CHAPTER : 11

BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

STUDY-NOTES

Biotechnology deals with the techniques of using live organisms or, enzymes from organisms to produce products and processes useful to humans.

PRINCIPLES OF BIOTECHNOLOGY

A. Genetic engineering

- It is the technique to modify the chemistry of genetic material (DNA and RNA).
- Later on these are introduced into the host organisms and thus changes the phenotype of the host organism.

B. Bioprocess engineering

- This is the maintenance of sterile (microbial contamination-free) environment in chemical engineering processes.
- It allows the growth of only the desired organisms in large quantities for the manufacture of products in large quantities. For example, antibiotics, vaccines, enzymes, etc.
- Asexual reproduction preserves the genetic information, while sexual reproduction documents variation.
- The **traditional hybridisation procedures** of plant and animal breeding, mostly leads to inclusion and multiplication of undesirable genes along with the desired genes. This limitation was overcome by genetic engineering processes.
- The process of **genetic engineering** includes creation of recombinant DNA, use of gene cloning and gene transfer methods. It permits to isolate and introduce only the desirable genes.
- The specific sequence in DNA called the **origin of replication** initiates the process replication. When an alien piece of DNA is linked with the origin of replication of host DNA, it also replicates with it and multiply itself in the host organism. This is called **cloning** or making multiple identical copies of any template DNA.
- The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native plasmid (autonomously replicating circular extra-chromosomal DNA) of *Salmonella typhimurium.*
- In 1972, Stanley Cohen and Herbert Boyer first made the recombinant DNA.
- The cutting of DNA at specific locations became possible with the discovery of the so-called '**molecular scissors**'- restriction enzymes.
- The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNA act as **vectors** to transfer the piece of DNA attached to it.
- A plasmid can be used as vector to deliver an alien piece of DNA into the host organism.
- The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme DNA ligase, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular autonomously replicating DNA created *in vitro* and is known as recombinant DNA.
- When this DNA is transferred into *Escherichia coli*, a bacterium closely related to *Salmonella,* it could replicate using the new host's DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in *E. coli* was called cloning of antibiotic resistance gene in *E. coli.*
- There are three simple steps for genetically modifying an organism –
  (i) Identification of DNA with desirable genes
  (ii) Introduction of identified DNA into the host
  (iii) Maintenance of introduced DNA in the host and transfer of the DNA to its progeny

TOOLS OF RECOMBINANT DNA TECHNOLOGY

A. Restriction Enzymes

- The first restriction endonuclease isolated was *Hind II.* Hind II always cut DNA molecules at a specific sequence, called recognition sequence. The recognition sequence is of six base pairs for *Hind II.*

B. Naming of Restriction Enzymes

(i) First letter of the name denotes genus and the second two letters denote the species from which they have been isolated. For example, e.g., *EcoRI* comes from *Escherichia coli* RY 13. The letter 'R' is derived from strain.

(ii) Roman numbers indicate the order in which the enzymes have been isolated from the strain.

Nucleases

Restriction enzymes belong to a larger class of enzymes called nucleases. Nucleases are of two types:

(i) Exonucleases: Exonucleases remove nucleotides from the ends of the DNA.

(ii) Endonucleases: Endonucleases make cuts at specific locations within the DNA sequence.

C. Function of restriction endonucleases

- Each restriction endonuclease checks the length of a DNA sequence.
- On finding its specific recognition sequence, it binds to DNA and cut each strands at specific points in sugar -phosphate backbones.
- Each restriction endonuclease recognises a specific palindromic nucleotide sequences in the DNA.
- Restriction enzymes cut the DNA strand little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. This leaves single stranded portions at both the ends. These are overhanging stretches called **sticky ends**.
- The sticky ends form hydrogen bonds with their complementary cut counterparts.
- **DNA ligase** joins the two sticky ends of DNA.

D. Palindromic sequence

- Palindrome is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.
- For example, the following sequences read the same on the two strands in 5′ →3′ direction. This is same if read in the 3′ → 5′ direction.

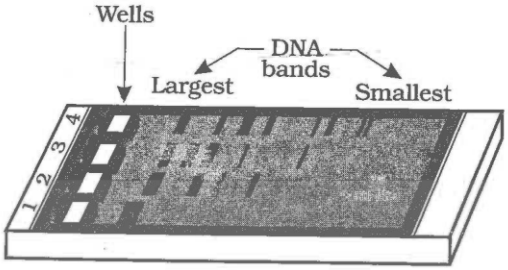
5'—GAATTC—3'
3'—CTTAAG—5'

E. Separation and isolation of DNA fragments

The restriction endonucleases cut DNA and forms DNA fragments. These fragments can be separated by **gel electrophoresis.**

Gel electrophoresis

- In gel electrophoresis, the DNA fragments are separated by applying electric force.
- Under the electric field, the negatively charged DNA fragments move towards the anode through a medium/ matrix.
- The most commonly used matrix is agarose. It is a natural polymer extracted from sea weeds.
- Agarose gel provides the sieving effect and separates the DNA fragments according to their size. The smaller DNA fragments move farther on the gel.
- The separated DNA fragments is visualised only after staining the DNA with ethidium bromide followed by exposure to UV rays. This step is performed because pure DNA fragments cannot be seen without staining and under the visible light.
- The bright orange coloured bands of DNA in ethidium bromide stained gel is observed under the UV light.
- The separated bands of DNA are cut from the agarose gel and extracted from the gel piece. This process is known as **elution.** The DNA fragments thus obtained is used in recombinant DNA technology.



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

F. Cloning Vectors

Types of cloning vectors

(a) Plasmids

- Plasmids may have only one or two copies per cell or may have 15-100 copies per cell.

(b) Bacteriophages

- Bacteriophages have very copy number of their genome within the bacterial cells.
- Both plasmids and bacteriophages replicate within bacterial cells which is independent of the control of chromosomal DNA.
- When any alien piece of DNA is linked with bacteriophage or plasmid DNA, it also multiplies its numbers equal to the copy number of the plasmid or bacteriophage.

G. Features of Cloning Vectors

(i) Origin of replication (ori)

- It is a sequence in DNA from where replication starts.
- Any piece of DNA when linked to *ori* sequence also replicates within the host cells. Hence, the *ori* sequence controls the copy number of the linked DNA also. Therefore, if we have to recover the many copies of target DNA, it should be cloned in a vector with high copy number.

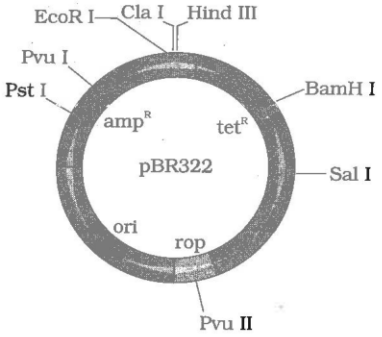
(ii) Selectable marker

- The selectable markers identify and eliminate the non-transformant cells. It allows growth of only the transformant cells.
- Transformation is a procedure through which a piece of DNA is introduced in a host bacterium.
- Usually, the genes that encodes for resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered suitable selectable markers for *E. coli.*
- The normal *E. coli* cells do not have resistance against any of these antibiotics. Therefore, these cells will not survive in presence of these antibiotics, whereas only transformed cells will grow in antibiotic containing culture medium.

(iii) Cloning sites

- The cloning vector should have single recognition sites for the commonly used restriction enzymes.
- Presence of more than one recognition sites within the vector generates several fragments, which complicates the gene cloning experiments.
- The foreign DNA is ligated at a restriction site which is present in one of the two antibiotic resistance genes.
- If a foreign DNA is ligated at the *BamHI* site of tetracycline resistance gene in the vector pBR322, the insertion of foreign DNA will cause the recombinant plasmids to lose resistance to tetracycline. However, it can be selected from non-recombinants by plating the transformants on tetracycline containing medium.
- The transformants growing on ampicillin containing medium are then transferred on a medium containing tetracycline. The recombinants will grow in ampicillin containing medium but not on the tetracycline containing medium.

- Selection of recombinants due to inactivation of antibiotics is troublesome because it requires simultaneous plating on two different plates with different antibiotics. Therefore, alternative selectable markers have been developed.
- The alternative marker differentiates recombinants from non-recombinants on the basis of the colour produced in presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. This inactivates the β-galactosidase gene, hence synthesis of this enzyme also does not occur. This is known as **insertional inactivation.**
- If the bacterial plasmids do not have any insert, it produces blue coloured colonies. This indicates that these are non-recombinant cells. On the other hand, the presence of DNA insert causes insertional inactivation of the β-galactosidase gene and the colonies do not produce any colour. These are recombinant colonies.



E. coli cloning vector pBR322 with restriction sites

(iv) Vectors for cloning genes in plants and animals

(a) Vectors for plants

- *Agrobacterium tumefaciens*, a pathogen of dicot plants. It delivers a piece of DNA known as 'T-DNA' to normal plant cells. Hence, transforms them into a tumor cells and direct the tumor cells to produce the chemicals which is required by the pathogen.
- The tumor inducing (Ti) plasmid of *Agrobacterium tumefaciens* is modified into a cloning vector which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants

(b) Vectors for animals

- Retroviruses also transform normal cells into cancerous cells. These are also disarmed and used to deliver desirable genes into animal cells.

H. Competent Host for Transformation with rDNA

Introduction of recombinant DNA into bacterial/host cells is performed through following methods-

(a) Treatment with divalent Calcium

- DNA is a hydrophilic molecule. Therefore, it cannot pass through cell membranes. Hence, bacteria are forced and made competent to take up the plasmid DNA.
- This is performed by treating bacterial cells with a specific concentration of a divalent cation, such as calcium. This creates pores in bacterial cell wall.
- Recombinant DNA can then be forced into such cells by incubating the bacterial cells with recombinant DNA on ice.
- It is then placed briefly at 42°C (heat shock), and then again putting them back on ice. This allows the bacteria to take up the recombinant DNA.

(b) Micro-injection method

- The recombinant DNA is directly injected into the nucleus of an animal cell through micro-injection.

(c) Biolistics or gene gun method

- It is suitable for plants.
- Micro-particles of gold or tungsten is coated with DNA and bombarded into cells with high velocity.

(d) Disarmed pathogen vectors

- These infect the cell and transfer the recombinant DNA into the host.

PROCESSES OF RECOMBINANT DNA TECHNOLOGY

A. Isolation of the DNA

(i) **Lysozyme:** From bacterial cells
(ii) **Cellulases:** treatment of plant cells
(iii) **Chitinases:** treatment of fungal cells
(iv) **RNAses:** RNAs is removed
(v) **Proteases:** Proteins removed
(vi) After all the above treatment, chilled ethanol is added. The purified DNA then precipitates out.

B. Cutting of DNA at Specific Locations

- Purified DNA is incubated with restriction enzymes at the optimal conditions (of that specific enzyme).
- Then the cut DNA is run through **agarose gel electrophoresis**. Being negatively charged, DNA moves towards the positive electrode (anode). This process is repeated with the vector DNA also.
- Then, the fragments of 'gene of interest' from source DNA as well as the vector DNA then mixed and DNA ligase is added. This results in the formation of recombinant DNA.

C. Amplification of Gene of Interest using PCR

- PCR (Polymerase Chain Reaction) was developed by **Kary Mulis** in 1985.
- In PCR, multiple copies of the gene (DNA) of interest is synthesised *in vitro*.
- In this reaction, two sets of primers (small chemically synthesised oligonucleotides complementary to the regions of DNA), thermostable enzyme DNA polymerase (isolated from a bacterium, *Thermus aquaticus*), and nucleotides are added in PCR machine.
- PCR has 3 steps:
  (i) **Denaturation** is performed at 95°C for 15 minutes. This step separates the two strands of dsDNA.
  (ii) **Annealing** occurs at 40-60°C. In this step the oligonucleotide primers anneal to each separated strands.
  (iii) **Extension** is performed by *Taq DNA polymerase* which uses the nucleotides and extend the primers by adding nucleotides, which is complementary to the template strand. The process of DNA replication is repeated many times; hence the desired segment of DNA is amplified with a short period of time. The amplified DNA fragment is used to ligate with a vector for further cloning.

D. Insertion of Recombinant DNA into the Host Cell/Organism

- The ligated DNA can be introduced into recipient cells by various methods.
- After being competent to receive, recipient cells take up DNA present in its surrounding. Therefore, in this way if a recombinant DNA with antibiotic (e.g., ampicillin) resistance gene is transferred into *E. coli*, it also become transformed into ampicillin-resistant cells.
- When we grow the transformed cells on agar plates containing ampicillin, only the transformant cells will grow.
- The untransformed cells will die. In this case, the ampicillin resistance gene is called the **selectable marker** gene.

E. Obtaining the Foreign Gene Product

(i) Cells grown in laboratory (batch) culture

- The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The desired protein can be extracted from such cells and then purified by using different separation techniques.

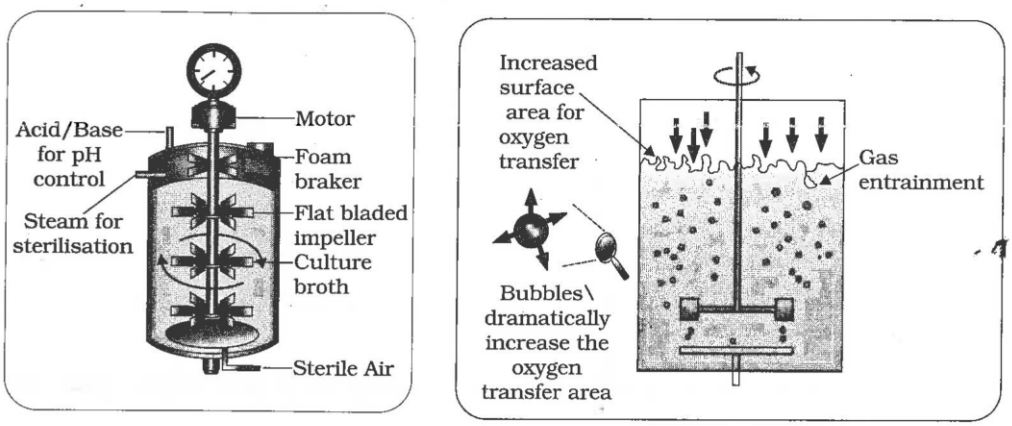
(ii) Cells grown in continuous culture

- The cells can also be multiplied in a continuous culture system.
- In the continuous culture system, the fresh medium is continuously added and the used medium is simultaneously drained from the other side.
- It maintains the cells in their log/exponential phase. It is the phase of most active growth of cells. Therefore, the continuous culture produces a larger biomass of cells and hence very high yields of desired protein.

(iii) Bioreactors

- Large quantities of products can be obtained by growing cells in bioreactors.
- In bioreactors, the large volumes (100-1000 litres) of culture can be processed.

- These are huge vessels in which raw materials are biologically converted into specific products, for example, enzymes, etc.,
- A bioreactor provides the optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen) to the cells.
- A stirred-tank reactor is cylindrical or with a curved base. It has stirrer inside the reactor which helps in even mixing of contents and availability of oxygen throughout the bioreactor.
- Alternatively, air can also be bubbled through the reactor.
- Bioreactor has an agitator system, an oxygen delivery system and a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.



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

F. Downstream Processing

- After the biosynthesis of the product, it is subjected to a series of processes before it is ready to sale in market as a finished product.
- These processes include separation and purification of products which are collectively known as **downstream processing**.
- The product is then formulated with suitable preservatives. Such formulations undergo clinical trials in case of drugs.
- Strict quality control testing for each product is also done.
- The downstream processing and quality control testing vary from product to product.

QUESTION BANK

MULTIPLE CHOICE QUESTIONS

1. Select the incorrect statement:

(a) The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native plasmid *Streptococcus.*

(b) Restriction enzymes are 'molecular scissors'.

(c) Plasmid DNA act as vectors to transfer the piece of DNA attached to it.

(d) The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme DNA ligase.

2. Consider the following statements.

A. Identification of DNA with desirable genes;

B. Introduction of the identified DNA into the host;

C. Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

Select the correct sequence of different steps for genetically modifying an organism.

(a) ABC (b) BAC (c) CAB (d) CBA

3. Select the method through which DNA is introduced into the host cell.

(a) Microinjection (b) Biolistic (c) Electroporation (d) All of these

4. The first restriction endonuclease was

(a) *Eco Ri* (b) *Bam HI* (c) *TaqI* (d) *Hind II*

5. The restriction endonuclease HindII recognises sequence of

(a) 2 base pairs (b) 3 base pairs (c) 4 base pairs (d) 6 base pairs

6. Which of the following is a palindromic sequence?

(a) 5′-CGTATG -3 ′
3′-CGAATG-5 ′

(b) 5′-CGAATG-3′
3′-GCATAC-5′

(c) 5′-GAATTC- 3′
3′-CTTAAG-5′

(d) 5′-CGAATG-3′
3′-CTTAAG-5

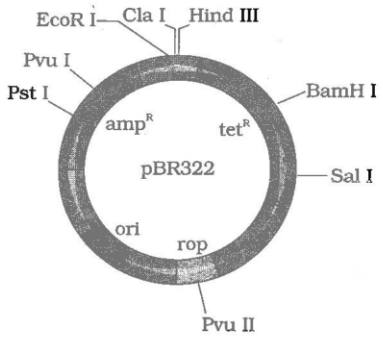
7. In *Escherichia coli RY 13*, the letter 'R' is derived from

(a) Name of strain (b) Type of strain (c) Name of species (d) Type of genus

8. Select the correct statements about sticky ends.

(a) These are overhanging stretches of DNA.
(b) They form hydrogen bonds with their complementary cut counterparts.
(c) This stickiness of the ends facilitates the action of the enzyme DNA ligase.
(d) All are correct.

9. The figure given below is the diagrammatic presentation of *E coli* vector pBR 322. Which one of the following correctly identifies its certain components?



(a) Ori-original restriction enzyme (b) rop-reduced osmotic pressure
(c) Hindi III, Eco RI-selectable markers (d) amp^R, tet^R-antibiotic resistance genes

10. In the naming procedure of restriction endonucleases, the Roman numbers following the names indicate

(a) the order in which the strain was isolated from bacteria
(b) the order in which the species were discovered
(c) the order in which the enzymes were isolated from that strain of bacteria
(d) the order in which the genus was cultured in laboratory

11. The enzyme used in the polymerase chain reaction is a

(a) DNA dependent RNA polymerase (b) RNA dependent DNA polymerase
(c) DNA dependent DNA polymerase (d) RNA dependent RNA polymerase

12. Select the character which is not required in a cloning vector.

(a) An inactive promoter
(b) An origin of replication
(c) Selectable markers such as genes for antibiotic resistance
(d) One or more unique restriction endonuclease sites

13. **A cloning vector has two antibiotic resistance genes – for tetracycline and ampicillin. A foreign DNA was inserted into the tetracycline gene. Non recombinants would survive on the medium containing**
    (a) Ampicillin but not tetracycline
    (b) Tetracycline but not ampicillin
    (c) Both tetracycline and ampicillin
    (d) None of these

14. **The function of calcium ion during the process of insertion of recombinant DNA is**
    (a) Binds to recombinant DNA for easy insertion
    (b) The plasma membrane becomes more permeable
    (c) Makes pores in the bacterial cell wall
    (d) The nucleus easily takes rDNA

15. **Which of the following cells grow on plates?**
    (a) Transformant (b) Non-transformant (c) Both (a) and (b) (d) None

16. **The colonies of recombinant bacteria appear white in contrast to blue colonies of non-recombinant bacteria because**
    (a) Non recombinant bacteria contain β galactosidase
    (b) Insertional inactivation of α galactosidase in non-recombinant bacteria
    (c) Insertional inactivation of β galactosidase in recombinant bacteria
    (d) Inactivation of galactosidase enzyme in recombinant bacteria

17. **Which of the following method of insertion of recombinant DNA is performed for animal cell?**
    (a) Microinjection
    (b) Biolistic
    (c) Electroporation
    (d) None of these

18. **Which plate is used in rDNA for DNA insertion?**
    (a) Agar plate containing DNA polymerase
    (b) Agar plate containing Cellulase and chitinase
    (c) Agar plate containing restriction endonuclease
    (d) Agar plate containing antibiotic

19. **Elution is**
    (a) Separating the restricted DNA fragments on agarose gel
    (b) Staining the separate DNA fragments with EtBr
    (c) Cutting out of the separated band of DNA from the agarose gel and extracting them from the gel
    (d) Constructing rDNA by joining the purified fragments to the cloning vector

20. **DNA is enclosed within the membranes. Which membrane has been referred here?**
    (a) Cell membrane (b) Nuclear membrane (c) Vacuolar membrane (d) None of these

21. **Which of the following is not correctly matched?**
    (a) Bacteria-lysozyme (b) Plant cells–cellulase (c) Algae–aldolase (d) Fungi–chitinase

22. **A bacterial cell is treated with _____ to remove DNA from its cell.**
    (a) Cellulase (b) Protease (c) RNAse (d) Lysozyme

23. **Chitinase is used for the isolation of DNA from**
    (a) Fungal cell (b) Plant cell (c) Animal cell (d) Bacterial cell

24. **A host cell normally does not take up a foreign DNA until it has been made competent to do so. This is due to**
    (a) DNA is a hydrophilic molecule.
    (b) DNA is very large.
    (c) There are no receptors for DNA molecule.
    (d) DNA is inert.

25. **DNA is extracted in**
    (a) Chilled ethanol (b) Chilled ether (c) Chilled ester (d) None of these

26. **For transformation, microparticles coated with DNA to be bombarded with gene gun are made up of**
    (a) Silver platinum (b) Zinc or gold (c) Silicon Tungsten (d) Gold Tungsten

27. Sticky-ends can be joined together by

(a) DNA ligases
(b) Restriction endonucleases
(c) DNA polymerases
(d) Exonucleases

28. Which of the following is not correct about restriction endonucleases?

(a) They recognize a specific base sequence.
(b) They are produced by bacterial cells as a primitive immune system.
(c) They digest DNA by removing nucleotides from a 3′ end.
(d) They often generate stick ends.

29. The histone proteins associated with DNA are found in

(a) Eukaryotes (b) Prokaryotes (c) Both (a) and (b) (d) None of these

30. Plasmid vectors are

(a) dsDNA molecule
(b) extrachromosomal molecule
(c) Present in bacteria
(d) All of these

31. Select the correct statement:

(a) EcoRI comes from *Escherichia coli* RY 13.
(b) Restriction endonuclease cut DNA strands of sugar-phosphate backbones.
(c) Bioprocess engineering involves Maintenance of sterile environment in chemical processes.
(d) All are correct

32. In the screening process during rDNA experiments, clones that metabolise β-gal turn

(a) Colourless (b) Blue (c) Yellow (d) Green

33. Biolistic gen gun method is suitable for

(a) Disarming pathogen vectors
(b) Transformation of plant cell
(c) Constructing recombinant DNA by joining vectors
(d) DNA fingerprinting

34. Biolistic gen gun method is suitable for

(a) Disarming pathogen vectors
(b) Transformation of plant cell
(c) Constructing recombinant DNA by joining vectors
(d) DNA fingerprinting

35. Restriction enzymes are synthesised by

(a) Bacteria only (b) Yeast and Bacteria (c) Eukaryotic cells (d) All types of cells

36. In EcoRI restriction endonuclease "I" denotes

(a) Species name (b) Genus name (c) Name of strain (d) Order of isolation

37. Plasmids are used as cloning vectors because they

(a) can be multiplied in culture
(b) self-replicate within bacterial cells
(c) can be multiplied in laboratories with the help of enzymes
(d) replicate freely outside the bacterial cells

38. Bacteriophages are

(a) Bacteria (b) Viruses (c) DNA (d) Plasmids

39. The first recombinant DNA was made from

(a) *Salmonella* (b) *E. coli* (c) *Agrobacterium* (d) *Haemophilus*

40. Which of the following is a vector?

(a) Plasmid (b) Bacteria (c) Virus (d) *Agrobacterium*

41. The sequence of basic steps while genetically modifying an organism is

A. Identification desirable genes;
B. Introduction of the identified DNA into the host;
C. Maintenance of introduced DNA in the host and
D. Transfer of the DNA to its progeny.

Select the correct option:

(a) BACD (b) CBAD (c) ABCD (d) ABDC

42. Restriction enzymes are _____ .

(a) Ligases (b) Sticky ends (c) Molecular scissors (d) Vectors

43. Which of the following is correctly matched?

(a) Restriction Endonuclease - Molecular scissors
(b) *Thermus aquaticus* - *Bt-gene*
(c) *Agrobacterium tumefaciens* – Eco RI
(d) *Hind II* - Plasmid vector

44. Which of the following enzymes remove nucleotides from the ends of the DNA?

(a) Exonuclease (b) Endonuclease (c) Ligase (d) Permease

45. In fungi, DNA can be isolated by using

(a) Ligase (b) Lysozyme (c) Cellulase (d) Chitinase

46. The DNA fragments possess sticky ends due to

(a) Unpaired bases (b) Phosphates (c) Deoxyribose sugar (d) None of these

47. Restriction Enzymes are

(a) Endonuclease which cleave DNA at specific sites
(b) Make DNA complementary to an existing DNA or RNA
(c) Cut or join DNA fragments
(d) Are required in vector less direct gene transfer

48. Restriction enzymes were discovered by

(a) Smith and Nathans (b) Alexander Fleming (c) Paul Berg (d) Watson and crick

49. Select the correct order of different steps in PCR.

(a) Extension, Priming, Annealing, Denaturation
(b) Denaturation, Priming, Annealing, Extension
(c) Priming, Extension, Annealing, Denaturation
(d) Priming. Annealing, Denaturation, Extension

50. How are transformant cells selected from non-transformant?

(a) Presence of more than one recognition sites in the vector DNA
(b) Presence of alien DNA into the vector DNA results into insertional inactivation of selectable marker
(c) Antibiotic resistance gene gets inactivated due to insertion of alien DNA
(d) Both (b) and (c)

51. _____ is used as a vector for cloning into higher organisms.

(a) *Retrovirus* (b) *Baculovirus* (c) *Salmonella typhimurium* (d) *Rhizopus nigricans*

52. On a large scale, the products from a recombinant gene can be obtained from

(a) Autoclave (b) Thermocycler (c) Bioreactors (d) Tissue culture

53. The source of *ECO RI* is

(a) *Escherichia coli* RI
(b) *Escherichia coli* RI 13
(c) *Escherichia coli* RY 13
(d) *Escherichia coli* RX 13

54. In agarose gel electrophoresis DNA fragments move according to

(a) Nature of gel applied (b) Positive charge (c) Negative charge (d) Size

55. Which of the following is used for visualising DNA?

(a) Ethidium chloride (b) Ethidium bromide (c) Ethyl bromide (d) Methyl bromide

56. Which of the following is used as a selectable marker?

(a) Ampicillin resistance gene
(b) Plasmid resistance gene
(c) Salmonella resistance gene
(d) Penicillin resistance gene

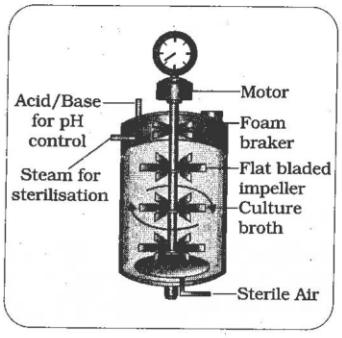
57. Technique associated with DNA amplification is

(a) DNA fingerprinting (b) PCR (c) Southern Blotting (d) Northern Blotting

58. Which of the following is the primary requirement of a vector?

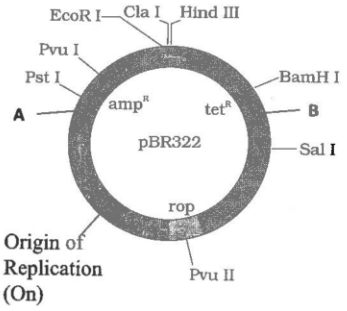
(a) *'ori'*, (b) Selectable marker (c) Cloning sites (d) All of these

59. The bioreactor shown in the diagram is



(a) Sparged stirred tank (b) Simple stirred tank (c) Hydrogen sparged tank (d) None of these

60. In the given figure, A and B denotes



(a) *Pvu II* and *Cla I* (b) *ROP* and *Sal I* (c) *amp* ^{R} and *tet* ^{R} (d) *tet* ^{R} and *Sal I*

61. Plasmid vectors are

(a) dsDNA molecule
(b) extrachromosomal molecule
(c) Present in bacteria
(d) All of these

62. Which one of the following palindromic base sequences in DNA can be easily cut at about the middle by some particular restriction enzyme?

(a) 5'—CGTTCG—3'
3'—ATGGTA—5'
(b) 5'—GATATG—3'
3'—CTACTA—5'
(c) 5'—GAATIC—3'
3'—CTTAAG—5'
(d) 5'—CACGTA—3'
3'—CTCAGT—5'

63. Selectable markers in vector

(a) are responsible for replication
(b) help in selecting transformation from non transformant
(c) code for proteins involved in the replicating plasmids
(d) contain unique recognition sites

64. *ori* is a specific DNA sequence which facilitates

(a) Attachment of primer
(b) Extension of DNA sequence
(c) Replication initiation
(d) Denaturation

65. Select the correct sequence of PCR.

(a) Extension, annealing, denaturation
(b) Denaturation, annealing, extension
(c) Denaturation, extension, annealing
(d) Annealing, denaturation, extension

66. A mixture of DNA fragments a, b, c, and d with molecular weight a+b=c, a>b and d> c was subjected to agarose gel electrophoresis. The position of these fragments from anode to cathode sides of the gel would be

(a) b a c d (b) a b c d (c) c b a d (d) b a c d

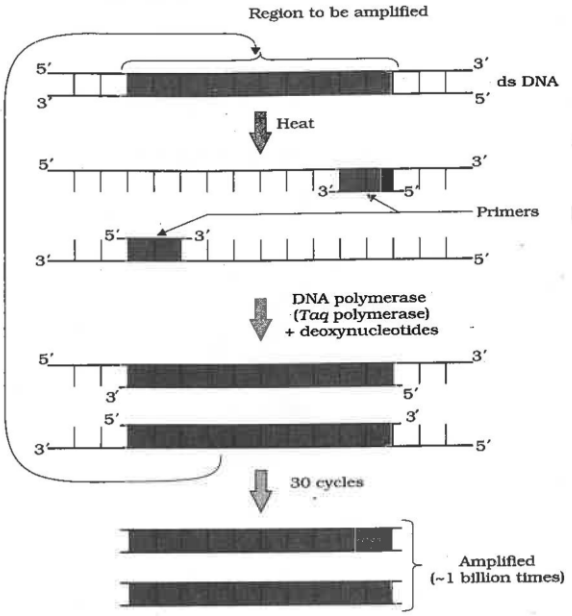
67. p in pBR 322 indicates

(a) Plasmid (b) Prokaryotic cell (c) Plant cell (d) Protozoan

68. The thermostable enzyme Taq polymerase used in PCR was discovered by

(a) Allec Jeffery (b) Karry Mullis (c) Temin and Baltimore (d) Rosalin Franklin

69. What is true about the steps of PCR shown in diagram given below?



I. Step A occurs at temperature of 95-98°C
II. Step B occurs at temperature of 55°C
III. Step C occurs at the temperature of 72°C

Select the correct answer

(a) I and II only (b) I and III only (c) II and III only (d) I, II and III only

70. pBR 322 is frequently used as a vector for cloning gene in *E coli* is a/an

(a) Original bacterial plasmid
(b) Modified bacterial plasmid
(c) Viral genome
(d) Transposon

71. How are transformants selected from non-transformants?

(a) Presence of more than one recognition site in the vector DNA
(b) Presence of alien DNA into the vector DNA results into insertional inactivation of selectable marker
(c) Antibiotic resistance gene gets inactivated due to insertion of alien DNA
(d) Both (b) and (c)

72. Which of the following is not a characteristic of a pBR322 vector?

(a) It is the first artificial cloning vector constructed in 1977 by Boliver and Rodriguez.
(b) It is the most widely used versatile and easily manipulated vector.
(c) It has two antibiotic resistance genes tet^R and amp^R.
(d) It does not have restriction site for Sal I.

73. Which of the following is not a component of downstream processing?

(a) Separation (b) Purification (c) Preservation (d) Expression

74. Which of the following restriction enzymes produces blunt ends?

(a) Sal I (b) Eco RV (c) X ho (d) Hind III

75. Which of the following is commonly used I transfer of foreign DNA into crop plants?

(a) *Trichoderma harzianum* (b) *Meloidogyne incognita*

(c) *Agrobacterium tumefaciens* (d) *Penicilium notatum*

76. The genes encoding resistance to antibiotics are considered as useful selectable markers for *E. coli*. Which of the following is/are correct?

(a) Chloramphenicol (b) Ampicllin (c) Tetracycline (d) All of these

77. Microbes are found to be very useful in genetic engineering are

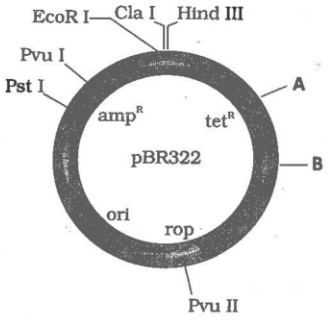
(a) *E. coli* and *A. tumefaciens* (b) *V. cholerae* and tailed bacteriophage

(c) *Dipococcus* sp. admn *Pseudomonas* sp. (d) None of these

78. cDNA probes are copied from the mRNA molecules with the help of

(a) Restriction enzymes (b) Reverse transcriptase (c) DNA polymerase (d) Peptidyl transferase

79. A and B in the pBR 322 shown in the diagram given below, respectively represent recognition sequences of



(a) Bam HI and Sma I (b) Hind II and Sma I (c) BamHI and Sal I (d) Sal I and Hind II

80. Which vector can clone only a small fragment of DNA?

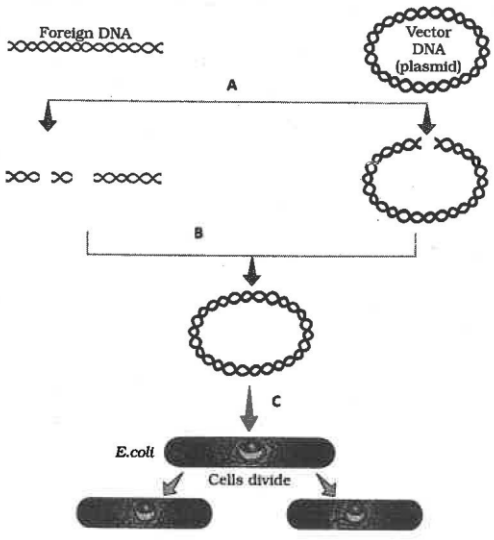
(a) Bacteria artificial chromosome (b) Yeast artificial chromosome

(c) Plasmid (d) Cosmid

81. A foreign DNA and plasmids cut by the same restriction endonuclease can be joined to form a recombinant plasmid using

(a) Eco RI (b) Taq polymerase (c) DNA polymerase III (d) Ligase

82.



I. At A some restriction enzyme is used to cut both foreign and vector DNA.
II. The enzyme used at B is DNA ligase.
III. Step C can be called as transformation

Select the correct answer.

(a) I and II only (b) I and III only (c) II and III only (d) All of these

83. The DNA fragments separated on an agarose gel can be visualised after staining with

(a) Bromophenol blue (b) Acetocarmine (c) Aniline blue (d) Ethidium bromide

84. Select the criterion for DNA fragments movement on agarose gel during electrophoresis.

(a) The larger the fragment size, farther it moves (b) The smaller the fragment size, farther it moves
(c) Positively charged fragment move to farther end (d) Negatively charged fragments do not move

85. The DNA molecule to which the gene of interest is integrated for cloning is called

(a) Vector (b) Template (c) Carrier (d) All of these

86. The process of separation and purification of expressed protein before marketing is called

(a) Upstream processing (b) Downstream processing
(c) Bioprocessing (d) None of these

INPUT-TEXT BASED QUESTIONS

Read the following paragraphs and answer the following questions.

I. The techniques of genetic engineering include creation of recombinant DNA, use of gene cloning and gene transfer, allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism. Genetic engineering or recombinant DNA technology can be accomplished only if we have the key tools, i.e., restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.

1. The ability to multiply copies of antibiotic resistance gene in *E. coli* is called

(a) Cloning (b) Bioprocess engineering
(c) Genetic engineering (d) Gene transfer

2. The first recombinant DNA was made from

(a) *E. coli* (b) *Salmonella typhimurium*
(c) *Agrobacterium tumefaciens* (d) *Haemophilus ducreyi*

3. Match Column I with Column II.

Column I	**Column II**
A. Restriction Endonuclease	1. Origin of replication
B. *Hind II*	2. First restriction enzyme
C. Alien DNA	3. Molecular scissors
D. DNA ligases	4. Sticky ends of DNA

Select the correct option:

(a) A1, B2, C3, D4 (b) A3, B2, C1, D4 (c) A2, B1, C3, D4 (d) A3, B4, C1, D2

4. The basic steps in sequence in genetically modifying an organism is

(i) Maintenance of introduced DNA in the host;
(ii) Introduction of the identified DNA into the host;
(iii) Transfer of the DNA to its progeny.
(iv) Identification desirable genes;

Select the correct option:

(a) (ii), (i), (iii) and (iv) (b) (iv), (ii), (i) and (iii)
(c) (iv), (i), (iii) and (ii) (d) (i), (ii), (iii) and (iv)

5. Palindrome is a base pairs sequence. It reads same on two strands when orientation of reading is kept the same.

Which of the following is a palindromic sequence?

(a) 5′-CGTATG-3′ / 3′-CGAATG-5′ (b) 5′-CGAATG-3′ / 3′-GCATAC-5′ (c) 5′-GAATTC-3′ / 3′-CTTAAG-5′ (d) 5′-CGAATG-3′ / 3′-CTTAAG-5′

II. In a reaction, multiple copies of the gene (or DNA) of interest is synthesised in vitro using sets of primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase. The enzyme extends the primers using the nucleotides provided in the reaction and the genomic DNA as template. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made.

1. This reaction is called

(a) Primer Chain Reaction (b) Recombination Reaction

(c) Polymerase Chain Reaction (d) Protein Chain Reaction

2. In the above reaction, there are some steps. Which of the following is not included?

(a) Extension of primers (b) Annealing of primers

(c) Recognition of cloning sites (d) Denaturation

3. Which of the following is associated with this technique?

(a) DNA fingerprinting (b) DNA amplification (c) Southern Blotting (d) Northern Blotting

4. Two statements are given.

I-DNA polymerase uses genomic DNA as template in the technique.

II-*Thermus aquaticus* is the bacterium which is used to isolate the enzyme.

Choose the correct option.

(a) I-True, II-False (b) I-True, II-True (c) I-False, II-True (d) I-False, II-False

5. Which of the following statements is/are true for the above reaction?

(i) Annealing is the first step of this reaction.

(ii) *Agrobacterium tumefaciens* tranfers a piece of DNA in the first step.

(iii) Thermostable DNA polymerase is used for repeated amplification.

(iv) Denaturation involves heating at 90-98 degree centigrade.

Choose the correct option.

(a) (i) and (iv) (b) (iii) and (iv) (c) (i) and (ii) (d) (i) and (iii)

ANSWERS

1. (a)	2. (a)	3. (d)	4. (d)	5. (d)	6. (c)	7. (a)	8. (d)	9. (d)	10. (c)
11. (c)	12. (a)	13. (c)	14. (c)	15. (a)	16. (c)	17. (a)	18. (c)	19. (c)	20. (b)
21. (c)	22. (d)	23. (a)	24. (a)	25. (a)	26. (d)	27. (a)	28. (c)	29. (a)	30. (d)
31. (d)	32. (b)	33. (b)	34. (b)	35. (a)	36. (d)	37. (b)	38. (b)	39. (a)	40. (a)
41. (c)	42. (c)	43. (a)	44. (a)	45. (d)	46. (a)	47. (a)	48. (a)	49. (b)	50. (d)
51. (a)	52. (c)	53. (c)	54. (d)	55. (b)	56. (a)	57. (b)	58. (d)	59. (b)	60. (c)
61. (d)	62. (c)	63. (b)	64. (b)	65. (b)	66. (a)	67. (a)	68. (b)	69. (d)	70. (b)
71. (d)	72. (d)	73. (d)	74. (b)	75. (c)	76. (d)	77. (d)	78. (b)	79. (c)	80. (c)
81. (a)	82. (d)	83. (d)	84. (b)	85. (a)	86. (b)				

EXPLANATION

1. The construction of the first recombinant DNA emerged from the possibility of linking a gene encoding antibiotic resistance with a native plasmid of *Salmonella typhimurium.*

7. In *EcoRI*, the letter 'R' is derived from the name of strain.

10. Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

11. DNA-dependent DNA polymerases are responsible for directing the synthesis of new DNA from deoxyribonucleotide triphosphates (dNTPs) opposite an existing DNA template.

14. Ca^{++} assist the transfer of the recombinant vector into a bacterial host cell.

27. DNA ligases joins breaks in the phosphodiester backbone of DNA.

29. Histones are basic proteins that associate with DNA in the nucleus and help condense it into chromatin. These are found in eukaryotes only.

30. A vector is often a virus or a plasmid that is used to transfer desired DNA sequence into a host cell.

35. Restriction enzymes, also called restriction endonucleases, are protein produced by bacteria that cleaves DNA at specific sites.

37. A plasmid is a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells.

42. A restriction enzyme, restriction endonuclease is an enzyme that cleaves DNA into fragments at or near specific recognition sites.

76. Palindromic sequences read the same on the two strands in 5′ to 3′ direction.

Input-Text Based Answers

I.	**1.** (a)	**2.** (b)	**3.** (b)	**4.** (b)	**5.** (c)
II.	**1.** (c)	**2.** (c)	**3.** (b)	**4.** (b)	**5.** (b)