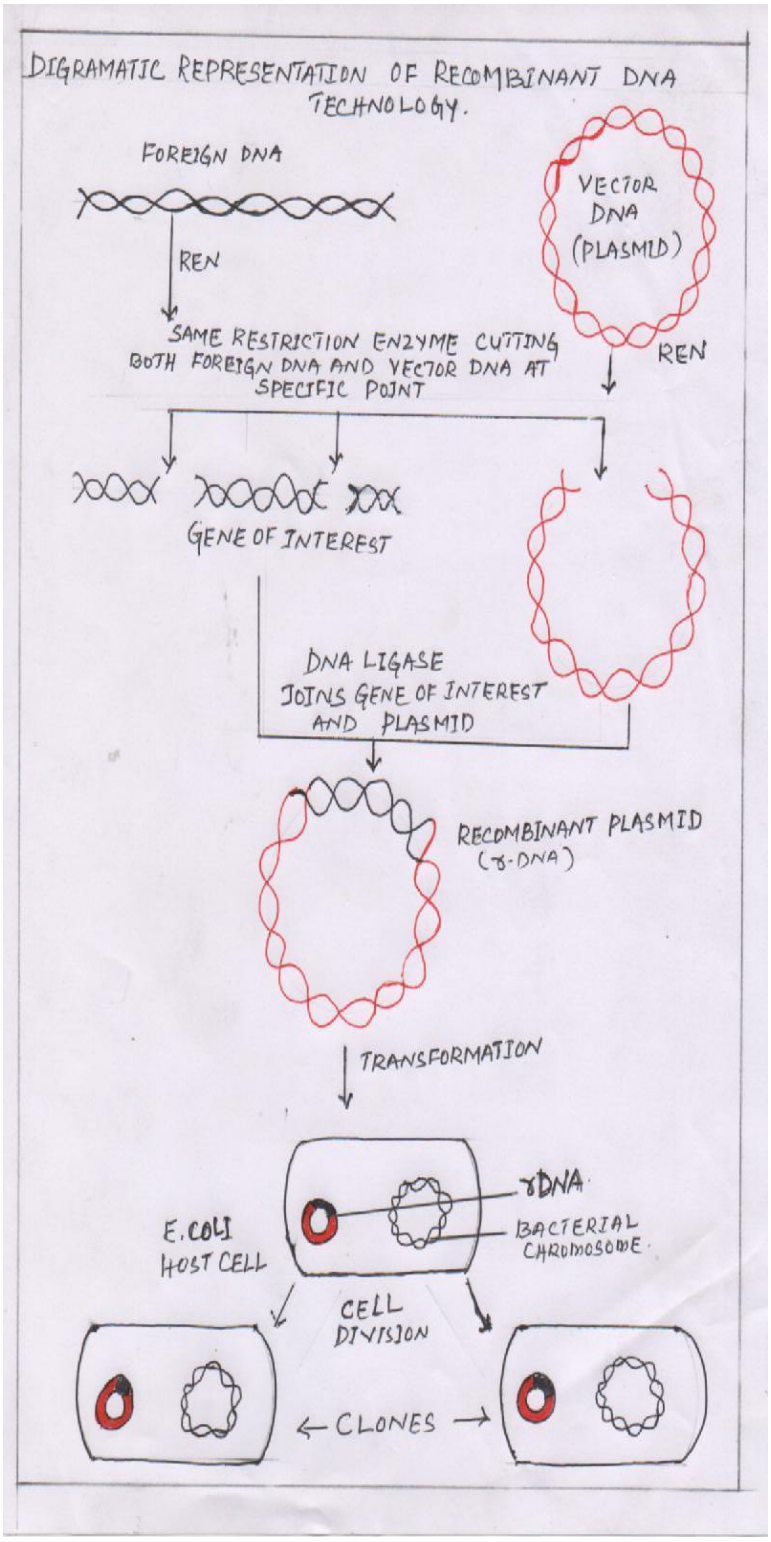
(b) Downstream processing.(2 marks)

(a) Bioreactor is a vessel in which raw materials are biologically converted in to specific products, individual enzymes, etc. A bioreactor provides the optimal growth conditions for achieving the desired product by providing optimum growth conditions .The most commonly used bioreactors are stirred tank reactor. It is cylindrical with curved base to facilitate the mixing of the reactor

contents. The stirrer facilitates even mixing and oxygen availability. Air also can be bubbled through the reactor. The reactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system , pH control system and sampling ports.

(b) Downstream processing: Separation, purification of the products obtained from recombinant DNA technique is called downstream processing (DSP). The product has to be formulated with suitable preservatives . Thorough clinical trials and strict quality control testing is also conducted before marketing of the product.

7. Give the diagrammatic representation of recombinant DNA technology.


DIGRAMATIC REPRESENTATION OF RECOMBINANT DNA TECHNOLOGY.
FOREIGN DNA
VECTOR DNA (PLASMID)
REN
SAME RESTRICTION ENZYME CUTTING BOTH FOREIGN DNA AND VECTOR DNA AT SPECIFIC POINT
REN
GENE OF INTEREST
DNA LIGASE JOINS GENE OF INTEREST AND PLASMID
RECOMBINANT PLASMID (γ-DNA)
TRANSFORMATION
γDNA
E.COLI HOST CELL
BACTERIAL CHROMOSOME
CELL DIVISION
← CLONES →