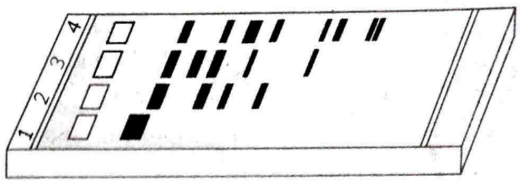
CHAPTER



9 Biotechnology: Principles and Processes

9.1. Principles of Biotechnology

1. In a chromosome, there is a specific DNA sequence, responsible for initiating replication. It is called as:
(A) recognition sequence (B) cloning site
(C) restriction site (D) *ori* site **[Re-NEET 2024]**

2. Which of the following is used in gene cloning?
(A) Nucleoids (B) Lomasomes
(C) Mesosomes (D) Plasmids
[AIPMT Mains 2010]

3. Manipulation of DNA in genetic engineering became possible due to the discovery of:
(A) restriction endonuclease
(B) DNA ligase
(C) transcriptase
(D) primase. **[AIPMT 2002]**

4. Two bacteria found to be very useful in genetic engineering experiments are:
(A) *Nitrobacter* and *Azotobacter*
(B) *Rhizobium* and *Diplococcus*
(C) *Nitrosomonas* and *Klebsiella*
(D) *Escherichia* and *Agrobacterium*. **[AIPMT 1998]**

5. When scientists make an animal superior by view of genotype, introducing some foreign genes in it, is called:
(A) immunisation (B) genetic engineering
(C) tissue culture (D) biotechnology.
[AIPMT 1996]

6. Organelle/organoid involved in genetic engineering is:
(A) plasmid (B) mitochondrion
(C) golgi apparatus (D) lomasome. **[AIPMT 1994]**

9.2. Tools of Recombinant DNA Technology

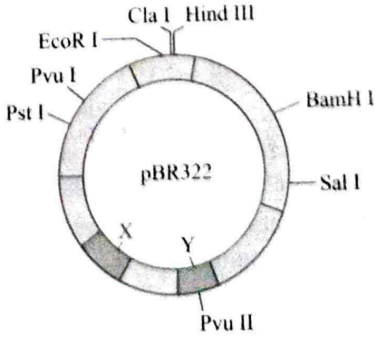
7. Identify the incorrect statement related to gel electrophoresis.
(A) Separated DNA fragments can be directly seen under UV radiation.
(B) Separated DNA can be extracted from get piece.
(C) Fragment of DNA moves toward anode.
(D) Sieving effect of agarose gel helps in separation of DNA fragments. **[Re-NEET 2024]**

8. Select the restriction endonuclease enzymes whose restriction sites are present for the tetracycline resistance (tet^R) gene in the pBR322 cloning vector.
(A) Bam HI and Sal I (B) Sal I and Pst I
(C) Pst I and Pvu I (D) Pvu I and Bam HI
[Re-NEET 2024]

9. Which of the following are correct about EcoRI?
(I) Cut the DNA with blunt end
(II) Cut the DNA with sticky end
(III) Recognises a specific palindromic sequence.
(IV) Cut the DNA between the base G and A when encounters the DNA sequence 'GAATTC'.
(V) Exonuclease
Choose the correct answer from the options given below:
(A) (II), (III), (V) only
(B) (I), (IV), (V) only
(C) (I), (III), (IV) only
(D) (II), (III), (IV) only **[Re-NEET 2024]**

10. Hind II always cuts DNA molecules at a particular point called recognition sequence and it consists of:
(A) 6 bp (B) 4 bp
(C) 10 bp (D) 8 bp **[NEET 2024]**

11. The following diagram showing restriction sites in *E. coli* cloning vector pBR322. Find the role of 'X' and 'Y' genes:



(A) The gene 'X' is responsible for controlling the copy number of the linked DNA and for protein involved in the replication of Plasmid.

(B) The gene 'X' is for protein involved replication of Plasmid and 'Y' for resistance to antibiotics.

(C) Gene 'X' is responsible for recognition sites and 'Y' is responsible for antibiotic resistance.

(D) The gene 'X' is responsible for resistance to antibiotics and 'Y' for protein involved in the replication of Plasmid. **[NEET 2024]**

12. The "Ti plasmid" of *Agrobacterium tumefaciens* stands for:

(A) Tumour independent plasmid

(B) Tumour inducing plasmid

(C) Temperature independent plasmid

(D) Tumour inhibiting plasmid **[NEET 2024]**

13. Upon exposure to UV radiation, DNA stained with ethidium bromide will show:

(A) Bright yellow colour (B) Bright orange colour

(C) Bright red colour (D) Bright blue colour

[NEET 2023, 21]

14. Which of the following is not a cloning vector?

(A) pBR322 (B) Probe

(C) BAC (D) YAC **[NEET 2023]**

15. Main steps in the formation of Recombinant DNA are given below. Arrange these steps in a correct sequence.

(I) Insertion of recombinant DNA into the host cell.

(II) Cutting of DNA at specific location by restriction enzyme.

(III) Isolation of desired DNA fragment.

(IV) Amplification of gene of interest using PCR.

Choose the correct answer from the options given below:

(A) (III), (II), (IV), (I) (B) (II), (IV), (I), (III)

(C) (II), (III), (IV), (I) (D) (III), (I), (II), (IV)

[NEET 2023]

16. During the purification process for recombinant DNA technology, addition of chilled ethanol precipitates out:

(A) Histones (B) Polysaccharides

(C) RNA (D) DNA **[NEET 2023]**

17. In gene gun method, which is used to introduce alien DNA into host cells, microparticles of _____ metal are used.

(A) tungsten or gold (B) silver

(C) copper (D) zinc **[NEET 2023]**

18. Which one of the following statements is not true regarding gel electrophoresis technique?

(A) The separated DNA fragments are stained by using ethidium bromide.

(B) The presence of chromogenic substrate gives blue coloured DNA bands on the gel.

(C) Bright orange coloured bands of DNA can be observed in the gel when exposed to UV light.

(D) The process of extraction of separated DNA strands from gel is called elution. **[NEET 2022]**

19. In the following palindromic base sequences of DNA, which one can be cut easily by particular restriction enzyme?

(A) 5' GAATTC 3'; 3' CTTAAG 5'

(B) 5' CTCAGT 3'; 3' GAGTCA 5'

(C) 5' GTATTC 3'; 3' CATAAG 5'

(D) 5' GATACT 3'; 3' CTATGA 5' **[NEET 2022]**

20. Given below are two statements:

Statement I: Restriction endonucleases recognise 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:

(A) Both statement I and statement II are incorrect.

(B) Statement I is correct but statement II is incorrect.

(C) Statement I is incorrect but statement II is correct.

(D) Both statement I and statement II are correct.

[NEET 2022]

21. Which of the following is not a desirable feature of a cloning vector?

(A) Presence of a marker gene

(B) Presence of single restriction enzyme site

(C) Presence of two or more recognition sites

(D) Presence of origin of replication **[NEET 2022]**

22. A specific recognition sequence identified by endonucleases to make cuts at specific positions within the DNA is:

(A) poly(A) tail sequences

(B) degenerate primer sequence

(C) okazaki sequences

(D) palindromic nucleotide sequences. **[NEET 2021]**

23. In gel electrophoresis, separated DNA fragments can be visualised with the help of:

(A) ethidium bromide in UV radiation

(B) acetocarmine in UV radiation

(C) ethidium bromide in infrared radiation

(D) acetocarmine in bright blue light. **[NEET 2020]**

24. The sequence that controls the copy number of the linked DNA in the vector, is termed as:

(A) *ori* site (B) palindromic sequence

(C) recognition site (D) selectable marker.

[NEET 2020]

25. Identify the wrong statement with regard to restriction enzymes.

(A) They cut the strand of DNA at palindromic sites.

(B) They are useful in genetic engineering.

(C) Sticky ends can be joined by using DNA ligases.

(D) Each restriction enzyme functions by inspecting the length of a DNA sequence. **[NEET 2020]**

26. Choose the correct pair from the following.

(A) Polymerases—Break the DNA into fragments

(B) Nucleases—Separate the two strands of DNA

(C) Exonucleases—Make cuts at specific positions within DNA

(D) Ligases—Join the two DNA molecules

[NEET 2020]

27. The specific palindromic sequence which is recognized by *Eco*RI is:

(A) 5' - GGAACC - 3'
3' - CCTTGG - 5'

(B) 5' - CTTAAG - 3'
3' - GAATTC - 5'

(C) 5' - GGATCC - 3'
3' - CCTAGG - 5'

(D) 5' - GAATTC - 3'
3' - CTTAAG - 5'.

[NEET 2020]

28. Given below are four statements pertaining to separation of DNA fragments using gel electrophoresis. Identify the incorrect statements.

(I) DNA is negatively charged molecule and so it is loaded on gel towards the anode terminal.

(II) DNA fragments travel along the surface of the gel whose concentration does not affect movement of DNA.

(III) Smaller the size of DNA fragment, larger is the distance it travels through it.

(IV) Pure DNA can be visualised directly by exposing to UV radiation.

Choose correct answer from the options given below:

(A) (I), (II) and (IV) (B) (I), (III) and (IV)

(C) (I), (II) and (III) (D) (II), (III) and (IV)

[NEET Odisha 2019]

29. The two antibiotic resistance genes on vector pBR322 are for:

(A) tetracycline and kanamycin

(B) ampicillin and tetracycline

(C) ampicillin and chloramphenicol

(D) chloramphenicol and tetracycline. **[NEET 2019]**

30. Following statements describe the characteristics of the enzyme restriction endonuclease. Identify the incorrect statement.

(A) The enzyme cuts DNA molecule at identified position within the DNA.

(B) The enzyme binds DNA at specific sites and cuts only one of the two strands.

(C) The enzyme cuts the sugar-phosphate backbone at specific sites on each strand.

(D) The enzyme recognizes a specific palindromic nucleotide sequence in the DNA. **[NEET 2019]**

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

(A) protease (B) DNA ligase

(C) endonuclease (D) exonuclease.

[NEET Odisha 2019]

32. A selectable marker is used to:

(A) mark a gene on a chromosome for isolation using restriction enzyme.

(B) help in eliminating the non-transformants, so that the transformants can be regenerated.

(C) identify the gene for a desired trait in an alien organism.

(D) select a suitable vector for transformation in a specific crop. **[NEET Odisha 2019]**

33. What is the criterion for DNA fragments movement on agarose gel during gel electrophoresis?

(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.

[NEET 2017]

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

(A) acetocarmine (B) aniline blue

(C) ethidium bromide (D) bromophenol blue

[NEET 2017]

35. A gene whose expression helps to identify transformed cell is known as:

(A) vector (B) plasmid

(C) structural gene (D) selectable marker.

[NEET 2017]

36. Which of the following is a restriction endonuclease?

(A) Hind II (B) Protease

(C) DNase I (D) RNase **[NEET 2016]**

37. Which of the following is not a feature of the plasmids?

(A) Independent replication

(B) Circular structure

(C) Transferable

(D) Single-stranded **[NEET 2016]**

38. The introduction of t-DNA into plants involves:

(A) altering the pH of the soil, then heat shocking the plants

(B) exposing the plants to cold for a brief period
(C) allowing the plant roots to stand in water
(D) infection of the plant by *Agrobacterium tumefaciens*.

[AIPMT 2015]

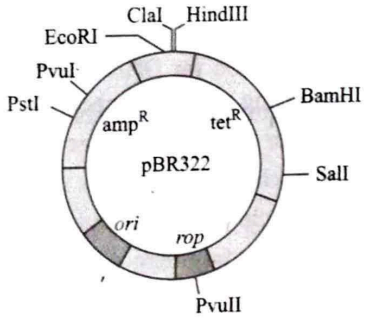
39. The DNA molecule to which the gene of interest is integrated for cloning is called:
(A) transformer (B) vector
(C) template (D) carrier.

[AIPMT Latest July 2015]

40. Genes of interest can be selected from a genomic library by using:
(A) cloning vectors (B) DNA probes
(C) gene targets (D) restriction enzymes.

[NEET Karnataka 2013]

41. The figure below is the diagrammatic representation of the *E. coli* vector pBR322. Which one of the given options correctly identifies its certain components?



(A) *Ori*-original restriction enzyme
(B) *rop*-reduced osmotic pressure
(C) Hind III, Eco RI - selectable markers
(D) amp^R, tet^R - antibiotic resistance genes

[AIPMT Screening 2012]

42. In genetic engineering, the antibiotics are used:
(A) as selectable markers
(B) to select healthy vectors
(C) to keep the cultures free of infection
(D) as sequences from where replication starts.

[AIPMT Mains 2012]

43. For transformation, micro-particles coated with DNA to be bombarded with gene gun are make up of:
(A) silver of platinum (B) platinum of zinc
(C) solicon or platinum (D) gold or tungsten

[AIPMT 2012]

44. Agarose extracted from sea weeds finds use in:
(A) spectrophotometry (B) tissue culture
(C) PCR (D) gel electrophoresis.

[AIPMT 2011]

45. There is a restriction endonuclease called *Eco*RI. What does '*co*' part in it stand for?
(A) Coelom (B) Coenzyme
(C) *coli* (D) Colon

[AIPMT Screening 2011]

46. Which one of the following is used as vector for cloning genes into higher organisms?
(A) Baculovirus (B) *Salmonella typhimurium*
(C) *Rhizopus nigricans* (D) Retrovirus

[AIPMT Screening 2010]

47. Polyethylene glycol method is used for:
(A) gene transfer without a vector
(B) biodiesel production
(C) seedless fruit production
(D) energy production from sewage.

[AIPMT Mains 2010, 09]

48. Restriction endonucleases are enzymes which:
(A) make cuts at specific positions within the DNA molecule
(B) recognize a specific nucleotide sequence for binding of DNA ligase
(C) restrict the action of the enzyme DNA polymerase
(D) remove nucleotides from the ends of the DNA molecule. **[AIPMT Screening 2010]**

49. In genetic engineering, a DNA segment (gene) of interest, is transferred to the host cell through a vector. Consider the following four agents (I-IV) in this regard and select the correct option about which one or more of these can be used as a vector/vectors.
(I) A bacterium (II) Plasmid
(III) Plasmodium (IV) Bacteriophage
Options:
(A) (I), (II) and (IV) only
(B) (I) only
(C) (I) and (III) only
(D) (II) and (IV) only **[AIPMT Mains 2010]**

50. ..... are the enzymes used to cut the DNA from specific site.
(A) Restriction endonuclease
(B) *Agrobacterium*
(C) *Bacillus*
(D) *Nucleotide sequence* **[AIPMT Mains 2009]**

51. Gel electrophoresis is used for:
(A) cutting of DNA into fragments
(B) separation of DNA fragments according to their size
(C) construction of recombinant DNA by joining with cloning vectors
(D) isolation of DNA molecule.

[AIPMT Screening 2008]

52. The linking of antibiotic resistance gene with the plasmid vector became possible with:
(A) DNA ligase (B) endonucleases
(C) DNA polymerase (D) exonucleases.
[AIPMT Screening 2008]

53. Restriction endonucleases:
(A) are present in mammalian cells for degradation of DNA when the cell dies.
(B) are used in genetic engineering for ligating two DNA molecule.
(C) are used for *in-vitro* DNA synthesis.
(D) are synthesized by bacteria as part of their defense mechanism. **[AIPMT 2004]**

54. The Ti plasmid, is often used for making transgenic plants. The plasmid is found in:
(A) *Azotobacter*
(B) *Rhizobium* of the roots of leguminous plants
(C) *Agrobacterium*
(D) Yeast as a 2 µm plasmid. **[AIPMT 2004]**

55. The most thoroughly studied of the known bacteria-plant interactions is the:
(A) cyanobacterial symbiosis with some aquatic ferns.
(B) gall formation on certain angiosperms by *Agrobacterium.*
(C) nodulation of *Sesbania* stems by nitrogen fixing bacteria.
(D) plant growth stimulation by phosphate-solubilising bacteria. **[AIPMT 2004]**

56. Maximum number of bases in plasmids discovered so far:
(A) 50 kilo base (B) 500 kilo base
(C) 5000 kilo base (D) 5 kilo base.
[AIPMT 2001]

9.3. Processes of Recombinant DNA Technology

57. Recombinant DNA molecule can be created normally by cutting the vector DNA and source DNA respectively with:
(A) Hind II, Hind II (B) Hind II, Alu I
(C) Hind II, Eco RI (D) Hind II, BamH I
[Re-NEET 2024]

58. Following are the steps involved in the process of PCR.
(I) Annealing
(II) Amplification (~ 1 billion times)
(III) Denaturation
(IV) Treatment with *Taq* polymerase and deoxynucleotides
(V) Extension
Choose the correct sequence of steps of PCR from the options given below:
(A) (III) → (I) → (IV) → (V) → (II)
(B) (I) → (II) → (V) → (IV) → (III)
(C) (I) → (III) → (V) → (IV) → (II)
(D) (IV) → (II) → (V) → (III) → (I) **[Re-NEET 2024]**

59. What is the fate of a piece of DNA carrying only gene of interest which is transferred into an alien organism?
(I) The piece of DNA would be able to multiply itself independently in the progeny cells of the organism.
(II) It may get integrated into the genome of the recipient.
(III) It may multiply and be inherited along with the host DNA.
(IV) The alien piece of DNA is not an integral part of chromosome.
(V) It shows ability to replicate.
Choose the correct answer from the options given below:
(A) (IV) and (V) only (B) (II) and (III) only
(C) (I) and (V) only (D) (I) and (II) only
[NEET 2024]

60. Which of the following statements is incorrect?
(A) Most commonly used bio-reactors are stirring type.
(B) Bio-reactors are used to produce small scale bacterial cultures.
(C) Bio-reactors have an agitator system, an oxygen delivery system and foam control system.
(D) A bio-reactor provides optimal growth conditions for achieving the desired product. **[NEET 2024]**

61. Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
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:
(A) Both (A) and (R) are correct but (R) is not the correct explanation of (A).
(B) (A) is correct but (R) is not correct.
(C) (A) is not correct but (R) is correct.
(D) Both (A) and (R) are correct and (R) is the correct explanation of (A). **[NEET 2022]**

62. Which of the following is a correct sequence of steps in a PCR (Polymerase Chain Reaction)?
(A) Denaturation, Annealing, Extension
(B) Denaturation, Extension, Annealing
(C) Extension, Denaturation, Annealing
(D) Annealing, Denaturation, Extension
[NEET 2021, 18]

63. Plasmid pBR322 has *Pst* I restriction enzyme site within gene amp^R that confers ampicillin resistance. If this enzyme is used for inserting a gene for β-galactosidase production and the recombinant plasmid is inserted in an *E.coli* strain:

(A) it will not be able to confer ampicillin resistance to the host cell

(B) the transformed cells will have the ability to resist ampicillin as well as produce β-galactosidase

(C) it will lead to lysis of host cell

(D) it will be able to produce a novel protein with dual ability. **[NEET 2021]**

64. 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?

(A) Annealing (B) Extension

(C) Denaturation (D) Ligation **[NEET 2021]**

65. Match the following techniques or instruments with their usage.

Column-I	Column-II
(a) Bioreactor	(i) Separation of DNA fragments
(b) Electrophoresis	(ii) Production of large quantities of products
(c) PCR	(iii) Detection of pathogen, based on antigen-antibody reaction
(d) ELISA	(iv) Amplification of nucleic acids

Select the correct option from following:

	(a)	(b)	(c)	(d)
(A)	(ii)	(i)	(iii)	(iv)
(B)	(iii)	(ii)	(iv)	(i)
(C)	(ii)	(i)	(iv)	(iii)
(D)	(iv)	(iii)	(ii)	(i)

[NEET Phase-II 2020]

66. Match the following enzymes with their functions:

Column-I	Column-II
(a) Restrictio endonuclease	(i) Joins the DNA fragments
(b) Restriction exonuclease	(ii) Extends primers on genomic 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:

	(a)	(b)	(c)	(d)
(A)	(ii)	(iv)	(i)	(iii)
(B)	(iii)	(i)	(iv)	(ii)
(C)	(iii)	(iv)	(i)	(ii)
(D)	(iv)	(iii)	(i)	(ii)

[NEET Odisha 2019]

67. DNA precipitation out of a mixture of biomolecules can be achieved by treatment with:

(A) isopropanol

(B) chilled ethanol

(C) methanol at room temperature

(D) chilled chloroform. **[NEET 2019]**

68. Which one of the following equipments is essentially required for growing microbes on a large scale, for industrial production of enzymes?

(A) BOD incubator

(B) Sludge digester

(C) Industrial oven

(D) Bioreactor **[NEET 2019]**

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

(A) downstream processing

(B) bioprocessing

(C) post production processing

(D) upstream processing. **[NEET 2017]**

70. During the process of isolation of DNA, chilled ethanol is added to:

(A) precipitate DNA

(B) break open the cell to release DNA

(C) facilitate action of restriction enzymes

(D) remove proteins such as histones.

[NEET Karnataka 2013]

71. Which one is a true statement regarding DNA polymerase used in PCR?

(A) It is used to ligate introduced DNA in recipient cells.

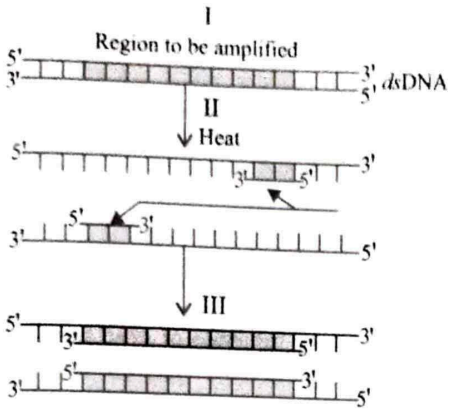
(B) It serves as a selectable marker.

(C) It is isolated from a virus.

(D) It remains active at high temperature.

[AIPMT Screening 2012]

72. The figure shows three steps (I, II, III) of Polymerase Chain Reaction (PCR). Select the option giving correct identification together with what it represents:



(A) II-Denaturation at a temperature of about 98°C separating the two DNA strands

(B) I-Denaturation at a temperature of about 50°C

(C) III-Extension in the presence of heat stable DNA polymerase

(D) I-Annealing with two sets of primers

[AIPMT Mains 2012]

SOLUTIONS

1. (D) The *ori* site (origin of replication) is a location where the duplication of DNA begins. DNA replication is bidirectional, and it operational away from the origin of replication in both directions.

2. (D) Plasmids are used in genetic engineering to amplify certain genes. In molecular cloning, a plasmid is a type of vector that can transport foreign genetic material from one cell to another cell, where the genes can be further expressed and replicated. Plasmids are useful in cloning short segments of DNA and can be used to replicate proteins, such as for insulin. Additionally, plasmids are being investigated as a way to transfer genes into human cells as part of gene therapy.

Related Theory

Chakravarthy bug is a super bug of Pseudomonas with multiple plasmids. Its scientific name is Pseudomonas putida, they are helpful in removing oil spills.

3. (A) Genetic engineering, also called recombinant DNA technology, involves the group of techniques used to cut (by restriction enzymes) and join together (*via* DNA ligase) genetic material, especially DNA from different biological species, and to introduce the resulting hybrid DNA into an organism in order to form new combinations of heritable genetic material.

Related Theory

Restriction endonucleases cut DNA molecules at specific places into many smaller fragments called restriction fragments.

4. (D) Many kinds of bacteria are used in genetic engineering, but the most important ones are *Escherichia coli (E. coli)* and *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens*, which is a naturally occurring bacteria, has the ability to infect plants and transfer some of its own DNA. *E. coli* has also been extensively used for genetic engineering in animals, like in production of humulin, somatotropin, *etc.*

Related Theory

The process of altering the genetic makeup of a host organism using recombinant DNA technology (rDNA technology) is termed as genetic engineering. It involves transferring isolated DNA from the donor organism to the host organism by means of a vector. Vectors should have the ability to replicate inside the host organism without any change in its own genetic makeup

5. (B) Genetic engineering or genetic modification, is the direct manipulation of an organism's genome using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.

6. (A) Small, self-replicating, non-essential chromosomal genetic/DNA components are known as plasmids. A plasmid is made up of a ring of double-stranded naked DNA that is circularly supercoiled and carries genes for replication and one or more extracellular activities. Since they are self-replicating, carry non-essential genes, and have a restriction site for one or more restriction endonucleases, they are excellent vectors for genetic engineering and gene cloning.

7. (A) Separated DNA fragments can not be directly seen under UV radiation as we can't see pure DNA without staining. For visualisation, it first stained with the ethidium bromide then exposed to the UV radiation.

8. (A) The pBR322 plasmid is a commonly used cloning vector in genetic engineering. It contains two antibiotic resistance genes, one for ampicillin (amp^R) and one for tetracycline (tet^R). The restriction enzyme sites are strategically located within these resistance genes. Restriction sites Bam HI and Sal I are within the tetracycline resistance gene (tet^R) of the pBR322 plasmid. Pst I is within the ampicillin resistance gene (amp^R), not the tetracycline resistance gene.

9. (D) Eco R I is a restriction endonuclease that recognises a specific palindromic sequence GAATTC and cuts between G and A in the sequence. This creates sticky ends, not blunt ends.

10. (A) The first restriction endonuclease, Hind II, always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs.

11. (A) Gene X represents origin of replication (*ori*), which is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is responsible for controlling the copy number of the linked DNA.

Gene Y represents *rop* that codes for the proteins involved in the replication of the plasmid.

12. (B) The tumor inducing (Ti) plasmid of *Agrobacterium tumefaciens* has now been modified into a cloning vector, which is no more pathogenic to the plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants.

13. (B) Upon exposure to UV radiation, DNA stained with ethidium bromide will show a bright orange color. This is because ethidium bromide intercalates between the base pairs of DNA, causing a fluorescence that can be visualized under UV light. The orange color is due to the interaction of ethidium bromide with the DNA.

14. (B) Probe is not a cloning vector. It is a fragment of DNA or RNA that can be labelled and used to identify complementary sequences.

15. (A) The correct sequence of steps in the formation of Recombinant DNA is as follows:

(1) Isolation of genetic material *i.e.*, isolation of DNA fragment or genes to be cloned.

(2) Cutting of DNA at specific locations by restriction enzyme.

(3) Amplification of gene of interest using PCR.

(4) Insertion of rDNA into the host cell.

(5) Obtaining foreign gene product *i.e.*, multiplication/expression of the introduced gene in the host.

(6) Downstream processing *i.e.*, separation and purification of the desired product.

16. (D) During the purification process for recombinant DNA technology, addition of chilled ethanol is used to precipitate out DNA from the solution. This is because DNA is insoluble in ethanol and precipitates out of the solution when ethanol is added, while other molecules such as histones and polysaccharides remain in solution.

Related Theory

DNases in the cytoplasm would destroy the DNA of viruses entering the cell. Cold ethanol helps the DNA to precipitate more quickly.

Caution

Students should remember that protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms.

17. (A) The choice of tungsten or gold microparticles is due to their high density and ability to withstand the physical stresses of the gene gun method. These metals are able to penetrate the cell walls and deliver the foreign DNA to the target cells without damaging the cells.

18. (B) Pure DNA pieces must be stained in order to be visible in daylight. Therefore, ethidium bromide is used to stain the isolated DNA fragments, which is followed by UV radiation. When exposed to UV light after staining, bright coloured orange bands of DNA are seen in the gel.

Related Theory

The separated bands of DNA are cut out from agarose gel and extracted from the gel piece. This process is known as elution.

19. (A) Palindromic sequences are a short sequence which means that they read the same both forward and backward. Each restriction enzyme has a different recognition sequence in DNA, which results in variations in the length, sequence and strand orientation (5' end or 3' end) of a sticky-end of an enzyme restriction. The DNA would be sliced exactly in the middle if it were cut with Eco RI. The parts would all be the same size, measuring 15 kb in length. Therefore, the palindrome sequence 5' GAATTC 3' ; 3' CTTAAG 5' can be easily cut at roughly the midpoint by Eco RI enzyme.

20. (D) Restriction endonucleases recognise a certain sequence known as a palindromic nucleotide sequence to cut DNA. When restriction endonuclease recognise the palindromic sequence within the DNA, the DNA strand is slightly cut by restriction endonucleases a little away from the palindromic site's centre.

21. (C) For cloning of DNA, foreign DNA fragments can be put into cloning vectors, which are tiny pieces of DNA. Due to more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning.

22. (D) A specific recognition sequence identified by restriction endonucleases to make cuts at specific site within the DNA is called palindromic nucleotide sequences.

23. (A) The separated DNA fragments after agarose gel electrophoresis can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation as we cannot see pure DNA fragments in the visible light and without staining.

24. (A) *Ori* refers to the sequence from where replication starts and regulates the number of copies of DNA linked with vector.

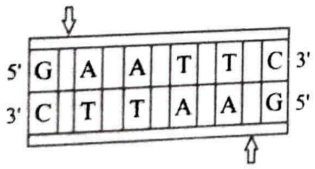
25. (D) Cuts in the DNA are made by restriction endonucleases at specified locations. They do not inspect the length of DNA sequence instead they recognize the palindrome sequence and cut at specific location.

When a restriction endonuclease attaches to DNA, it cuts the double helix's two strands at particular locations in their sugar-phosphate backbones. They help create recombinant DNA molecules in genetic engineering.

DNA ligases reconnect the broken pieces of DNA.

26. (D) Polymerase synthesize the DNA strand. Nucleases break the DNA into fragments. Exonucleases a special type of nucleases enzymes that make cut at the end of the DNA. Ligases are the enzymes that join the two DNA molecules.

27. (D) Eco RI is the restriction enzyme which recognises 6 base pair palindromic sequence and cuts both the strands of DNA at the following sequence:

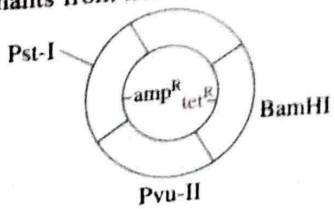


28. (A) DNA fragments are negatively charged molecules, separated by forcing them to move towards positive electrode *i.e.*, anode.

Increasing the concentration of a gel reduces the migration speed of DNA.

DNA fragment can only be visualised under UV light after staining with ethidium bromide and not directly.

29. (B) *E.coli* cloning vector pBR322 contains antibiotic resistance genes for ampicillin and tetracycline. Antibiotic reistance gene are used for selection of transformants from non-transformants.



30. (B) Restriction enzymes cut DNA molecules at a particular point by recognising a specific sequence. 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 backbone.

31. (D) Exonuclease is the enzyme that catalyses the nucleotide removal from the ends of DNA. Exonuclease is the enzyme that breaks phosphodiester links at any location inside the DNA. Endonuclease is an enzyme that breaks phosphodiester links at particular locations inside DNA. Protease is an enzyme that breaks down protein into smaller polypeptides or simple amino acids. Ligases joins two DNA fragments.

32. (B) Selectable markers enable the selective growth of transformants while assisting in the detection and eradication of non-transformants.

33. (A) Agarose gel electrophoresis involves separation of DNA fragments according to the size through sieving effect provided by the agarose gel. The smaller the fragment size, the farther it moves is the criterion for DNA fragments movement on agarose gel during gel electrophoresis.

34. (C) Agarose gel electrophoresis is used to segregate DNA fragments according to the mass and size. Ethidium bromide is the fluorescent stain used in this technique. Ethidium bromide, when exposed to ultraviolet light, produces a fluorescent effect. Hence, the DNA tagged by it can be traced quickly on the transparent gel.

35. (D) Selectable markers in recombinant DNA technology are the particular genes that are employed to separate the transformants from the non-transformants following the recombination process. These genes are used to determine whether a nucleic acid sequence has successfully been incorporated into an organism's DNA. These flag genes are employed in cloning vectors and are resistant to many drugs.

36. (A) A type II restriction enzyme is Hind II. On the basis of subunit composition, cleavage position, sequence specificity, and cofactor requirements, restriction enzymes are often divided into three types- restriction type I, II and III enzyme.

37. (D) Small, circular, double-stranded extrachromosomal DNA is known as a plasmid. It is present in some eukaryotes as well as bacterial cells. Plasmids frequently carry genes that give bacteria genetic advantages like antibiotic resistance. A plasmid is a circular, self-replicating, autonomous dsDNA molecule that only contains a small number of genes.

38. (D) *Agrobacterium tumefaciens*, a gram-negative soil bacterium, infects plants when t-DNA is introduced, leading to crown gall disease. This bacterium is pathogen of several dicot plants.

39. (B) Vector is an agent that can carry a DNA fragment into a host cell. The DNA molecule with gene of interest is integrated with vector for cloning. It is also used for expressing certain gene in the DNA fragment.

Related Theory

A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, that can be stably maintained in an organism and into which a foreign DNA fragment can be inserted for cloning purposes. An ideal cloning vector must have an origin of replication (ori) and should be small in size less than 10 kB. It must be self-replicating inside host cell and must possess restriction site for restriction endonuclease enzymes. It must have the cloning sites for the ligation of the alien DNA which is carried out at the recognition site.

40. (B) Gene bank or genomic library is a complete collection of cloned DNA fragments which comprises the entire genome of an organism. Probes with DNA sequence complementary to the gene to be isolated are used. They bind with the desired gene making it visible and help in isolating it from the library. Cloning vectors are utilized to insert foreign DNA into another cell and create multiple copies of the same. A restriction enzyme is an enzyme that cleaves DNA into fragments at or near specific recognition sites within molecules.

Related Theory

In order to analyse a Southern Blot, a radioactive genetic probe is used in a hybridisation reaction with the DNA. If an X-ray is taken of the Southern Blot after a radioactive probe has been allowed to bind with the denatured DNA on the paper, only the areas where the radioactive probe binds will show themselves on the film (autoradiography). This allow researchers to identify, in a particular person's DNA, the occurrence and frequency of the particular genetic pattern contained in the probe.

41. (D) Origin of Replication (*Ori*) is the DNA sequence where initiation of replication starts. Many antibiotic-resistant genes in bacteria are present in plasmids, which act as a selectable marker for selecting bacteria containing desired plasmid. Multiple Cloning Sites (MCS) are recognition sites to insert foreign DNA fragment by using restriction enzymes, like Hind III, Eco RI.

Related Theory

Vectors produce some colour after reacting with a chromogenic substance. The alternative markers are used for the ease of differentiating recombinants from non-recombinants, e.g., gene coding for β-galactosidase In the presence of a chromogenic substrate, non-recombinants form blue colour colonies and recombinants form colourless colonies.

Caution

Students should remember that in pBR322, 322 stands for the number assigned to segregate it from other type of plasmid or it stands for order of synthesis.

42. (A) In order to distinguish recombinant cells from non-recombinant ones, vectors carry an antibiotic-resistant gene. Only recombinant cells would display antibiotic resistance and would be able to survive on antibiotic-rich medium. Selectable markers are those genes that distinguish recombinant cells from non-recombinant ones and that the host cell needs for growth under specific conditions.

43. (D) Devices used for introducing alien DNA into host cells, include gene guns and biolistic particle delivery systems, which were initially developed for plant transformation. The payload is a plasmid DNA-coated elemental particle of a heavy metal, such as gold or tungsten. Frequently, this method is referred to as a biolistics or a bioballistics.

44. (D) Agar is used to make agarose. Red sea algae like *Gelidium* and *Gracilaria* are used to make agar. In agarose gel electrophoresis, agarose is used as matrix which is a natural polymer extracted from sea weeds.

45. (C) The convention for naming restriction enzymes is the first letter of the name which comes from the bacterial genus; the second two letters come from the species, and the fourth letter from strain, *e.g.*, Eco RI comes from *Escherichia coli* RY13. Roman numbers following the names indicate the order in which the enzymes were isolated.

46. (D) Retroviruses in animals have the ability to transform normal cells into cancerous cells and used as vectors for delivering genes of interest to humans. When a gene or a DNA fragment has been ligated into a suitable retroviral vector it is transferred into a bacterial, plant or animal host (where it multiplies). *Salmonella typhi* is most suitable as a vaccine vector carrying antigens of other pathogens. Baculoviruses is mainly used for high-level transient protein expression in insects and insect cells. *Rhizopus nigricans* is used as host vector system for mass synthesis of protein encoded by gene cloned in plasmid vectors.

47. (A) Polyethylene glycol helps in direct gene transfer method by DNA uptake and utilises the interaction between PEG, naked DNA, salts and the protoplast membrane to effect transport of the DNA into the cytoplasm.

Related Theory

Polyethylene glycol, polyvinyl alcohol and calcium phosphate enhance the uptake of DNA by plant's protoplast. PEG and calcium phosphate are thought to precipitate the DNA onto the outer surface of plasmalemma and the precipitate is taken up by the endocytosis.

48. (A) Restriction endonuclease is a type of restriction enzyme that cuts within the DNA at a specific site. These enzymes cut the strard of DNA a little away from the centre of the palindromic sites, but between the same two bases on the opposite strands. This leaves single stranded portions at the ends.

49. (D) Vectors serve as a medium or vehicle to carry recombinant DNA into the host cell. The most commonly used vectors are plasmids, cosmids and bacteriophages.

Related Theory

Cosmids act as vectors to insert DNA sequences (genes) into the genome of a bacteria, much like a plasmid. Cosmids in recombinant DNA technology, utilize phage (bacterial viruses) to insert DNA sequences of genes with up to 44,000 base pairs while normal plasmids are only able to efficiently carry 10,000 base pairs.

50. (A) Restriction endonuclease cleaves DNA into fragments at or close to particular recognition sites with molecules called restriction site.

51. (B) DNA has a partial negative charge that is attracted to the positive end of the electrical current. Hence, DNA fragments move towards the positive terminal, separating on the basis of their size. Isolation of DNA molecules is done by using restriction enzymes which cut DNA at specific sites. Ligase enzyme is involved in joining the two DNA fragments.

Related Theory

When electric field is applied across two electrodes that are totally submerged in a colloidal solution, the particles (colloid) tend to move towards the electrode depending on their electric charge. This movement of particles under the effect of electric field is known as electrophoresis. When extracted DNA is subjected to electrophoresis following points are observed:

(1) DNA fragments are separated by agarose gel electrophoresis.

(2) DNA fragments being negatively charged move towards positive electrode.

(3) DNA fragments are separated according to size/ charge ratio.

(4) Separated fragments, when stained with ethidium bromide and exposed to UV rays, they form orange-coloured bands.

(5) The bands formed are extracted by elution or southern blotting.

52. (A) DNA ligase is used to seal the nicks that remain in recombinant DNA molecule by joining together the neighbouring nucleotides and forming a phosphodiester bond. Endonucleases are the enzymes that produce internal cuts called cleavage in DNA molecules. Exonucleases are enzymes that remove one or more nucleotide from the free ends. DNA polymerase is the enzyme, which causes polymerisation of nucleotides during DNA replication.

53. (D) Restriction enzymes are degradative enzymes which recognizes and cuts up DNA that is foreign to a cell. These enzymes protect bacteria against intruding DNA from other organisms such as virus or other bacterial cells.

Related Theory

Restriction endonuclease cuts DNA at specific site, where complementary DNA binds. Multiple restriction enzyme increases the specificity of DNA binding sites, giving more reliable results.

54. (C) Ti plasmid is isolated from *Agrobacterium tumefaciens* and has been modified into a cloning vector which is not pathogenic to plant but still is able to use the mechanisms to deliver genes of interest into plants.

55. (B) The *Agrobacterium* is extensively used as natural genetic engineer in plant genetic engineering. It is a pathogen of several dicot plants that deliver a piece of DNA called T-DNA and transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen.

56. (B) Bacterial plasmids are the extrachromosomal DNA present in bacteria. It is capable of replication. The size of plasmids varies from 1- 500 kilobases.

Related Theory

The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952. A plasmid is a double stranded DNA molecule separate from the chromosomal DNA. It usually occurs in bacteria, and is sometimes found in eukaryotic organisms. The size of plasmids varies from 1 to over 400 kilobase pairs (kbp). There may be one copy, for large plasmids, to hundreds of copies of the same plasmid in a single cell.

57. (A) The recombinant DNA molecule can be created normally when we cut the vector and the source DNA with the same restriction enzyme so the Hind II and Hind II is the correct answer, while rest the restriction enzymes are different for cutting the vector and source DNA.

58. (A) The process of Polymerase Chain Reaction (PCR) involves the following steps in the correct sequence:

(1) Denaturation: The double-stranded DNA is heated to separate it into two single strands.

(2) Annealing: The temperature is lowered to allow primers to attach (anneal) to the single-stranded DNA.

(3) Treatment with *Taq* polymerase and deoxynucleotides: This is implicitly included in the extension step, where *Taq* polymerase adds nucleotides to the growing DNA strand.

(4) Extension: *Taq* polymerase synthesises the new DNA strand by adding nucleotides to the primer.

(5) **Amplification (~1 billion times):** This step is the repeated cycling of the denaturation, annealing, and extension to amplify the DNA.

Caution

➥ *Students should remember the significance of temperature, at each step of PCR technique and the specific reason for using Taq polymerase (temperature resistance).*

59. (B) If a piece of DNA carrying only a gene of interest transferred into alien DNA, then the piece of DNA would not be able to multiply itself in the progeny cells of the organism.

But, when it gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA. This is because the alien piece of DNA has become part of a chromosome, which has the ability to replicate, due to the presence of a specific DNA sequence called the origin of replication in the chromosome.

60. (B) Bioreactors are used to process large volumes (100-1000 litres) of bacterial culture to produce the desired product.

Related Theory

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

61. (A) DNA amplification uses the polymerase chain reaction in order to make millions to billions of copies of a specific DNA sample rapidly. By choosing transformants, the ampicillin resistance gene serves as a selectable marker that aids in preventing transformation, identifying and eliminating non-transformants and selectively permitting the growth of transformants.

62. (A) PCR is based on three simple steps required for any DNA synthesis reaction:

(1) Denaturation of the template into single strands.
(2) Annealing of primers to each original strand for new strand synthesis.
(3) Extension of the new DNA strands from the primers.

Related Theory

➥ *PCR is used in molecular biology to make many copies (amplify) of small sections of DNA or a gene.*

63. (A) Insertional inactivation refers to the inactivation of a gene due to the insertion of a DNA segment within its sequence. When a gene coding for β-galactosidase is inserted into the plasmid pBR322 at the Pst I site within the amp^R gene, the ampicillin resistance gene will be inactivated as a result of which it will fail to confer ampicillin resistance to its host. However, β-galactosidase will be produced in the cell as the gene encoding the same will be intact.

64. (C) PCR (Polymerase chain reaction) involves three steps: (i) Denaturation (ii) Annealing and (iii) extension. The initial denaturation step is commonly performed at 94–98°C for 1–3 minutes. The time and temperature of this step can vary depending on the nature of the template DNA and salt concentrations of buffer.

Related Theory

➥ *Initial denaturation occurs for 2 minutes at 94°C. This initiation step heats the double stranded DNA template strand to the point where the strands start denaturing and the hydrogen bonds are broken between the nucleotide base pairs. The initial denaturation step is commonly performed at 94–98°C.*

Caution

➥ *Students should remember that gene amplification is an increase in the number of copies of a gene sequence. Cancer cells sometimes produce multiple copies of genes in response to signals from other cells or their environment.*

65. (C) In order to produce specific gene products for use in industry, cells (from plants, animals, or microorganisms) are cultured on a large scale in bioreactors.

A laboratory technique called gel electrophoresis is based on the idea that charged particles would migrate toward electrodes with opposing charges when an electric field is present.

Short segments of a DNA (nucleic acid) molecule can be amplified using the polymerase chain reaction, which is a technique used in molecular biology.

Enzyme Linked Immuno-sorbent Assay, or ELISA, is the fundamental assay method that is founded on the idea of antigen-antibody interaction.

66. (C) Restriction endonuclease cuts DNA at specific position. Restriction exonuclease removes nucleotides from the ends of DNA. DNA ligase joins the DNA fragments. In PCR *Taq* polymerase extends primers using the nucleotides provided in the reaction and genomic DNA as template.

67. (B) During the isolation of desired gene, chilled ethanol is used for the precipitation of DNA. This can be seen as collection of fine threads in the suspension process called spooling.

68. (D) Bioreactor is essentially required for growing microbes on a large scale for industrial production of enzymes. In bioreactors, raw materials are converted biologically into specific products using microbial plant, animal or human cells.

69. (A) Downstream processing helps in the recovery and purification of biosynthetic products. It is mainly used during large scale production of metabolites.

70. (A) Chilled ethanol is used to precipitate DNA out of a mixture of biomolecules. Low temperature protects DNA by slowing down the activity of enzymes that could break DNA apart while ethanol, being a dehydrating agent removes water molecules bound to DNA and making it visible. Chloroform is used in the extraction process of DNA to remove proteins bound to DNA.

Related Theory

- *There are five basic steps of DNA extraction that are consistent across all the possible DNA purification chemistries:*
  (1) disruption of the cellular structure to create a lysate,
  (2) separation of the soluble DNA from cell debris and other insoluble material,
  (3) binding the DNA of interest to a purification matrix,
  (4) washing proteins and other contaminants away from the matrix and
  (5) elution of the DNA.

71. (D) DNA polymerase is a heat stable enzyme, used for polymerisation or amplification of DNA sequence. It is not used to ligate DNA, instead DNA ligase is used for amplification of DNA, *Taq* polymerase is a thermostable enzyme isolated from bacterium *Thermus aquaticus*.

Related Theory

- *Taq DNA polymerase, isolated from Thermus aquaticus, is a thermostable DNA polymerase that catalyses the primer-dependent incorporation of nucleotides into duplex DNA in the 5′ → 3′ direction in the presence of Mg^{2+}. It is the standard thermostable DNA polymerase used in PCR applications. Taq does not possess 3′ → 5′ exonuclease activity but has 5′ → 3′ exonuclease activity.*

72. (C) Polymerase chain reaction involves *in-vitro* synthesis of DNA using two sets of primers and enzyme DNA polymerase. There are three steps in polymerase chain reaction namely.
(1) Denaturation at 94°C for DNA strand separation.
(2) Annealing for binding of primer at 60°C.
(3) Extension of primers in presence of DNA polymerase which is heat stable at 72°C.

Related Theory

- *In contrast to cellular DNA replication, which amplifies all of a cell's DNA during a replication cycle, PCR targets amplification to replicate only a segment of DNA bounded by the two primers that determine where DNA polymerase begins replication. PCR essentially mimics cellular DNA replication in the test tube, repeatedly copying the target DNA over and over, to produce large quantities of the desired DNA.*