BIOLOGY

Chapter 9: Biotechnology Principal and Processes

Biotechnology Principal and Processes

Biotechnology Principles and Processes:

Biotechnology is the field of biology which is used to develop various technologies that help in the production of certain products that result in the welfare of human beings. It consists of various applications in different fields that include therapeutics, processed food, diagnostics, waste management, genetically modified crops, energy production, etc. The definition of biotechnology given by the European Federation of Biotechnology states that "The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services."

Principles of Biotechnology:

Modern biotechnology is based on two core techniques that are:

Genetic Engineering: Genetic engineering is the direct manipulation of an organism's gene by the use of biotechnology which is used to change the genetic makeup of the cell. The set of technologies are used for the genetic makeup of the cells which includes the transfer of genes in the species boundaries for the production of improved organisms, most importantly called clones resulting in gene cloning.

Maintenance of a Sterile Environment in Chemical Engineering Processes: It helps in the growth of only those microbes that are required and this process helps in the manufacturing of vaccines, antibiotics, drugs, etc.

Basic Principles of Biotechnology:

Genetic engineering involves the isolation and introduction of only those genes into an organism that is desired and does not introduce undesirable genes. The steps involved in genetic engineering are:

- Development of recombinant DNA (rDNA).
- Cloning of the desired gene.
- Transfer of the cloned gene into the suitable host organism.

Origin of Replication (ori): The sequence of chromosomes in the DNA that helps in the initiation of the relocation of DNA. The foreign DNA that is inserted into the host organism needs to be attached to the origin of relocation and this results in the formation of multiple copies of the DNA while if the foreign gene is not attached to the origin of replication then it may not result in the multiplication of DNA.

Cloning: The process of formation of several identical copies of the DNA template.

Plasmid: An extrachromosomal, circular DNA material that helps in the replication of DNA. they are used as cloning vectors and also helps in the process of gene expression. Here, a foreign gene is inserted into the plasmid which then multiplies and results in the formation of several copies of the desired gene.

Antibiotic Resistance Gene: In the case of certain microorganisms there are several genes that have the ability to grow when there is a specific antibiotic present while the genes provide resistance against them. These genes are found to be located on the plasmids and are used in the process of cloning and transformation.

Restriction Enzymes: These enzymes are responsible for the cutting of DNA fragments at specific sites, thus they are called the “molecular scissors”. These enzymes cut the DNA at a particular site that is specific for each restriction enzyme. They help in the process of cutting the sedated gene which is then inserted into the specific locations of the vector or the host DNA.

Vectors: They are the plasmids that help in the process of multiplication and then the transfer of genes from one organism to the other.

Ligase: They are those enzymes that joined together the fragrant of DNA that contains the desired gene and the DNA of the host. They help in the sticking of fragments of DNA together.

The basic steps in the genetic modification of an organism:

- Identification of desired DNA fragments.
- Introduction of desired DNA fragments into a suitable host.
- Maintaining foreign DNA in the host and its transfer to the progeny.

Tools for Genetic Engineering (Recombinant DNA Technology):

Restriction enzymes also called molecular scissors are used to simply cut the DNA which is then inserted into the vector. These restriction enzymes help in the addition of the methyl groups to the DNA that results in the restriction of the digestion of their own DNA. These enzymes cut DNA fragments at their particular recognition sequences.

Recognition Sequences: The bases of the DNA sequence that are specific for each restriction enzyme and act as the site for restriction or cutting resulting in the formation of the palindromic sequences.

There are two types of restriction enzymes: endonucleases and exonucleases.

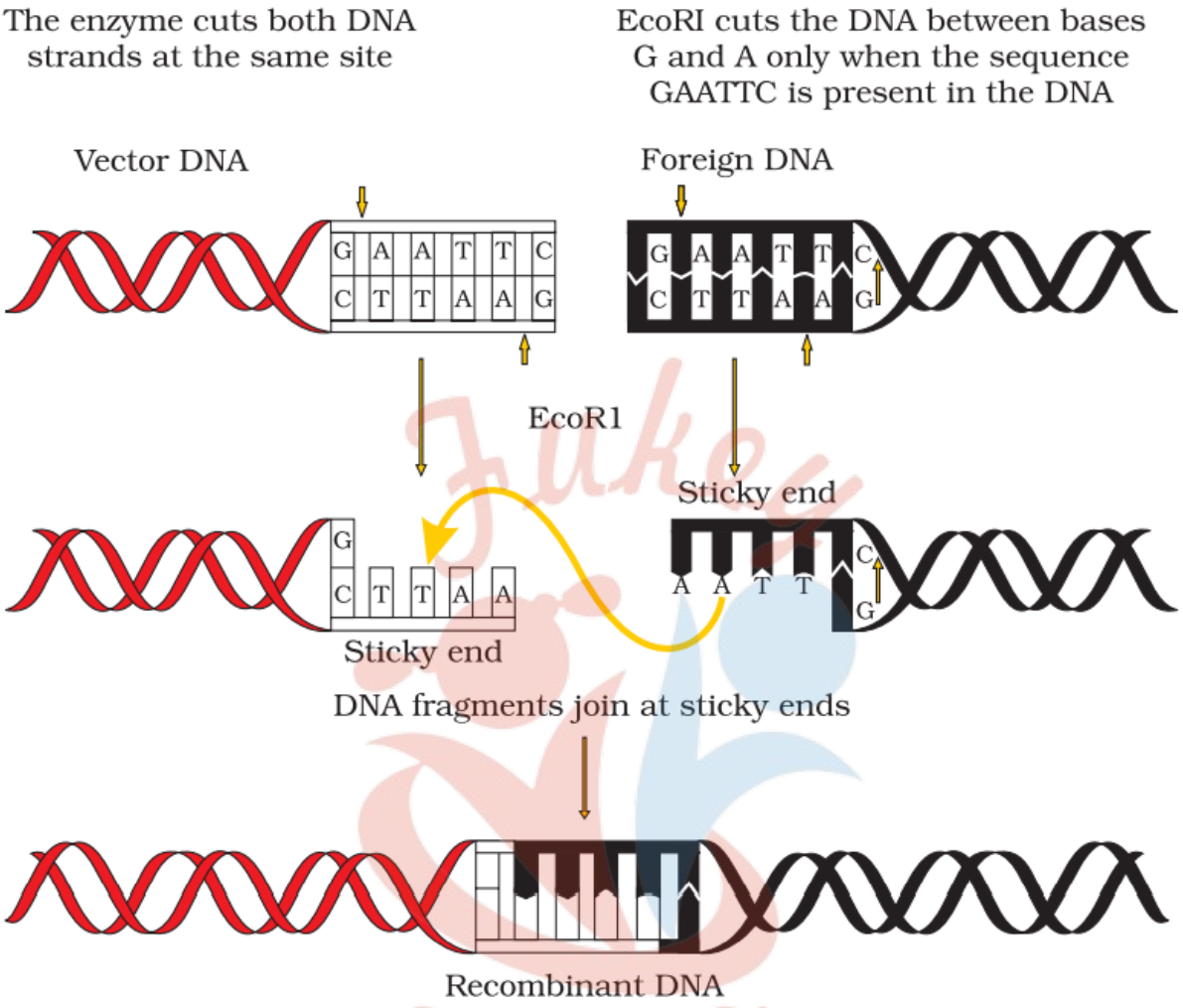
Endonucleases: These enzymes are responsible for the cutting of the DNA in the middle while the exonucleases enzymes are responsible for the cutting of the DNA at the ends. Examples of restriction endonucleases are ECoR1, Hind III, etc. Restriction enzymes cut the DNA molecule at a specific site that is known as a restriction site. Each endonuclease characterized the restriction site by a specific recognition sequence. Each restriction endonuclease is responsible for the identification of the specific palindromic nucleotide sequence in the DNA. The Palindromic DNA sequence of the base pairs is present on the two strands of DNA in the same order when the orientation of reading is kept the same.

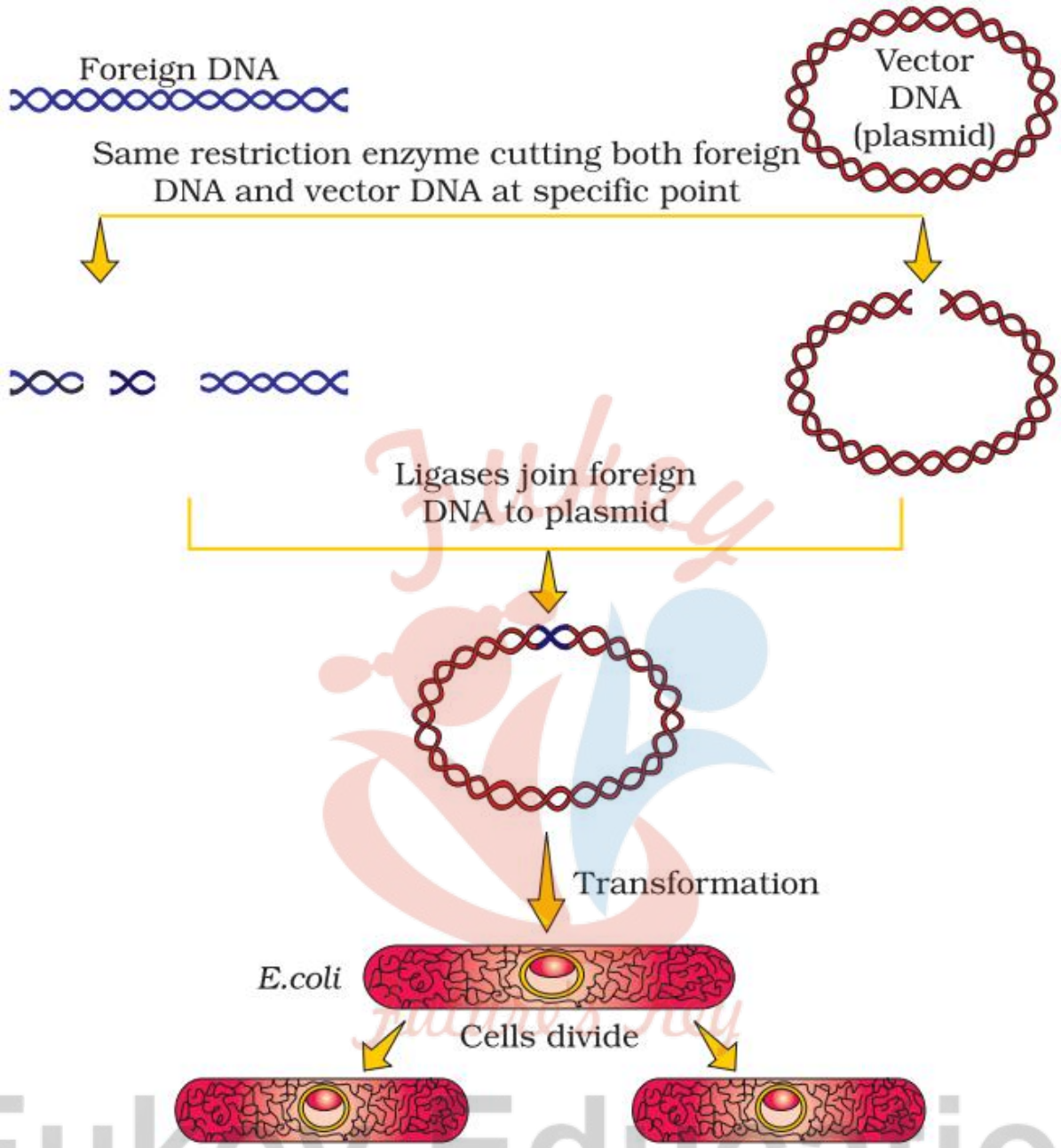
Ligases: Ligases are the enzyme that is responsible for the joining of the two DNA fragments. The process of ligation occurs in the presence of sticky ends (they are the similar overhanging sequences formed due to the action of the same restriction enzyme).

Palindromic nucleotide sequences: Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it will bind to the DNA and cut each of the two strands of the double helix at specific points in their sugar-phosphate backbones Each restriction endonuclease recognizes a specific palindromic nucleotide sequences in the DNA.

Restriction Enzymes: the two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated. One of these added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease.

Action of Restriction enzyme





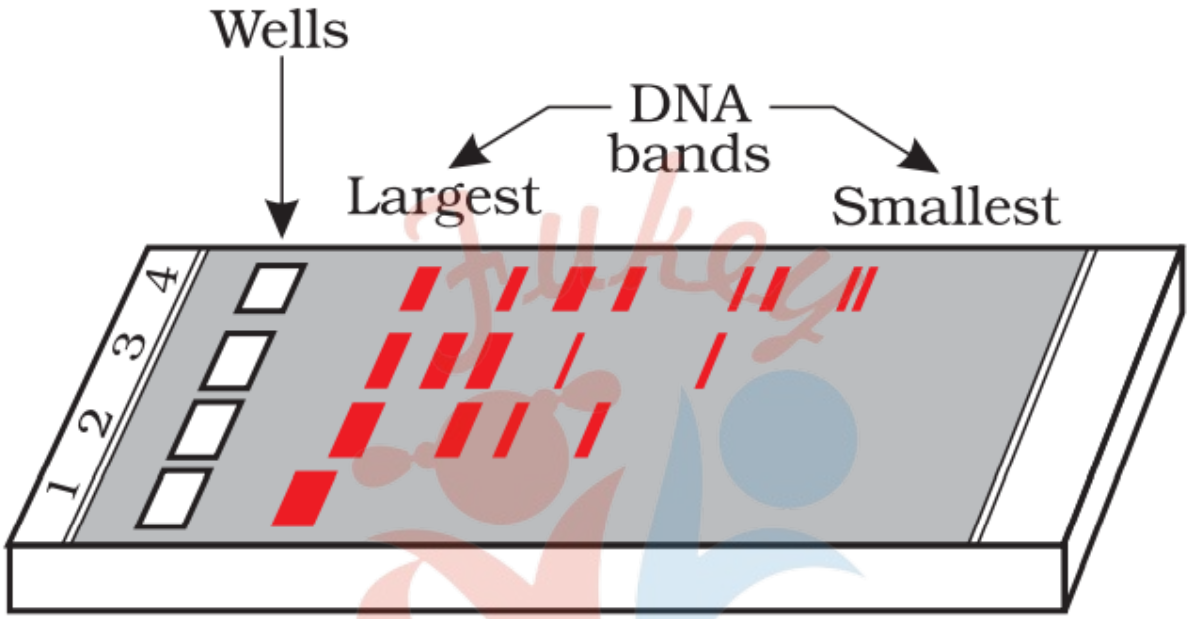
Separation and Isolation of DNA Fragments:

The technique called gel electrophoresis is responsible for the separation of the DNA fragments obtained through restriction.

Gel Electrophoresis: The process of migration of negatively charged DNA towards the positively charged electrode through a porous polymer gel matrix when the electric current is passed in an electric field. The DNA fragments will then start to move in the gel and will separate or resolve based on their size as well as the pore size of the gel. The smaller DNA fragments will be able to cover the larger distance while the larger DNA fragments will cover a smaller distance. They commonly use gel matrix for the process of DNA electrophoresis is agarose which is obtained from seaweeds.

Visualization: To observe the DNA fragments they first need to be stained by the compound called ethidium bromide (EtBr) since they cannot be observed directly and are then exposed to the UV light this will result in the fluoresces of DNA.

Elution: The process of elution involves the purification of the desired DNA fragments using various methods from the gel.



Cloning Vectors:

Vector is any DNA molecule that is responsible for the carrying of the desired gene that needs to be inserted into the host organism. For example, plasmid. The plasmid is an extrachromosomal autonomously replicating genetic content that is present in the bacteria and is different from the other chromosomal DNA. It helps in the transfer of desired genes into the host cell. Plasmids consist of an origin of replication, it is the site responsible for the replication as soon as the gene of interest enters the host cell. It also contains the antibiotic resistance gene.

Following features are required for a cloning vector:

Origin of Replication: This is known as ori. This helps in the replication of DNA fragments into the host cell and results in the maintenance of the number of copies of DNA.

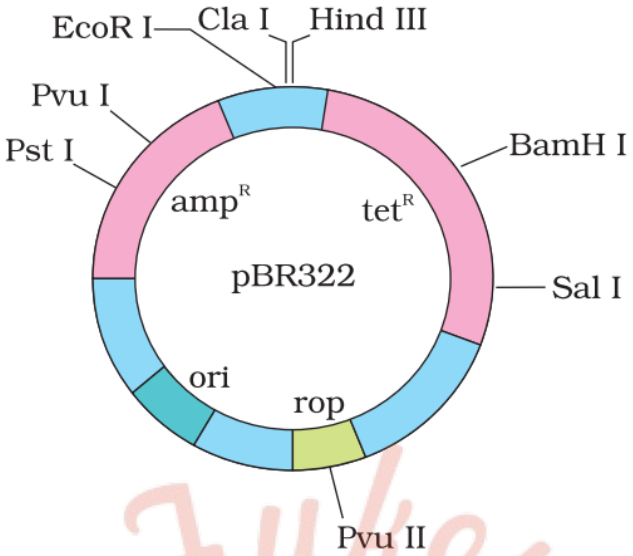
Selectable Marker to Identify Transformed Cells: The process of introduction of a piece of DNA into the host cells is known as the transformation. The genes that encode resistance towards certain antibiotics such as ampicillin, chloramphenicol, tetracycline, or kanamycin, etc. are some of the useful selectable markers for E. coli and in the absence of these selectable markers, the normal E. coli cells do not show any resistance against any of these antibiotics.

There Should be a Cloning Site in the Cloning Vector: There must be one cloning site present so as not to complicate the process of cloning. The antibiotic resistance gene present as the restriction sites are responsible for the ligation of the foreign DNA. When the desired gene is introduced at the site of the antibiotic resistance gene resulting in the loss of antibiotic resistance. This results in the loss of antibiotic resistance in the recombinant plasmid. So, recombinants can be selected from the non-recombinants. Another method is insertional inactivation which is used to find out the transformed cells. This is based on the ability to produce colour when the chromogenic substrate is present. In this technique, the recombinant DNA is introduced into the coding sequence of an enzyme, β-galactosidase. Beta-galactosidase converts galactose into lactose. If a gene is introduced into this region, the formation of the β-galactosidase will not, and thus there will be no formation of lactose resulting in the inactivation of the enzyme which is called insertional inactivation. The blue colour of the non-transformed colonies occurs due to the presence of a chromogenic substrate while no colour is produced in the colonies if the insertional inactivation of the galactosidase occurs due to the presence of the gene of interest. These colonies can be named recombinant colonies.

Insertional Inactivation: The process of introduction of the desired gene in the coding region of DNA that results in the inactivation of an enzyme.

Vectors for Cloning in Plants:

A pathogen of various dicot plants, Agrobacterium tumefaciens is used as a vector for the plants. It is responsible for carrying the piece of DNA known as 'T-DNA' that results in the transformation of the normal plant cells into a tumor which then results in the production of the chemicals that are required by the pathogen. The desired gene is introduced along with the other required genes into the T-DNA that result in the transformation of the plant cells. The tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens is modified into a cloning vector which is no more pathogenic to the plants. In plasmids, the growth regulator is the coding genes of the cytokinin and auxin. The sources of energy are the gene codes responsible for the catabolism of opine. The transfer of T-DNA into the required host plant cell requires the right and left borders. Similarly, in the case of animal cells, the retroviruses have been modified to act as vectors.



Competent Host:

The bacterial cells need to be competent in order to take up the DNA which can be achieved by treating the cells with a specific concentration of divalent ions such as calcium ions, which results in the formation of pores in the cell wall of the bacteria. These bacteria are prone to heat shock. In this method, the calcium-treated competent cells are kept on ice, then they are incubated briefly at 42◦C for 1-2 minutes, and then immediately placed in ice. This converts the rDNA into the competent cell. Other methods used for the insertion of DNA into the host cells are microinjection, biolistic, gene gun, etc. By the method of microinjection, the DNA can be inserted directly into the nucleus of the host cell while in the case of biolistic, a high-velocity microparticle of gold or tungsten coated with DNA is required.

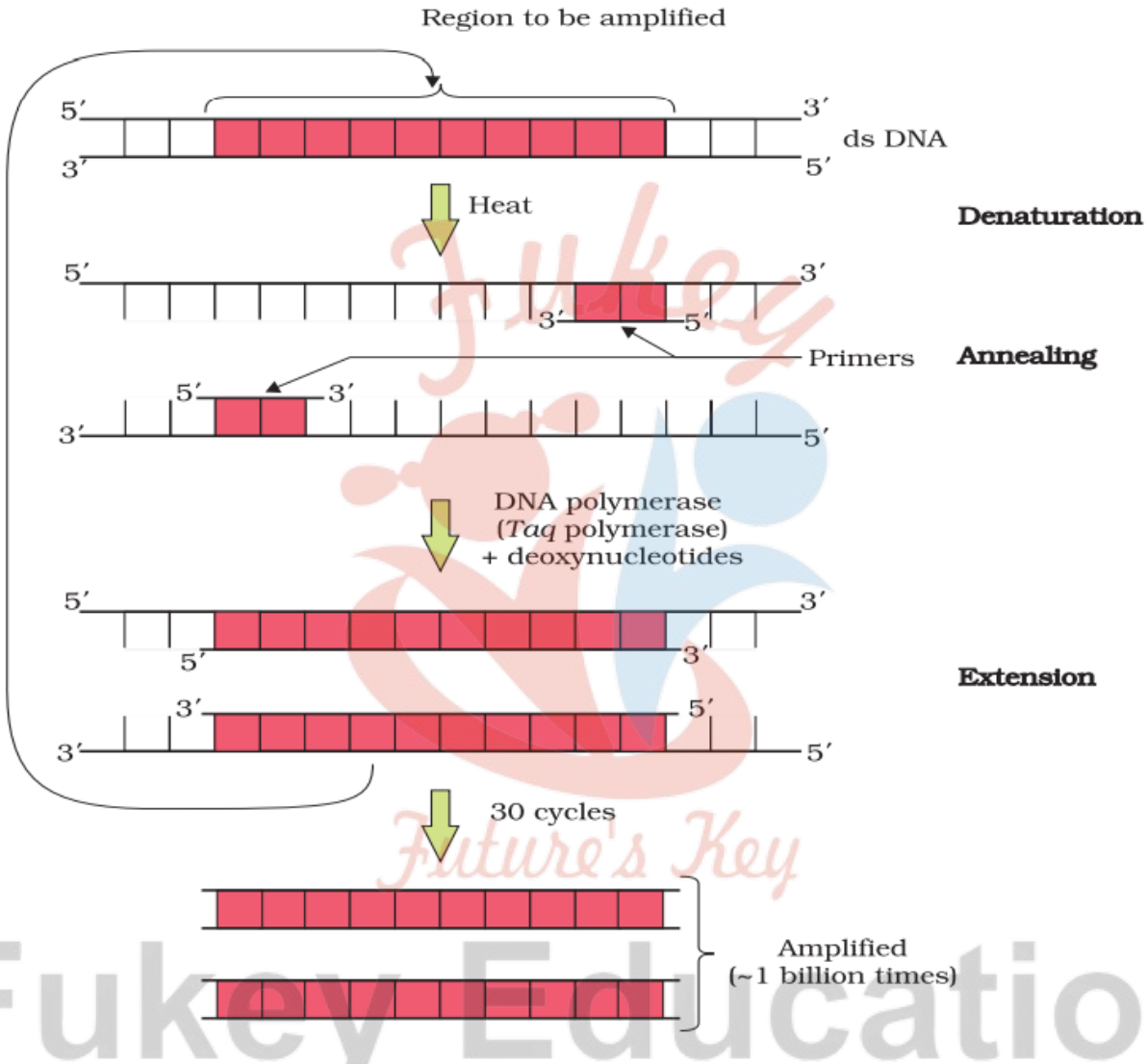
Process of Recombinant DNA Technology:

There are several steps involved in the process of recombinant DNA technology.

Isolation of the Genetic Material: The membrane surrounding the DNA needs to be removed to isolate the DNA. This can be done with the help of lysozyme enzymes that result in the breaking of the cell walls of the cells of bacteria, breaks cellulase (in case of plant cells), and chitinase (in case of fungus). The RNA can be isolated with the help of ribonucleases while proteins can be removed using proteases. Lastly, the DNA obtained is treated with ethanol so as to remove the remaining impurities. DNA is then obtained as fine threads in suspension.

Restriction Digestion of the Isolated DNA: The restriction digestion of the DNA is progressed with the help of the agarose gel electrophoresis. The desired gene is then introduced into the specific vector and is joined with the help of an enzyme known as a ligase which results in the formation of the recombinant DNA molecule.

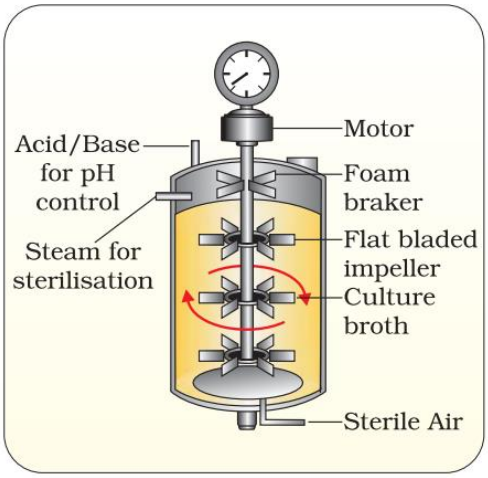
Amplification of Gene of Interest Using PCR: The amplification of the desired gene of the DNA can be done by the process of the Polymerase chain reaction (PCR). There are two sets of primers required that are the forward primer and the reverse primer. The DNA amplification is done with the help of the DNA polymerase enzyme. Taq polymerase is the most commonly used polymerase during PCR.

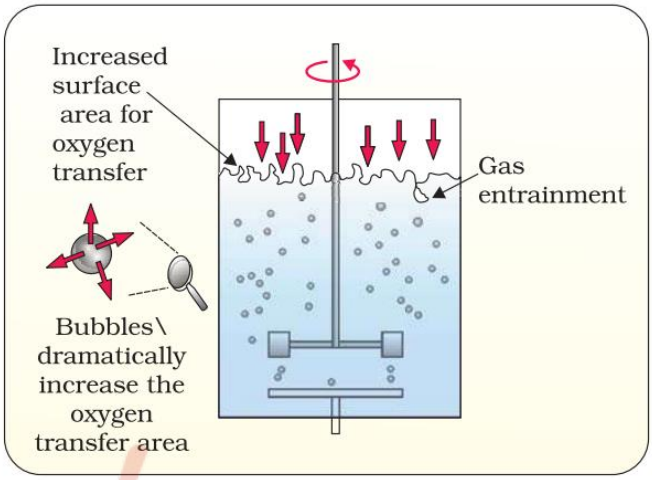


Insertion of Recombinant DNA Into Host Cell or Organism: The host cells need to be more competitive so as to receive the recombinant DNA.

Expression of Desired Protein: The main aim of the recombinant DNA technology is to obtain desired protein of interest. Thus, the protein which is obtained is known as a recombinant protein.

Bioreactors: Bioreactors are the large vessels that are used to produce large quantities of recombinant protein. To achieve the desired product the optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen) are provided by the bioreactors.





Basic Parts of a Bioreactor:

- Agitator
- Oxygen Control system
- Foam control system
- Temperature control
- pH control
- Sampling port
- Inlet
- Outlet

There are mainly two types of bioreactors: Stirred type and the sparger type.

Stirring Type Bioreactor:

The stirrer type of bioreactor consists of a stirrer that are having a curved base and functions in the better mixing of the contents. It also improves the aeration of the medium.

Sparger Type Bioreactor:

In the sparger type of bioreactor, the air is bubbled that is generated from the base of the bioreactor which results in the mixing as well as aeration of the contents.

Downstream Processing:

The downstream processing involves those processes and methods that are responsible for the separation and purification of the desired product. The products produced in the case of drugs need to be formulated suitably and also the drugs need to be tested before they are made available commercially.

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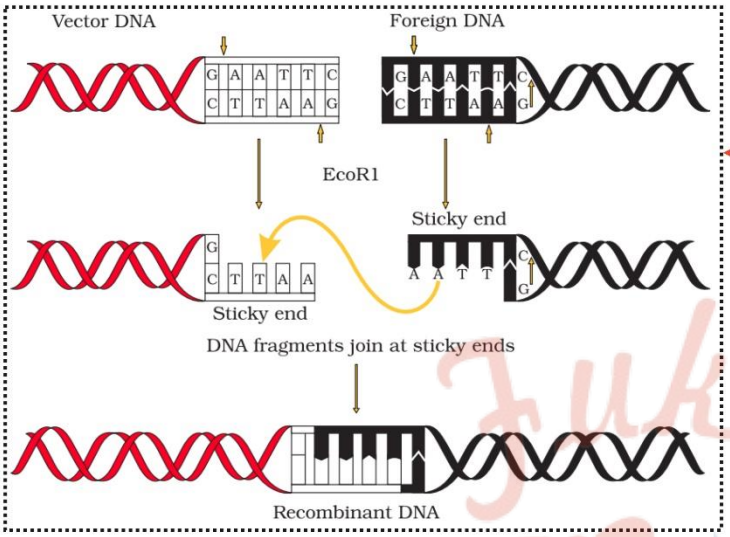


Restriction Enzymes

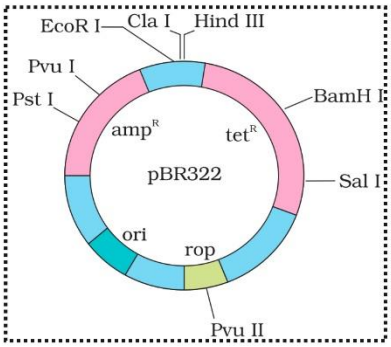
Biolistics
Cell are bombarded with high velocity micro particles of gold or tungsten coated with DNA

Micro injection
rDNA is directly injected into the nucleus of an animal cell

Disarmed pathogen vector
Vectors like Agrobacterium when infects the cell transfer the recombinant DNA into the host

Host is made competent by:

Competent host



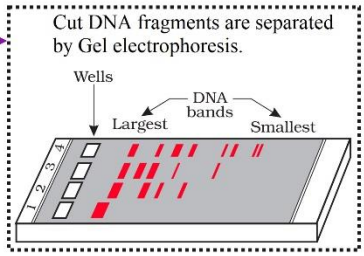
Cloning vector

Features of vector

- Has Ori
- Has Selectable Markers
- Has fewer cloning sites

Tools of rDNA Technology

It is the integration of natural science and organisms, cells, parts thereof, and molecular analogues for product and services



Biotechnology Principles and Processes

Cutting of DNA at specific locations by restriction enzymes

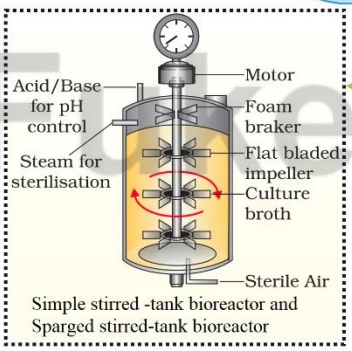
Processes of rDNA Technology

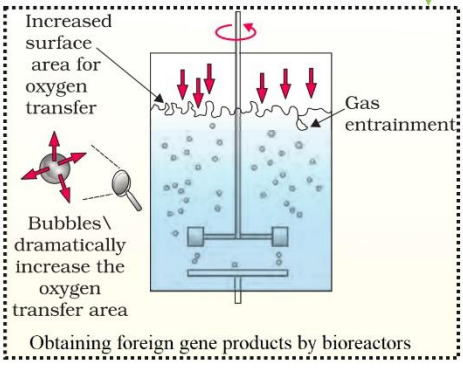
Transfer of rDNA into the Host cell

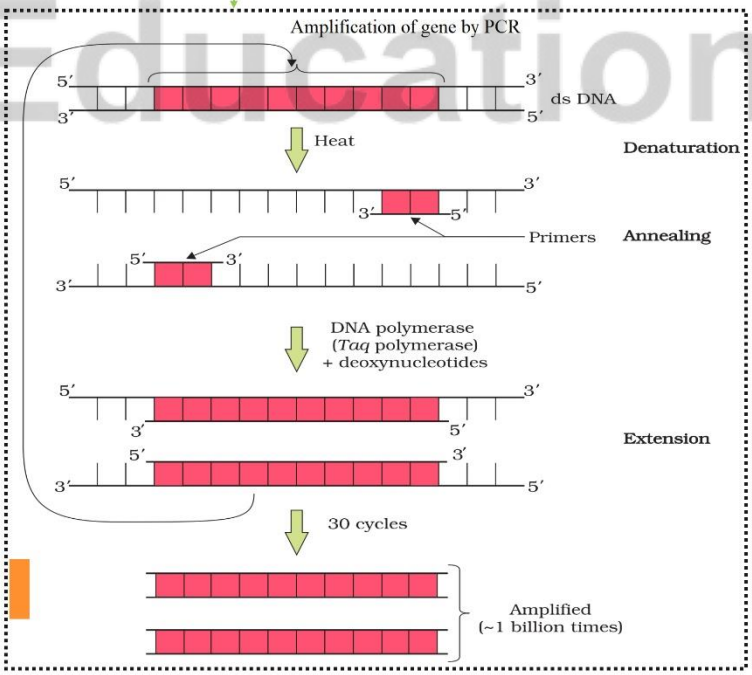
Obtaining foreign gene product by bioreactors

- rDNA
- Gene cloning
- Gene transfer

Principles of Genetic engineering







Important Questions

➢ Multiple Choice Questions:

1. Rising of dough is due to

   (a) Multiplication of yeast
   (b) Production of CO_2
   (c) Emulsification
   (d) Hydrolysis of wheat flour starch into sugars.

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

   (a) endonuclease
   (b) exonuclease
   (c) DNAligase
   (d) Hind-ll.

3. The transfer of genetic material from one bacterium to another through the mediation of a vector-like virus is termed as:

   (a) Transduction
   (b) Conjugation
   (c) Transformation
   (d) Translation.

4. Which of the given statement is correct in the context of observing DNA separated by agarose gel electrophoresis?

   (a) DNA can be seen in visible light
   (b) DNA can be seen without staining in visible light
   (c) Ethidium bromide stained DNA can be seen in visible light
   (d) Ethidium bromide stained DNA can be seen under exposure to UV light.

5. 'Restriction' in Restriction enzyme refers to:

   (a) Cleaving of phosphodiester bond in DNA by the enzyme
   (b) Cutting of DNA at specific position only
   (c) Prevention of the multiplication of bacteriophage in bacteria
   (d) All of the above.

6. A recombinant DNA molecule can be produced in the absence of the following:

   (a) Restriction endonuclease
   (b) DNA ligase
   (c) DNA fragments
   (d) E. coli.

7. 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 the above.

8. The most important feature in a plasmid to be used as a vector is:

(a) Origin of replication (ori)
(b) Presence of a selectable marker
(c) Presence of sites for restriction endonuclease
(d) Its size.

9. While isolating DNA from bacteria, which of the following enzymes is not used?

(a) Lyozyme
(b) Ribonuclease
(c) Deoxyribonuclease
(d) Protease.

10. 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.

11. An antibiotic resistance gene in a vector usually helps in the selection of:

(a) Competent cells
(b) Transformed cells
(c) Recombinant cells
(d) None of the above.

12. Significance of 'heat shock' method in bacterial transformation is to facilitate:

(a) Binding of DNA to the cell wall
(b) Uptake of DNA through membrane transport proteins
(c) Uptake of DNA through transisent pores in the bacterial cell wall and plasma membrane
(d) Expression of antibiotic resistance gene.

13. A biotechnologist wanted to create a colony of E.coli possessing the plasmid pBR322, sensitive to Tetracycline. Which one of the following restriction sites would he use to ligate a foreign DNA?

(a) Sal I
(b) Pvu I
(c) EcoRI

(d) Hind III

14. Which of the following steps are catalysed by Taq polymerase in a PCR reaction?

(a) Denaturation of template DNA
(b) Annealing of primers to template DNA
(c) Extension of primer end on the template DNA
(d) All of the above.

15. A bacterial cell was transformed with a recombinant DNA that was generated using a human gene. However, the transformed cells did not produce the desired protein. Reasons could be:

(a) Human gene may have intron which bacteria cannot process
(b) Amino acid codon for humans and bacteria are different
(c) Human protein is formed but degraded by bacteria
(d) All of the above.

➢ Very Short Question:

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 EcoRI.

5. A linear DNA fragment and a plasmid has three restriction sites for EcoRIhow 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. Name the substance used as a medium in gel electrophoresis.

8. What is Bioconversion?

9. Name the bacterium that yields thermostable DNA polymerase.

10. Which enzymes are known as “molecular Scissors”?

➢ Short Questions:

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

2. What are ‘Selectable marker’? What is their use in genetic engineering?

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

4. What is "Insertional Inactivation"?
5. What are the two basic techniques involved in modern Biotechnology?
6. Differentiate between plasmid DNA and chromosomal DNA?
7. What is the role of enzyme "Ligase" in genetic Engineering?
8. Name the components a bioreactor must possess to achieve the desired product?

➢ Long Questions:

1. How is recombinant DNA transferred to host?
2. Explain any three methods of vector less gene transfer.
3. Write a note on the cloning vector.

➢ Assertion and Reason Questions:

1. Two statements are given-one labelled Assertion and the other labelled Reason. 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.

Assertion: The insertion of DNA fragment into pBR 322 plasmid using enzyme Pst I or Pvu I make ampicillin resistant gene non functional.

Reason: Bacterial cells containing recombinant pBR322 is unable to grow in the presence of ampicillin.

2. Two statements are given-one labelled Assertion and the other labelled Reason. 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.

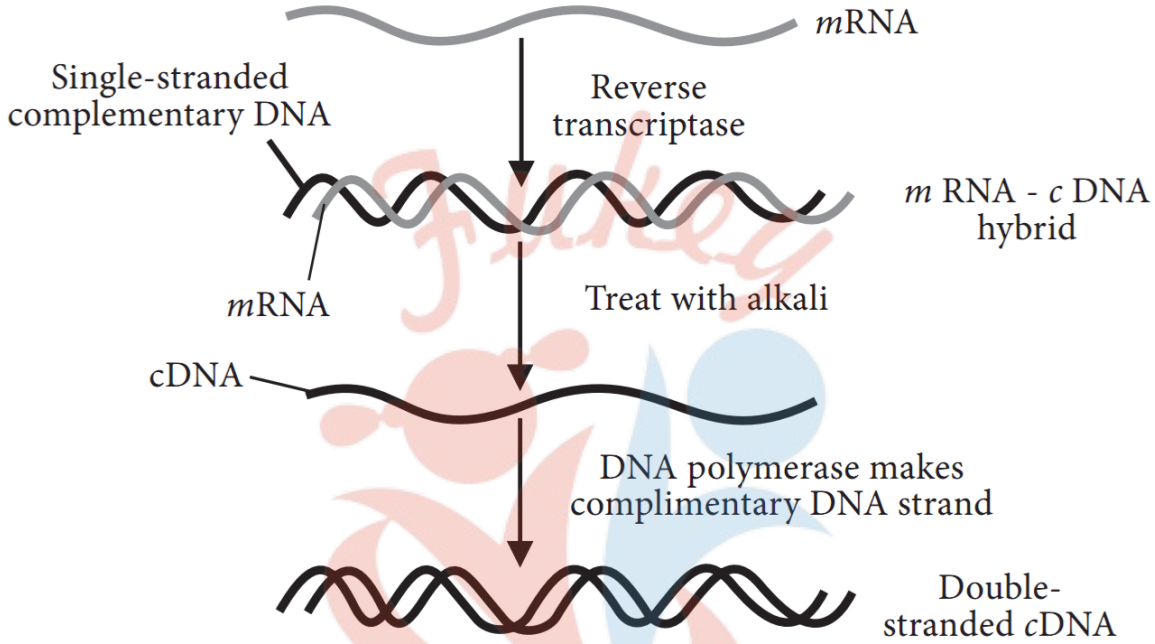
Assertion: Soil inhabiting bacterium Agrobacterium tumefaciens is called a natural plant genetic engineer.

Reason: Agrobacterium tumefaciens produce crown galls in several dicot plants.

➢ Case Study Questions:

1. Read the following and answer any four questions from (i) to (v) given below:

The DNA, which is transferred from one organism into another by joining it with the vehicle DNA is called passenger or foreign DNA. Generally three types of passenger DNAs are used. These are complementary DNA (cDNA), synthetic DNA (sDNA) and random DNA. Complementary DNA (cDNA) is synthesized on RNA template (usually mRNA) with the help of reverse transcriptase. Synthetic DNA (sDNA) is synthesized on DNA template or without a template. Random DNA are small fragments fanned by breaking a chromosome of an organism in the presence of restriction endonucleases.



(i) Reverse transcriptase enzyme was discovered by:

a) Temin and Baltimore.

b) Cohen and Boyer.

c) Arber and Nathan.

d) Paul Berg.

(ii) During cDNA formation, what would happen if DNA formed by reverse transcriptase is not treated with the alkali?

a) cDNA will not be digested.

b) mRNA will not be digested.

c) Hydrogen bonds will not form between base pairs.

d) rnRNA will not be formed.

(iii) Enzyme that helps in the formation of double stranded cDNA is:

a) DNA synthetase

b) Ligase

c) DNA polymerase

d) Helicase.

(iv) DNA polymerase can be obtained form:

a) Retrovirus.

b) Agrobacteriurn.

c) Tobacco mosaic virus.

d) Thermus aquaticus.

(v) DNA synthesised without a template is referred to as:

a) Complementary DNA.

b) Random DNA.

c) Synthetic DNA.

d) Z-DNA..

2. Read the following and answer any four questions from 9(i) to 9(v) given below:

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 these.

(iii) Assertion**:** The stirred-tank is well suited for large scale production of microorganisms under aseptic conditions.

Reason: In sparged stirred tank bioreactor, surface area for oxygen transfer is increased.

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.

(iv) 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.

(v) Vessels in which raw materials are biologically converted into specific products are.

a. Bioreactors.

b. Fermentors.

c. Gene guns.

d. Both (a) and (b).

✓ Answer Key-

➢ Multiple Choice Answers:

1. (b) Production of CO_2
2. (b) exonuclease
3. (a) Transduction
4. (d) Ethidium bromide stained DNA can be seen under exposure to UV light.
5. (d) All of the above.
6. (d) E. coli.
7. (b) Size only
8. (a) Origin of replication (ori)
9. (c) Deoxyribonuclease
10. (d) Availability of 'Thermostable' DNA polymerase.
11. (b) Transformed cells
12. (c) Uptake of DNA through transisent pores in the bacterial cell wall and plasma membrane
13. (a) Sal I
14. (c) Extension of primer end on the template DNA
15. (a) Human gene may have intron which bacteria cannot process

➢ Very Short Answers:

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. Agarose
8. Bioconversion refers to the process by which raw material are converted to specific product by microbial, plant or animal cell.
9. Thermusaquaticus.
10. Restriction Endonuclease.

➢ Short Answer:

1. 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. A selectable marker is a gene which helps in selecting those host cells which contains thevector & eliminating the non–transformanteg – gene encoding resistance to antibiotics are usefulSelectable markers as they allow Selective growth of transformants only.

3. The product obtained from genetic engineering is subjected to a series of processes collectivelycalled downstream processing before it made into final processes involved in downstreamprocessing are :- Separation & purification.

4. If a recombinant DNA is inserted within the coding Sequence of enzyme B–galactosidase. Thisresults into inactivation of enzyme which is referred to as “Insertional Inactivation”. The presenceof chromogenic Substrate gives blue–coloured colonies if the plasmid in bacteria does not have aninsert presence of insert results into insertional inactivation &the colonies do not produce anycolor.

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

   i. Genetic Engineering is the technique of altering the nature of genetic material or introduction of it into another host organism to change its phenotype.

   ii. Techniques to facilitate the growth & multiplication of only the desired microbes or cells in large number under sterile conditions for manufacture

6. Plasmid DNA is extranuclear DNA, found in protoplasmic whereas chromosomal DNA is the nuclear or genetic DNA which is found within the nucleus.

7. 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.

8. 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.

➢ Long Answer:

1. Transfer of recombinant DNA into the host:

   i. The bacterial cells must be made competent to take up DNA; this is done by treating them with a specific concentration of calcium, that increases the efficiency with which DNA enters the cell through the pores in its cell wall.

   ii. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice followed by placing them at 42°C and then putting them back on ice (heat shock treatment),

iii. Microinjection is a method in which the recombinant DNA is directly injected into the nucleus of the animal cell with the help of microneedles or micropipettes.

iv. Gene gun or biolistics is a method suitable for plant cells, where cells are bombarded with high-velocity microparticles of gold or tungsten coated with DNA.

v. Disarmed pathogens are used as vectors; when they are allowed to infect the cell, they transfer the recombinant DNA into the host.

2. Vectors of gene transfer. Following are common methods of vectors gene transfer.

i. Microinjection: Microinjection is the process/technique of introducing foreign genes into a host cell by injecting the DNA directly into the nucleus by using microneedle or micropipette.

ii. Electroporation: Electroporation is the process by which transient holes are produced in the plasma membrane of the (host) cell to facilitate entry of foreign DNA.

iii. Gene Gun: Gene gun is the technique of bombarding microprojectiles (gold or tungsten particles) coated with foreign DNA with great velocity into the target cell.

3. Cloning vectors:

i. Plasmids and bacteriophages are the commonly used vectors

ii. Presently genetically engineered/ synthetic vectors are also used for easily linking the foreign DNA and selection of recombinants from non-recombinants.

iii. The following features are required to facilitate cloning in a vector:

(a) Origin of replication (Ori)

(b) Selectable marker

(c) Cloning (Recognition) site

(d) Small size of the vector.

➢ Assertion and Reason Answers:

1. (b) Both assertion and reason are true but reason is not the correct explanation of assertion.

Explanation:

Plasmid pBR322 has a variety of unique restriction sites for restriction endonucleases. Two unique sites, Pst I and Pvu /are located within the amp' gene and Bamrll, Sal I etc. are within tet[1] gene. The presence of restriction sites within the marker tef and amp' permits an easy selection for cell transformed with the recombinant pBR322. Insertion of the DNA fragment into the plasmid using enzyme Pst I and Pvu I places the DNA insert within the gene amp' and make it non functional.

2. (b) Both assertion and reason are true but reason is not the correct explanation of assertion.

➢ Case Study Answers:

1.

(i) (a) Temin and Baltimore.

(ii) (b) mRNA will not be digested.

Explanation:

The cDNA formation involves the alkaline denaturation of the mRNA-cDNA hybrid. The double stranded DNA molecule formed after the activity of reverse transcriptase is treated with alkali to digest mRNA.

In a bioreactor, all operations must be carried under sterile conditions to avoid contamination.

(iii) (c) DNA polymerase

Explanation:

A cDNA strand is formed on the separated single stranded DNA template with the help of DNA polymerase enzyme.

(iv) (d) Thermus aquaticus.

(v) (c) Synthetic DNA.

2.

(i) (c) Processing large volume of culture.

Explanation:

Bioreactors are considered as vessels in which raw molecules are biologically converted into specific products.

(ii) (b) Sterile condition.

Explanation:

In a bioreactor, all operations must be carried under sterile conditions to avoid contamination.

(iii) (b) Both assertion and reason are true but reason is not the correct explanation of assertion.

(iv) (d) Temperature, pH and oxygen supply.

Explanation:

A bioreactor provides the optimal growth conditions such as temperature, pH, substrate, vitamins, oxygen and salts for obtaining the desired product.

(v) (d) Both (a) and (b).