For BioResire students

NEET Biology Material

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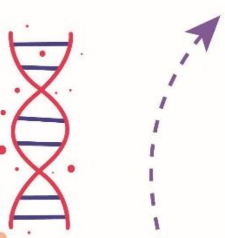
BIOTECHNOLOGY: PRINCIPLES & PROCESSES



PRINCIPLES OF BIOTECHNOLOGY

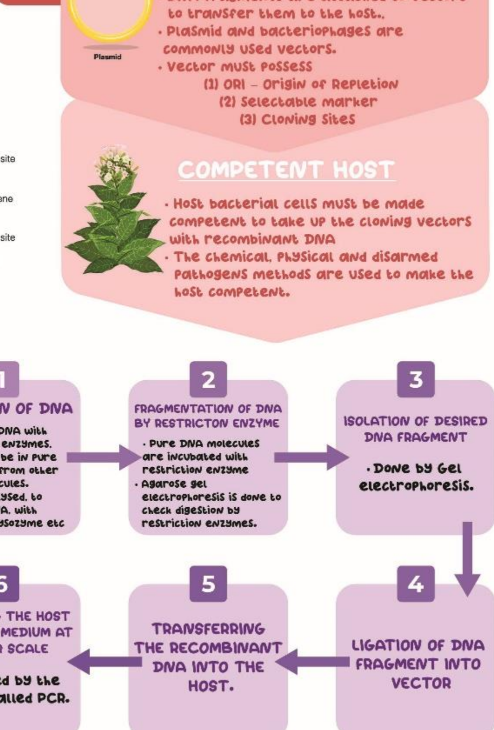
BIOPROCESS ENGINEERING

It includes rDNA. use of gene cloning and gene transfer to overcome the limitation of multiplication of undesirable genes during hybridization

GENETIC ENGINEERING

It involves the maintenance of a sterile ambience in chemical engineering processes to enable the growth of only desired cell in large number

TOOLS



RESTRICTION ENZYMES

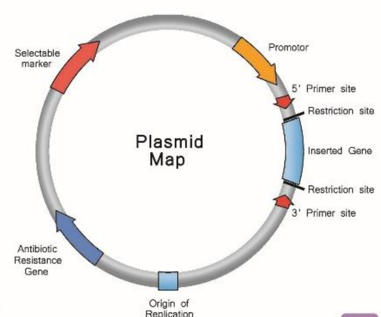
- Also celled molecular scissors.
- Cut the DNA at specific site into fragments.
- Two types :-
  (1) Exonuclease – cut from ends
  (2) Endonuclease – cut within these strand

CLONING VECTORS

- Vehicles of DNA fragments.
- DNA fragments are attached to vectors to transfer them to the host..
- Plasmid and bacteriophages are commonly used vectors.
- Vector must possess
  (1) ORI – Origin of Repletion
  (2) Selectable marker
  (3) Cloning sites

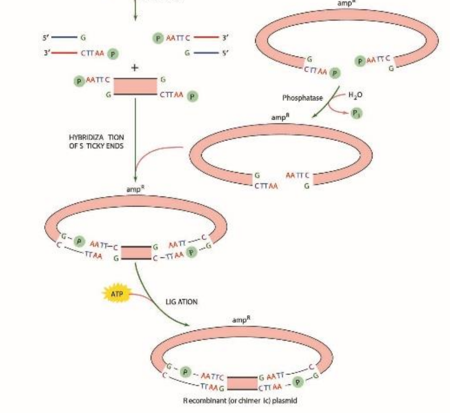
COMPETENT HOST

- Host bacterial cells must be made competent to take up the cloning vectors with recombinant DNA
- The chemical, physical and disarmed pathogens methods are used to make the host competent.

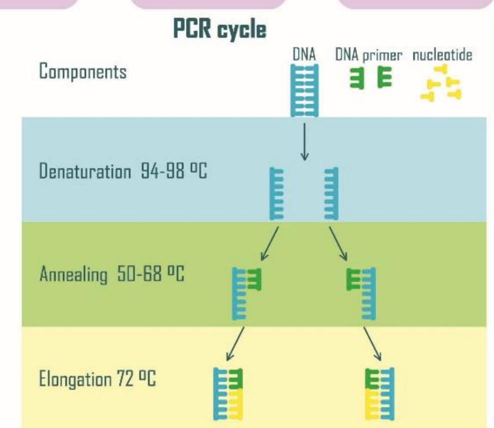


PROCESSES OF rDNA TECHNOLOGY

1. **ISOLATION OF DNA**
   - To cut the DNA with restriction enzymes, it needs to be in pure form free from other macromolecules.
   - The cell is lysed, to release DNA, with chitinase, lysozyme etc
2. **FRAGMENTATION OF DNA BY RESTRICTON ENZYME**
   - Pure DNA molecules are incubated with restriction enzyme
   - Agarose gel electrophoresis is done to check digestion by restriction enzymes.
3. **ISOLATION OF DESIRED DNA FRAGMENT**
   - Done by Gel electrophoresis.
4. **LIGATION OF DNA FRAGMENT INTO VECTOR**
5. **TRANSFERRING THE RECOMBINANT DNA INTO THE HOST.**
6. **CULTURING THE HOST CELLS IN A MEDIUM AT LARGER SCALE**
   - Amplified by the process called PCR.
7. **EXTRACTION OF DESIRED PRODUCT.**

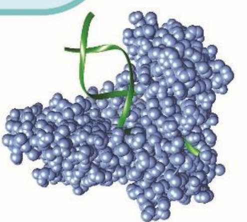


PCR cycle



TAQ POLYMERAESE

Synthesizes DNA region between the primers, using deoxynucleotide triphosphates and Mg2+



OBTAINING THE FOREIGN GENE PRODUCT

BIOREACTORS

Bioreacotors are the vessels in which raw materials are biologically converted into specific products, individual enzymes etc. using microbial plant, animal, or human cells

STIRRING TYPE BIOREACTOR

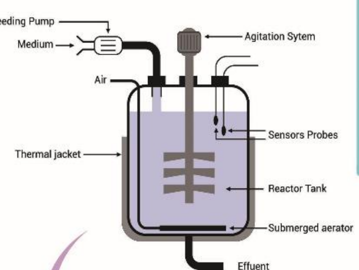
- Most commonly used bioreactors
- Usually cylindrical or with curved base to facilitate the mixing of reactor contents.

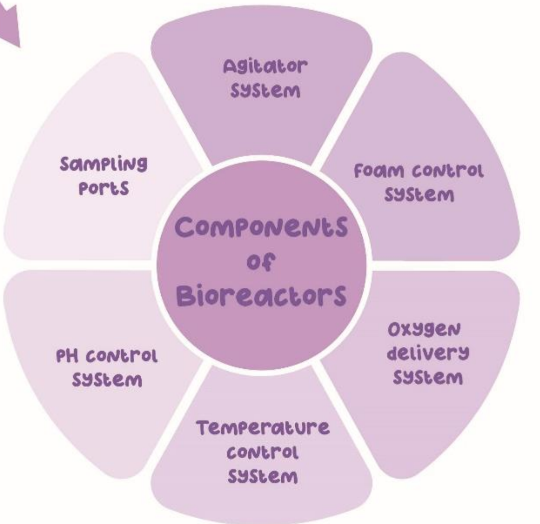
Simple Stirred tank

Simple moving type reactor

Sparged Stirred tank

Gas is bubbled through a liquid to remove the other dissolved gas





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.

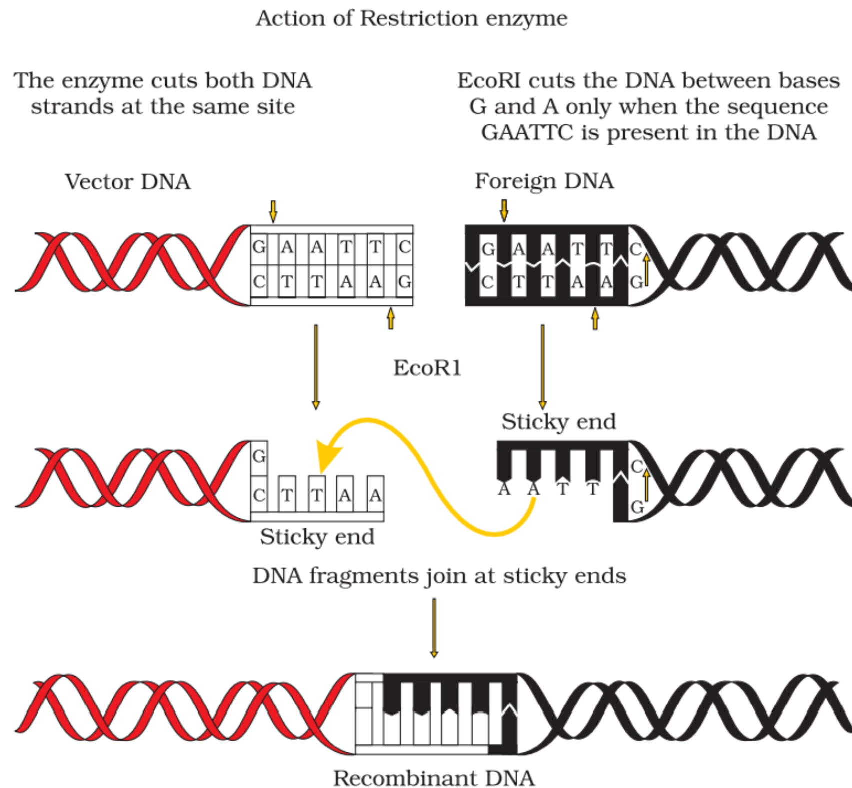
Endonucleases: These enzymes are responsible for the cutting of the DNA in the middle while the exonucleases enzymes are responsible for the cutting of the DNA at the ends. Examples of restriction endonucleases are ECoR1, Hind III, etc. Restriction enzymes cut the DNA molecule at a specific site that 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.

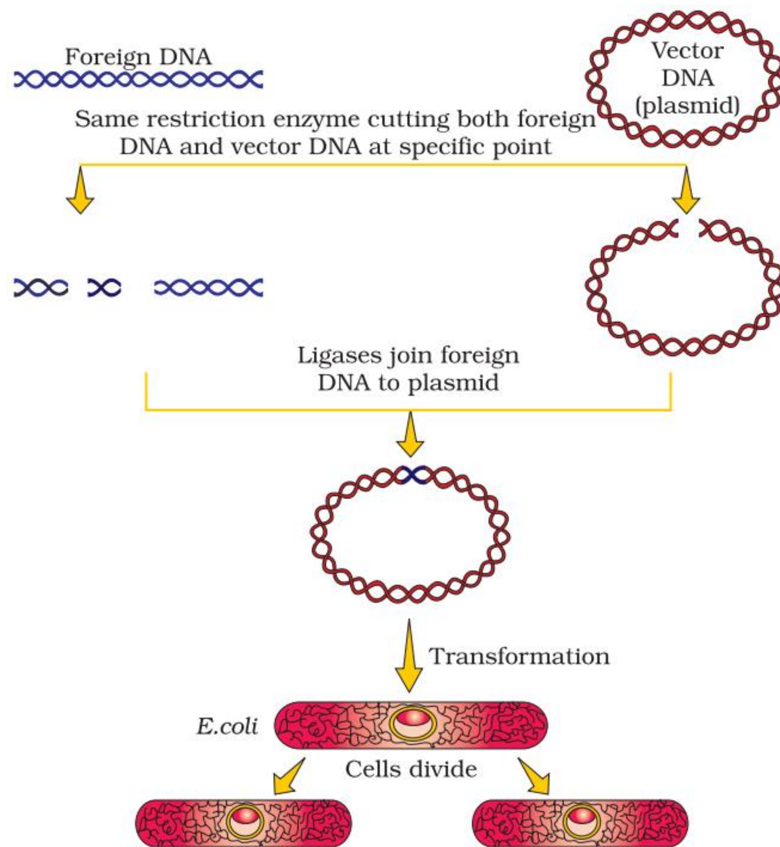
Ligases: Ligases are the enzyme that is responsible for the joining of the two DNA fragments. The process of ligation occurs in the presence of sticky ends (they are the similar overhanging sequences formed due to the action of the same

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.





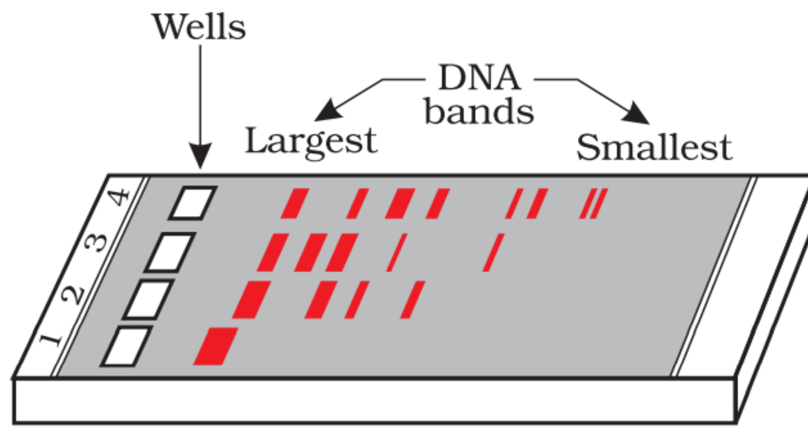
Separation and Isolation of DNA Fragments:

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.

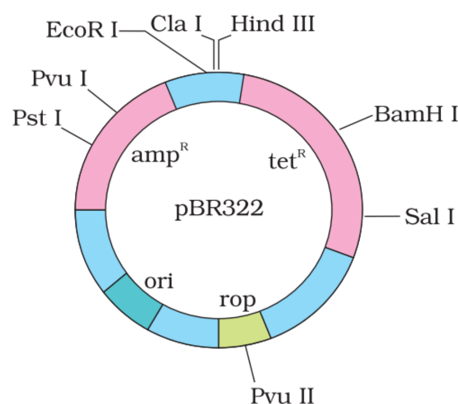
There Should be a Cloning Site in the Cloning Vector: There must be one cloning site present so as not to complicate the process of cloning. The antibiotic resistance gene present as the restriction sites are responsible for the ligation of the foreign DNA. When the desired gene is introduced at the 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:

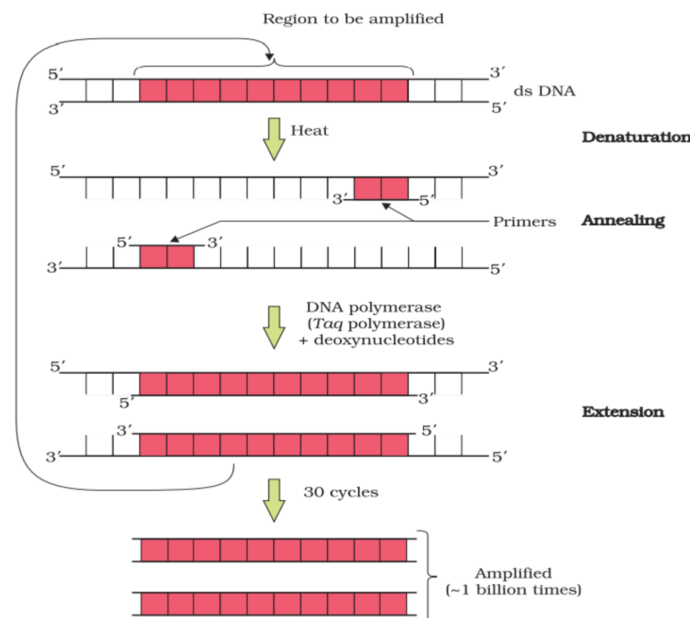
There are several steps involved in the process of recombinant DNA

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.

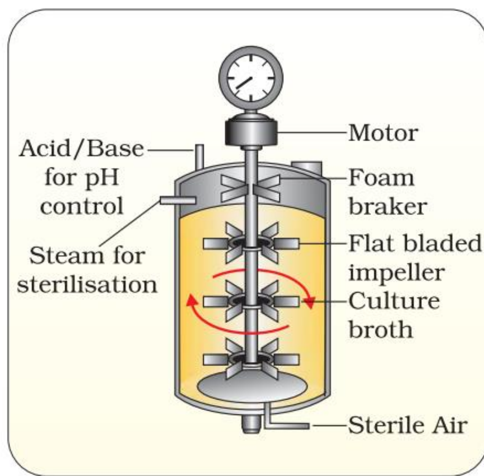
Amplification of Gene of Interest Using PCR: The amplification of the desired gene of the DNA can be done by the process of the Polymerase chain reaction (PCR). There are two sets of primers required that are the forward primer and the reverse primer. The DNA amplification is done with the help of the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

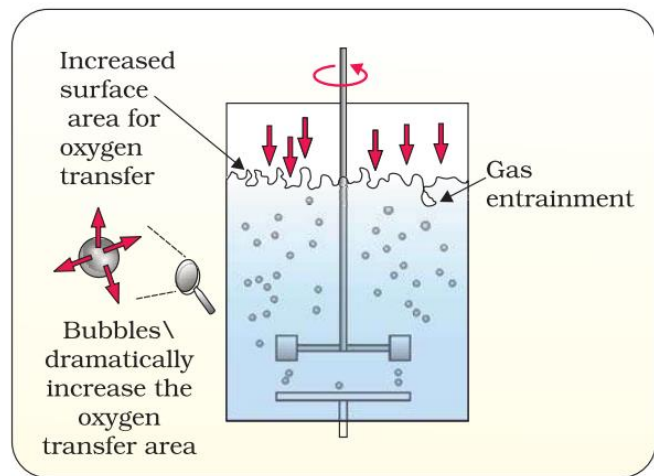


Insertion of Recombinant DNA Into Host Cell or Organism: The host cells need to be more competitive so as to receive the recombinant DNA.

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.

NCERT LINE BY LINE QUESTIONS

1. EFB stands for **[Pg-193,E]**
(A) English Federation of Biology
(B) European federation of Biology
(C) English Federation of Biotechnology
(D) European federation of Biotechnology

PARAGRAPH - 11.1 PRINCIPLES OF BIOTECHNOLOGY

2. Two core techniques that enabled birth of modern biotechnology are **[Pg-193,E]**
(A) Physical & biological engineering
(B) Bioprocess & genetic engineering
(C) Molecular & cellular genetics
(D) None of these
3. Biotechnology uses techniques to alter chemistry of **[Pg- 193,E]**
(A) Protein & Lipid (B) Protein & RNA
(C) Lipid & DNA (D) RNA & DNA
4. In chemical engineering processes, it is important to maintain **[Pg-194,E]**
(A) maintain microbe-free environment
(B) microbe-full environment
(C) sterile environment
(D) more than one option
5. Unique combinations of genetic setup is naturally provided by **[Pg-194,E]**
(A) Sexual reproduction (B) Asexual reproduction
(C) Biotechnology (D) More than one option
6. All genetic changes occurring naturally are **[Pg-194,M]**
(A) harmful to organism & its population
(B) beneficial for organism & its population
(C) not harmful for organism & its population
(D) Both A & C
7. Genetic information is preserved by **[Pg-194,E]**
(A) sexual reproduction (B) asexual reproduction
(C) Both of these (D) none of these
8. When a piece of DNA is transferred to an alien organism as it is **[Pg-194,M]**
(A) it will multiply itself
(B) it will not be able to multiply itself
(C) it will be present in progeny cells of organism.
(D) Both (A) & (C)
9. Chromosome replication is initiated at **[Pg-194,M]**
(A) gateway of replication a specific RNA sequence
(B) origin of replication a specific RNA sequence
(C) path of replication a specific RNA sequence
(D) None of these
10. For alien DNA to replicate it needs to be a part of **[Pg-194,H]**
(A) chromosome without origin of replication site
(B) mitochondrial DNA with origin of replication site
(C) chromosome with origin of replication site
(D) cytoplasmic DNA with origin of replication site
11. Plasmid is- **[Pg-194,E]**
(A) autonomously replicating, extra chromosomal
(B) non- autonomously replicating extra chromosomal
(C) autonomously replicating chromosomal
(D) non-autonomously replicating extrachromosomal

12. Plasmid is **[Pg-194,E]**
(A) Linear RNA (B) Circular RNA
(C) Linear DNA (D) Circular DNA

13. First recombinant DNA involved native plasmid of **[Pg-194,E]**
(A) *Escherichia coli*
(B) *Salmonella typhimurium*
(C) *Streptococcus pneumonia*
(D) *Clostridium butylicom*

14. First recombinant DNA was made by **[Pg194,E]**
(A) Herbert Cohen & Stanley Boyer, 1972
(B) Stanley Cohen & Herbert Boyer, 1992
(C) Stanley Cohen & Herbert Boyer, 1972
(D) Herbert Cohen & Stanley Boyer, 1992

15. The recombinant DNA was made **[Pg-194,195,H]**
(A) before discovery of DNA cutting restriction enzymes
(B) after discovery of DNA cutting restriction enzymes
(C) after discovery of DNA cutting Ligases
(D) before discovery of DNA cutting Ligases

16. The plasmid DNA linked with cut piece of DNA acts as **[Pg-195,M]**
(A) host (B) vector
(C) medium to transfer the DNA piece
(D) more than one option

17. Linking of antibiotic resistance gene with plasmid is done using enzyme **[Pg-195,M]**
(A) Ligase (B) Lyase (C) Hydrolase (D) Nuclease

18. The plasmid joined with required DNA of interest is transferred into........ by Boyer. **[Pg-195,E]**
(A) Escherichia coli (B) Salmonella typhimurium
(C) Streptococcus pneumonia (D) Clostridium butylicom

PARAGRAPH-11.2 TOOLS OF RECOMBINANT DNA TECHNOLOGY

19. The key tools for recombinant DNA technology are **[Pg-195,E]**
(A) Restrication enzyme, polymerase, hydrolase, vectors
(B) Recognition enzyme, polymerase, ligase, vector
(C) Restriction endonuclease, polymerase, ligase, vector
(D) Restriction enzyme, polymerase, dehydrogenase vector

PARAGRAPH-11.2.1 RESTRICTION ENZYME

20. In 1963, two restriction endonucleases were isolated in E. Coli that restricted growth of bacteriophage by **[Pg-195,M]**
(A) cutting DNA (B) adding methyl group to DNA
(C) removing methyl group to DNA (D) more than one option

21. The first restriction endonuclease was **[Pg-195,E]**
(A) Hind-III (B) Hind-II (C) Hind-I (D) Hind-IV

22. EcoRI comes from **[Pg-195,E]**
(A) genus Eichhonia (B) species coli
(C) genus Echinus (D) species crispus

23. Recognition sequence is **[Pg-195,H]**
(A) Specific sugar sequence in DNA which is recognized by restriction endonuclease
(B) Specific protein sequence which is recognized by restriction endonuclease
(C) Specific lipase sequence which is recognized by restriction endonuclease
(D) Specific base sequence in DNA which is recognized by restriction enconulcease

24. The convention for naming restriction endonucleases is **[Pg-195,H]**
(A) First two letters come from genus & third from species of prokaryotic cell from which they were isolated.
(B) First two letters come from species & third from genus of prokaryotic cell from which they were isolated.

(C) First letter come from genus & second two from species of prokaryotic cell from which they were isolated.
(D) First letter come from species & second two from genus of prokaryotic cell from which they were isolated

25. Roman number indicate **[Pg-196,E]**
(A) order in which enzyme were isolated
(B) strain of bacteria
(C) lab number in which enzyme was isolated
(D) none of these

26. Restriction enzymes belong to **[Pg-196,E]**
(A) Exonucleases (B) Endonucleases
(C) Both (D) None

27. Exonuclease cuts DNA from **[Pg-196,E]**
(A) specific position within DNA (B) ends of DNA
(C) Both (A) & (B) (D) None of these

28. Restriction enzyme recognize **[Pg-196,M]**
(A) Paleondromic sequence of nucleoside in DNA
(B) Palindromic sequence of nucleoside in DNA
(C) Paleondromic sequence of nucleotide in DNA
(D) Palindromic sequence of nucleotide in DNA

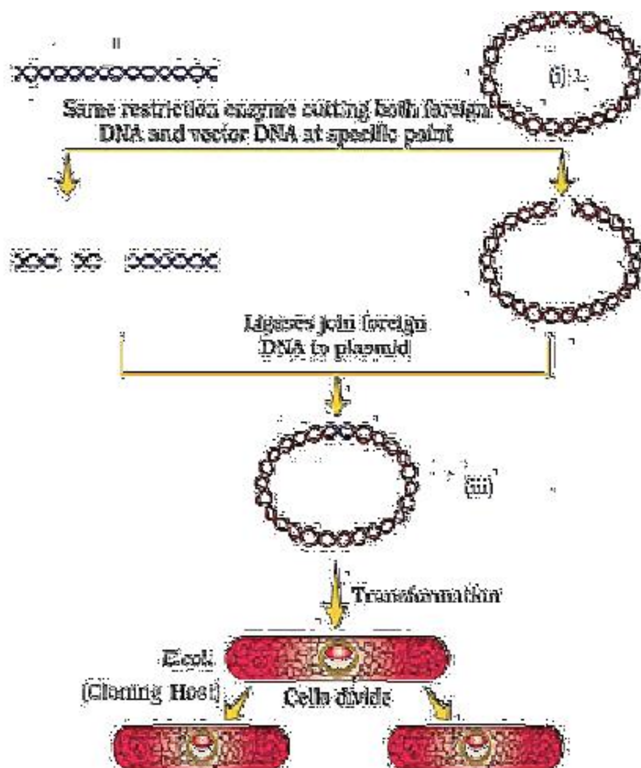
29. ECoRI cuts DNA at **[Pg-196,H]**

30. Which of the following is a palindrome? **[Pg-197,H]**
(A) 5' – GAATAC – 3'
3' – CTTATG – 5'
(B) 5' – GATATAC – 3'
3' – CTATATG – 5'
(C) 5' – GAATTC – 3'
3' – CTTAAG – 5'
(D) All of these

31. Restriction enzyme cuts DNA **[Pg-197,H]**
(A) between same two bases on opposite strands, in centre of DNA sequence recognized
(B) between same two bases on opposite strands, a little away from centre of DNA sequence recognized
(C) between different two bases on opposite strands, in centre of DNA sequence recognized
(D) between different two bases on opposite strands, living away from centre of DNA sequence recognized.

32. Same restriction enzyme produce **[Pg-197,M]**
(A) same kind sticky ends joined using endonucleases
(B) different kinds of sticky ends joined using ligase
(C) same kind of sticky ends joined using ligase
(D) different kind of sticky ends joined using endonucleases

33. [Pg-197,M]



Identify correct labeling

(i)	(ii)	(iii)
(A) vector plasmid	Recombinant DNA	Foreign DNA
(B) Foreign DNA	Vector plasmid	Recombinant DNA
(C) Recombinant DNA	Vector plasmid	Foreign DNA
(D) vector Plasmid	Foreign DNA	Recombinant DNA

34. The process of 'Transformation' is taking place when [Pg-197,M]
(A) bacteria replicates and makes copies of rDNA with it
(B) bacteria picks up rDNA
(C) foreign gene is added to cloning host prokaryote cell
(D) more than one option

SEPARATION & ISOLATION OF DNA FRAGMENTS

35. Technique used for separation of DNA fragments are [Pg-198,M]
(A) Gel electrophoresis (B) DNA fingerprinting
(C) PCR (D) DNA cloning

36. DNA fragments are [Pg-198,E]
(A) negatively charged (B) positively charged
(C) neutral (D) none of these

37. In gel electrophoresis, DNA are forced to move towards [Pg-198,M]
(A) anode under magnetic field (B) cathode under magnetic field
(C) anode under electric field (D) cathode under electric field

38. Matrix used in electrophoresis is [Pg-198,E]
(A) ethidium bromide (B) agarose gel
(C) natural polymer extracted from sea weeds
(D) more than one option

39. Ethidium bromide is used to stain because [Pg-198,H]
(A) DNA fragments are visible without staining

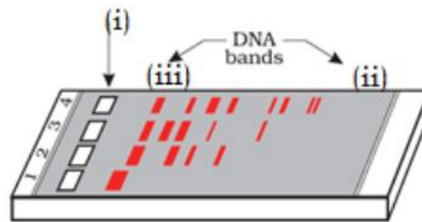
(B) DNA fragments are not visible under staining
(C) DNA fragments are not visible without staining
(D) DNA fragments are visible under staining

40. Stained DNA is exposed to **[Pg-198,H]**
(A) visible light (B) UV light (C) IR light (D) Radio wave

41. Colour of DNA visible under UV light after Ethidium bromide staining is **[Pg-198,H]**
(A) blue (B) black (C) orange (D) green

42. The extraction of separated bands of DNA from agarose gel are **[Pg-198,H]**
(A) Dilution (B) Elition (C) Elution (D) Delution

43. **[Pg-197,E]**



(i)	(ii)	(iii)
(A) Largest DNA band	Smallest DNA band	Wells
(B) Wells	Largest DNA bands	Smallest DNA bands
(C) Smallest DNA bands	Largest DNA bands	Wells
(D) Smallest DNA bands	Wells	Largest DNA bands

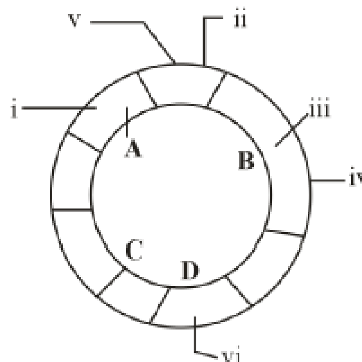
PARAGRAPH-11.2.2 CLONING VECTORS

44. Plasmids in bacterial cells replicate **[Pg-197,M]**
(A) depending on chromosomal DNA
(B) independent of chromosomal DNA
(C) depending on extra-nuclear DNA
(D) more than one option

45. Bacteriophages **[Pg-197,E]**
(A) replicate independent of other organisms
(B) replicate inside bacterial cell, controlled by chromosomal DNA of bacteria.
(C) replicate inside bacterial cell autonomously
(D) more than one option

46. Bacteriophages serve as ____ in biotechnology. **[Pg-197,E]**
(A) host (B) vector
(C) molecular marker (D) enzyme

Figure for question. (47 to 53)



47. Identify Bam HI in given plasmid figure **[Pg-199,E]**
(A) (i) (B) (ii) (C) (iii) (D) (iv)

48. Identify antibiotic resistance gene in figure **[Pg-199,E]**
(A) Sal I (B) EcoRI (C) *ampR* (D) pBR322

49. Identify ECoRI in the plasmid **[Pg-199,E]**
(A) (iv) (B) (v) (C) (iii) (D) (ii)

50. 'A' & 'B' in figure are **[Pg-199E]**
(A) ampR & tetR (B) ori & ampR
(C) tetR & ampR (D) rop & tetR

51. 'rop' codes for i & is shown in figure by ii **[Pg-199,M]**
(A) proteins involved in replication ; D
(B) proteins involved in transcription, C
(C) proteins involved in transcription, D
(D) proteins involved in replication, C

52. 'Ori' means ____ & is shown in figure by **[Pg-199,E]**
(A) origin of translocation; C (B) origin of replication ; D
(C) origin of translation ; D (D) origin of replication; C

53. Identify pvu II in given figure of plasmid **[Pg-199,E]**
(A) i (B) ii (C) vi (D) iv

54. Which of the following is correct? **[Pg-199,M]**
(A) Any piece of DNA linked to ori gene will be replicated
(B) Number of replication copies is under control of recognition site
(C) Vector should not be chosen based on number of copies supported by it
(D) More than one option

55. Transformants include **[Pg-199,M]**
(A) cells which have picked vector with foreign DNA ligated to it.
(B) cells which have picked up vector without foreign DNA ligated to it
(C) cells which have not picked up vector
(D) Both (A) & (B)

56. Recombinants are **[Pg-199,M]**
(A) cells which have picked vector with foreign DNA ligated to it.
(B) cells which have picked up vector without foreign DNA ligated to it
(C) cells which have not picked up vector
(D) Both (A) & (B)

57. Which is true about recombinant & transformant? **[Pg-199,H]**
(A) All transformants are recombinants
(B) All recombinants are transformants
(C) no relation between these two
(D) Both are same thing

58. Normal E.coli cell- **[Pg-199,M]**
(A) Carries resistance against antibiotics ampicillin, tetracycline and kanamycin
(B) Does not carry resistance against antibiotics ampicillin, tetracycline and kanamycin
(C) Carries resistance against ampicillin but not tetracycline and kanamycin
(D) Carries resistance against tetracycline but not ampicillin and kanamycin

59. In order to link alien DNA, vector needs to have ____ recognition sites for commonly used restriction enzymes. **[Pg-199,E]**
(A) very few (B) preferably single
(C) many (D) more than one option

60. Assertion- Vector should have many recognition sites for commonly used restriction enzymes.
Reason- Lot of recognition sites generate several fragments, which make gene cloning easy. **[Pg-200,H]**
(A) Assertion and Reason are both correct and Reason is correct explanation for Assertion
(B) Assertion and Reason are both correct but Reason is not correct explanation for Assertion
(C) Assertion and Reason both are incorrect
(D) Assertion is correct but Reason is incorrect

61. If a foreign gene is ligated at Bam HI site of vector PBR322, then the resistance for ____. **[Pg-199,M]**

(A) tetracycline is lost
(B) ampicillin is lost
(C) tetracycline is not lost
(D) more than one option

62. The recombinants mentioned previous question non-recombinants by- **[Pg-199,M]**

(A) Plating the transformants on tetracycline
(B) Planting the transformants on ampicillin
(C) Both of these are necessary
(D) None of these

63. Recombinants mentioned in 'If a foreign gene is ligated at Bam HI site of vector PBR322' will- **[Pg-199,H]**

(A) Grow in ampicillin and tetracycline both
(B) Grow in ampicillin but not tetracycline
(C) Grow in tetracycline but not ampicillin
(D) Grow neither in tetracycline nor in ampicillin

64. Non-recombinants transformants will **[Pg-199,M]**

(A) Grow in ampicillin and tetracycline both
(B) Grow in ampicillin but not tetracycline
(C) Grow in tetracycline but not ampicillin
(D) Grow neither in tetracycline nor in ampicillin

65. Non-transformants E.coli will- **[Pg-199,M]**

(A) Grow in ampicillin and tetracycline both
(B) Grow in ampicillin but not tetracycline
(C) Grow in tetracycline but not ampicillin
(D) Grow neither in tetracycline nor in ampicillin

66. When rDNA is inserted in coding sequence of β-galactosidase, **[Pg-200,H]**

(A) The enzyme gets synthesized
(B) Blue coloured colonies are produced
(C) Colourless colonies are produced
(D) Orange colonies are produced

67. Ti-plasmid stands for ____ and are present in ____. **[Pg-200,E]**

(A) Tumor inhibiting, Agrobacterium speciense
(B) Tumor inducing, Agrobacterium speciense
(C) Tumor inhibiting, Agrobacterium tumifaciens
(D) Tumor inducing, Agrobacterium tumifaciens

68. The Ti-plasmid being used as cloning vector- **[Pg-200,M]**

(A) causes crown gall disease
(B) is not pathogenic
(C) is pathogenic
(D) More than one option

PARAGRAPH-11.2.3 COMPETENT HOST
(For transformation with recombinant DNA)

69. DNA is- **[Pg-200,E]**

(A) hydrophilic and can pass through cell membrane
(B) hydrophobic and can pass through cell membrane
(C) hydrophilic and cannot pass through cell membrane
(D) hydrophobic and cannot pass through cell membrane

70. Bacterial host cells are made competent to take up rDNA by- **[Pg-200,H]**

(A) Treating with $Na+$
(B) Treating with $Al3+$
(C) Treating with $Ca2+$
(D) More than one options

71. Choose the correct sequence to be followed to enable bacteria to take up rDNA. **[Pg-201, 202,M]**

(i) Treating with divalent cation.
(ii) Heat shock (42ºC).
(iii) Incubating on ice.

(A) i-ii-iii-ii (B) i-iii-ii-iii
(C) ii-iii-i-ii (D) iii-ii-i-iii

72. Other methods for introducing foreign DNA into host cells are- **[Pg-201,E]**
(A) Micro-injection for animal cells (B) Gene gun for plant cells
(C) Disarmed pathogens (D) All of these

73. In micro-injection technique, rDNA is injected into- **[Pg-201,E]**
(A) Cytoplasm (B) Nucleus
(C) Cell membrane (D) Lysosomes

74. In biolistics, cells are bombarded with high velocity- **[Pg-201,E]**
(A) Micro-particles of iron
(B) Macro-particles of tungsten
(C) Micro-particles of gold
(D) More than one option

PARAGRAPH-11.3 PROCESSES OF RECOMBINANT DNA TECHNOLOGY

75. Identity correct sequence of process of rDNA technology : **[Pg-201,M]**
(i) transferring rDNA into host
(ii) isolation of DNA fragment desired
(iii) isolation of DNA
(iv) culturing host cells in medium at large scale
(v) fragmentation of DNA by restriction enzyme
(vi) ligation of DNA fragment into a vector
(vii) extraction of desired product
(A) (iii) – (ii) – (v) – (vi) – (i) – (iv) – (vii)
(B) (iii) – (v) – (i) – (vi) – (ii) – (iv) – (vii)
(C) (iii) – (v) – (ii) – (vi) – (i) – (iv) – (vii)
(D) (iii) – (v) – (vi) – (i) – (ii) – (iv) – (vii)

PARAGRAPH-11.3.1 ISOLATION OF THE GENETIC MATERIAL (DNA)

76. Nucleic acid is genetic material of: **[Pg-201,E]**
(A) some organisms (B) no organism
(C) all organisms without exception
(D) most organisms with some exception

77. How many of given enzymes involved in extraction of genetic material from cell of organisms are: **[Pg-201,M]**
(i) cellulase (ii) chitinase (iii) lysozyme (iv) Ribonuclease
(v) protease (vi) deoxyribonuclease
(A) 3 (B) 2 (C) 5 (D) 6

78. Match the following**:** **[Pg-201,E]**

A	**B**
(i) cellulase	I. plant
(ii) chitinase	II. Bacteria
(iii) lysozyme	III. Fungi

(i) (ii) (iii) **(i) (ii) (iii)**
(A) I III II (B) II III I
(C) III I II (D) I II III

79. Purified DNA is precipitated out by addition of: **[Pg-201,H]**
(A) warm acetic acid (B) chilled acetic acid
(C) warm ethanol (D) chilled ethanol

80. **[Pg-201,E]**

The figure shows DNA separated out, removed by :

(A) spooning (B) spooling (C) spilling (D) speeling

81. The precipitated DNA is seen as : **[Pg- 201,M]**

(A) collection of fine threads in suspension

(B) collection of fine threads in solution

(C) coagulated mass in suspension

(D) coagulated mass in solution

PARAGRAPH-11.3.2 CUTTING OF DNA AT SPECIFIC LOCATION

82. To check the progression of restriction enzyme digestion, ________ is used. **[Pg-202,M]**

(A) PCR (B) gel electrophoresis

(C) DNA fingerprinting (D) Selectable marker gene

83. Preparation of rDNA involves the enzymes: **[Pg-202,E]**

(A) specific restriction enzyme (B) gene of interest

(C) vector DNA (D) all of these

PARAGRAPH-11.3.3 AMPLIFICATION OF GENE OF INTEREST USING PCR

84. PCR stands for: **[Pg-202,E]**

(A) Polynuclease chain reaction (B) Polylipase chain reaction

(C) Polyamide chain reaction (D) None of these

85. PCR is an: **[Pg-202,E]**

A) in vitro process B) in vivo process

C) both D) none

86. How many sets of primers are used in PCR? **[Pg-202,E]**

(A) 1 (B) 2 (C) 3 (D) 4

87. Enzyme involved in PCR is: **[Pg-203,E]**

(A) DNA endonuclease (B) RNA polymerase

(C) DNA polymerase (D) DNase

88. The enzyme involved in PCR with thermostability is isolated from: **[Pg-203,E]**

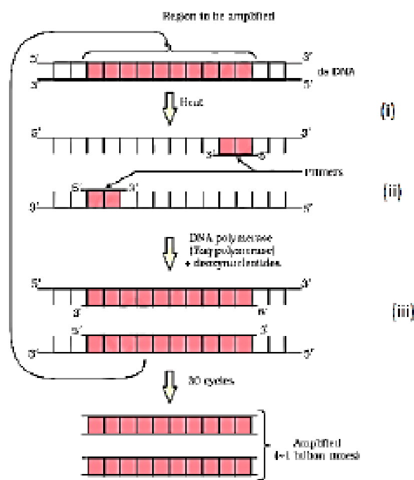
(A) Thermus aquaticus fungi

(B) Escherechia coli bacteria

(C) Agrobacterium tumefaciense bacteria

(D) None of these

89.



[Pg-202,E]

Identify correct labeling of sequence:

(i)	(ii)	(iii)
(A) Annealing	Denaturation	Extension
(B) Denaturation	Extension	Annealing
(C) Denaturation	Annealing	Extension
(D) Extension	Annealing	Denaturation

PARAGRAPH-11.3.4
INSERTION OF RECOMBINANT DNA INTO THE HOST CELL / ORGANISM

90. A-Ampicillin resistance gene is called selectable marker in case E.coli is made to take up rDNA bearing ampicillin resistance gene.
B-Such E.coli coil grow on amplicillin containing agar plates.
Choose right option with regards to above statements. **[Pg-203,H]**
(A) Both are correct (B) Only A is correct
(C) Only B is correct (D) None is correct

PARAGRAPH-11.3.5
PARAGRAPH- 11.3.5 OBTAINING FOREIGN GENE PRODUCT

91. If a protein encoding gene is expressed in a heterologous host, it is called: **[Pg-203,M]**
(A) secondary protein (B) recombinant protein
(C) transmitted protein (D) tertiary protein

92. In continuous culture system: **[Pg-203,M]**
(A) used medium is drained at the end
(B) used medium is drained twice in the whole process
(C) used medium is continuously drained out
(D) none of these

93. Bioreactors are: **[Pg-204,E]**
(A) large vessels
(B) used for large quantity production
(C) used for biological conversion of raw materials into products
(D) all of these

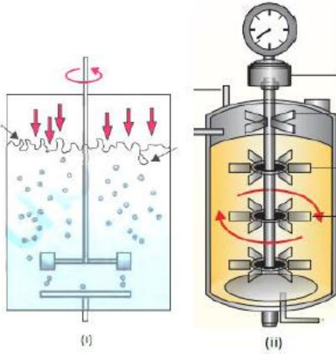
PARAGRAPH-11.3.6 DOWNSTREAM PROCESSING

94. Downstream processing includes : **[Pg-205,E]**
(A) separation (B) purification
(C) both the above (D) none of these

95. A- Suitable preservatives are added B- These formulations need clinical trials.
C- Quality control testing is uniform for all the products.
How many of the above statements is incorrect? **[Pg-205,M]**
(A) 0 (B) 1 (C) 2 (D) 3

96. Optimal conditions for growth include. How many of the following- **[Pg-205,H]**
pH, Salt, Temperature, Vitamin, Oxygen
(A) 5 (B) 6 (C) 7 (D) 4

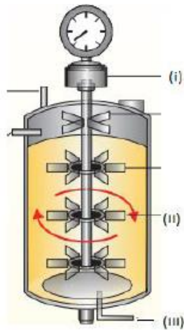
97. **[Pg-204,E]**



Identify types of stirred-tank bioreactor-

(i)	(ii)
(A) Simple stirred-tank bioreactor	complex stirredtank bioreactor
(B) Complex stirred-tank bioreactor	simple stirredtank bioreactor
(C) Simple	Sparged
(D) Sparged	Simple

98. **[Pg-204,E]**



Identify the correct labels-

(i)	**(ii)**	**(iii)**
(A) Motor	Culture broth	Sterile air
(B) Culture broth	Motor	Sterile air
(C) Motor	Sterile air	Culture broth
(D) Sterile air	Culture broth	Motor

99. Samling ports are mainly required to- **[Pg-204,M]**
(A) Keep adding samples into Bioreactors
(B) Withdraw small volume of culture
(C) Add Acid/Base for pH control (D) All of these

100. Sterile air bubbles are sprayed in the biovector in a type of bioreactor. That is because- **[Pg-204,M]**
(A) air bubbles makes it easier to agitate the system
(B) air bubbles increase surface area for oxygen transfer
(C) air bubbles enable microbes to grow (D) none of these

NEET PREVIOUS YEARS QUESTIONS

1. The correct order of steps in Polymerase Chain Reaction (PCR) is : **[2018]**
(a) Extension, Denaturation, Annealing (b) Annealing, Extension, Denaturation
(c) Denaturation, Annealing, Extension (d) Denaturation, Extension, Annealing

2. Which of the following is commonly used as a vector for introducing a DNA fragment in human lymphocytes? **[2018]**
(a) Retrovirus (b) *Ti* plasmid (c) *pBR* 322 (d) l phage

3. The DNA fragments separated on an agarose gel can be visualised after staining with: **[2017]**
(a) Acetocarmine (b) Aniline blue (c) Ethidium bromide (d) Bromophenol blue

4. A gene whose expression helps to identify transformed cell is known as : **[2017]**
(a) Vector (b) Plasmid (c) Structural gene (d) Selectable marker

5. What is the criterion for DNA fragments movement on agarose gel during gel electrophoresis? **[2017]**
(a) The smaller the fragment size, the farther it moves.
(b) Positively charged fragments move to farther end.
(c) Negatively charged fragments do not move.
(d) The larger the fragment size, the farther it moves.

6. The process of separation and purification of expressed protein before marketing is called **: [2017]**
(a) Downstream processing (b) Bioprocessing
(c) Post production processing (d) Upstream processing

7. Which of the following is not a feature of the plasmids? **[2016]**
(a) Independent replication (b) Circular structure
(c) Transferable (d) Single - stranded

8. The *Taq* polymerase enzyme is obtained from **[2016]**
(a) *Thermus aquaticus* (b) *Thiobacillus ferroxidans*
(c) *Bacillus subtilis* (d) *Pseudomonas putida*

9. Which of the following is a restriction endonuclease? **[2016]**
(a) *Hind* II (b) Protease (c) DNase I (d) RNase

10. The cutting of DNA at specific locations became possible with the discovery of : **[2015]**
(a) Probes (b) Selectable markers (c) Ligases (d) Restriction enzymes

11. The DNA molecule to which the gene of interest is integrated for cloning is called : **[2015]**
(a) Vector (b) Template (c) Carrier (d) Transformer

12. An analysis of chromosomal DNA using the Southern hybridization technique does not use : **[2014]**
(a) Electrophoresis (b) Blotting (c) Autoradiography (d) PCR

13. *In vitro* clonal propagation in plants is characterised by : **[2014]**
(a) PCR and RAPD (b) Northern blotting (c) Electrophoresis and HPLC (d) Microscopy

14. Which vector can clone only a small fragment of DNA? **[2014]**
(a) Bacterial artificial chromosome (b) Yeast artificial chromosome
(c) Plasmid (d) Cosmid

15. Commonly used vectors for human genome sequencing are: **[2014]**
(a) T-DNA (b) BAC and YAC (c) Expression Vectors (d) T/A Cloning vectors

16. Following statements describe the characteristics of the enzyme Restriction endonuclease. Identify the incorrect statement. **[NEET-2019]**
(1) The enzyme cuts DNA molecule at identified position within the DNA
(2) The enzyme binds DNA at specific sites and cuts only one of the two strands.
(3) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand.
(4) The enzyme recognizes a specific palindromic nucleotide sequence in the DNA

17. DNA precipitation out of a mixture of biomolecules can be achieved by treatment with : **[NEET-2019]**
(1) Isopropanol (2) Chilled ethanol
(3) Methanol at room temperature (4) Chilled chloroform

18. Match the following enzymes with their functions : **[NEET-2019 ODISSA]**
(a) Restriction endonuclease (i) Joins the DNA fragments
(b) Restriction exonuclease (ii) Extends primers on genomic DNA template
(c) DNA ligase (iii) Cuts DNA at specific position
(d) Taq polymerase (iv) Removes nucleotides from the ends of DNA
Select the correct option from the following :
(1) a-iii, b-i, c-iv d-ii (2) a-iii, b-iv, c-i, d-ii
(3) a-iv, b-iii, c-i, d-ii (4) a-ii, b-iv, c-i, d-iii

19. The two antibiotic resistance genes on vector pBR322 are :- **[NEET-2019 ODISSA]**

(1) Ampicillin and Tetracycline (2) Ampicillin and Chloramphenicol
(3) Chloramphenicol and Tetracycline (4) Tetracycline and Kanamycin

20. A selectable marker is used to: **[NEET-2019 ODISSA]**
(1) help in eliminating the non-transformants, so that the transformants can be regenerated
(2) identify the gene for a desired trait in an alien organism
(3) select a suitable vector for transformation in a specific crop
(4) mark a gene on a chromosome for isolation using restriction enzyme

21. Given below are four statements pertaining to separation of DNA fragments using gel electrophoresis. Identify the incorrect statements. **[NEET-2019 ODISSA]**
(a) DNA is negatively charged molecule and so it is loaded on gel towards the Anode terminal
(b) DNA fragments travel along the surface of the gel whose concentration does not affect movement of DNA.
(c) Smaller the size of DNA fragment larger is the distance it travels through it.
(d) Pure DNA can be visualized directly by exposing UV radiation.
Choose correct answer from the options given below
(1) (a), (c) and (d) (2) (a), (b) and (c) (3) (b), (c) and (d) (4) (a), (b) and (d)

22. An enzyme catalysing the removal of nucleotides from ends of DNA is: **[NEET-2019 ODISSA]**
(1) DNA ligase (2) Endonuclease (3) Exonuclease (4) Protease

23. First discovered restriction endonuclease that always cuts DNA molecule at a particular point by recognising a specific sequence of six base pairs is: **[NEET-2020 COVID]**
(1) EcoR1 (2) Adenosine deaminase (3) Thermostable DNA polymerase (4) Hind II

24. In Recombinant DNA technology antibiotics are used : **[NEET-2020 COVID]**
(1) to keep medium bacteria-free (2) to detect alien DNA
(3) to impart disease-resistance to the host plant (4) as selectable markers

25. In a mixture, DNA fragments are separated by :- **[NEET-2020 COVID]**
(1) Bioprocess engineering (2) Restriction digestion
(3) Electrophoresis (4) Polymerase chain reaction

26. The specific palindromic sequence which is recognized by EcoRI is **[NEET-2020]**
1) 5' – GGATCC – 3', 3' – CCTAGG – 5'
2) 5' – GAATTC – 3', 3' – CTTAAG – 5'
3) 5' – GGAACC – 3' , 3' – CCTTGG – 5'
4) 5' = CTTAAG – 3' 3' – GAATTC – 5'

27. Identify the wrong statement with regard to restriction enzymes **[NEET-2020]**
1) Sticky ends can be joined by using DNA ligases
2) Each restriction enzyme functions by inspecting the length of DNA sequence
3) They cut the strand of DNA at palindromic sites
4) They are useful in genetic engineering

28. In gel electrophoresis, separated DNA fragments can be visualized with the help of **[NEET-2020]**
1) Ethidium bromide in infrared radiation 2) Acetocarmine in bright blue light
3) Ethidium bromide in UV radiation 4) Acetocarmine in UV radiation

29. Choose the correct pair from the following **[NEET-2020]**
1) Exonucleases – Make cuts at specific positions within DNA
2) Ligases – Join the two DNA molecules
3) Polymerases – Break the DNA into fragments
4) Nucleases – Separate the two strands of DNA

30. The sequence that controls the copy number of the linked DNA in vector, is termed **[NEET-2020]**
1) Recognition site 2) Selectable marker 3) Ori site 4) Palindromic sequence

31. DNA strands on a gel stained with ethidium bromide when viewed under UV radiation, appear as : **[NEET-2021]**
(1) Bright orange bands (2) Dark red bands
(3) Bright blue bands (4) Yellow bands

32. Which of the following is not an application of PCR (Polymerase Chain reaction) ?**[NEET-2021]**
1) Gene amplification 2) Purification of isolated protein

3) Detection of gene mutation 4) Molecular diagnosis

33. During the purification process for recombinant DNA technology, addition of chilled ethanol precipitates out: **[NEET-2021]**

1) DNA 2) Histones 3) Polysaccharides 4) RNA

34. Which of the following is a **correct** sequence of steps in a PCR(Polymerase Chain reaction)?**[NEET21]**

1) Denaturation, Extension, Annealing 2) Extension, Denaturation, Annealing
3) Annealing, Denaturation, Extension 4) Denaturation, Annealing, Extension

35. Plasmid pBR322 has PstI restriction enzyme site within gene amp^R that confers ampicillin resistance. If this enzyme is used for inserting a gene for β-galactoside production and the recombinant plasmid is inserted in an *E.Coli* strain **[NEET-2021]**

1) The transformed cells will have the ability to resist ampicillin as well as produce β-galactoside.
2) It will lead to lysis of host cell.
3) It will be able to produce a novel protein with dual ability
4) It will not be able to confer ampicillin resistance to the host cell.

36. During the process of gene amplification using PCR , if very high temperature is not maintained in the beginning , then which of the following steps of PCR will be affected first ? **[NEET-2021]**

1. Extension 2. Denaturation 3. Ligation 4. Annealing

37. Given below are two statements: on is labelled as Assertion (A) and the other is labelled as Reason (R) **[NEET-2022]**

Assertion (A): Polymerase chain reaction is used in DNA amplification
Reason (R): The ampicillin resistant gene is used as a selectable marker to check transformation
In the light of the above statements, choose the correct answer from the options given below.

1) Both (A) and (R) are correct and (R) is the correct explanation of (A)
2) Bothe (A) and (R) are correct but (R) is not the correct explanation of (A)
3) (A) is correct but (R) is not correct
4) (A) is not correct but (R) is correct

38. Which one of the following statements is not true regarding gel electrophoresis technique? **[NEET-2022]**

1) The process of extraction of separated DNA strands from gel is called elution
2) The separated DNA fragments are stained by using ethidium bromide
3) The presence of chromogenic substrate gives blue coloured DNA bands on the gel
4) Bright orange coloured bands of DNA can be observed in the gel when exposed to UV light

39. In the following palindromic base sequences of DNA, which one can be cut easily by particular restriction enzyme? **[NEET-2022]**

1) 5' G A T A C T 3'; 3' C T A T G A 5'
2) 5' G A A T T C 3' ; 3' C T T A A G 5'
3) 5' C T C A G T 3' ; 3' G A G T C A 5'
4) 5' G T A T T C 3' ; 3' C A T A A G 5'

40. Given below are two statements: **[NEET-2022]**

Statement I: Restriction endonucleases recognize specific sequence to cut DNA known as palindromic nucleotide sequence.
Statement II: Restriction endonucleases cut the DNA strand a little away from the centre of the palindromic site.
In the light of the above statements, choose the most appropriate answer from the options given below:

1) Both Statements I and Statement II are correct
2) Both Statement I and Statement II are incorrect
3) Statement I is correct but Statement II is incorrect
4) Statement I is incorrect but Statement II is correct

41. Which of the following is not a desirable feature of a cloning vector? **[NEET-2022]**

1) Presence of origin of replication
2) Presence of a marker gene
3) Presence of single restriction enzyme site
4) Presence of two or more recognition sites

42. What is the fate of a piece of DNA carrying only gene of interest which is transferred into an alien organism?

A. The piece of DNA would be able to multiply itself independently in the progeny cells of the organism.
B. It may get integrated into the genome of the recipient.
C. It may multiply and be inherited along with the host DNA.
D. The alien piece of DNA is not an integral part of chromosome.
E. It shows ability to replicate.
Choose the correct answer from the options given below:
(a) A and B only
(b) D and E only
(c) B and C only
(d) A and E only

[NEET 2024]

43. Hind II always cuts DNA molecules at a particular point called recognition sequence and it consists of:
(a) 8 bp (b) 6 bp
(c) 4 bp (d) 10bp

[NEET 2024]

44. Ligation of foreign DNA at which of the following site will result in loss of tetracyclin resistance of pBR322:
(a) Pstl (b) Pvul
(c) EcoRI (d) BamHI

[NEET 2023 Manipur]

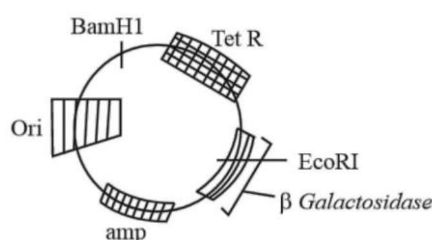
45. Which of the following is not a cloning vector?
(a)YAC (b)pBR 322
(c) Probe (d) BAC

[NEET 2023]

46. Polymerase chain reaction (PCR) amplifies DNA following the equation.
(a) N^2
(b) 2^n
(c) $2n + 1$
(d) $2\ N^2$

[NEET 2025]

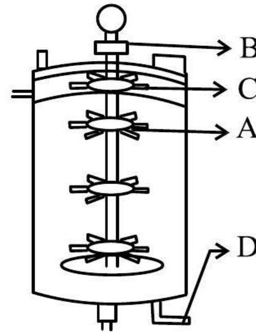
47.



In the above represented plasmid an alien piece of DNA is inserted at EcoRI site. Which of the following strategies will be chosen to select the recombinant colonies?
(a) Using ampicillin & tetracyclin containing medium plate.
(b) Blue color colonies will be selected.
(c) White color colonies will be selected.
(d) Blue color colonies grown on ampicillin plates can be selected.

[NEET 2025]

48. Identify the part of a bio-reactor which is used as a foam braker from the given figure.



(a) A
(b) B
(c) D
(d) C

[NEET 2025]

49. Given below are two statements:
Statement I: The DNA fragments extracted from gel electrophoresis can be used in construction of recombinant DNA.
Statement II: Smaller size DNA fragments are observed near anode while larger fragments are found near the wells in an agarose gel.
In the light of the above statements, choose the most appropriate answer from the options given below:
(a) Both statement I and statement II are correct
(b) Both statement I and statement II are incorrect
(c) Statement I is correct but statement II is incorrect
(d) Statement I is incorrect but statement II is correct

[NEET 2025]

50. Which of the following enzyme(s) are NOT essential for gene cloning?
A. Restriction enzymes
B. DNA ligase
C. DNA mutase
D. DNA recombinase
E. DNA polymerase
Choose the correct answer from the options given below :
(a) C and D only
(b) A and B only
(c) D and E only
(d) B and C only **[NEET 2025]**

51. The blue and white selectable markers have been developed which differentiate recombinant colonies from non-recombinant colonies on the basis of their ability to produce colour in the presence of a chromogenic substrate.
Given below are two statements about this method:
Statement I: The blue coloured colonies have DNA insert in the plasmid and they are identified as recombinant colonies.
Statement II: The colonies without blue colour have DNA insert in the plasmid and are identified as recombinant colonies.
In the light of the above statements, choose the most appropriate answer from the options given below:
(a) Both Statement I and Statement II are correct
(b) Both Statement I and Statement II are incorrect
(c) Statement I is correct but Statement II is incorrect
(d) Statement I is incorrect but Statement II is correct

[NEET 2025]

NCERT LINE BY LINE QUESTIONS – ANSWERS

1) D	2) B	3) D	4) D	5) A	6) C	7) B	8) B	9) D	10) C
11) A	12) D	13) B	14) C	15) B	16) B	17) A	18) B	19) C	20) D
21) B	22) B	23) D	24) C	25) A	26) B	27) B	28) D	29) A	30) C
31) B	32) C	33) D	34) D	35) A	36) A	37) C	38) B	39) C	40) B
41) C	42) C	43) B	44) B	45) C	46) B	47) C	48) C	49) B	50) A
51) A	52) D	53) C	54) A	55) D	56) A	57) B	58) B	59) D	60) C
61) A	62) A	63) B	64) A	65) D	66) B	67) D	68) B	69) A	70) C
71) B	72) D	73) B	74) C	75) C	76) C	77) C	78) A	79) D	80) B
81) A	82) B	83) D	84) D	85) A	86) B	87) C	88) D	89) C	90) A
91) B	92) C	93) D	94) C	95) B	96) A	97) C	98) A	99) B	100) B

NEET PREVIOUS YEARS QUESTIONS-ANSWERS

1 (c) **2** (a) **3** (c) **4** (d) **5** (a) **6** (a) **7** (d) **8** (a) **9** (a) **10** (d)
11 (a) **12** (d) **13** (a) **14** (c) **15** (b) **16** (2) **17** (2) **18** (2) **19** (1) **20** (1)
21 (4) **22** (3) **23** (4) **24** (4) **25** (3) **26** (2) **27** (1) **28** (3) **29** (2) **30** (3)
31 (1) **32** (2) **33** (1) **34** (4) **35** (4) **36** (3) **37** (2) **38** (3) **39** (2) **40** (1) **41** (4)
42(C) 43(B) 44(D) 45(C)46(b)47(c)48(d)49(a)50(a)51(d)

NEET PREVIOUS YEARS QUESTIONS-EXPLANATIONS

1. (c) PCR is based on three simple steps required for any DNA synthesis reaction: (i) denaturation of the template into single strands; (ii) annealing of primers to each original strand for new strand synthesis; and
(iii) extension of the new DNA strands from the primers.
2. (a) Retrovirus is commonly used as vector for introducing a DNA fragment in human lymphocyte.
3. (c) Ethidium bromide (Et Br) is used to stain DNA fragments and will appear as orange coloured bands when kept under UV light.
4. (d) Selectable markers in recombinant DNA technology, help in identification and elimination of non transformants and selectively permits the growth of the transformants.
5. (a) DNA fragments during gel electrophoresis, separate (resolve) according to their size due to sieving effect
provided by agarose gel.

6. (a) The various stages of processing that occur after the completion of fermentation or biosynthetic stage which include separation and purification of product are called downstream processing.
7. (d) Plasmid has an extra chromosomal, double stranded circular DNA.
8. (a) The *Taq* polymerase enzyme is obtained from *Thermus aquaticus* which lives in hot springs.
9. (a) A restriction enzyme or restriction endonuclease is an enzyme that cuts DNA at or near specific recognition nucleotide sequences known as restriction sites. *Hind* II among these is a type of restriction endonuclease.
10. (d) Restriction enzymes are used to cut DNA at specific locations.
11. (a) A vector is a DNA molecule which is used as a vehicle to carry the gene of interest to another cell.
12. (d) PCR is a technique for enzymatically replicating DNA without using a living organism such as *E.coli* or Yeast. It is commonly used in medical and biological research labs for a variety of tasks like detection of hereditary diseases, identification of genetic fingerprints etc.
13. (a) Now a days, PCR and RAPD technique are used for the characterisation of *in vitro* clonal propagation in
plants.
14. (c) Plasmids are small extranuclear circular DNAs which carry extrachromosomal genes in bacteria and some fungi. They replicate independently. The best known vectors which are also available commercially are *pBR* 322 and *pUC*-18.
15. (b)
26. Palindromic sequence is a specific sequence of nitrogen basis in DNA molecule which red same and both the strands, if the reading polarity is same
5' – GAATTC – 3',
3' – CTTAAG – 5'
27. Sticky ends can be joined by using DNA ligases, it not related to restriction enzymes
28. In gel electrophoresis DNA fragments are stained by ethidium bromide in UV radiation
29. Ligases join the two DNA molecules and useful for rDNA preparation
30. Ori site is the place of origin of replication of DNA and it also controls copy number
31. After the bands are stained, they are viewed in UV light. The bands appear bright orange in colour.Ethidium bromide is the intercalating agent that stacks in between the nitrogenous bases.
32. Gene amplification
33. During the purification process for recombinant DNA technology, addition of chilled ethanol precipitates out – DNA
34. Denaturation, Annealing, extension
35. It will not be able to confer ampicillin resistance to host cells
36. In high temperature $\left(>90^{0}C\right)$ is not maintained denaturation not possible which leads to failure of annealin

37. Assertion is about PCR, whereas Reason is about SELECTABLE MARKER hence it is not the correct explanation

NCERT - XII Page No- 202 & 199

38. The presence of chromogenic substrate gives blue coloured DNA bands on the gel is related to selection of recombinant cells from Non – Recombinants

39. $5^{!}$ GAATTC $3^{!}$

$3^{!}$ CTTAAG $5^{!}$ Is a palindrome and the rest are not palindromes
40. Both statements are correct
41. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning and is not a desirable feature of a cloning vector hence the statement is false

42.Ans.(c)

Explanation

Correct answer is option (c) because
The fate of a piece of DNA carrying only gene of interest which is transferred into an alien organism are:
(b) It may get integrated into the genome of the recipient
(c) It may multiply and be inherited along with the host DNA
⇒ This piece of DNA would not be able to multiply itself in the progeny cells of the organism but when gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA.

43.Ans. (b)
Explanation
The correct answer is option (b).

The first restriction endonuclease - Hind II, whose functioning depends on a specific DNA nucleotide sequence was isolated. It was found that Hind II always cut DNA molecules at a particular point by recognising sequence of six base pairs.

Option (a), (c) and (d) are incorrect because they have either more than 6 or less than 6bp.

44.Ans.(d)
Explanation
In pBR322, a commonly used plasmid in genetic engineering, certain restriction sites are present within the antibiotic resistance genes, which provide resistance to tetracycline and ampicillin. Here's what would happen if foreign DNA is inserted at each of the following restriction sites:
Option a : Pst I - The PstI site is present in the ampR gene (ampicillin resistance gene). Insertion of DNA here would disrupt the ampicillin resistance gene, causing a loss of ampicillin resistance, but it would not affect tetracycline resistance.
Option b : Pvu I - Similar to PstI, the PvuI site is also present in the ampR gene. Therefore, insertion of DNA here would cause a loss of ampicillin resistance, but it wouldn't affect the plasmid's tetracycline resistance.
Option c : EcoR I - The EcoRI site is not present within either the ampR or tetR genes in pBR322. Inserting DNA at this site would not disrupt either the ampicillin or tetracycline resistance genes.
Option d : BamH I - The BamH I site is within the tetR gene. If foreign DNA is inserted at this site, it would disrupt the tetracycline resistance gene, causing a loss of tetracycline resistance.
So, the correct answer to the question "Ligation of foreign DNA at which of the following site will result in loss of tetracycline resistance of pBR322?" is Option d : BamH I.

45.Ans.(c)
Explanation
Option (c) is correct answer because a single stranded DNA or RNA tagged with a radioactive molecule is called a probe and it helps in the detection of mutated gene.
Option (a), (b) and (d) are not correct because YAC, BAC, pBR 322 are vectors
46.Ans. (b)
Explanation
In Polymerase chain reaction, each cycle of amplification doubles the amount of DNA. So, if we start with one molecule of DNA, after:

- 1 cycle → 2 molecules
- 2 cycles → 4 molecules
- 3 cycles → 8 molecules
- ...and so on.

This pattern is exponential and follows the
formula:

Therefore, amount of DNA after n cycles $= 2^n$
Where n = number of cycles
And 2^n = fold increase in DNA quantity

47.Ans. (c)
Explanation
In insertional inactivation process, a recombinant DNA is inserted into the β-galactosidase gene, which inactivates the enzyme.

- When no insert is present, the enzyme works, and colonies turn blue due to a chromogenic substrate.
- When the insert is present, the enzyme is inactivated, and white(no colour) appears, identifying the colonies as recombinant.

48.Ans. (d)
Explanation

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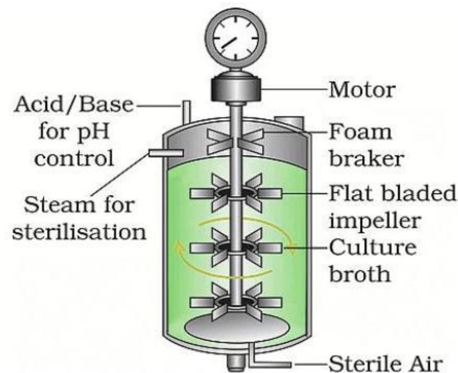

49.Ans. (a)
Explanation

- The DNA fragments extracted from the gel electrophoresis can be used in the construction of recombinant DNA molecules by joining them with cloning vectors.
- Since DNA fragments are negatively charged molecules, therefore they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix.
- The DNA fragments separate according to their size through sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther it moves.
- Therefore, smaller fragments will be observed at the anode whereas the larger DNA fragments near the wells in agarose gel.

50.Ans. (a)
Explanation

The enzymes essential for gene cloning are: Restriction enzyme - It recognises a specific sequence of nucleotides in double stranded DNA and cuts the DNA at a specific location. DNA ligase - They join broken pieces of DNA strand together during DNA replication. DNA polymerase - They function by replicating DNA by synthesizing new DNA strands using existing DNA strands as templates.
DNA mutase and DNA recombinase are not essential for gene cloning. As DNA mutase are enzymes that catalyse the movement of a functional group within a single molecule, effectively rearranging the molecular structure. DNA recombinase facilitates the exchange of DNA strands between two segments that share partial sequence homology.

51.Ans. (d)
Explanation
Selectable markers have been developed which differentiate recombinants from nonrecombinants 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.

This results into inactivation of the gene for synthesis of this 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 gene and the colonies do not produce any colour, these are identified as recombinant colonies.

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"The future belongs to those who explore the unseen — where biology meets innovation and discovery begins."