

BIOTECHNOLOGY-PRINCIPLES & PROCESSES

IMPORTANT CONCEPTS AND DEFINITIONS-

1. **Biotechnology** deals with techniques of using live organisms or enzymes from organisms to
2. produce products and processes useful to humans
3. **Biotechnology** - The *definition* given by EFB is as follows:
'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'
4. **Genetic engineering** : Techniques to alter the chemistry of genetic material (DNA and RNA),
to introduce these into host organisms and thus change the phenotype of the host organism.
5. **Restriction enzymes**: Enzymes that are used to cut DNA segment at a specific site are called
restriction enzymes.
6. **Exonucleases** remove nucleotides from the ends of the DNA molecule.
7. **Endonucleases** make cuts at specific positions within the DNA molecule.
8. **Plasmid**: autonomously replicating circular extra-chromosomal DNA of any bacteria.
9. **Origin of replication** - a specific DNA sequence which is responsible for initiating replication is called **origin of replication**.
10. **Vectors** - These are plasmid DNA or viruses act as vehicle to transfer the piece of DNA attached to it.
11. **Palindromic Nucleotide Sequences** - 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.
12. **Gel electrophoresis** - a technique which is used to separate the fragments of DNA is known as **gel electrophoresis**.
13. **Transformation** is a procedure through which a piece of DNA is introduced in a host bacterium
14. **Insertional inactivation** – The procedure of inserting a recombinant DNA within coding sequence of a functional gene which makes that gene inactive (unable to express) is called insertional inactivation.
15. **Selectable marker**- the gene encoding desirable information useful in identifying and eliminating nontransformants and selectively permitting the growth of the transformants is called **selectable marker**.
16. **Micro-injection**- a technique in which recombinant DNA is directly injected into the nucleus of an animal cell.
17. **Biolistics** or **Gene gun** - plant cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as **biolistics** or **gene gun**.
18. **Bioreactors** - 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.

IMPORTANT POINTS, PRINCIPLES AND THEORY-

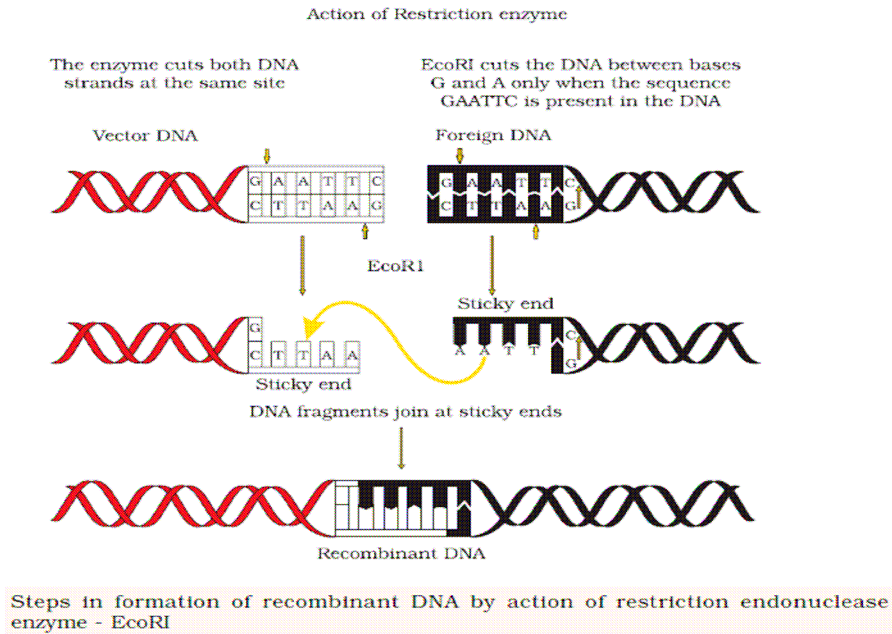
1. Genetic engineering : Techniques to alter the chemistry of genetic material (DNA and RNA), to introduce these into host organisms and thus change the phenotype of the host organism.
2. Maintenance of sterile (microbial contamination-free) ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.
3. Three basic steps in genetically modifying an organism —
 - (i) identification of DNA with desirable genes;
 - (ii) introduction of the identified DNA into the host;
 - (iii) maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

TOOLS OF RECOMBINANT DNA TECHNOLOGY---

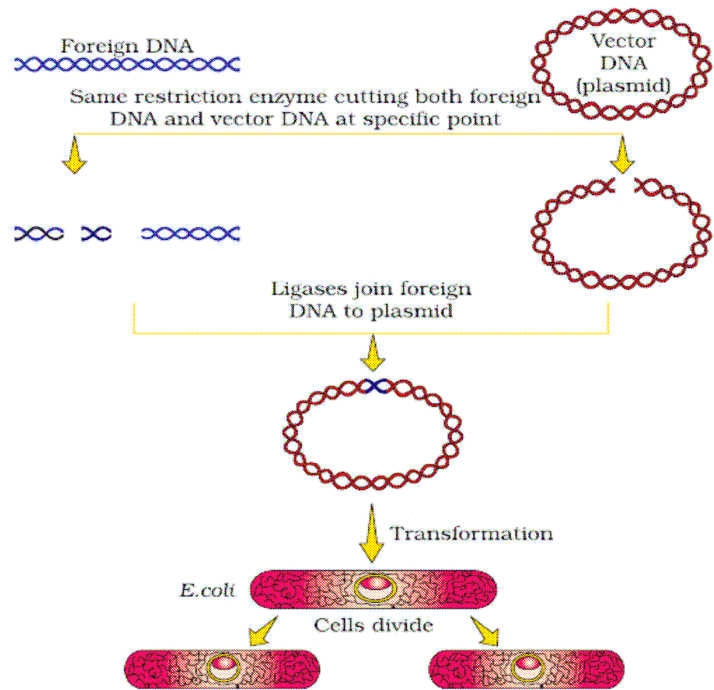
1. DNA technology can be accomplished only if we have the key tools, i.e., restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.
2. The first restriction endonuclease—Hind II
3. Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the recognition sequence for Hind II.
4. The convention for naming these enzymes is the first letter of the name comes from the genes and the second two letters come from the species of the prokaryotic cell from which they were isolated, e.g., EcoRI comes from Escherichia coli RY 13.
5. In EcoRI, 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.
6. Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.
7. 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 reads the same on the two strands in 5' → 3' direction. This is also true if read in the 3' → 5' direction.

5' — GAATTC — 3'

3' — CTTAAG — 5'



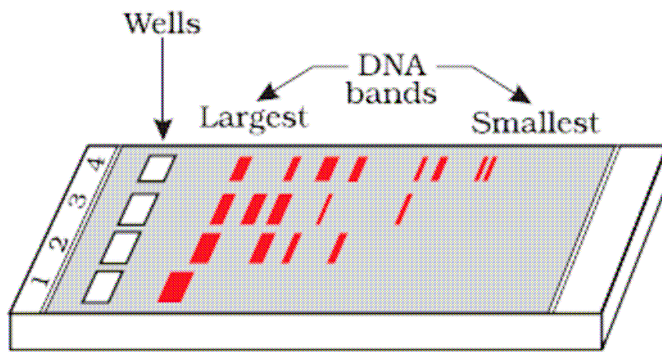
8. Restriction endonucleases are used in genetic engineering to form 'recombinant' molecules of DNA, which are composed of DNA from different sources/genomes.
9. When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky- ends' and, these can be joined together (end-to-end) using DNA ligases



Diagrammatic representation of recombinant DNA technology

(A) Separation and isolation of DNA fragments : The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as gel electrophoresis. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix. Nowadays the most commonly used matrix is agarose which is a natural polymer extracted from sea weeds. The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.

The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (you cannot see pure DNA fragments in the visible light and without staining). You can see bright orange coloured bands of DNA in a ethidium bromide stained gel exposed to UV light. The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as elution. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.



A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

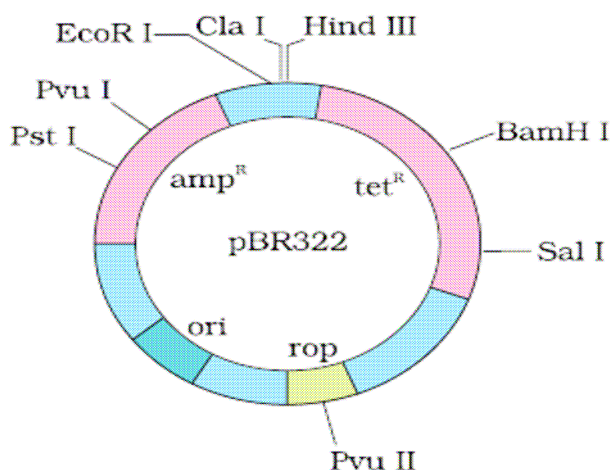
B) Cloning Vectors: Making many copies of rDNA is possible through multiplying the vector to which it has aligned. We are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage.

(C) The following are the features that are required to facilitate cloning into a vector.

(i) 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.

(ii) Selectable marker : In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating nontransformants and selectively permitting the growth of the transformants. Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E. coli. The normal E. coli cells do not carry resistance against any of these antibiotics.

(iii) Cloning sites: In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning.



111

E. coli cloning vector pBR322 showing restriction sites (Hind III, EcoR I, BamH I, Sal 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.

Alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β -galactosidase (gene gets 'inactivated due to insertion' of alien DNA). This results into inactivation of the enzyme, which is referred to as **insertional inactivation**. 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.

(iv) Vectors for cloning genes in plants and animals: The tumor inducing (Ti) plasmid of **Agrobacterium tumifaciens** has now been modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants. Similarly, **retroviruses** have also been disarmed and are now used to deliver desirable genes into animal cells. So, once a gene or a DNA fragment has been ligated into a suitable vector it is transferred into a bacterial, plant or animal host (where it multiplies).

(D) Competent Host (For Transformation with Recombinant DNA)

In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium, 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 briefly at 42°C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

Other Methods:-

1. In a method known as micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell.
2. In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biolistics or gene gun.
3. Another method uses 'disarmed pathogen' vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host

(E) Isolation and purification of the Genetic Material (DNA)-

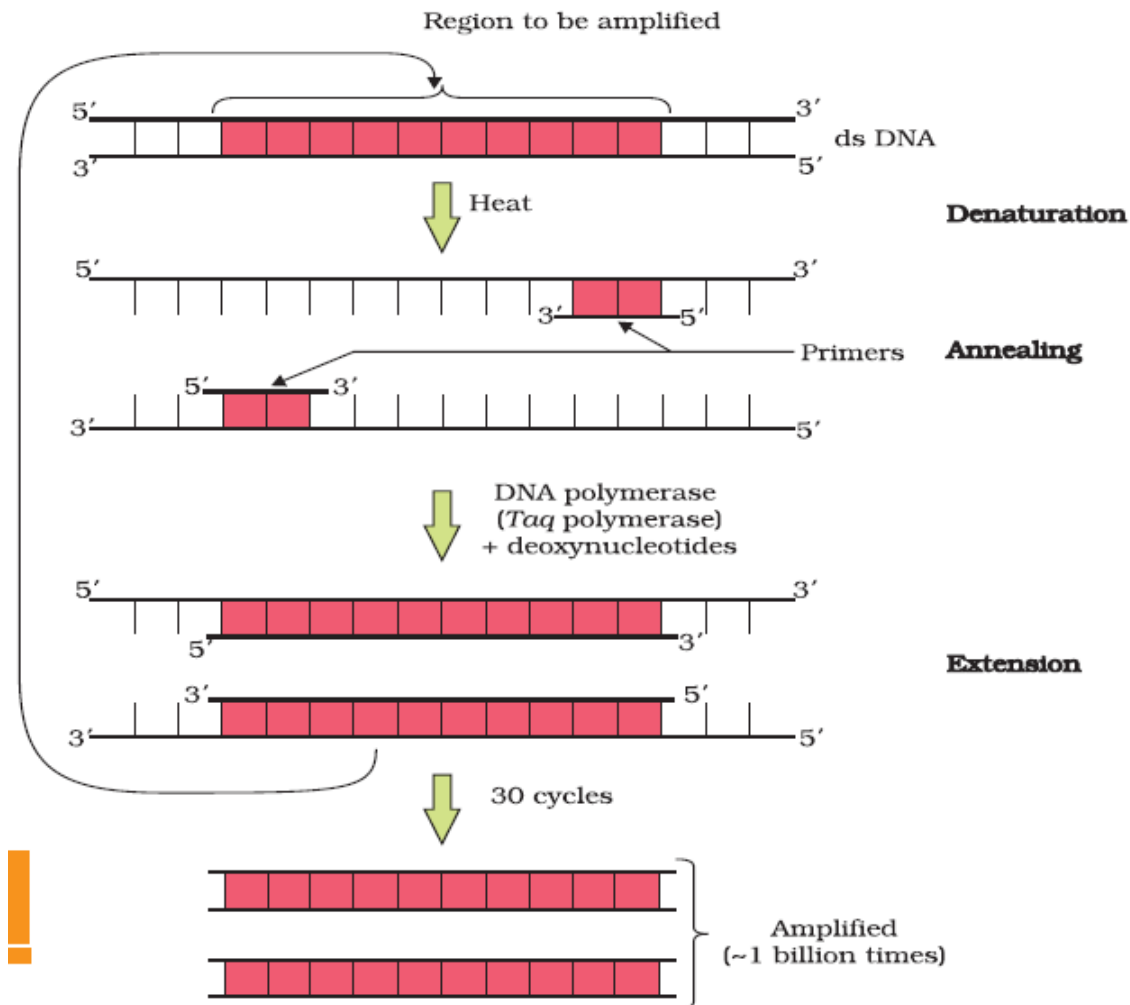
Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. This can be achieved by treating the bacterial cells/plant or animal tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus).

Genes are located on long molecules of DNA intertwined with proteins such as histones. The RNA can be removed by treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments and purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as collection of fine threads in the suspension by spooling.

(F) Amplification of Gene of Interest using PCR-

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest is synthesised in vitro using two sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase. The enzyme extends the primers using

the nucleotides provided in the reaction and the genomic DNA as template. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. Such repeated amplification is achieved by the use of a thermostable DNA polymerase (isolated from a bacterium, *Thermus aquaticus*), which remain active during the high temperature induced denaturation of double stranded DNA. The amplified fragment if desired can now be used to ligate with a vector for further cloning.



Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

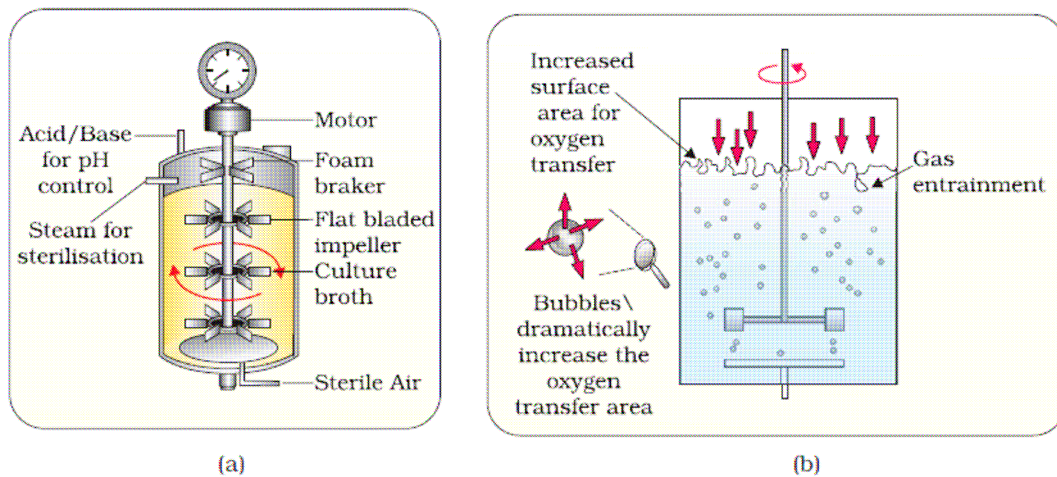
(G) Obtaining the Foreign Gene Product-

After having cloned the gene of interest and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale. If any protein encoding gene is expressed in a heterologous host, is called a recombinant protein.

The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

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.

Bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).



(a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

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. Alternatively

air can be bubbled 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.

(H) Downstream Processing-

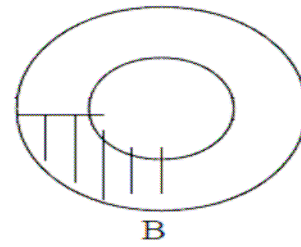
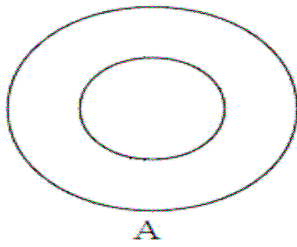
After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives. Such formulation has to undergo thorough clinical trials as in case of drugs. Strict quality control testing for each product is also required. The downstream processing and quality control testing vary from product to product.

Question Bank

1 mark Questions:

1 Restriction Enzymes are called "Molecular scissors". In what context they are referred so?

2 In what way the following two plasmids differ? 1



3. What is the source of thermostable DNA polymerase and Name that polymerase. Why thermostable DNA polymerase is essential in PCR? 1

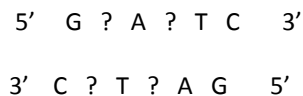
4 “The prophase I of meiosis plays a vital role in r-DNA formation” Justify the statement.1

5 Eukaryotes do not have restriction endonuclease, then how they manage with normal endonuclease enzyme?1

6 What special feature do prokaryotes have to defend themselves from bacteriophages?1

7 _____ = Denaturation + _____ + Extension.1

8. Complete the following palindrome sequence and name the restriction endonuclease that recognizes this.



9. Which is not a tool of Recombinant DNA technology?

- a) Restriction enzyme
- b) Vector
- c) Bioreactor

10. In what stage of meiosis recombination of DNA takes place?

11. How has EFB defined biotechnology?

12. Which group of enzymes known as molecular scissors?

13. From which organism restriction endonuclease was first extracted?

14. How is a DNA fragment amplified?

15. Name the common substance used as matrix for gel electrophoresis.

16. Which of the following DNA sequences would a Restriction enzyme recognize and cut?

- a. (a) ATGCAC (b) GATATC (c) TAGATA (d) AATATA
- i. TACGTG CTATAG ATCTAT TTATAT

17. Who isolated the antibiotic resistance genes from bacteria?

18. What are recognition sequences?

19. What is elution in the process of separation of DNA fragments?

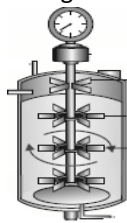
20. Name the chemical used to stain DNA in gel to view them.

21. What is Rop in cloning vector pBR322?

22. Which enzyme is used to treat the fungal cells during isolation of DNA?

23. Identify the type of bioreactor given in below figure:-

11

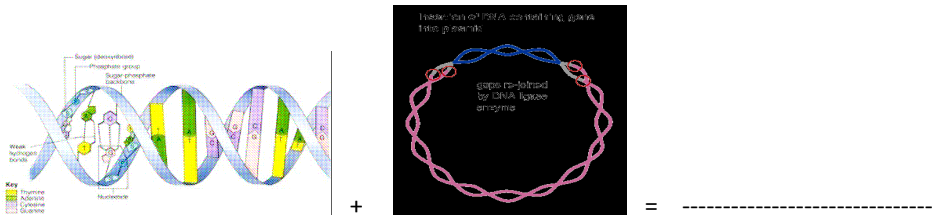


24. Why does DNA move towards the anode in gel electrophoresis?
25. What is gene gun?

2marks

1. Is advisable to use different restriction endonucleases to cut the vector DNA and source DNA? Why?
2. What will happen if more than one recognition sites are present in a vector?
3. Uncontrolled recombinant DNA technology experiments is dangerous to mankind. Comment on it.

4.



Foreign DNA + plasmid =??.....

Complete the above sequence of diagrammatic representation and name it.

5. (a) Which is the most commonly used matrix in gel electrophoresis ?

(b) What is the source of it?

6 Find the 'odd one out and write why that is 'odd'

(a) Sal I, Pst I, Cla I, BamH I, pBR 322

(b) Bacteria, Virus, Gene-gun, Fungi

7 Detect the mismatch from the following and replace the wrong match with a right one

- (a) ECOR I – Gel electrophoresis
- (b) Ethidium Bromide - Bacteria
- (c) Lysozyme - Restriction enzyme
- (d) Palindrome sequence - Fungi

8. Write the use of the following in Biotechnology.

- (a) Chilled ethanol (b) Microinjection
- (c) Bioreactor (d) plasmid

9. Is there any difference between recombinant DNA and recombinant protein? Support your answer.

10. What are selectable markers? Give two examples.

11. What are the optimal conditions bioreactor provides for the achieving the desired product of gene?

12. Few gaps have been left in the following table showing certain terms and their meanings.

Sl.No	Terms	Meanings
1.	-----	any protein encoding gene is expressed in a heterologous host
2.	lysozyme	-----
3.	-----	In this recombinant DNA is directly injected into the nucleus of an animal cell.
4.	Ti	-----

13. How you overcome inclusion and multiplication of undesirable genes along with the desired genes by hybridization procedures used in plants? What techniques are included in this process?

14. A scientist cultured the recombinant DNA bearing gene for resistance to an antibiotic ampicillin is transferred into bacterial cells on agar plates containing ampicillin. Which one will grow on this medium? What you call such type genes in bacterial cells?

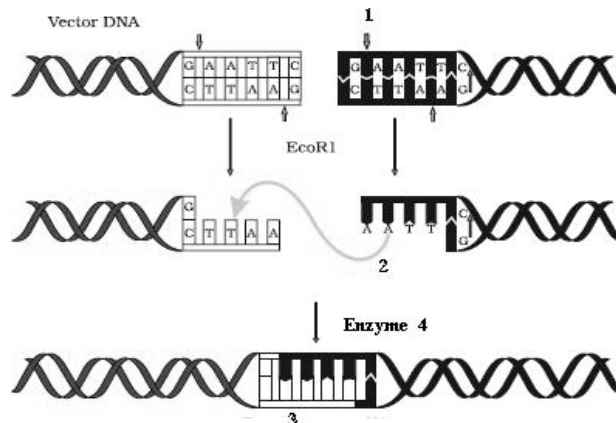
15. How you Isolate the Genetic Material (DNA) from plant cells?

16. Why the bacterial cells must first be made 'competent' to take up DNA? List the methods used to achieve this Process.

17. How has *Agrobacterium tumifaciens* been suitably modified to act as a cloning vector?

18. Name the 1,2,3 And Enzyme 4 in given below diagram.

11



19. What are three basic steps in genetically modifying an organism?

3 MARKS

1. Give the correct term for the following; -

- (a) Replacing a defective mutant allele with a normal functional allele /gene.
- (b) Increasing the copies (content) of a gene
- (c) Preventing m-RNA translation
- (d) Specific pattern of base pairs that are recognized by restriction enzymes.
- (e) Mobile genetic element
- (f) One DNA copies itself and produces two

2. Name the enzyme involved in the following process:

- (a) Repeated amplification of DNA fragments.
- (b) Formation of short piece of RNA strand for annealing.
- (c) Breaking of bacterial cell to release DNA and other macromolecules.
- (d) Cutting and rejoining DNA fragments.
- (e) Formation of m-RNA.
- (f) Joining of foreign DNA fragments with plasmid.

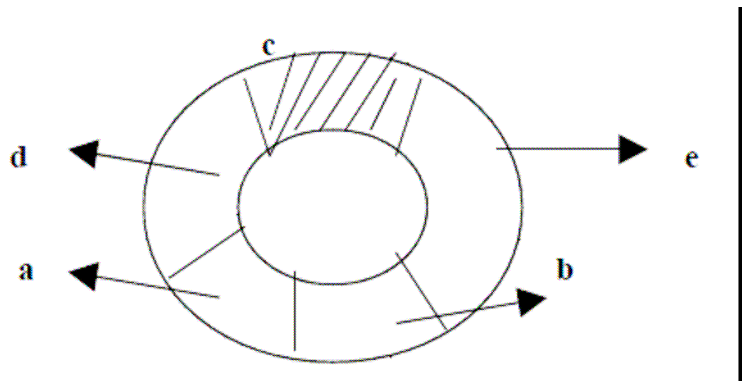
3. Explain the following, emphasizing their formation.

- (a) rDNA

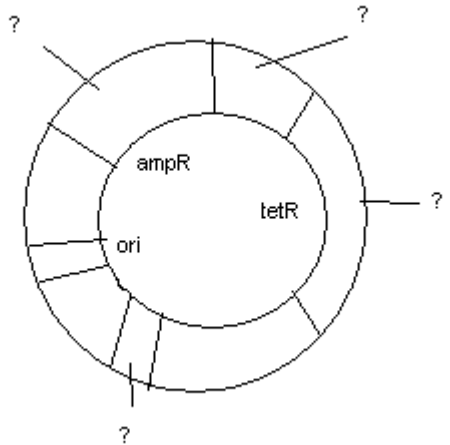
(b) cDNA

(c) dsRNA

4. Name the diagram and label the parts 'a' to 'e'.



5. (i) Label the following diagram & Identify the selectable markers (2)



(ii) What is the role of polymerase? (1)

6. Two of the steps involved in producing rDNA are mentioned below. Write the missing steps in its proper sequence?

a. Isolation of DNA,

b.

c.

d.

e. ligation of the DNA fragment into a vector,

f.-----

g.-----.

7. Describe the naming of restriction enzymes with an example.
8. What is insertional inactivation? Which enzyme is used in this process? What type of colour it gives in its reaction?
9. What Polymerase Chain Reaction? Name the steps involved in this process? Name the bacteria used to isolate thermostable polymerase enzyme.

5 MARKS

1. (a) Explain how recombinants and non- recombinants are differentiated on the basis of colour production in the presence of a chromogenic substrate. Name that procedure.

(b) Describe the temperature treatment (-3 step-) that enhances the bacteria to take up the rDNA.
2. What features are required to facilitate as cloning vector? Describe in brief.
3. Diagrammatically represent the experimental set up in cloning and expressing a human genome like growth hormone in to a bacterium E.coli

Questions with hints of model answers

Section - A: 1 Mark Questions [To be answered in one word in one sentence]

1. What is plasmid?
Answer: Plasmid is autonomously replicating circular extra chromosomal DNA.
2. Write any two ways of introducing foreign DNA into host cell.
Answer:
 - i) Micro-injection
 - ii) biolistics/ gene gun.
3. Which compound is used for staining DNA in gel?
Answer: Ethidium Bromide.
4. Name the enzyme that digest bacterial cell wall in genetic engineering.
Answer: Lysozyme.

Section – B: 2 Marks Questions [To be answered in 20 to 30 words approximately]

1. A farmer found that agrochemicals cause pollution of soil and water and are too expensive. What alternative should be use?

Answer: Farmer should use genetically modified crops and could have organic farming as a possible solution for given problems.

2. What are the key tools needed for the recombinant technology?

Answer: The key tools needed for the recombinant technology to be accomplished are:-

- i) cell culture with desired DNA
- ii) restriction enzymes
- iii) DNA polymerase
- iv) Ligases
- v) Vector
- vi) Host organism/ Cell

3. To which class of enzyme restriction enzyme belong and write its types.

Answer: Restriction enzyme belong to a class of enzyme, called nuclease. There are following two kinds of restriction enzymes:-

- a) Exonucleases, which remove nucleotides from two ends of DNA.
- b) Endonucleases, which cut the DNA at specific position anywhere in its length.

8. What is palindrome nucleotide sequence?

Answer: The recognition sequence is a palindrome, where the sequence of base pairs reads the same on both the DNA strands.

Example- 5'- GAATTC- 3'
 3'- CTTAAG- 5'

4. What is vector in genetic engineering? Write its one property.

Answer: Plasmid and bacteriophages are the commonly used vectors, the vector should have a few (or) at least one unique recognition site, to link the foreign/ alien DNA.

5. What is marker?

Answer: A marker is a gene, which helps in selecting those host cells which contain the vector (transformant) and eliminating the non-transformants.

Section – C: 3 Marks Questions [To be answered in 30 to 50 words approximately]

1. Why are cloning vectors necessary in cloning? Name any two such vectors that are used in experiments with Escherichia coli.

Answer: Cloning vectors are necessary in cloning because these are the vehicle for cloning. The vector carries a foreign DNA sequence into a given host cell. Bacterial plasmids and bacteriophages are considered the most useful.

Name of two vectors are:-

- i) PBR 322 and
- ii) PUC (named after University of California) is reconstructed plasmid vector.

2. What is genetic engineering? List the steps involved in rDNA technology?

Answer: Genetic engineering is a technique of molecular biology which includes deliberate manipulation of genetic make up of an organism, synthesis of new DNA, repair of DNA etc.

Recombinant DNA (rDNA) technology involves the following steps:-

- i) Isolation of DNA.
- ii) Fragmentation of DNA by restriction endonuclease
- iii) Isolation of the desired DNA fragment.
- iv) Amplification of the gene of interest.
- v) Ligation of two DNA fragment into a vector using DNA ligase.

4. Which convention do you use to write name of restriction enzymes?

Answer: first of all scientific name, strain and then order of enzyme isolation is written in roman. Example- EcoRI where,

E- Escherichia (Genus) Co- coli (species)

R- Strain of organism I- order of enzyme isolation.

5. What is palindrome in DNA? Give one example.

Answer: Palindrome in DNA is a sequence of base pairs that pairs that reads same in two strands when orientation of reading is kept the same.

5'- GAATTC- 3'

3'- CTTAAG- 5'

These two strands read same in 5'-3' direction as well as in 3'-5' direction.

6. Define the terms:

- i) Recombinant DNA
- ii) sticky ends
- iii) Plasmid
- iv) gene gun
- v) Transgenic
- vi) Bioconversion

Answer:

- i) Recombinant DNA: It is the DNA formed by combining DNA from two different organisms.
- ii) Sticky ends: refers to the single stranded proteins at the ends of DNA when cut by a restriction enzyme.
- iii) Plasmid: extra chromosomal fragments of DNA present in bacteria which is circular.
- iv) Gene Gun: bombarding micro projectiles coated with foreign DNA with great velocity into the target cell.
- v) Transgenic: genetically modified organisms that contain a foreign gene.

- vi) Bio conversion: raw materials are biologically converted into specific products using microbial, plant or animal cells.

Section – D: 5 Marks Questions [To be answered in 80 to 120 words approximately]

1. What are Bioreactors? Write its types and describe them.

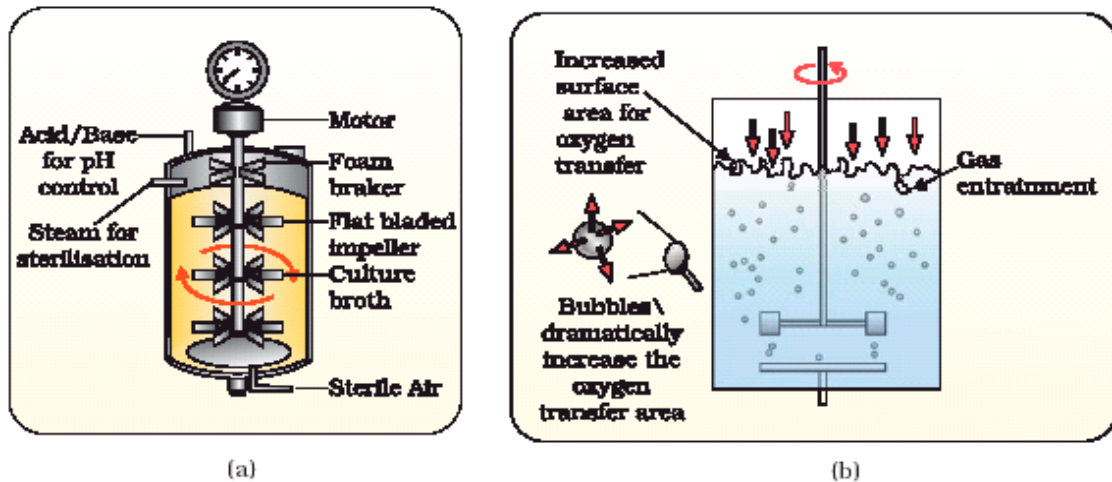
Answer: Bioreactors: These bio appliances could be considered as vessels in which certain raw materials are biologically converted into specific products by microbes (or) plants/ animals cells and/or their enzymes.

Two common types of bioreactors are the following:-

- a) Simple stirred tank bioreactor
- b) Sparged stirred tank bioreactor

Stirred tank bioreactors are the most commonly used bioreactors. In a simple stirred tank bioreactor, there is usually a cylindrical vessel (or) a vessel with a curved base to facilitate mixing of the content.

But in the sparged stirred tank bioreactor sterile air bubble are sparged.



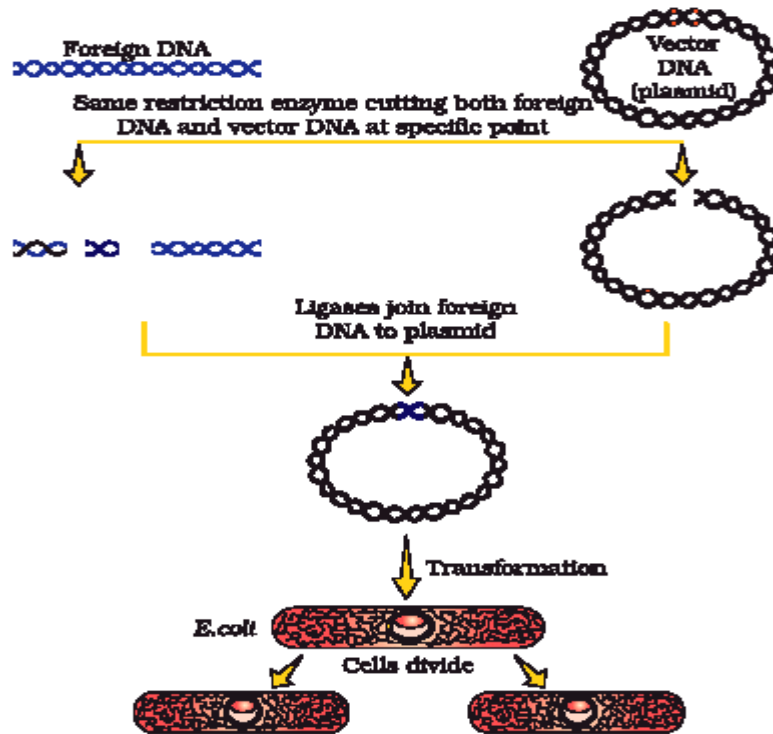
(a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

2. What is rDNA? Explain the technique of cloning DNA.

Answer: To alter the chemistry of genetic material by gene cloning and gene transfer.

Techniques involved in Recombinant DNA:-

- i) Isolation of DNA.
- ii) Fragments of DNA by restriction Endonucleases.
- iii) Isolation of desired DNA fragments.
- iv) Amplification of the gene.
- v) Ligation of the DNA fragments.
- vi) Transfer of recombinant DNA into the host.
- vii) Culturing the host cells.
- viii) Extraction of desired product.



Diagrammatic representation of recombinant DNA technology

HOTS Questions:

1. Each restriction enzyme cuts the DNA at a specific nucleotide sequence. Name such a sequence.
2. Traditional Hybridization procedures to produce hybrid varieties of plants and animals, used to introduce one or more undesirable genes along with the desirable ones. How is this problem overcome by biotechnology?
3. One or very few recognition sites are preferred in a vector than many. Give reason.
4. What is the role of enzymes like Chitinase, Lysozymes and Cellulase in genetic engineering?
5. To use the restriction enzymes for cutting the DNA, The DNA must be in the pure form, i.e., it should be free from associated compounds like proteins and RNA. How is it achieved?
6. 5'- GAATTC-3'
3'-CTTAAG-5' is the palindromic sequence of a restriction enzyme. Name the enzyme and indicate the site where it cuts this palindromic sequence.
7. Why do prokaryote (Bacteria) has restriction enzymes whereas eukaryotes do not have? Explain.
8. What are the techniques included under biotechnology?
9. Restriction enzyme that cuts the DNA strands a little away from the centre of the recognition / palindromic sequence are more useful in rDNA construction. Justify with examples.

has

